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Polarized light in communication and behavior of two fish species

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Polarized light in communication and behavior of two fish species

by

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Abstract

Polarized light in communication and behavior of two fish species

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Abstract

Many animals can see polarization of light (a property to which humans are visually insensitive) and use polarization for a variety of behavioral tasks such as navigation and foraging. The polarized light environment is spatially and temporally complex, presenting a unique challenge for signaling or crypsis in animals with polarization vision. Some invertebrates have polarization body patterning that may be used in communication, but only in one species has polarization body patterning been shown to affect receiver behavior, and polarization communication has never been investigated in vertebrates. Many species of fish see polarized light and the aquatic environment is highly polarized; body patterning in visual communication is also common in fish. We measured polarization patterning in the northern swordtail (*Xiphophorus nigrensis*) and used behavioral assays to measure response to polarization cues of social stimuli in the swordtail and in the rockhind (*Epinephelus adscensionis*).

We found that swordtails have sexually dimorphic polarization patterning. By manipulating the light environment of stimulus males in a two-choice female preference test, we presented females a highly-polarized male and a male with reduced polarization patterning. Females preferred the polarized male, indicating that polarization patterning

functions as a sexual signal in swordtails. We measured polarization patterning of swordtails alone and in social contexts, and did not find evidence that swordtails modulate their polarization patterning according to social condition.

Rockhinds use color patterning in social dominance interactions and live in highly polarized environments in the Gulf of Mexico. We presented rockhinds with social stimulus images (e.g. images of displaying males, females) and measured behavioral response in two assays. In one assay, the images were not manipulated and thus composed of color, luminance and polarization contrast (as is typical of images displayed with LCD monitors). In the other assay, we manipulated the monitor to remove color and luminance contrast, leaving images of only polarization contrast (invisible to humans and other viewers without polarization vision). Rockhind behavior differed between control conditions (no image displayed) and treatment (social images displayed) for both the complete visual information assay and the polarization-only assay, indicating that they can respond to social stimuli when only polarization cues are present. For most behaviors, response did not differ between the two assay types. Rockhinds responded differently to the different social images for both assays. We find evidence that both swordtails and rockhinds use polarization cues in social behavior, and that polarization patterning functions as a sexual signal in swordtails.

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Chapter 1: General Introduction

Understanding how an animal perceives its environment is crucial to understanding the function and evolution of its signaling and communication (Endler 1992, Bradbury and Vehrencamp 2011). By quantifying signal production, environmental transmission, and perception, researchers have elucidated how selection by receivers' sensory and neural processing system features (Ryan and Rand 1990), phylogenetic history (Basolo 1996), and environmental properties (Gomez 2004, Seehausen et al. 2008) contribute to the evolution of signals. Research on the perception of visual signals in particular has benefitted from a variety of approaches, including electrophysiological recording that has allowed us to understand how visual information is processed in the retina (Blackstrom and Reuter 1974), the optic tectum (O'Benar 1976), and the visual cortex (Hubel and Wiesel 1959, 1962); visual modeling that uses this processing information as well as measures of the light environment to predict how signals are detected in different environments and by different viewers (Vorobyev and Osorio 1998; Crothers and Cummings 2013); and manipulative experiments that test signal function in natural behaviors such as mate choice and male rivalry contests (Cummings et al. 2003, Crothers et al. 2011). While the study of color and brightness in visual signaling has benefitted enormously from these approaches, polarization in animal communication has been studied only a handful of times and remains poorly understood (Mathgar et al. 2009).

Polarization refers to the organization of the orientation of the waveform of light; if all the light reaching a detector is oscillating in the same plane, the light is fully polarized, whereas if the plane of oscillation is random, the light is unpolarized (Fig. 1). In nature, partially polarized light is common in environments where small particles scatter sunlight (for example, aquatic environments [Waterman 1954, Ivanoff and Waterman 1958, Cronin and Shashar 2001, You et al. 2011] and the sky as seen from below [Gal et al. 2001, Cronin and Marshall 2011]). Animals of many taxa can detect polarization; this ability is common among invertebrate taxa (bees: von Frisch 1949;

flies: von Phillipsborn and Labhart 1990, Horvath et al. 2008; beetles: Warrant 2010; butterflies: Sweeney et al. 2003, Douglas et al. 2007; odonates: Kriska et al. 2009; mayflies: Kriska et al. 2007; locusts: Shashar et al. 2005; daphnia: Schwind 1999; cephalopods: Shashar et al. 2000; shrimp: Goddard and Forward 1991; Ritz 1991; stomatopods: Marshall et al. 1999, Chiou et al. 2008) but has been found in several vertebrate taxa as well (salamanders: Taylor & Adler 1973; birds: Muheim et al. 2006; and fish: Parkyn and Hawryshyn 1993).

Animals use polarization vision for a variety of behavioral tasks. Because Rayleigh scattering in the sky creates a polarization pattern dependent on solar angle, many animals navigate using this pattern (Goddard and Forward 1991, Ritz 1991, Schwind 1999, Shashar et al. 2005) or use it to calibrate their magnetic compass (Muheim et al. 2006). The polarization differences between the near-shore and limnetic light environment are used by *Daphnia* to remain in the center of the lake (a behavior known as 'shore flight': Schwind 1999), and the highly polarized light reflected from water bodies is used by odonates to locate these sites (Wildermuth 1998). Polarization cues are used during foraging by juvenile rainbow trout (Flamarique & Browman 2001) and in cuttlefish (Shashar et al. 2000). Some animals may use polarization vision and patterns in communication (Sweeney et al. 2003), but research in this area has been inconclusive in many cases (Mathgar et al. 2009). Without studying polarization patterning and communication in animals that see polarized light, we may be missing or misunderstanding important behavioral features. By analogy, Blue Tits were long thought to have sexually monomorphic plumage coloration, but when ultraviolet (UV) plumage reflectance was measured, Andersson et al. (1998) found sexually dimorphic plumage on the crown, and that Blue Tits were mating assortatively with respect to this trait. The study of polarization patterning and communication for animals that see polarized light may be even more revelatory. While UV reflectance and vision is important in communication of many species (Cummings et al. 2003; Obara et al. 2008; Alonso-Alvarez et al. 2004; Whiting et al. 2006), such studies are an extension of an existing literature and experimental framework of color vision and signaling. The

polarized light environment, in contrast, is much more spatially complex and variable than the color-brightness light environment (Cronin and Marshall 2011, Brady et al. 2013), and little is known about polarization visual processing in vertebrates (Horvath and Varju 2004, Kamermans and Hawryshyn 2011; but see Flamarique and Harosi 2002, Flamarique 2011 for an exception).

Polarized light environments, such as the aquatic environment, present unique challenges for signaling and crypsis due to their spatial complexity (Brady et al. 2013; Fig. 2). Because the angle of polarization (the angle of the plane in which most of the light waves are oscillating) is determined by the angle between the sun and the scattering particle or reflecting surface, and the angle between the scattering particle or reflecting surface and the viewer, the amount and the angle of background polarization seen by a viewer in an aquatic environment changes throughout the day as the solar position changes, and background polarization also changes with slight changes in viewing angle (Waterman 1954, Cronin and Marshall 2011, Brady et al. 2013; Fig. 2). At midday conditions, the polarized light environment is axially symmetric in water: as a viewer moves around an object in the horizontal plane, background polarization features are consistent (Fig. 2A). However, if the viewer moves out of the horizontal plane, the amount of background polarization decreases (Fig. 2A). Thus, slight changes in viewing angle can cause an object that was cryptic with respect to the polarization background to suddenly be conspicuous, or vice versa. The polarization background is much more complex at low solar angles such as crepuscular times (Cronin and Marshall 2011, Brady et al. 2013; Fig. 2B). At these times the polarization background is not axially symmetric, and as a viewer moves around an object in the horizontal plane, the amount of background polarization increases and decreases drastically, while the angle of polarization also changes across 360 degrees (Brady et al. 2013; Fig. 2B).

While highly complex, this polarization background is predictable: the angle and amount of polarization in the background water column can be predicted given the position of the viewer and the viewing angle relative to the sun (Cronin and Marhsall 2011). How animals respond to the challenge of crypsis in this environment is just

beginning to be studied, and a complex, non-random polarized reflecting surface that reduces contrast measured across all viewing angles has been found in the one fish species (*Selene vomer*: Brady et al. 2013). In addition to this spatially complex polarization background, changes in the angle of a signaler with respect to a viewer will change the polarization angle seen by the viewer, similar to the way viewers see different colors from iridescent signalers as they move. Thus, maintaining contrast with the spatially complex polarization background while signaling to a viewer could be extremely challenging, and even more so at crepuscular time periods. The evolution of signals is often affected by selection from multiple viewers (Tuttle and Ryan 1981; Cummings and Crothers 2013), including selection for signals that are cryptic to predators in some cases (Cummings et al. 2003; Sieback 2004). [Distinguishing between signals, which evolved under selection from receiver response, and cues, which affect receiver response but may have evolved through processes other than selection from receivers, is often difficult. Because little is known about the evolutionary history of polarization body patterning, we will refer to such patterns as signals if they affect receiver response in a way that is relevant to sender fitness]. Looking for polarization patterning and communication in aquatic animals may provide insight into how animals have responded to selection from predators and conspecifics in this highly variable, spatially complex light environment.

Polarization patterning has been documented in a number of invertebrate species and has been suggested to function in communication (Stomatopods: Marshall et al. 1999, Chiou et al. 2008; Cephalopods: Shashar and Hanlon 1997, Boal et al. 2004, see Mathger et al. 2009 for review; Butterflies: Sweeney et al. 2003). Because signals evolve due to selection from receiver responses, many definitions of communication signals include that signals affect receiver behavior (Alcock 1984, Owren et al. 2010, Mathger et al. 2009). An effect of polarization pattern on receiver response has only been found in one species, *Heliconius cydno,* in which males visited female wing specimens more frequently when polarization patterning was intact than when it was removed by a filter (Sweeney et al. 2003). Some stomatopods have sexually dimorphic polarization

patterning on the telson, and have the ability to discriminate different polarization angles including handedness of circular polarization, but the function of this patterning in a communication context has never been tested (Marshall et al. 1999; Chiou et al. 2008). Cephalopods show striking and dynamic polarization body patterns that can be rapidly changed or turned on or off (Shashar et al. 1996; Shashar and Hanlon 1997; Boal et al. 2004) but this patterning has not been demonstrated to function in communication. Boal et al. (2004) found no differences in the polarization patterns displayed by cuttlefish when alone versus with a receiver, and no difference in behaviors of pairs of cuttlefish that viewed each other through barriers that distorted polarization patterns and barriers that left polarization patterns intact (Boal et al. 2004). They did, however, find an association between polarization patterns and the color patterns characterized as 'cryptic' by human observers (Boal et al. 2004). While the polarization patterns of stomatopods and cepahlopods are frequently referred to as communication signals in literature (Mathger and Hanlon 2006; Mathger et al. 2009) it is possible that these patterns function to enhance crypsis, or are incidentally produced by the physical arrangement of chromatophores and skin tissues to create cryptic color and texture patterning.

Polarization patterning and communication have not previously been demonstrated in vertebrates. While vertebrates from several taxa are sensitive to polarized light (Taylor & Adler 1973, Muheim et al. 2006, Parkyn and Hawryshyn 1993), polarization-mediated behavior is less well studied in vertebrates. This may be because polarization vision seems to be less prevalent in vertebrates, or because the mechanisms by which they see and process polarized light are poorly understood (Horvath and Varju 2004). In invertebrate eyes, rhabdomeric photoreceptors in the dorsal rim area are specialized such that photopigment molecules are aligned parallel to one another within the microvilli, with two orthogonal orientations present in each ommatidium (Menzel and Snyder 1974; Schinz 1975; Labhart & Meyer 1999). This arrangement creates orthogonal polarization detectors within an ommatidium, allowing an ommatidium to respond preferentially to a particular angle of polarization (Labhart & Meyer 1999). [Interestingly, there is often correspondence between the color sensitivity and the

polarization angle sensitivity of ommatidia, which has caused speculation about whether achromatic polarized light might cause stimulate color visual pathways, causing insects to experience polarization 'false color' vision (Kelber 1999, Kelber et al. 2001)].

In vertebrates, photopigments are typically aligned randomly within the plane of the discs, making the photoreceptor insensitive to polarization angle of incoming light, but possibly sensitive to polarization angle of light striking perpendicular to the plane of the discs [but see the anchovy (Flamarique 2011) for an exception]. Most of the mechanistic study of polarization vision has been done in teleosts. Physiological evidence for polarization sensitivity has been observed in at least five teleost families (Carangidae, Centrarchidae, Cichlidae, Cyprinidae, and Salmonidae: summarized in Parkyn and Hawryshyn 1993) using a variety of techniques including heart-rate conditioning, single-unit recording in the optic tectum, and optic ganglion cell recording. Behavioral evidence for polarization sensitivity in the form of operant conditioning to orient to particular polarization angles (Pomacentridae: Parkyn et al. 2003; Cichlidae: Davitz and MacKaye 1978; Salmonidae: Hawryshyn et al. 1990, Hawryshyn and Bolger 1990, Ramsden et al. 2008) and innate orientation (Cyprinidae: Hawryshyn and McFarland 1987; Hemiramphidae: Forward and Waterman 1972, Forward et al. 1972) further supports polarization sensitivity in fishes. While the mechanism of polarization sensitivity at the level of the photoreceptor is not conclusively know in cases other than the anchovy (Flamarique 2011), electrophysiological measurements indicate that different cone classes are sensitive to particular polarization angles (Parkyn and Hawryshyn 1993), and polarization sensitivity has also been observed at higher processing levels, including polarization sensitivity in the optic tectum (Cyprinidae: Waterman and Aoki 1974). Flamarique et al. (1998) suggest that retinal polarization sensitivity in salmonids arises from the matrix arrangement of UV cones and red-green double cones. In this model, light is partially refracted by the bulge in the red-green double cone and obliquely strikes neighboring photoreceptors, with the angle of refraction—and thus the class of photoreceptor that the refracted light strikes—dependent on the angle of polarization (Flamarique et al. 1998). Polarization sensitivity in the

salmonid *O. mykiss* has also been observed from both electroretinograms (ERG) and compound action potentials (CAP) in the optic nerve (Ramsden et al. 2008). These measurements, combined with pharmacological manipulation, reveal opponent and non-opponent processing channels that create sensitivity to specific polarization angles (Ramsden et al. 2008), and support earlier findings that different cone classes are sensitive to different polarization angles (Parkyn and Hawryshyn 1993). ERG and CAP measurements in another salmonid, *Salmo salar* (Hawryshyn et al. 2010) reveal similar polarization angle sensitivities for the cone classes, and similar opponent and non-opponent processing channels. Polarization sensitivity in salmonids is used for a variety of behavioral tasks including orientation (Hawryshyn et al. 1990, Hawryshyn and Bolger 1990), navigation (Hawryshyn 2010), and foraging (Flamarique and Browman 2001).

Given the evidence for polarization sensitivity (Parkyn and Hawryshyn 1993) and behavioral use (Hawryshyn et al. 1990, Hawryshyn and Bolger 1990, Flamarique and Browman 2001, Hawryshyn 2010) in teleosts, the complex yet predictable aquatic polarization environment (Cronin and Marshall 2011, Brady et al. 2013), and the widespread use of visual communication in fishes (Rowland et al. 1995, Kodric-Brown and Nicoletto 2001, Cummings et al. 2003, Carleton et al. 2006, Karion 2010, Kline et al. 2011**)**, it is reasonable to ask whether fish use polarized light in visual communication. I studied polarization patterning and its use in communication in two fish species, the freshwater guppy *Xiphophorus nigrensis* and the marine grouper *Epinephelus adscensionis*. *X. nigrensis* is a model organism in behavioral ecology, wherein courting males use visual displays and ornaments to attract females (Ryan and Rosenthal 2001). *E. adscensionis* is a protogynous grouper in which males defend territories and their position in the social hierarchy with dynamic pattern displays (Kline et al. 2011). In each swordtails, I measured polarization patterning in social and asocial conditions, and both swordtails and rockhinds I used behavioral assays to test whether polarization cues affect receiver behavior in signaling interactions. Swordtails exhibit sexual dimorphism in polarization body patterning, and female preference for males with enhanced polarization patterning vs. diminished polarization patterning. I found no evidence that male

swordtails modulate their polarization patterning among social contexts over short time periods. Rockhinds use polarization vision to respond to a variety of social stimuli, and respond similarly when using only polarization vision (color and brightness contrast removed from stimulus images, leaving only polarization contrast) and when using all visual information (stimulus images composed of color, brightness and polarization contrast).

Chapter 2: Polarization Signaling in Swordtails Alters Female Mate Preference[1]

ABSTRACT

Polarization of light —and visual sensitivity to it—is pervasive across aquatic and terrestrial environments. Documentation of invertebrate use of polarized light is widespread from navigation and foraging to mate-recognition. However, polarization body patterning has rarely been shown to act as a signal by affecting receiver behavior, and studies of polarization patterning and communication in vertebrates are conspicuously missing. Here we investigate polarization-mediated communication by northern swordtails, *Xiphophorus nigrensis*, using a custom-built videopolarimeter to measure polarization signals and a novel experimental paradigm that manipulates polarization signals without modifying their brightness or color. We conducted mate choice trials in an experimental tank that illuminates a pair of males with light passed through a polarization and a diffusion filter. By alternating the order of these filters between males, we presented females with live males that differed in polarization reflectance by > 200%, but with intensity and color differences below detection thresholds (≤6%). Combining videopolarimetry and polarization-manipulated mate choice trials, we found sexually dimorphic polarized reflectance and polarization-dependent mate choice behavior. Male swordtails exhibit greater polarization contrast than females, and females preferentially associate with high polarization-reflecting males. However, we found no evidence that males also adjust polarization signals based on social context over short timescales. Polarization cues in mate choice contexts may provide aquatic vertebrates with enhanced detection of specific display features (e.g.,

[1]This chapter was in review at the time of publication of this thesis. The citation for this chapter in review is:

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movements or angular information), as well as a signaling mechanism that may enhance detection by intended viewers while minimizing detection by others.

INTRODUCTION

When sensory systems evolve to detect environmental properties, the opportunity arises for the evolution of signals that utilize these properties (Endler 1992, Endler and Basolo 1998, Ryan and Cummings 2013). The complex interaction of light with atmospheric and underwater particles leads to predictable polarization backgrounds in terrestrial and aquatic environments (Waterman 1954, Ivanoff and Waterman 1958, Shashar et al. 1998, Conin and Shashar 2001, Treibitz and Shechner 2009, You et al. 2011). In brief, the term 'polarization' refers to the vibrational behavior of the electromagnetic field, with unpolarized light describing photons vibrating in all possible directions (e.g. sunlight prior to entering our atmosphere), and plane polarized light occurring when one particular orientation is more prevalent (e.g. light interacting with water vapor in our atmosphere). Karl von Frisch (1949) was the first to demonstrate that bees use polarization gradients in the sky as a sky compass. Since 1949, researchers have determined that many invertebrates use polarization cues for celestial orientation (Wehner 1989, Dacke et al. 2003, Horvath and Varju 2004), navigation (Goddard and Forward 1991, Ritz 1991, Schwind 1999, Shashar et al. 2005), foraging (Shashar et al. 2000), and mate recognition (Sweeney et al. 2003), and have identified an angular distribution of linear polarized detectors that is responsible for polarization sensitivity in many invertebrate eyes (Labhart and Meyer 1999). While polarization body patterning has been measured in a variety of taxa [cephalopods (Shashar and Cronin 1996, Shashar and Hanlon 1997, Boal et al. 2004, Mathger and Hanlon 2006); stomatopods (Marshall et al. 1999, Chiou et al. 2008); butterflies (Sweeney et al. 2003)], polarization patterning has only been shown to affect receiver behavior, and thus function as a signal, in one species (Sweeney et al. 2003).

In contrast to the widespread documentation of polarization-mediated behavior by invertebrates, research into polarization-mediated behavior by vertebrates is more

limited, despite ample behavioral evidence for vertebrate polarization sensitivity (Taylor and Adler 1973, Cameron and Pugh 1991, Parkyn and Hawryshyn 1993, Flamarique and Browman 2001, Muheim et al. 2006). This may be due in part to the current lack of a mechanistic understanding for vertebrate polarization sensitivity at the level of the photoreceptor, or that vertebrates do not rely on polarization to the same degree that invertebrates do. In general, vertebrate eyes do not share the unique geometric arrangements that compound eyes afford invertebrates for plane polarization detection. Vertebrate photopigments are randomly oriented within parallel photoreceptors [with the exception of the anchovy, (Flamarique 2011)], hence vertebrate polarization sensitivity likely employs a different mechanism for detection than invertebrates [see (Hawryshyn 2010, Roberts et al. 2011) for reviews of current hypotheses]. Despite differences in mechanisms, there is ample behavioral evidence that many non-mammalian vertebrates, particularly fish, have polarization sensitivity from behavioral and physiological training experiments (Taylor and Adler 1973, Davitz and McKaye 1978, Hawryshyn and Mcfarland 1987, Hawryshyn et al. 1990, Hawryshyn and Bolger 1990, Parkyn et al. 2003, Mussi et al. 2005), as well as direct cellular recordings in the retina (Ramsden et al. 2008, Hawryshyn et al. 2010), optic nerve (Parkyn and Hawryshyn 1993, Ramsden et al. 2008, Hawryshyn et al. 2010), and optic tectum (Waterman and Hashimoto 1974, Waterman and Aoki 1974). However, there have been no studies to date of vertebrates relying on polarization cues to communicate.

Here we investigate whether a fish that inhabits the near-surface freshwater environment uses polarization-mediated signaling in mate choice contexts. The aquatic environment of the northern swordtail, *Xiphophorus nigrensis*, shares many polarization features with the sky, as both environments develop a polarization gradient due to scattering interactions between light and water molecules that is dependent on the location of the sun (Waterman 2006). The near-surface underwater environment is characterized by a high Degree of Linear Polarization [DoLP; the fraction of light that is polarized (Shashar et al. 2004)], that varies in terms of its plane of orientation (You et al. 2011). To evaluate the plane of polarized light, we use two Stokes parameters, Q and U.

Q measures the polarization along the horizontal-vertical axes, and U measures the polarization associated with the axes rotated 45° from the horizontal-vertical axes (Fig. 1). These parameters provide angular information of the polarization light field and are detected by comparing output from orthogonal polarization detectors; which is consistent with the retinal opponency processing measured in fish stimulated with polarized light (Ramsden et al. 2008). For instance, salmonid photoreceptors are sensitive to light polarized at +Q or -Q, and horizontal cells integrate input from these two classes to produce retinal sensitivity to light polarized at +U and -U (Ramsden et al. 2008).

Swordtails are a highly tractable system for studying visual mate preference behavior in the lab (Cummings 2012). For decades, researchers have successfully tracked female preferences for male visual stimuli in swordtails (Ryan and Wagner 1987, Basolo 1990, Rosenthal and Evans 1998, Cummings et al. 2003) using a simple measure of association time that significantly predicts female mating intent (Walling et al. 2010). *Xiphophorus nigrensis* exhibit a female-choice-dominant mating system with three male phenotypes: large, ornamented males that court females; small, non-ornamented males that rely on chase copulations; and intermediate-size males that employ a combination court-chase strategy (Ryan and Causey 1989). Female *X. nigrensis* prefer large male *X. nigrensis* over the small male class (Ryan and Causey 1989, Cummings and Mollaghan 2006) and prefer large males with UV ornamentation over large males without UV ornamentation (Cummings et al. 2003). Hence, the northern swordtail is an ideal model system to characterize whether males have polarization ornamentation, and if present, to manipulate such ornamentation and quantify the behavioral results in terms of female mate preference.

Determining whether polarization ornamentation serves a signaling function in an organism requires showing (1) polarization signal production by a sender, (2) detection of the signal by a receiver, and (3) change in receiver behavior that is adaptive to sender or receiver (Mathger et al. 2009). While polarization body patterning (step 1) has been described in several invertebrate species [cephalopods: see (Shashar and Hanlon 1997, Boal et al. 2004, Mathger and Hanlon 2006), and (Mathger et al. 2009) for a review;

stomatopods (Marshall et al. 1999, Chiou te al. 2008); butterflies (Sweeney et al. 2003, Douglas et al. 2007)], evidence for adaptive behavioral responses by receivers (steps 2 and 3) has been limited in invertebrates (Sweeney et al. 2003), and none of these steps has been addressed in vertebrates. In the present study, we used a combination of physical measurements and behavioral experiments to identify polarization signaling in *X. nigrensis*.

To determine whether *X. nigrensis* use polarization cues for communication, we first compared polarization patterning between males and females with a custom-built videopolarimeter (Gruev et al. 2010), calculating polarization contrast for DoLP, Q and U (Fig. 3). We then quantified female mate preference response to large males with altered polarization-reflecting ornamentation using a mate choice assay that predicts female mating intent (Walling et al. 2010). We tested whether females prefer males with high polarization ornamentation over males with low polarization ornamentation by using a combination of linear polarizers and diffusion tanks to manipulate the polarization of males' light environment. The high-polarization treatment significantly increases the polarization of males by 220% to 286% relative to the low polarization treatment (see Fig. 4A, Table 1) while altering signal color and intensity below detection thresholds (Table 1, median hue, saturation and intensity differences (≤6%; differences of this magnitude are not detectable in fish [Hawryshyn 1991]). By significantly altering the polarization of signaling males, while keeping variation in signal color and intensity below visual detection thresholds (Hawryshyn 1991), we can isolate the female mating response to differences in the polarization features of the male. Finally, we measured the polarization contrast features of large males swimming alone relative to social conditions to determine whether polarization features differed by social context. We found evidence that *X. nigrensis* use polarization cues for communication with our measurements of

sexually dimorphic polarized ornamentation and differential female response towards males with high polarization contrast, but did not find evidence that these signals change across communication contexts (Fig. 5).

METHODS

Swordtails were collected from Brackenridge Field Laboratory populations stocked with *X. nigrensis* from Nacimiento Choy, San Luis Potosí, Mexico.

Reflectance measurements

Large males (n=12), intermediate males (n=3), small males (n=2), and females (n=17) were filmed with a videopolarimeter for 5-min trials while illuminated with front- and side-welling light horizontally polarized by a filter (polarization.com) to mimic midday underwater conditions (high DoLP, high Q, and minimal U) (You et al. 2011). Black felt lining the tank reduced spurious Fresnel reflections. The videopolarimeter was positioned 25° to the normal of the tank wall with black cloth blocking light from the front-welling source to minimize glare and polarization artifacts. Each large male and female was filmed alone, and large male was filmed with a stimulus fish of each phenotype. Median DoLP ($\frac{\sqrt{Q^2 + U^2}}{I}$, where I = total intensity), Q (the proportion of polarization along the horizontal-vertical axes), and U (the proportion of polarization associated with the axes rotated 45° from the horizontal-vertical axes) for fish regions and background were calculated with custom IGOR-PRO programs from selected frames (averaging up to 5 frames per video) that met positional criteria (fish's long axis perpendicular to camera and within 15° of horizontal). Values from the frames selected within a single trial, for an individual fish, were averaged. Contrast for a polarization parameter (DoLP, Q or U) was calculated as the difference in that parameter between two regions (either two body regions or a body region and a background region). Comparisons of each polarization parameter were made between large males and females for each body region (Fig. 3A, Fig. 5, Table 2A) and for each contrast measure (Fig. 3B-D, Table 2B). Within the reflectance data for large males, comparisons of each

polarization parameter and contrast measure were made across social conditions (Fig. 5; Fig. 7; Fig. 8; Table 3). *t*-Tests were used for all comparisons; and corrected for multiple comparisons with a Benjamini-Hochberg correction.

Behavioral experiment

Swordtails (n=28 female subjects; 9 large males as stimuli) were isolated for at least one week prior to preference testing to ensure motivation to mate. Males were size-matched to form 6 pairs, each of which was used to test 3-7 females.

Females were presented with two side-by-side male chambers (Fig. 4B). Each male was illuminated from the front and side by a visible-range bulb (Sylvania-Capsylite 120W/120V Spot) and a UV-visible-range bulb (Reptile-UV 160W/120V MegaRay Zoologist). Diffusion tanks (2-gallon tank of an aqueous dispersion of magnesium hydroxide, a 1:277 dilution of Maalox) depolarized source light; UV-transmissive horizontal polarizers (Bolder Vision Optik, Boulder, CO) polarized source light. For high-DoLP illumination, the polarizer was placed in front of the diffusion tank such that light was diffused and subsequently polarized before reaching the experimental tank (Fig. 4B, left side; polarization standard mean DoLP=23.5±11.2%, max DoLP=61.7%, for polarization standard across five tank regions and three viewing angles). For low-DoLP illumination, the polarizer was placed behind the diffusion tank so that light was polarized but subsequently depolarized before reaching the experimental tank (Fig. 4B, right side; polarization standard mean DoLP=5.38±2.43%, max DoLP=11.2%).

To determine how the high- and low- DoLP illumination conditions affected male swordtail visual signals, we measured hue, saturation, luminance, and DoLP values of body regions from two stationary large *X. nigrensis* males in high-DoLP and low-DoLP illumination conditions with a videopolarimeter (images analyzed in custom IGOR programs) and an Olympus Stylus Tough TG-830 underwater camera (images analyzed in ImageJ). Males were positioned to mimic their 'lateral display' during courtship bouts, with the fish's long axis perpendicular to a potential viewer; hence, these measurements

should represent one of the most biologically relevant views to females. Each male was recorded in the high-DoLP condition and low-DoLP conditions 4 times (alternating the order) for up to one minute. Our measurements indicate that hue, saturation, and luminance were statistically invariant across high- and low-DoLP illumination for all measures except two (luminance of dorsum and hue of fin base), which differed significantly, but differences were below detection thresholds (Table 1; Hawryshyn 1991). DoLP values differed significantly for each body region, and at differences much higher than probable detection thresholds (>200% change; Table 1).

DoLP condition alternated sides between trials, as did the side of each male in a pair. Males could not see one another; females could swim throughout association, neutral, and back zones and interact with males through a glass barrier. Females were given a 10-min control period (during which males were behind opaque barriers) to test for preference of polarization conditions in absence of male stimuli, followed by a 10-min preference test (males in front of barriers and visible to females). Trials were filmed and videos scored for time females spent in each zone and males' interaction time (time spent moving within the front portion of his chamber—the 8-cm portion directly adjacent to the female chamber in Fig. 4B), blind to polarization condition.

RESULTS

Sexual dimorphism in polarization patterning

We filmed free swimming females (n=17) and courter males (n=12) with a videopolarimeter under horizontally polarized illumination (natural shallow-water polarization conditions [9]) in order to quantify DoLP, Q and U reflectance from the fish and from background. Because polarization properties are strongly influenced by position of sender and receiver, we controlled for male position by analyzing frames in which the fish was perpendicular to the camera (such that his full lateral flank was visible) and the fish's long axis was within 15° of horizontal (see Fig. 3A for an

example). These positioning criteria mimic the most behaviorally relevant position (large males perform lateral displays during courtship; citation) while controlling for the effect of body positioning on polarization characteristics. Comparing the DoLP, Q and U components of male and female fish revealed that the large courting male phenotype reflected higher DoLP than females on the lateral line (Fig. 3A; Fig. 6B; Table 2A). These sexually dimorphic differences in DoLP reflectance resulted in greater polarization contrast for large males relative to females in within-body contrast (lateral line and adjacent ventral flank area; highest DoLP reflecting vs. lowest DoLP region; fig. 3B; Table 2B), as well as body to background contrast (highest DoLP-reflecting body region and the gravel background, fig. 3B; Table 2B). Furthermore, we observed sexual dimorphism in U measurements from the operculum and eye (fig. 3A; Fig. 6D; Table 2A), as well as sexually dimorphic contrast differences in Q and U (Fig. 3C,D; Table 2B). Across all three polarization parameters (DoLP, Q and U), sexually dimorphic polarization reflectance resulted in higher male within-body and body-to-background contrast measurements, suggesting that large males are easier to detect than females for a polarization-sensitive viewer (Fig. 3B-D; Table 2B).

Female preference for high-DoLP reflecting males

We tested female preference for male polarized ornamentation in an experimental tank that allowed us to manipulate the polarization of incident light while keeping intensity and color differences below detection thresholds (Fig. 4A,B; Table 1). Females viewed a pair of males in which one male was illuminated with high-DoLP light and the other with low-DoLP light, and could move freely between association, neutral and back zones (Fig. 4A-B). The two experimental conditions significantly altered male DoLP reflectance ($p << 0.01$, Table 1) with males illuminated in the high-DoLP condition exhibiting more than double their DoLP reflectance than when illuminated under low-DoLP conditions (Fig 4A; Table 1). The relative gain in male DoLP reflectance under high-DoLP conditions varied by body region over a range of a 220% increase in DoLP (fin base) to a 286% increase in DoLP (dorsum); Fig 4A; Table 1A). To ensure that the experimental

manipulation did not significantly alter non-polarization features of the male display, such as the luminance (intensity) or hue (color) features, we directly measured the change in large male swordtail polarization, intensity and color features between the high- and low-DoLP conditions. We found no significant change in fish-reflected saturation between males illuminated in the High DoLP condition relative to the Low DoLP condition (difference in saturation p >0.07 across all body regions; avg. p = 0.5; Table 1A). While luminance and hue did not significantly differ across treatment for 7 of the 8 fish regions (Table 1A), hue of the fin base and luminance of the dorsum significantly differed across the two treatments, but by a 2% and 6% relative difference, respectively, which is likely undetectable by fish (Hawryshyn 1991). Within-body contrast (maximum – minimum of luminance, hue, saturation, or DoLP) of the fish did not differ significantly between illumination conditions for luminance, hue, or saturation (p>0.10; Table 1), while within-body contrast in polarization differed by greater than 200% (p<<.01; Table 1).

Females exhibited no preference for polarization environments when males were absent (control trials): there was no significant difference in time spent in high-DoLP versus low-DoLP association zones (Fig. 4C; mean ± SEM in high-DoLP = 96±27 sec, low DoLP =91±24 sec; paired $t_{df=27}$=0.12; p=.90; Shapiro-Wilk normality test W=0.94, p=0.11) or in time spent in all high-DoLP versus all low-DoLP zones (high-DoLP association + back =247±36 sec.; low-DoLP association + back = 244± 36 sec; paired $t_{df=27}$=0.04, p=.97; Shapiro-Wilk W=0.95, p=.18). However, when males were present, females spent significantly more time in the high-DoLP association zone than in the low-DoLP association zone (Fig. 4C; high-DoLP =178±31 sec, low-DoLP=78±13 sec; paired $t_{df=27}$=2.76, p=.01; Shapiro-Wilk W=0.94, p=0.11) but did not differ in time spent in high-DoLP and low-DoLP back zones (high-DoLP=121±21 sec, low-DoLP=101±21 sec; paired $t_{df=27}$=.61, p=.55; Shapiro-Wilk W=0.99. p=0.98). These results indicate that females have no preference for the polarization state of the environment, but a preference for associating with polarization-ornamented males. Stimulus males did not alter

interaction time (time spent swimming in the front of the chamber) based on the presence or absence of polarized light (Fig. 4C; paired $t_{df=27}$=–0.19, p=.85; Shapiro Wilk W=0.98, p=0.87), which suggests that female preference for males under high-DoLP illumination is a result of the polarization ornamentation itself, rather than an effect of polarization on male display behavior. Polarization treatment (high or low DoLP) of a male was a significant explanatory variable for time a female spent with a male (ANOVA,; F=7.83; $df_{polarization_treatment=}$1, df_{error}=16, p=.013), but neither male pair (F=1.01, $df_{pair\_identity}$=5, df_{error}=16, p=0.44) nor the interaction of polarization treatment*male pair (F=2.16, $df_{interaction}$=5, df_{error}=16, p=0.11) were significant explanatory variables.

Social modulation of polarization patterning

We used videopolarimetry of large males in social and asocial contexts to measure DoLP, Q and U across 8 body regions, and calculated within-body and body-background contrasts in these properties (Fig. 5; Fig. 7; Fig. 8; Table 3). Large males (n=12) were filmed alone or with one other individual (female, another large male, intermediate male, or small male). DoLP reflectance did not differ among social conditions for any body region (Fig. 7; Table 3A). Large males had significantly higher within-body DoLP contrast (maximum vs. minimum DoLP contrast; Fig. 5, Fig. 8B, Table 3B) and U contrast (lateral line vs. adjacent dorsal flank; Fig. 8D, Table 3B) when in the presence of a small male than alone. However, no differences in absolute values or contrast of DoLP, Q or U were observed between asocial conditions and courting (female present) or male rivalry (large male present) conditions (Fig. 7, Fig. 8, Table 3).

DISCUSSION

X. nigrensis swordtails meet the evidential criteria for polarization signaling outlined in Mathger et al. (2009). Male swordtails (1) produce a polarization signal (sexually dimorphic polarization patterns, Fig 2) that is (2) sensed by receivers (females increase association time in the presence of the signal, Fig 4C) and which (3) alters behavior in an adaptive manner—female association time is specifically predictive of

reproductive success in swordtails (Walling et al. 2010). This is the first demonstration, to our knowledge, of polarization communication in a vertebrate.

Male and female *X. nigrensis* are significantly dimorphic in both the overall degree and angular components of polarization reflectance, and females prefer polarization-reflecting males (Figs 3,4). Female preference for males exhibiting higher polarization contrast ornamentation may be due to the increased conspicuousness that polarization ornamentation provides, since contrast between patches within an animal, or between an animal and background, increases the detectability of signals (Endler 1992). Contrast detection is a key feature of processing visual stimuli in the brain (Lettvin et al. 1959, Hubel and Wiesel 1959, 1962). As such it is not surprising that both within body and body to background contrast measures from other visual signal components (e.g. color or luminance contrast) have been shown to be an important measure for sexual selection studies (Endler 1983, Endler and Thery 1996, Andersson 1998, Bougman 2001, Gomez 2004, Pauers et al. 2004, Brooks and Endler 2007, Cummings 2007, Stuart-Fox and Moussalli 2008, Cummings and Crothers 2013). Polarization contrast signaling may provide more opportunities for facultative signaling than color and brightness, for two reasons. First, angular features of polarization reflectance—Q and U—depend on the angle between the signaling region and the receiver, meaning signalers can rotate to change the polarization angle that the receiver sees (Brady et al. 2013). In this way, polarization signals may increase the resolution of angular display features similar to the way iridescence allows viewers to detect flexure and motion of a signaler (Rutowski et al. 2010). Second, the aquatic polarization environment is not axially symmetric at low solar angles (Waterman 1954, You et al. 2011, Brady et al. 2013, Cronin and Marshall 2011). The relative position of the sun to the signaler and receiver affects the background DoLP, Q and U observed by the receiver, so a signaler can enhance or mute contrast with the background by strategic orientation with respect to the sun. Thus by signaling in the polarization modality, signalers have the potential to facultatively change polarization features and contrast with background through rotation or repositioning (Brady et al.

2013), allowing swordtails to customize signaling features to accommodate signaling microenvironments and multiple viewers. Future studies should investigate whether or not polarization signalers strategically vary their display position in their environment.

Comparison of polarization attributes of large males in social and asocial contexts reveals that polarization signaling may not be socially modulated in swordtails over the short time scales (<1 hr) used during testing. While courter males showed greater DoLP and Q contrast in the presence of small males (Fig. 5, Fig. 8, Table 3B), no social modulation was observed in contexts in which courter males commonly utilize visual displays (courting and male-male rivalry). While our strict positioning criteria for analysis controlled for the effects of body position on polarization features, they prevented detection of any effect of movement and positioning differences across social contexts on polarization signaling. Receiver-dependent differences in polarization signaling could be achieved by alterations in the male's body position during social interactions, and warrants further behavioral experimentation, as such dynamic signaling capabilities could allow animals to reduce the costs of signaling by modulating characteristics (e.g., conspicuousness) to be context-appropriate.

Our assays depart from previous studies of polarization communication in ways that we hope will provide advances in detecting polarization signals. Using the same pair of optical filters (polarizer and diffuser), and manipulating only the order of the filters while controlling for Fresnel effects, strongly manipulates the amount of polarization of illuminating light without fish-detectable differences in luminance or color between high-DoLP and low-DoLP conditions (Fig 4). The visual system is capable of simultaneously processing different visual cues (e.g. hue, saturation, intensity) so it is essential that behavioral studies isolate the component of interest without manipulating other important features of the stimuli. The ability to significantly alter polarization properties of male stimuli (by >200%; Table 1A) while minimally altering the intensity and color attributes of males below visual detection thresholds [≤ 6%, Table 1A; (56)] is a critically important advancement for behavioral studies in polarization.

While evidence for vertebrate polarization-sensitivity has been well documented for decades (Hawryshyn and Mcfarland 1987), the current study presents evidence for polarization signaling in a vertebrate by isolating and manipulating polarization reflectance of males and testing its effect on female mate choice preferences. Our findings with freshwater swordtails open exciting possibilities for mechanistic studies of polarization communication. While behavioral testing of polarization signals in vertebrates is nascent, further work, across diverse species and environments, will provide insights into a sensory modality that lies beyond human perception and at the frontiers of current knowledge.

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Chapter 3: Behavioral Evidence of Polarization Vision in Rockhinds

ABSTRACT

Vertebrates of many classes have polarization vision, but the mechanisms of this vision vary across species and are poorly understood. As such, determining whether vertebrates can see polarized light has proven difficult and often requires behavioral evidence. Here we take advantage of a social signaling vertebrate and of polarization properties of commercial technology to implement a behavioral test for polarization vision. Rockhinds, a highly social fish, live in high-polarization environments in the Gulf of Mexico. To assess whether rockhinds use polarization vision, we assayed their behavioral responses to social stimuli in the presence and absence of polarization information. Specifically, we exposed rockhinds to social images or control (no image) with a modified LCD monitor that displayed color-brightness contrast images or polarization contrast images. Whereas humans can see the color-brightness images, the polarization-only images are not visible to human viewers, as humans lack polarization vision. We utilize this assay to determine whether rockhinds have polarization vision, and whether they can use it in response to social stimuli. We found significantly different levels of rockhind activity, proximity to screen, and frequencies of screen hits and surfacing between social image exposure and control (no image) trials. Importantly, we found no difference in rockhind activity levels or proximity to screen between the color-brightness assay and the polarization-only assay, suggesting that rockhinds have polarization-sensitive vision. Furthermore, we found rockhinds respond differently to different social images, and with the exception of surfacing, there was no difference in frequencies of discrete behaviors performed between the color-brightness and polarization assays. Rockhinds have the capability to use polarization vision; whether polarization is a feature of their communication system is currently unknown.

INTRODUCTION

Many invertebrates have specialized photoreceptors that detect polarized light (Labhart and Meyer 1999) and innate orientation and navigation to polarized light fields

is common among terrestrial and aquatic invertebrates (Schwind 1999, Dacke et al. 2003, Horvath et al. 2008, Kriska et al. 2009). In contrast, the retinal mechanism of polarization sensitivity in vertebrates is poorly understood (Kamermans and Hawryshyn 2011) and differs across species (Flamarique and Harosi 2002, Kamermans and Hawryshyn 2011), meaning it is often not possible to determine whether a vertebrate sees polarization by examining the retina. While some vertebrate species innately orient to angles of polarization (Forward and Waterman 1972, Kleerekoper et al. 1973), assessing polarization vision in vertebrates often requires many techniques of varying complexity, such as electrophysiology (Waterman and Hashimoto 1974, Ramsden et al. 2008), heart rate conditioning (Cameron and Pugh 1991, Hawryshyn and McFarland 1987), and training experiments (Mussi et al. 2005, Parkyn et al. 2003). Among vertebrates, the most substantial body of evidence supporting polarization vision is in fish (Parkyn and Hawryshyn 1993, Horvath and Varju 2004, Kamermans and Hawryshyn 2011). Because many fish respond to video playback of social stimuli (Green swordtails: Rosenthal et al. 1996, Siamese bettas: Allen and Nicoletto 1997; Trinidadian guppies: Kodric-Brown and Nicoletto 1997; Sticklebacks: Boylard and Rowland 1996), we use behavioral response to social images presented in polarization contrast vs. images presented in color and brightness contrast to test polarization vision in the rockhind grouper (*Epinephelus adscenscionis*).

Groupers are a widespread subfamily of reef fish in which complex social behavior is common, including social control of sex (Quinitio et al. 1997, Kline et al. 2011), inter- and intra-specific dominance hierarchies (Zabala et al. 1997, Shpigel and Fishelson 2006, Kline et al. 2011) sex- and dominance-specific behaviors and color patterns (Gilmore and Jones 1992, Donaldson 1995, Zabala et al. 1997, Kline et al. 2011), and interspecific cooperative hunting and signaling (Bshary et al. 2006). Rockhinds are protogynous and live in social groups of one dominant male and several females (Kline et al. 2011). If a dominant male is removed, the largest male will transition to female (Kline et al. 2011). Dominance behaviors such as patrolling, chasing, biting, and display of the ‘tuxedo’ pattern (Fig. 10A) are directed at females by the dominant male, and

females may direct aggression at smaller females (but the tuxedo pattern is only displayed by males or intersex individuals transitioning to male; Kline et al. 2011). Rockhinds live in near-shore marine environments which have a high degree of polarization in the light environment (Shashar et al. 2004, You et al. 2011), and many aquatic animals use polarized light for behavioral tasks such as navigation (Goddard and Forward 1991, Schwind 1999, Hawryshyn 2010) and foraging (Flamarique and Browman 2001, Shashar et al. 2000). Here we test for the first time 1) whether rockhinds differentially respond to blank screen vs social image displays, and 2) whether response can be elicited by polarization information alone (no access to color or brightness information). Positive responses to both of these queries would provide evidence for polarization vision in this system.

We first examine whether rockinds show differential behavioral response to social images (images of conspecifics) relative to a blank LCD screen (white screen). We then determine whether rockhinds have polarization vision by comparing their behavioral responses to social images during color-brightness image assays relative to polarization-only image assays. We presented rockhinds with images of conspecifics on an LCD monitor that had its front linear polarizer removed, resulting in display images without color or brightness contrast (and thus invisible to viewers without polarization vision; Fig. 9A), but composed of polarization contrast (modified after Pignatelli et al. 2011). Polarization contrast is present in these images while color and brightness are absent because LCD monitors use the front linear polarizer to selectively transmit polarization angle differences from a liquid crystal matrix that have a specific polarization angle-to-color transformation. Without the front linear polarizer, the transformation to color does not occur and only angular polarization contrast is emitted (Fig. 9A). We measured response to images in this 'polarization-only assay' by scoring behaviors and using motion-tracking software. We repeated this assay with the front linear polarizer replaced, producing color-brightness images typical of an LCD computer monitor (Fig. 9B), to measure response to images in color and brightness contrast ('color-brightness assay'). Differences in behaviors performed during social image playback and control (no image)

indicate that rockhinds can see and respond to the images. If rockhinds have polarization vision, we predict behavioral response to the images will not differ between polarization-only and color-brightness assays.

METHODS

Rockhinds (n=5; 4 males and 1 female) were obtained by a collaborator (R. Kline) from the Gulf of Mexico near Brownsville, TX via line-fishing and were housed in the Cummings Lab at the University of Texas, in individual tanks.

Each rockhind was filmed in a 100-gallon testing tank (Fig. 10) and was scored for behavioral responses in 4 trials: brightness-color-control; color-brightness assay; polarization-only-control; and polarization-only assay. Rockhinds were acclimated for 10 minutes in the testing tank. They were then filmed for a 24 minute control period, in which the monitor was turned on displaying a white background with no images, to measure behavioral responses to the testing environment and the presence of the monitor. After the control period, rockhinds were exposed to a monitor displaying a slideshow of 4 images: a male rockhind in cryptic pattern, a female rockhind, a male rockhind in territorial 'tuxedo' pattern, and a coral reef (Fig. 10A). Images were displayed on the monitor for 90 seconds each, in a random order with no consecutive repeats, for a treatment period of 24 minutes. The display program (custom python script by R. Etheredge) automatically recorded the display time and identity of each image.

Rockhinds' behavioral responses to the images were analyzed from video. The linear polarizing filter on the front of the display monitor was manually removed for the polarization-only assay (Fig. 9A), such that the images displayed have no color or brightness contrast, but are composed of polarization angle contrast only. This is because LCD monitors produce color contrast images by inducing polarization angle differences among colors at a pixel via a liquid crystal matrix, then using the front linear polarizer to selectively absorb light of a particular polarization angle, and thus of a particular color. Thus, images displayed on the monitor during the polarization-only assay are not visible to observers without polarization vision, unless a linear polarizer is replaced in front of

the monitor (Fig. 9C). We exposed rockhinds to the image slideshow twice, once with polarization-only images (no linear polarizer on the monitor, Fig. 9A), and once with color-brightness images (linear polarizer placed in front of the monitor, Fig. 9B). The order of these two assays was randomized, and assays were separated by at least 48 hours.

As polarized light travels through materials with different indices of refraction, internal reflection and partial refraction can cause polarization artefacts and images called Fresnel reflections. These Fresnel reflections can cause light without color or brightness contrast to form color images if the angle of polarization differs across the color spectrum (as it does for images displayed on an LCD screen). While glass and water have similar indices of refraction (≈1.5 and 1.33 respectively), air has a much lower index of refraction (1.00) and the air interface between the screen and the fish can induce Fresnel reflections (personal observation). To prevent Fresnel reflections of the displayed polarization images, the monitor was placed in a separate compartment inside the testing tank and submerged in mineral oil (Fig. 10B). Mineral oil has a similar index of refraction to water and glass (≈1.48), but is non-conductive and does not interfere with the function of the monitor. White cloth lined the inside of the fish chamber on all sides except the front (through which the monitor was visible) to further reduce Fresnel reflections. We used an underwater video camera (Olympus Stylus Tough TG-830 underwater camera) to film the interior of the testing tank from all viewing angles to confirm the absence of Fresnel reflections during image display.

Rockhinds were filmed from three camera angles throughout the assay (Fig. 10B,C) with webcams (Microsoft 1080p HD Sensor Widescreen Autofocus) and a custom recording program (by R. Etheredge) that records the timestamp for each frame. Videos from the overhead camera (Fig. 10C, center panel) were analyzed in a custom motion tracking program (R. Etheredge) that tracks the position of manually placed points. Three points along the dorsal surface were used to obtain total activity (path length), average speed (pathlength /(50 frames = 5 seconds)), median orientation (angle of linear regression of tracking points), and average distance from the screen. Videos

were also scored for frequencies of discrete behaviors (described in Table 4). The image displayed at the time interval of each behavioral or motion tracking data point was determined by matching the timestamp of the image display with the timestamp of the video frame. ANOVA in R statistical software was used to test for significant effects of assay and display image on activity, average distance from screen, average speed, and median orientation. Interactions between display image and assay were dropped from the model if not significant. Post-hoc Welch two-sample t-tests in R were used to examine significant effects detected by ANOVA. Poisson models were generated using glm in R to determine relationships between frequency of behaviors and assay type (polarization-only or color-brightness) and display image (Fig. 10A).

RESULTS

Motion tracking

Rockhinds were significantly more active ($p = 0.001$, Table 5) and closer to the display screen ($p < 0.001$, Table 5) during treatments (images displayed in polarization only and color-brightness assays) than control (no image displayed on monitor; Fig. 11-12, Table 5). They did not differ in average speed ($p = 0.54$) or in orientation ($p = 0.19$) between treatment and control (Table 5). There was no significant difference between assay type (polarization-only or color-brightness) for any of these behavioral measures (activity $p = 0.60$; distance to screen $p = 0.15$; speed $p = 0.62$; orientation $p = 0.28$; Table 5).

Discrete behaviors

For each of the behaviors in Table 4 (rear wiggles, screen hits, up-downs, face digs, and surfaces), a Poisson model with a log-link was estimated using the glm function in R (Table 8). To test for differences between treatment and control, a simple model including only assay type (polarization-only or color-brightness) and control vs. treatment as predictor variables (Table 8) was estimated. To test for differences in

behaviors between control and each of the display images, a model including assay type and image type as predictor variables was estimated (Table 9). The default assay was the polarization-only assay, and the second assay level was color brightness; the default image was control (no image), and the other image levels were 1) cryptic male, 2) female 3) territorial male, and 4) coral reef (Fig. 10A). Thus, a significant negative coefficient for assay indicates that fish were more likely to perform the behavior during the polarization-only assay, and a significant positive coefficient for assay indicates fish were more likely to perform the behavior during the color-brightness assay (Table 8-9). A significant negative coefficient for a display image indicates the fish were more likely to perform the behavior during the control than during the display image, and a significant positive coefficient for image indicates fish were more likely to perform the behavior during the display image than during the control (Table 8-9).

Rockhinds performed fewer screen hits and more surfacing during treatment assays than during controls (screen hits: $p<.001$, Control-vs-Treatment coefficient = -1.87; surfaces: $p=0.02$, Control-vs-Treatment coefficient=1.68; Table 8, Fig. 13A,B). Only one behavior—surfacing—was significantly affected by assay type; rockhinds were more likely to surface during the polarization assay than the color brightness assay ($p<.001$, Assay Type coefficient = -2.33, Table 8, Fig. 13B). Rear wiggles, screen hits, and surfaces all showed differences in behavior across display image (Table 9, Fig. 14). Rockhinds were more likely to perform rear wiggles while viewing a female compared to viewing no image (control: $p=.014$, coefficient =0.85, Table 9, Fig. 14C), but were less likely to perform rear wiggles when viewing a cryptic male ($p=0.019$, coefficient= -1.79; Table 9, Fig. 14C). They were less likely to perform screen hits during any of the three fish images than during the control (cryptic male: $p=0.014$, coefficient = -1.87; female: $p=0.022$, coefficient = -1.46; displaying male: $p=0.022$, coefficient = -1.46; Table 9, Fig. 14A), but did not differ in this behavior when viewing coral vs. the control ($p=1.00$, coefficient=5.4E-16, Table 9, Fig. 14A). Rockhinds also surfaced more during images of a female ($p<.01$, coefficient=2.35; Table 9, Fig. 14B) and of coral than during the control ($p=0.004$, coefficient=2.14; Table 9, Fig. 14B, while surfaces during male images did not

differ significantly from control (cryptic male: p=0.57, coefficient= -0.69; displaying male: p=0.42, coefficient=0.69;Table 9, Fig. 14A).

DISCUSSION

Rockhinds are responsive to social images displayed on LCD monitors and they respond to these images whether the image is transmitted via polarization only or brightness-color channels. In both polarization-only and brightness-color assays, rockhinds responded to viewing images by moving closer to the display screen and increasing overall motion compared to the control (no image on the display screen; Table 5-6). They also differed in numbers of discrete behaviors performed when viewing images compared to control (Table 8). Specifically, they increased rear-wiggles in response to female images and decreased them in response to cryptic male images, surfaced more in response to female images, and decreased screen hits in response to images of males and females (but not coral; Table 9). Thus, the image assay was successful in eliciting behavioral response. Importantly, with the exception of surfacing, these behavioral responses did not differ between the color-brightness assay (images visible to viewers without polarization vision) and the polarization-only assay (images composed of polarization contrast, and not visible to viewers without polarization vision; Table 5-8). The finding that rockhinds respond similarly to color-brightness images and polarization-only images provides behavioral support for the hypothesis that rockhinds have polarization vision, and have the ability to use it in response to social stimuli.

Increased proximity to the screen and increased activity during the polarization and color-brightness assays compared to controls may reflect increased attentiveness or stimulation. The discrete behaviors observed in this study (Table 4) have not been previously documented (although they had been occasionally observed in home tanks; personal observation) and thus cannot be definitively related to dominance or territorial behavior. However, incidence of rear wiggles, screen hits, and surfacing differed depending on the image displayed to the rockhinds (Table 9). Further experiments are needed to place these behaviors in context, but it is particularly intriguing that fish

perform rear wiggles more frequently while viewing images of females, and less frequently while viewing images of males.

Removing the front linear polarizer of an LCD monitor was used by Pignatelli et al. (2011) to test fish and cephalopods (*Carassius aurautus* and *Danio rerio*) for polarization vision; yet they found no response to this assay in fish (Pignatelli et al. 2011). It is possible that these fish species do not respond to polarization images; however, it is also possible that their assay did not employ a stimulus that fish are likely to encounter in nature (the image displayed on the altered monitor was a black circle that increased in size to simulate the approach of a predator). With our polarization-only assay, we employed images of conspecifics and other ecologically-relevant features. Use of ecologically-relevant features in behavioral polarization assays may be necessary to elicit a significant behavioral response, as well as provide insight into the behavioral contexts in which fish use polarization vision.

Polarization vision can enhance visual contrast to improve feature detection (Johnsen et al. 2011, How and Marshall 2014), and is used for navigation and foraging in a variety of aquatic species (Goddard and Forward 1991, Schwind 1999, Shashar et al. 2000, Flamarique and Browman 2001, Hawryshyn 2010). While a number of species with polarization vision have polarization components to their body patterning (Marshall et al. 1999, Chiou et al. 2008, Shashar and Hanlon 1997), evidence that polarization cues affect receiver behavior during signaling interactions is less common (Boal et al. 2004; Mathger et al. 2009; but see Sweeney et al. 2003). Rockhinds can use polarization vision to respond to artificial social stimuli (images of conspecifics), but whether this capability is part of their communication in nature, or an extension of polarization vision typically used for other tasks, requires further investigation.

The rockhind is unusual among groupers in that dominant males display a rapid-onset aggressive color pattern (Kline et al. 2011), but rockhinds are infrequently studied. Rockhinds use dynamic color displays in territorial defense interactions (such as the 'tuxedo' pattern; Kline et al. 2011), and pilot data suggest these patterns have polarization contrast components. The male-specific aggressive display pattern

('tuxedo') documented by Kline et al. (2011) was not observed in the study subjects, either during behavioral trials or at any point during housing and care. Importantly, tuxedo patterns are only displayed in dominant individuals (males, and females transitioning to male; Kline et al. 2011). In nature rockhinds live in harems of one male and several females and establish dominance hierarchies, with males occasionally holding satellite territories or living solitarily (Kline et al. 2011). Fish were housed individually due to space limitations, but were in close proximity and could presumably see each other. It is possible that natural housing conditions are necessary to establish dominance hierarches and elicit tuxedo display. Measuring the polarization properties of patterns displayed during live territorial intrusions, and recording receiver response to these displays in the presence and absence of polarized illumination, will elucidate whether rockhinds have evolved to integrate polarization vision into their communication.

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Table 1

A.

Measure	Statistic	Lateral Line	Upper Flank	Dorsum	Lower Flank	Ventrum	Fin base	Operculum	Eye	Whole-fish contrast
Luminance	p-value	0.15	0.25	**0.01**	0.64	0.11	0.16	0.25	0.15	0.24
	Mean luminance: High DoLP	48.02	62.41	68.05	74.04	76.66	74.03	82.29	57.14	45.24
	Mean luminance: Low DoLP	45.16	60.36	64.30	73.56	74.42	70.96	83.64	54.57	47.10
	Relative Difference	0.06	0.03	0.06	0.01	0.03	0.04	-0.02	0.05	-0.04
Hue	p-value	0.63	0.35	0.51	0.70	0.71	**0.01**	0.83	0.37	0.37
	Mean Hue: High DoLP	41.01	39.88	39.93	44.01	43.81	47.25	46.18	45.47	10.95
	Mean Hue: Low DoLP	41.45	40.60	40.31	44.28	44.00	48.26	46.29	46.92	12.36
	Relative Difference	-0.01	-0.02	-0.01	-0.01	0.00	-0.02	<0.01	-0.03	-0.11
Saturation	p-value	0.64	0.44	0.89	0.33	0.32	0.55	0.07	0.72	0.10
	Mean Saturation: High DoLP	0.92	0.91	0.94	0.85	0.90	0.90	0.75	0.88	0.23
	Mean Saturation: Low DoLP	0.92	0.90	0.94	0.83	0.89	0.90	0.72	0.88	0.25
	Relative Difference	0.01	0.01	<0.01	0.02	0.01	0.01	0.04	<0.01	-0.10
DoLP	p-value	**1.08E-04**	**2.60E-04**	**1.28E-03**	**7.71E-05**	**5.89E-05**	**2.59E-05**	**1.02E-05**	**3.69E-05**	**9.14E-6**
	Mean DoLP: High DoLP	0.32	0.27	0.22	0.23	0.21	0.37	0.35	0.40	0.26
	Mean DoLP: Low DoLP	0.09	0.07	0.07	0.06	0.06	0.11	0.09	0.12	0.08
	Relative Difference	2.41	2.86	2.24	2.74	2.53	2.20	2.80	2.23	2.03

Table 1 Continued

B.

Measure	Statistic	Blue	Gold	Rose	Green	Orange	Pink	Red	White	Yellow
Luminance	p-value	0.80	0.22	0.24	0.50	**0.01**	0.09	0.42	**0.02**	**0.02**
	Mean luminance: High DoLP	47.36	85.85	87.33	54.73	82.29	87.38	69.26	123.98	93.49
	Mean luminance: Low DoLP	47.78	87.87	89.07	67.45	85.44	89.63	70.43	127.79	96.34
	Relative Difference	-0.01	-0.02	-0.02	-0.19	-0.04	-0.03	-0.02	-0.03	-0.03
Hue	p-value	**0.01**	0.77	0.80	0.31	**0.01**	**0.004**	0.91	**0.002**	**0.02**
	Mean Hue: High DoLP	216.31	42.38	19.25	91.09	31.09	8.06	10.87	51.65	38.55
	Mean Hue: Low DoLP	218.63	42.44	19.30	82.21	31.53	7.49	10.84	50.24	38.12
	Relative Difference	-0.01	<0.01	<0.01	0.11	-0.01	0.08	<0.01	0.03	0.01
Saturation	p-value	0.10	0.17	0.19	0.39	0.29	0.20	0.24	**0.03**	**0.02**
	Mean Saturation: High DoLP	0.28	0.93	0.77	0.86	0.97	0.68	0.90	0.58	0.90
	Mean Saturation: Low DoLP	0.30	0.94	0.77	0.87	0.98	0.67	0.90	0.56	0.89
	Relative Difference	-0.06	<0.01	0.01	-0.01	-0.01	0.01	-0.01	0.04	0.01

Table 1: Color, luminance and polarization properties of fish regions and color standards between the high- and low-DoLP experimental conditions.

Male *X. nigrensis* (n=10) were illuminated in a stationary position across 4 low DoLP and 4 high DoLP treatments while filmed for 10 seconds with a standard color video camera (Olympus Stylus Tough TG-830 underwater camera) for color (hue, saturation) and luminance (intensity) values as well as with the videopolarimeter to evaluate changes in DoLP (See Fig 4A). P-values indicate the results of paired t-tests measured under high-DoLP and low-DoLP illumination conditions, with significant values ($p<.05$) in bold. Whole fish contrast was calculated as difference between the maximum region – and the minimum region in luminance, hue, saturation or DoLP. Relative difference is computed as the quantity ((high-DoLP illumination Mean - low-DoLP illumination Mean)/ low-DoLP illumination Mean). While DoLP is significantly higher for all fish regions under high-DoLP illumination, and at differences greater than 200% between high and low DoLP treatments. There were only two significant differences among illumination conditions in hue, saturation, or luminance: luminance of the dorsum and hue of the caudal fin base, both of which were very small relative differences (6% and 2%, respectively). Lower limits to difference detection by visual systems are described by the Weber fraction, which in fish is around 5% (Hawryshyn 1991), indicating that luminance and color differences between the treatments are likely not detectable by fish. Measurements of a color standard (X-rite Colorchecker MSCCPPCC0113; Table 1B) reveal some significant differences between high- and low-DoLP illumination, all of which are below a 10% relative difference between treatment conditions.

A.

Measure	Phenotype	Statistic	Lateral Line	Upper Flank	Dorsum	Lower Flank	Ventrum	Fin base	Operculum	Eye
DoLP	LM	mean ± SE	0.16±0.02	0.12±0.02	0.11±0.03	0.09±0.02	0.09±0.04	0.23±0.07	0.25±0.05	0.30±0.05
	F	Mean ± SE	0.14±0.02	0.10±0.02	0.11±0.03	0.10±0.02	0.11±0.05	0.21±0.06	0.22±0.04	0.28±0.05
	LM vs F	P-value	**0.02**	0.09	0.71	0.45	0.21	0.40	0.13	0.12
Q	LM	Mean ± SE	0.05±0.02	0.03±0.01	0.02±0.01	0.03±0.01	0.03±0.02	0.06±0.05	0.09±0.04	0.11±0.03
	F	Mean ± SE	0.04±0.02	0.02±0.01	0.02±0.01	0.03±0.02	0.04±0.02	0.04±0.03	0.07±0.03	0.10±0.04
	LM vs F	P-value	0.06	0.16	0.30	0.99	0.51	0.23	0.07	0.72
U	LM	Mean ± SE	0.01±0.01	0.01±0.01	0.01±4.66E-03	0.02±0.01	0.01±0.01	0.01±0.02	0.02±0.01	0.03±0.01
	F	Mean ± SE	0.01±0.01	0.02±4.56E-03	0.01±4.09E-03	0.02±0.01	1.72E-03±0.01	4.35E-03±0.01	0.01±0.01	0.02±0.01
	LM vs F	P-value	0.17	0.55	0.59	0.69	0.12	0.26	**4.36E-04**	**0.03**

B.

Measure	Phenotype	Statistic	Lateral Line vs. Lower Flank	Lateral Line vs. Upper Flank	Lower Flank vs. Fin Base	Max vs. Min within Fish	Max Fish vs. Gravel	Max Fish vs. Water Above	Max fish vs. Water Below
DoLP	LM	Mean ± SE	0.07±0.01	0.04±0.01	-0.01±0.16	0.27±0.02	0.25±0.03	-0.02±0.14	0.06±0.09
	F	Mean ± SE	0.04±0.02	0.03±0.01	0.05±0.12	0.24±0.04	0.22±0.04	-0.02±0.10	0.04±0.08
	LM vs F	P-value	**2.53E-04**	0.19	0.25	**0.02**	**0.01**	0.88	0.48
Q	LM	Mean ± SE	0.02±0.01	0.02±0.01	-0.03±0.05	0.13±0.05	0.12±0.05	0.14±0.05	0.14±0.05
	F	Mean ± SE	0.01±0.02	0.01±0.01	-0.01±0.02	0.11±0.04	0.10±0.04	0.11±0.04	0.11±0.03
	LM vs F	P-value	**0.04**	0.14	0.19	0.15	0.14	0.13	0.07
U	LM	Mean ± SE	-0.01±0.01	-3.98E-03±0.01	0.01±0.01	0.05±0.02	0.03±0.02	0.05±0.02	0.05±0.02
	F	Mean ± SE	-0.01±0.01	-0.01±0.01	0.01±0.01	0.04±0.01	0.02±0.01	0.03±0.01	0.03±0.01
	LM vs F	P-value	0.29	0.07	0.36	0.13	0.06	**0.03**	**0.03**

Table 2: Comparison of polarization features and polarization contrast between large males and females.

Degree of Linear Polarization (DoLP), Q, and U were measured for large males (LM) and females (F) from 8 body regions (Table 2A). Contrast between two regions (Table 2B) was calculated as the difference between the two indicated regions (e.g., Lateral Line DoLP –Lower Flank DoLP). For each polarization measure (2A) or contrast measure (2B), means and standard errors are given for each sex x body region. P-values indicate results of t-tests for differences between two phenotypes. Significant differences ($p<.05$ after Benjamini-Hochberg multiple comparison corrections) are indicated in bold.

Table 3

A.

Measure	Social Partner	Statistic	Lateral Line	Upper Flank	Dorsum	Lower Flank	Ventrum	Fin base	Operculum	Eye
DoLP	Alone	Mean ± SE	0.14±0.03	0.11±0.03	0.10±0.03	0.09±0.04	0.09±0.04	0.32±0.09	0.26±0.06	0.32±0.06
	LM	Mean ± SE	0.16±0.04	0.12±0.03	0.11±0.03	0.09±0.03	0.08±0.04	0.32±0.07	0.24±0.04	0.29±0.05
	IM	Mean ± SE	0.16±0.03	0.11±0.03	0.11±0.04	0.09±0.03	0.10±0.04	0.31±0.04	0.23±0.03	0.29±0.02
	SM	Mean ± SE	0.17±0.05	0.13±0.03	0.12±0.03	0.10±0.03	0.09±0.03	0.35±0.13	0.26±0.07	0.30±0.07
	F	Mean ± SE	0.15±0.03	0.12±0.03	0.11±0.04	0.09±0.03	0.09±0.04	0.35±0.12	0.24±0.05	0.31±0.03
	Alone vs LM	P-value	0.60	0.96	0.91	0.99	0.96	0.95	0.84	0.50
	Alone vs IM	P-value	0.60	0.96	0.91	0.99	0.96	0.95	0.76	0.50
	Alone vs SM	P-value	0.60	0.96	0.91	0.99	0.96	0.93	0.98	0.77
	Alone vs F	P-value	0.62	0.96	0.91	0.99	0.98	0.93	0.87	0.79
	LM vs IM	P-value	0.81	0.96	0.91	0.99	0.96	0.93	0.84	0.96
	LM vs SM	P-value	0.76	0.96	0.91	0.99	0.96	0.93	0.87	0.90
	LM vs F	P-value	0.77	0.96	0.91	0.99	0.96	0.93	0.99	0.38
	IM vs SM	P-value	0.69	0.96	0.91	0.99	0.96	0.78	0.87	0.77
	IM vs F	P-value	0.81	0.96	0.99	0.99	0.96	0.76	0.87	0.27
	SM vs F	P-value	0.62	0.96	0.91	0.99	0.96	0.98	0.87	0.79
Q	Alone	Mean ± SE	0.06±0.03	0.04±0.03	0.03±0.03	0.03±0.02	0.04±0.04	0.06±0.09	0.11±0.11	0.13±0.08
	LM	Mean ± SE	0.04±0.02	0.03±0.01	0.02±0.01	0.03±0.01	0.03±0.01	0.05±0.06	0.10±0.08	0.10±0.05
	IM	Mean ± SE	0.05±0.03	0.03±0.01	0.02±0.01	0.02±0.01	0.02±0.01	0.02±0.02	0.06±0.04	0.09±0.03

Table 3 Continued

	SM	Mean ± SE	0.05±0.02	0.03±0.01	0.02±0.01	0.03±0.01	0.03±0.02	0.09±0.13	0.10±0.07	0.11±0.03
	F	Mean ± SE	0.05±0.02	0.03±0.02	0.02±0.02	0.03±0.01	0.03±0.02	0.07±0.08	0.08±0.05	0.10±0.03
	Alone vs LM	P-value	0.84	0.76	0.83	0.62	0.53	0.78	0.86	0.69
	Alone vs IM	P-value	0.84	0.76	0.83	0.62	0.53	0.64	0.44	0.69
	Alone vs SM	P-value	0.84	0.76	0.83	0.62	0.53	0.76	0.86	0.69
	Alone vs F	P-value	0.84	0.76	0.83	0.62	0.53	0.78	0.57	0.69
	LM vs IM	P-value	0.89	0.88	0.83	0.62	0.53	0.55	0.44	0.75
	LM vs SM	P-value	0.84	0.88	0.83	0.87	0.92	0.74	0.94	0.95
	LM vs F	P-value	0.84	0.88	0.83	0.87	0.72	0.76	0.57	0.95
	IM vs SM	P-value	0.84	0.88	0.83	0.62	0.53	0.55	0.44	0.69
	IM vs F	P-value	0.84	0.88	0.83	0.62	0.53	0.55	0.57	0.75
	SM vs F	P-value	0.84	0.88	0.83	0.87	0.74	0.78	0.57	0.95
U	Alone	Mean ± SE	0.02±0.02	0.02±0.01	0.02±0.01	0.02±0.02	0.01±0.02	0.02±0.05	0.04±0.03	0.03±0.02
	LM	Mean ± SE	0.01±0.01	0.01±0.01	0.01±4.39 E-03	0.02±0.01	0.01±0.01	0.01±0.01	0.03±0.03	0.04±0.04
	IM	Mean ± SE	2.82E-03±0.02	0.01±0.01	0.01±0.01	0.01±0.01	-2.07E-03±0.01	-2.33E-03±0.01	0.01±0.02	0.02±0.02
	SM	Mean ± SE	0.01±0.01	0.02±0.01	0.01±0.01	0.02±0.01	0.01±0.01	0.02±0.04	0.03±0.01	0.03±0.02
	F	Mean ± SE	0.01±0.01	0.01±0.01	0.01±4.92 E-03	0.01±0.01	0.01±0.01	0.01±0.02	0.02±0.02	0.03±0.02
	Alone vs LM	P-value	0.78	0.31	0.50	0.67	0.75	0.64	0.71	0.72
	Alone vs IM	P-value	0.27	0.31	0.50	0.05	0.25	0.36	0.17	0.67
	Alone vs SM	P-value	0.54	0.54	0.57	0.31	0.79	0.85	0.58	0.83
	Alone vs F	P-value	0.53	0.31	0.53	0.18	0.75	0.80	0.18	0.81
	LM vs IM	P-value	0.27	0.58	0.71	**0.01**	0.25	**2.47E-03**	0.18	0.67

Table 3 Continued

	LM vs SM	P-value	0.63	0.54	0.67	0.38	0.87	0.64	0.84	0.67
	LM vs F	P-value	0.54	0.95	0.73	0.18	0.87	0.64	0.26	0.67
	IM vs SM	P-value	0.51	0.51	0.57	0.14	0.25	0.36	0.17	0.67
	IM vs F	P-value	0.51	0.58	0.64	0.14	0.25	0.17	0.71	0.72
	SM vs F	P-value	0.84	0.54	0.75	0.69	0.87	0.84	0.20	0.83

B.

Measure	Social Partner	Statistic	Lateral Line vs. Lower Flank	Lateral Line vs. Upper Flank	Lower Flank vs. Fin Base	Max vs. Min within Fish	Max Fish vs. Gravel	Max Fish vs. Water Above	Max fish vs. Water Below
DoLP	Alone	Mean ± SE	0.05±0.02	0.03±0.02	-0.03±0.11	0.23±0.04	0.21±0.05	-0.03±0.30	0.07±0.17
	LM	Mean ± SE	0.07±0.02	0.04±0.03	-0.04±0.17	0.28±0.06	0.26±0.06	-0.07±0.20	0.03±0.12
	IM	Mean ± SE	0.07±0.01	0.04±0.02	0.01±0.16	0.27±0.05	0.25±0.06	-0.02±0.15	0.05±0.12
	SM	Mean ± SE	0.07±0.03	0.04±0.03	-0.01±0.22	0.30±0.06	0.28±0.07	-0.01±0.14	0.09±0.12
	F	Mean ± SE	0.07±0.02	0.04±0.03	-0.01±0.17	0.27±0.03	0.25±0.04	-3.09E-03±0.16	0.07±0.09
	Alone vs LM	P-value	0.17	0.30	0.97	0.09	0.11	0.96	0.90
	Alone vs IM	P-value	0.17	0.30	0.97	0.23	0.17	0.96	0.90
	Alone vs SM	P-value	0.17	0.30	0.97	**0.04**	0.11	0.96	0.90
	Alone vs F	P-value	0.31	0.40	0.97	0.06	0.11	0.96	0.94
	LM vs IM	P-value	0.89	0.98	0.97	0.69	0.78	0.96	0.90
	LM vs SM	P-value	0.89	0.98	0.97	0.54	0.58	0.96	0.90
	LM vs F	P-value	0.89	0.98	0.97	0.70	0.78	0.96	0.90
	IM vs SM	P-value	0.91	0.98	0.97	0.24	0.39	0.96	0.90

Table 3 Continued

	IM vs F	P-value	0.89	0.98	0.97	0.79	0.90	0.96	0.90
	SM vs F	P-value	0.89	0.98	0.97	0.24	0.39	0.96	0.90
Q	Alone	Mean ± SE	0.02±0.02	0.02±0.02	-0.02±0.07	0.15±0.11	0.15±0.12	0.16±0.12	0.16±0.12
	LM	Mean ± SE	0.02±0.01	0.02±0.01	-0.02±0.06	0.13±0.08	0.12±0.09	0.14±0.08	0.14±0.08
	IM	Mean ± SE	0.02±0.02	0.02±0.02	-2.44E-03±0.02	0.09±0.04	0.08±0.05	0.09±0.04	0.09±0.04
	SM	Mean ± SE	0.03±0.01	0.02±0.02	-0.06±0.13	0.16±0.12	0.15±0.13	0.17±0.12	0.17±0.12
	F	Mean ± SE	0.02±0.02	0.02±0.02	-0.03±0.07	0.12±0.07	0.12±0.07	0.13±0.08	0.13±0.08
	Alone vs LM	P-value	0.85	1.00	0.94	0.76	0.72	0.69	0.72
	Alone vs IM	P-value	0.97	1.00	0.65	0.29	0.45	0.29	0.27
	Alone vs SM	P-value	0.88	1.00	0.65	0.88	0.96	0.96	0.94
	Alone vs F	P-value	0.88	1.00	0.84	0.75	0.72	0.69	0.72
	LM vs IM	P-value	0.85	1.00	0.65	0.29	0.45	0.29	0.27
	LM vs SM	P-value	0.85	1.00	0.65	0.75	0.72	0.69	0.72
	LM vs F	P-value	0.85	1.00	0.84	0.86	0.95	0.90	0.88
	IM vs SM	P-value	0.88	1.00	0.65	0.29	0.45	0.29	0.27
	IM vs F	P-value	0.88	1.00	0.65	0.37	0.45	0.42	0.41
	SM vs F	P-value	0.85	1.00	0.75	0.75	0.72	0.69	0.72
U	Alone	Mean ± SE	-0.01±0.01	-4.20E-03±0.02	3.95E-03±0.04	0.06±0.04	0.05±0.04	0.06±0.05	0.06±0.05
	LM	Mean ± SE	-0.01±0.01	1.10E-03±0.01	0.01±0.01	0.06±0.04	0.04±0.05	0.06±0.04	0.05±0.04
	IM	Mean ± SE	-4.78E-03±0.01	-0.01±0.02	0.01±0.01	0.04±0.01	0.02±0.02	0.03±0.01	0.03±0.01

Table 3 Continued

	SM	Mean ± SE	-0.01±0.01	-0.01±0.01	-7.28E-04±0.03	0.06±0.03	0.04±0.03	0.05±0.03	0.05±0.03
	F	Mean ± SE	-4.42E-03±0.01	-3.11E-03±0.01	4.46E-03±0.02	0.05±0.02	0.03±0.03	0.04±0.02	0.04±0.02
	Alone vs LM	P-value	0.95	0.71	0.73	0.84	0.72	0.87	0.90
	Alone vs IM	P-value	0.95	0.77	0.79	0.46	0.38	0.23	0.22
	Alone vs SM	P-value	0.95	**0.02**	0.84	0.78	0.65	0.84	0.88
	Alone vs F	P-value	0.95	0.86	0.97	0.64	0.53	0.52	0.53
	LM vs IM	P-value	0.95	0.71	0.73	0.46	0.38	0.23	0.22
	LM vs SM	P-value	0.95	0.71	0.73	0.84	0.79	0.87	0.88
	LM vs F	P-value	0.95	0.71	0.73	0.76	0.65	0.52	0.53
	IM vs SM	P-value	0.95	0.77	0.73	0.46	0.38	0.23	0.22
	IM vs F	P-value	0.95	0.71	0.73	0.52	0.56	0.41	0.41
	SM vs F	P-value	0.95	0.77	0.79	0.78	0.71	0.62	0.63

Table 3: Comparison of large male polarization features and contrast across social conditions.

Degree of Linear Polarization (DoLP), Q, and U were measured from 8 body regions for large males in each of 5 social conditions (Alone; LM: with a Large Male; IM: with an intermediate male; SM: with a small male; F: with a Female). Contrast between two regions (Table 3B) was calculated as the difference between the two indicated regions (e.g., Lateral Line DoLP –Lower Flank DoLP). For each polarization measure or contrast measure, means and standard errors are given for each large male body region in each social condition. P-values indicate results of t-tests for differences between large males in two social conditions. Significant differences ($p<.05$ after Benjamini-Hochberg multiple comparison corrections) are indicated in bold.

Behavior	**Description**
Rear Wiggle	Undulates rear body and tail vigorously on substrate, disturbing sand.
Screen Hits	Collides face with tank; only observed at the glass panel separating tank from display screen.
Up-down	Travels in an upwards and downwards motion with face in contact with tank. Only observed at the glass panel separating tank from display screen. 1 Up-Down = (1 upwards + 1 downwards motion).
Face dig	Rubs face and front of body on substrate.
Surface	Fish breaks surface of water.

Table 4: Description of rockhind behaviors observed during polarization and color-brightness assays.

Activity ~ Assay_Type + Control_vs_Treatment		
	F	Pr(>F)
Effect of Assay Type	0.276	0.60207
Effect of Control vs. Treatment	11.823	**0.00124**
Average distance from screen ~ Assay_Type + Control_vs_Treatment		
	F	Pr(>F)
Effect of Assay Type	2.153	0.149
Effect of Control vs. Treatment	58.620	**8.33E-10**
Average speed ~ Assay_Type + Control_vs_Treatment		
	F	Pr(>F)
Effect of Assay Type	0.256	0.615
Effect of Control vs. Treatment	0.372	0.545
Median orientation ~ Assay_Type + Control_vs_Treatment		
	F	Pr(>F)
Effect of Assay Type	1.198	0.279
Effect of Control vs. Treatment	1.169	0.190

Table 5: Effect of treatment vs. control on motion tracking metrics.

ANOVA models were generated in R to test the effect of treatment vs. control and assay type on rockhind motion behavior. Treatment levels included treatment (stimulus images displayed to rockhind) and control (no image displayed to rockhind); assay levels included color-brightness (images displayed with color-brightness contrast) and polarization-only (images displayed with only polarization contrast). Treatment had a significant effect on rockhind activity and average distance from the screen, indicating rockhinds respond to displayed images differently than to a blank screen for these behaviors. Assay type had no effect on any motion-tracking metrics, indicating rockhinds respond similarly to color and polarization images.

Activity ~ Assay_Type + Display_Image		
	F	Pr(>F)
Effect of Assay Type	0.259	0.6137
Effect of Display Image	2.796	**0.0374**
Average distance from screen ~ Assay_Type + Display_Image + Assay_Type*Display_Image		
	F	Pr(>F)
Effect of Assay Type	0.394	0.1297
Effect of Display Image	16.292	**5.37E-8**
Interaction	3.061	**.0272**
Average speed ~ Assay_Type + Display_Image		
	F	Pr(>F)
Effect of Assay Type	0.240	0.627
Effect of Display Image	0.103	0.981
Median orientation ~ Assay_Type + Display_Image		
	F	Pr(>F)
Effect of Assay Type	1.182	0.283
Effect of Display Image	1.026	0.404

Table 6: Effect of assay type and individual display image on motion tracking metrics.

ANOVA models were generated in R to test the effect of assay type and individual display image vs. control on rockhind motion behavior. Display image levels included control (no image/blank screen), Image 1 (image of cryptic male), Image 2 (female), Image 3 (Displaying male), and Image 4 (Coral). Assay levels included color-brightness (images displayed with color-brightness contrast) and polarization-only (images displayed with only polarization contrast). Activity during controls (24 minutes) was normalized to activity during each treatment image (6 minutes total for each image) by dividing control activity by four. Display image had a significant effect on rockhind activity and average distance from the screen. Assay type had no effect on any motion-tracking metrics, indicating rockhinds respond similarly to color and polarization images.

A.

Activity		
	t	P
Cryptic male vs Female	-0.0827	0.935
Cryptic male vs Displaying male	0.1447	0.8865
Cryptic male vs Coral	-0.1062	0.9166
Cryptic male vs Control	3.375	**0.008181**
Female vs Displaying male	0.2327	0.8186
Female vs Coral	-0.031	0.9756
Female vs Control	3.7006	**0.004907**
Displaying male vs Coral	-0.2439	0.8101
Displaying male vs Control	3.2453	**0.01006**
Coral vs Control	3.1745	**0.01127**

B.

Distance from Screen		
Polarization Assay	t	p
Cryptic male vs Female	0.2988	0.7728
Cryptic male vs Displaying male	-0.1042	0.9196
Cryptic male vs Coral	0.5085	0.6251
Cryptic male vs Control	-3.8239	**0.01778**
Female vs Displaying male	-0.3802	0.7144
Female vs Coral	0.222	0.8299
Female vs Control	-3.8766	**0.01718**
Displaying male vs Coral	0.5703	0.5855
Displaying male vs Control	-3.7948	**0.0179**
Coral vs Control	-3.9096	**0.01673**
Color-Brightness Assay	t	p
Cryptic male vs Female	-0.1982	0.848
Cryptic male vs Displaying male	-0.217	0.8336
Cryptic male vs Coral	0.1981	0.8479
Cryptic male vs Control	-3.0378	**0.03845**
Female vs Displaying male	-0.0334	0.9742
Female vs Coral	0.4069	0.6953
Female vs Control	-3.0335	**0.03863**
Displaying male vs Coral	0.4137	0.69
Displaying male vs Control	-3.0326	**0.03865**
Coral vs Control	-3.0428	**0.03827**

Table 7: Post-hoc t-tests of activity and distance from screen across display image.

A) Average ±SE of Activity for each display image are as follows: Cryptic Male: 3397.90 ±3138.0; Female: 3510.71 ± 2958.18; Displaying male: 3197.06±3067.69; Coral: 3555.63±3493.47; Control 48.02±68.49. Activity was significantly greater for each display image than for the control (no image), but did not differ significantly among display images. B) Average ±SE of distance from screen for each display image are as follows. Polarization Assay: Cryptic Male: 242.22±133.16; Female: 218.50±117.31l; Displaying male: 251.81±156.85; Coral: 202.18± 115.14; Control: 2181.85 ± 1126.39. Color-brightness Assay: Cryptic Male:157.66 ±63.17; Female:164.97± 52.88; Displaying male:166.17±60.71; Coral:=149.56±66.10; Control:5101.24±3638.28. Rockhinds were significantly closer to the screen during all display images compared to control, but did not differ in distance from screen among display images.

Frequency of Rear Wiggle ~ Assay_Type + Control_vs_Treatment			
Coefficient	Estimate ±SE	Z	Pr (>|z|)
Intercept	0.002981±0.32268	0.009	0.993
Effect of Assay Type	0.331357±0.2477	1.338	0.181
Effect of Control vs.Treatment	0.136132±0.318614	0.427	0.669
Frequency of Screen Hits ~ Assay_Type + Control_vs_Treatment			
Coefficient	Estimate ±SE	Z	Pr (>|z|)
Intercept	0.5500±0.3174	1.733	0.0831
Effect of Assay Type	-0.6931±0.4629	-1.497	0.1343
Effect of Control vs.Treatment	-1.8718±0.4493	-4.166	**3.1E-5**
Frequency of Up-downs ~ Assay_Type + Control_vs_Treatment			
Coefficient	Estimate ±SE	Z	Pr (>|z|)
Intercept	-0.1431±0.3789	-0.378	0.7504
Effect of Assay Type	0.2683±0.3684	0.728	0.4665
Effect of Control vs.Treatment	-0.6931±0.3873	-1.790	0.0735
Frequency of Face digs ~ Assay_Type + Control_vs_Treatment			
Coefficient	Estimate ±SE	Z	Pr (>|z|)
Intercept	0.2344±0.2583	0.822	0.4113
Effect of Assay Type	0.4261±0.2273	1.875	0.0608
Effect of Control vs.Treatment	0.0155±0.2791	0.056	0.9557
Frequency of Surfaces ~ Assay_Type + Control_vs_Treatment			
Coefficient	Estimate ±SE	Z	Pr (>|z|)
Intercept	-1.0094±0.7086	-1.424	0.1543
Effect of Assay Type	-2.3273±0.5238	-4.443	**8.88E-06**
Effect of Control vs.Treatment	1.6818±0.7234	2.325	**0.0201**

Table 8: Effect of treatment vs. control on frequency of discrete behaviors.

Poisson models with a log link were generated in R using the GLM function to test the effect of treatment vs. control on rockhind motion behavior. Treatment levels included control (default level in model; no image displayed to rockhind) and treatment (stimulus images displayed to rockhind); assay levels included color-brightness (images displayed with color-brightness contrast) and polarization-only (default level in model; images displayed with only polarization contrast). Treatment had a significant effect on the frequency of screen hits and surfaces, indicating rockhinds respond to displayed images differently than to a blank screen for these behaviors. Assay type had an effect on the frequency of surfaces, but no other behaviors.

Table 9

Frequency of Rear Wiggle ~ Assay_Type + Display_Image			
Coefficient	Estimate ±SE	Z	Pr (>|z|)
Intercept	2.98E-3 ± 0.323	.009	0.993
Assay: color-brightness	0.31 ± 0.248	1.338	0.181
Image: cryptic male	-1.79 ± 0.763	-2.346	**0.019**
Image: female	0.847±0.345	2.456	**0.014**
Image: displaying male	0.080±0.400	0.200	0.8415
Image: coral	5.44E-16±0.408	0.000	1.000
Frequency of Screen Hits ~ Assay_Type + Display_Image			
Coefficient	Estimate ±SE	Z	Pr (>|z|)
Intercept	0.550±0.317	1.733	0.083
Assay: color-brightness	-0.693±0.462	-1.497	0.134
Image: cryptic male	-1.872±0.760	-2.464	**0.014**
Image: female	-1.463±0.641	-2.289	**0.022**
Image: displaying male	-1.463±0.641	-2.289	**0.022**
Image: coral	-18.425±1686	-0.011	0.991
Frequency of Up-downs ~ Assay_Type + Display_Image			
Coefficient	Estimate ±SE	Z	Pr (>|z|)
Intercept	-0.143±0.379	-0.378	0.706
Assay: color-brightness	0.2683±0.368	0.728	0.467
Image: cryptic male	-0.916±0.592	-1.549	0.121
Image: female	-0.357±0.493	-0.724	0.469
Image: displaying male	-1.204±0.658	-1.829	0.067
Image: coral	-0.511±0.516	-0.989	0.323
Frequency of Face digs ~ Assay_Type + Display_Image			
oefficient	Estimate ±SE	Z	Pr (>|z|)
Intercept	0.234±0.285	0.822	0.411
Assay: color-brightness	0.426±0.227	1.875	0.061
Image: cryptic male	-0.065±0.359	-0.180	0.858
Image: female	0.318±0.329	0.969	0.332
Image: displaying male	0.118±0.343	0.343	0.732
Image: coral	-0.470±0.403	-1.166	0.244
Frequency of Surfaces ~ assay + image			
Coefficient	Estimate ±SE	Z	Pr (>|z|)
Intercept	-1.009±0.709	-1.424	0.154
Assay: color-brightness	-2.327±0.524	-4.443	**8.87E-6**
Image: cryptic male	-0.693±1.225	-0.566	0.571

Table 9 Continued

Image: female	2.351±0.740	3.177	**1.49E-3**
Image: displaying male	0.693±0.866	0.800	0.423
Image: coral	2.140±0.748	2.863	**0.004**

Table 9: Effect of display image and assay type on frequencies of discrete behaviors.

Behavior during controls (24 minutes) was normalized to behavior during each treatment image (6 minutes total for each image) by dividing frequency of control behaviors by four. Poisson models with a log link were generated in R using the GLM function to test the effect of individual display image vs. control on frequencies of discrete behaviors. The default assay was the polarization assay, and the second assay level was color-brightness. A significant positive coefficient for assay indicates fish were more likely to perform the behavior during the color brightness assay, and a significant negative coefficient for assay indicates that fish were more likely to perform the behavior during the polarization assay. The default image was control (no image), and the other image levels were 1) cryptic male, 2) female 3) displaying male, and 4) coral. A significant negative coefficient for a display image indicates the fish were more likely to perform the behavior during the control than during the display image, and a significant positive coefficient for image indicates fish were more likely to perform the behavior during the display image than during the control.

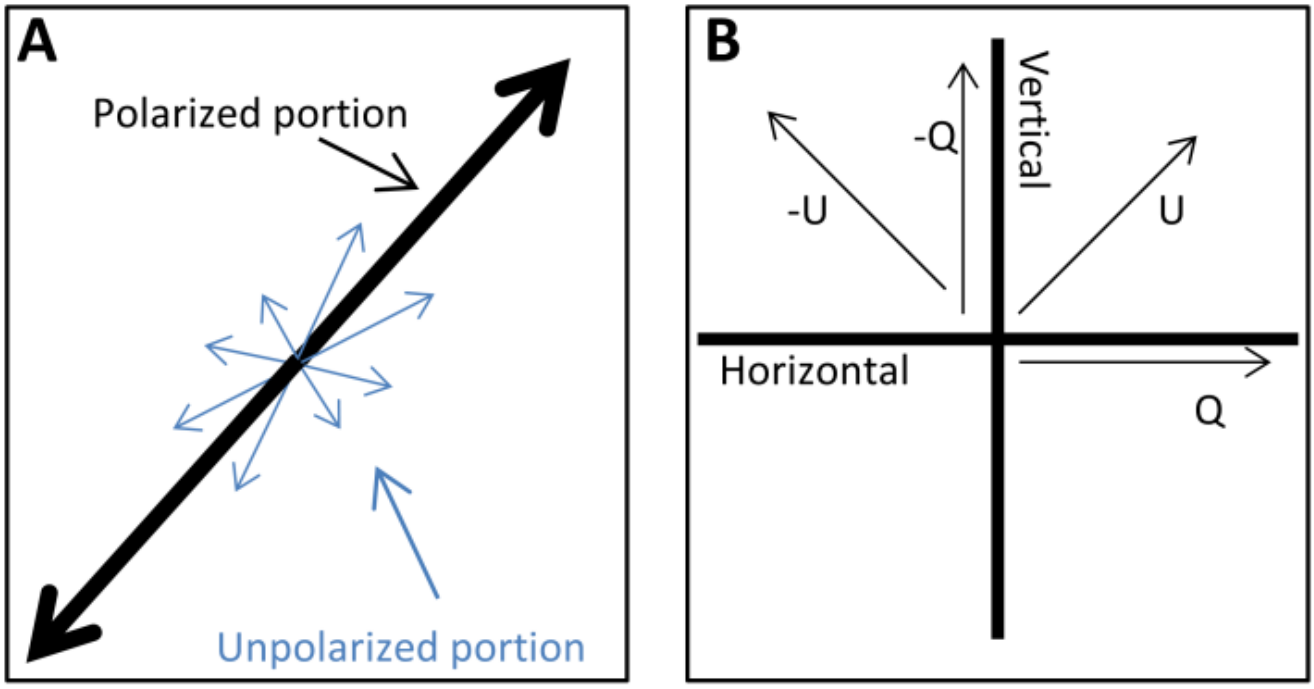


Figure 1: Schematic of polarization of light.

A) Graphical description of the degree of polarized light or the amount that is polarized. Arrows indicate the direction of the electric field of light, and thickness indicates the amount of light with electric field aligned in that direction. Unpolarized light is equivalent to light with a randomized electric field direction (light blue), and the portion that is not random is the polarized portion of light (black). B) Graphical depiction of the quantities Q and U where Q is a parameter quantifying the amount of polarization in the horizontal-vertical axes of the environment and U is a parameter quantifying the amount of polarization associated with the axes rotated 45°from the horizontal-vertical axes. The angle of polarization can be calculated from the quantities Q and U.

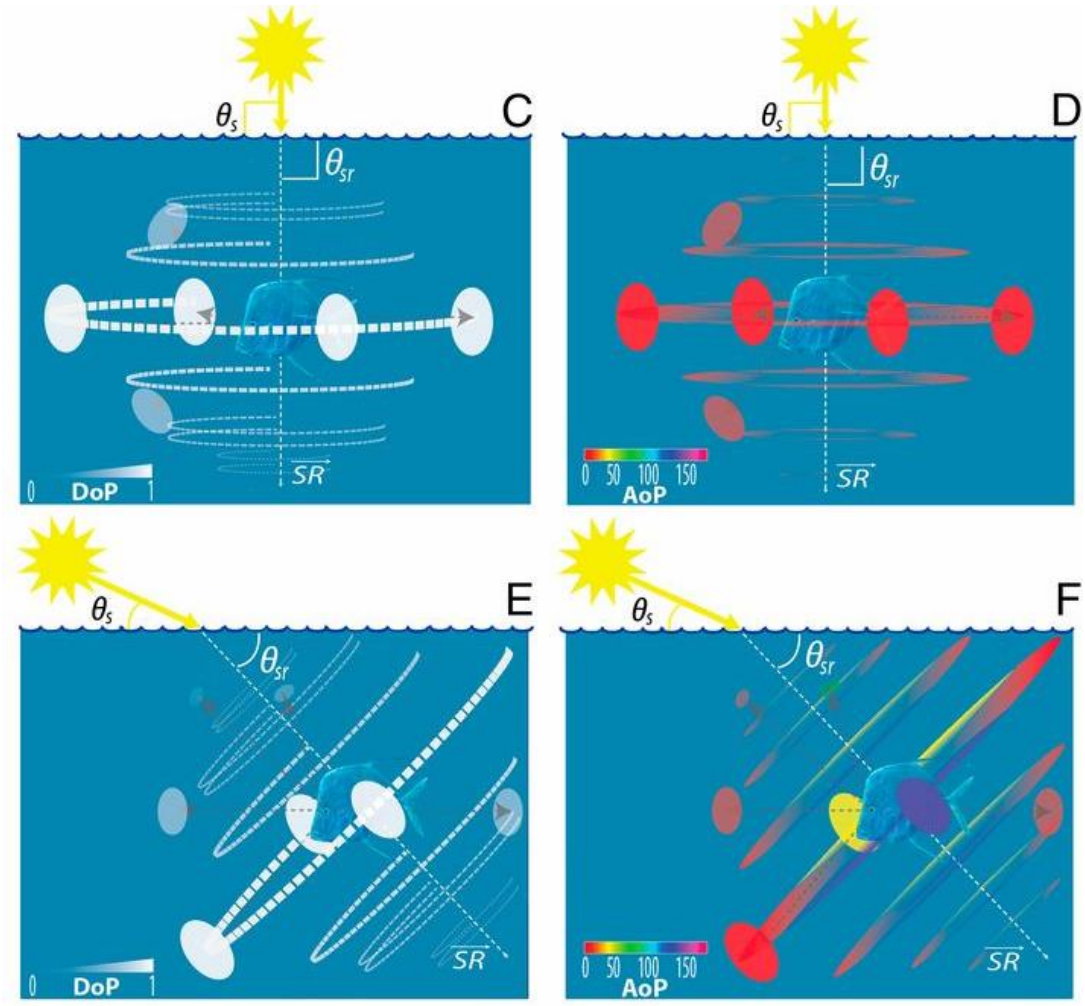


Figure. 2: Complexity of underwater polarization fields in relation to camouflage (Adapted from Brady et al. 2013).

Panels *C–F* represent the 3D viewing environment in high solar inclination angle environments (*C* and *D*) and low solar inclination angle environments (*E* and*F*). θ_{sr} is the refracted solar angle in the water, and SR is the SR propagation direction. The dashed (*C* and *E*) or colored (*D* and *F*) planes perpendicular to SR represent the DoP (*C* and *E*) and AoP (*D* and *F*). The gray-scale (*C* and *E*) or colored (*D* and *F*) disks represent the visual DoP and AoP background properties in different viewing directions from the fish's frame of reference. Concentric ring radii and disk orientation and size are arbitrary.

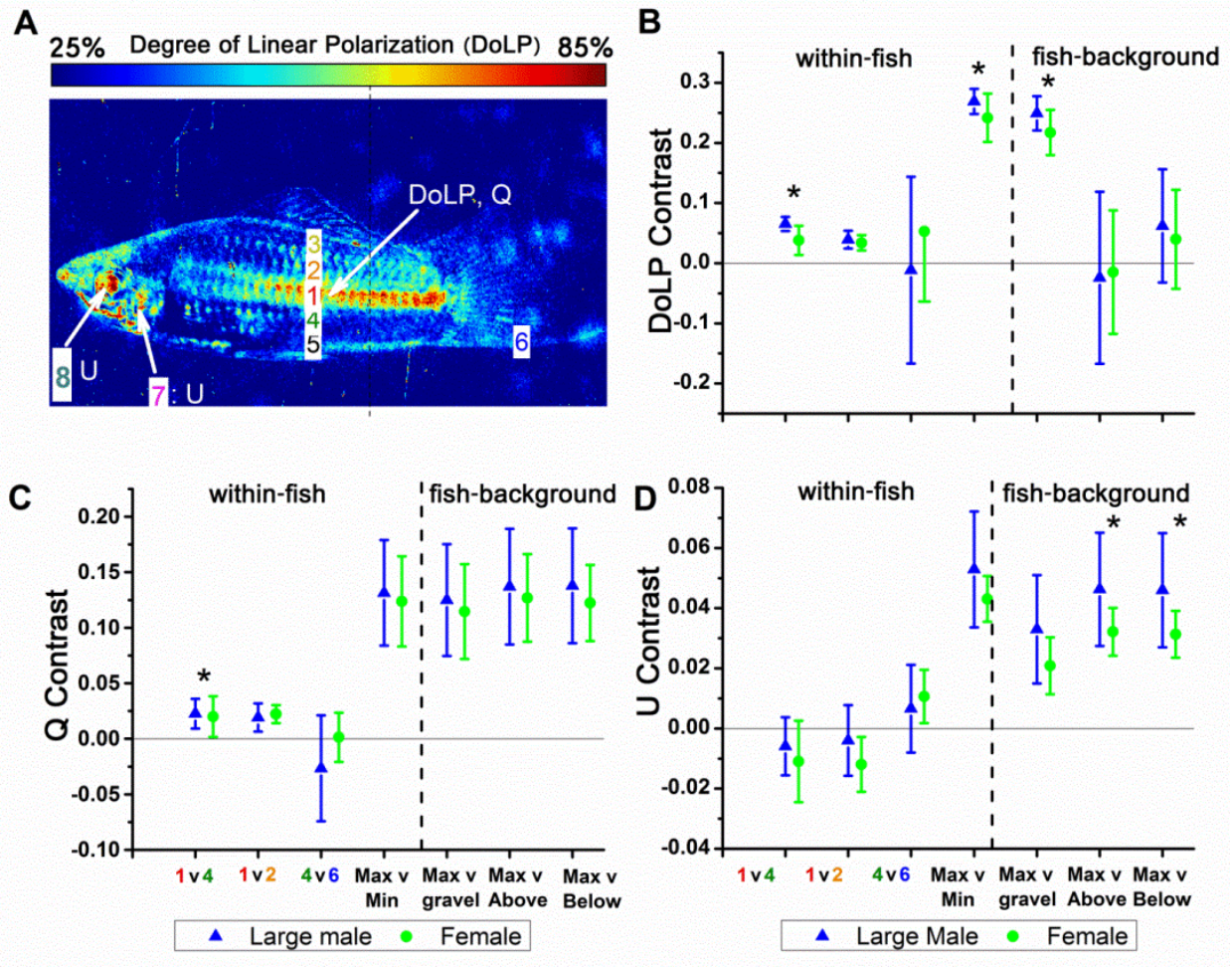


Figure 3: Sexual dimorphism in polarization contrast.

A) Videopolarimetry image of a large male in false color showing Degree of Linear Polarization (DoLP) reflectance. Colored numbers indicate regions analyzed: 1, lateral line; 2, upper flank; 3, dorsum; 4, lower flank; 5, ventrum; 6, caudal fin base; 7, operculum; 8, eye. Arrows indicate body regions with statistically significant sexual dimorphism in DoLP, Q, or U. **B-D**) Polarization contrast [within-body and body-to-background; mean contrast ± 1 SEM of large males (n=12, df=11) and females (n=17, df=16)] for DoLP (**B**), Q (**C**), and U (**D**). Contrast values are calculated as the difference between two body regions as labeled in (**A**) or as the difference between the indicated body region and the maximum-DoLP (Max) or minimum-DoLP (Min) region of the fish, the gravel substrate , or the water column above the fish. . Asterisks indicate significant differences (between males (n=12) and females (n=17) in Welch's two-sample *t*-tests (*=p<.05, after Benjamini-Hochberg correction for multiple comparisons).

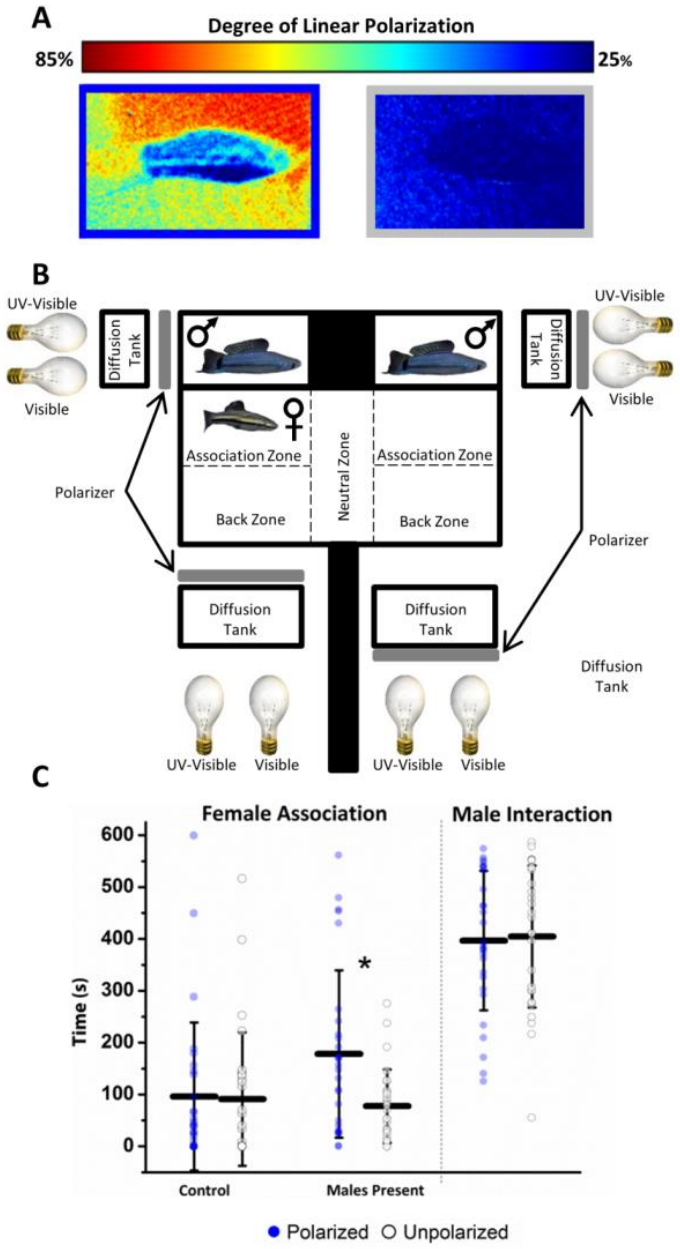


Figure 4: Female mate preference experiment.

A) Videopolarimetry image showing the DoLP reflectance of a large male in the high-DoLP experimental condition (left) and the same male in the low-DoLP experimental condition (right). **B**) Schematic of female mate-preference experiment. Females were presented with two side-by-side male chambers. Each male was illuminated from front and side by visible-range and UV-visible bulbs. Diffusion tanks depolarized source light; UV-transmissive horizontal polarizers polarized source light. Placement of polarizers relative to diffusion tanks provided high-DoLP light incident on a male (left side of figure) or low-DoLP light (right side of figure), without differences in light intensity between polarization conditions (see Table 1). Males were prevented from seeing each other by placement of an opaque barrier between male chambers. Females could swim throughout association, neutral, and back zones and interact with males through a glass barrier. **C**) Female preference for polarized males. Data points show time in polarized (blue) and unpolarized (gray) conditions, with center line showing mean and error bars showing ±1 SD. Females (n=28) spent significantly more time associating with males in polarized relative to unpolarized conditions (* p=.01, paired $t_{df=27}$=2.76), while showing no difference in male-absent controls (p=.90, paired $t_{df=27}$=0.12). Male interaction time did not differ between polarized and unpolarized conditions (p=.85, paired $t_{df=27}$=–0.19).

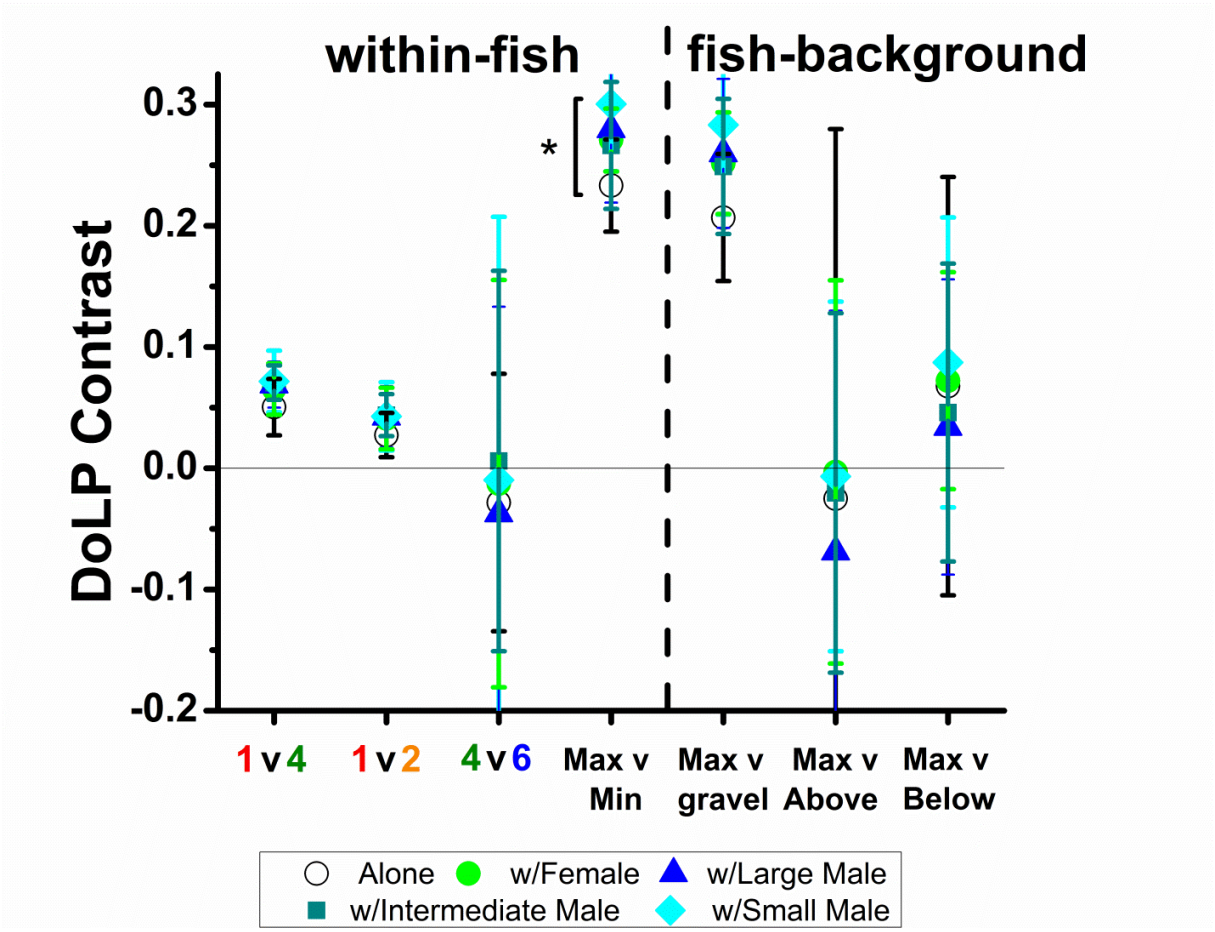


Figure 5: Effect of social partner on large male.

DoLP contrast values [mean contrast ± 1 SEM of large males (n=12, df=11)] are plotted for contrasts of body regions as labeled in Fig. 1A, or between the indicated body region and maximum-DoLP (Max) or minimum-DoLP (Min) fish region, gravel substrate , or water column DoLP measured above or below the fish. Asterisks indicate significant differences between reflectances in different social environments of males (n=12) in Welch's two-sample *t*-tests (*=$p<.05$, after Benjamini-Hochberg correction for multiple comparisons).

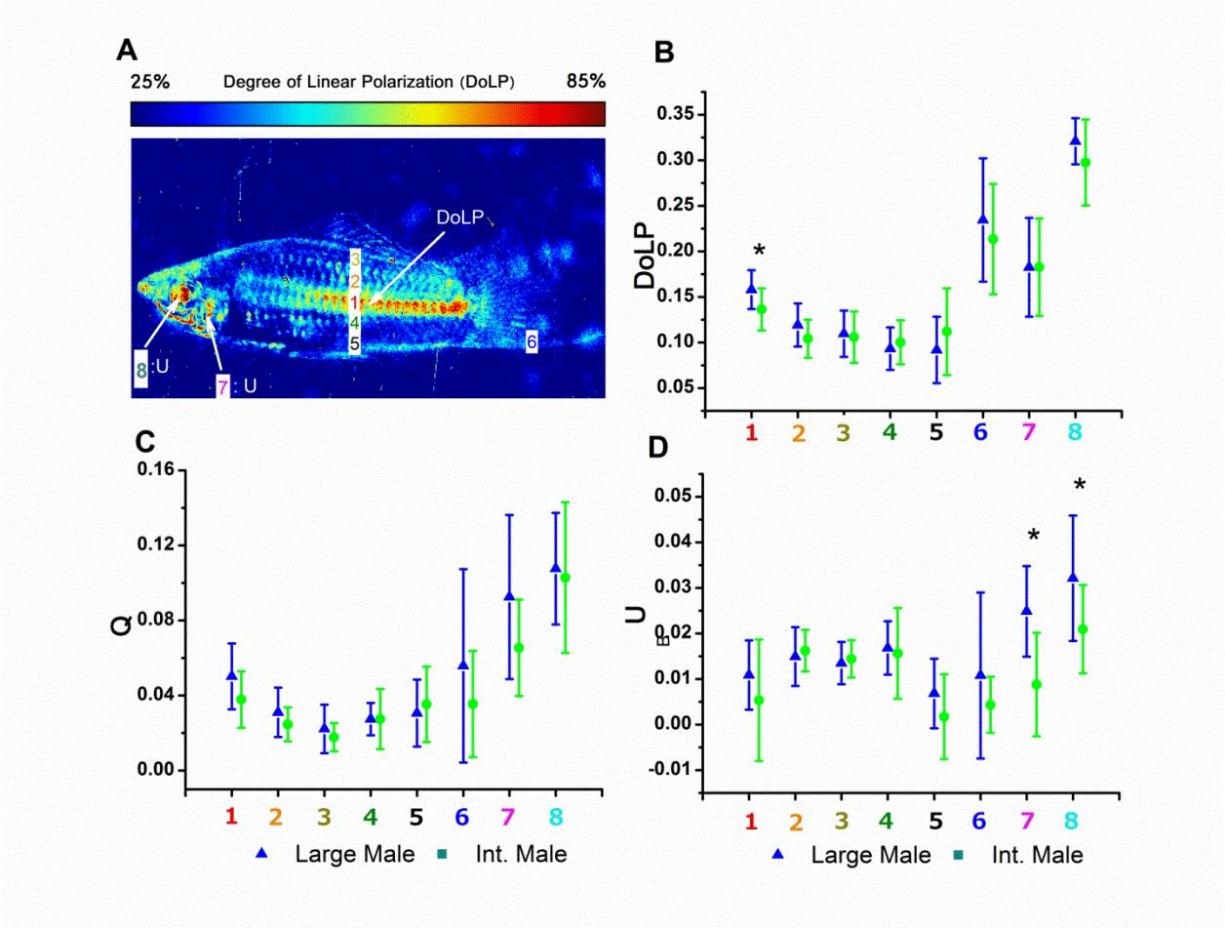


Figure 6: Differences in large male and female polarization reflectance.

A) Videopolarimetry image of a large male *X. nigrensis* in false color showing Degree of Linear Polarization (DoLP) reflectance. Colored numbers indicate body regions analyzed: 1, lateral line; 2, upper flank; 3, dorsum; 4, lower flank; 5, ventrum; 6, caudal fin base; 7, operculum; 8, eye. Arrows indicate areas sexually dimorphic in DoLP, Q, or U. **B-D**) DoLP (**B**), Q (**C**), and U (**D**) of the body region given on the x-axis, plotted as mean ± 1 SEM for large males and females. Fish were illuminated with horizontally polarized light to mimic midday underwater polarization conditions (You et al. 2011). Asterisks indicate significant difference between the pair of phenotypes indicated by brackets in two-tailed *t*-tests: *=p<.05 after correction for multiple comparisons using the Benjamini-Hochberg method (see Table 2A).

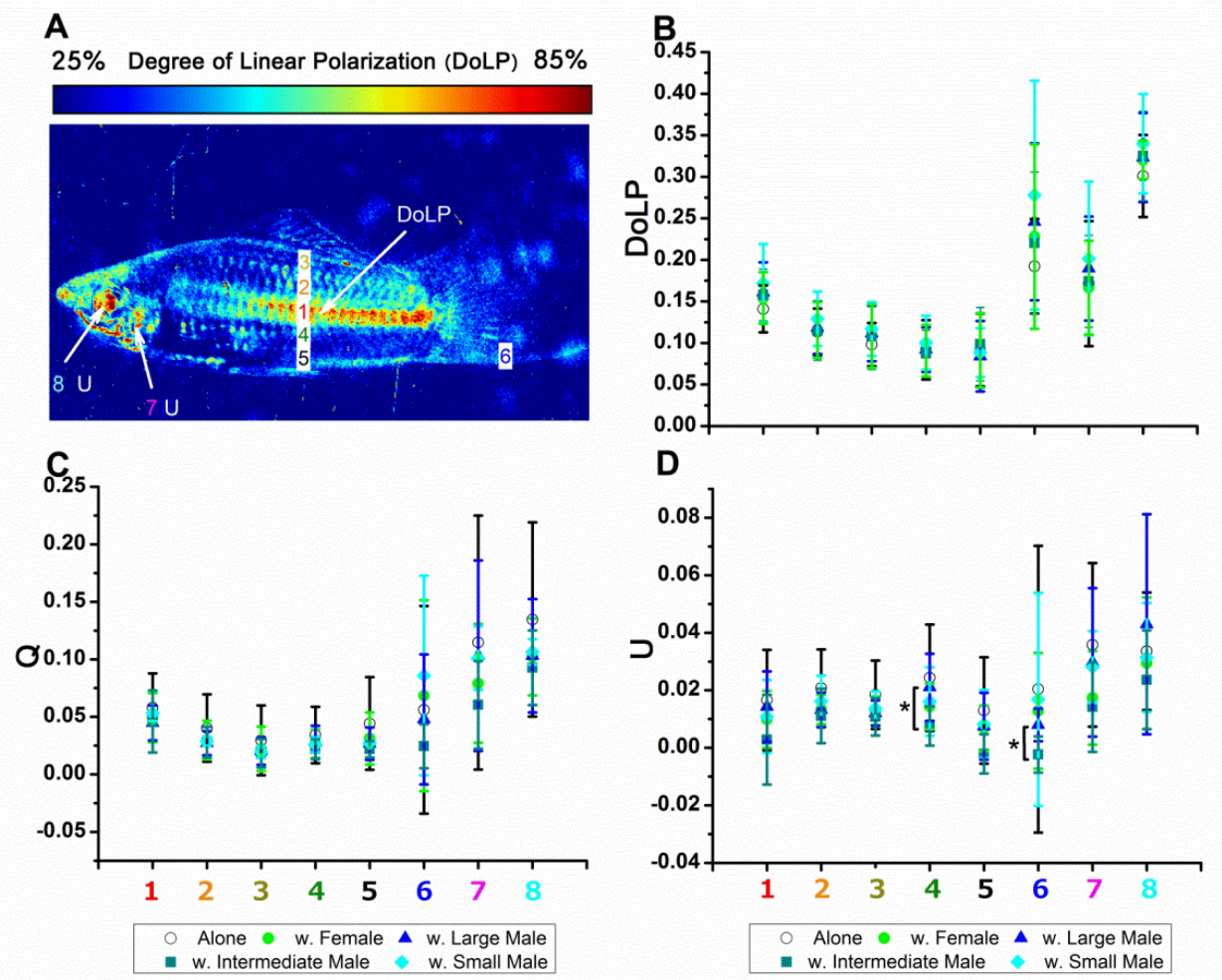


Figure 7: Effect of social partner on large male polarization reflectance.

A) Videopolarimetry image of a large male *X. nigrensis* in false color showing degree of linear polarization (DoLP) reflectance. Colored numbers indicate body regions analyzed: 1, lateral line; 2, upper flank; 3, dorsum; 4, lower flank; 5, ventrum; 6, caudal fin base; 7, operculum; 8, eye. Arrows indicate areas sexually dimorphic in DoLP, Q, or U. **B-D**) Differences (mean ± 1 SEM) in large males' polarization reflectance according to social condition for DoLP (**B**), Q (**C**), and U (**D**), plotted for each body region and type of social partner. Fish were illuminated with horizontally polarized light to mimic midday underwater polarization conditions (You et al. 2011). Asterisks indicate significant differences between the pair of social conditions indicated by brackets in two-tailed *t*-tests: *=p<.05 after correction for multiple comparisons using the Benjamini-Hochberg method (see Table 3A).

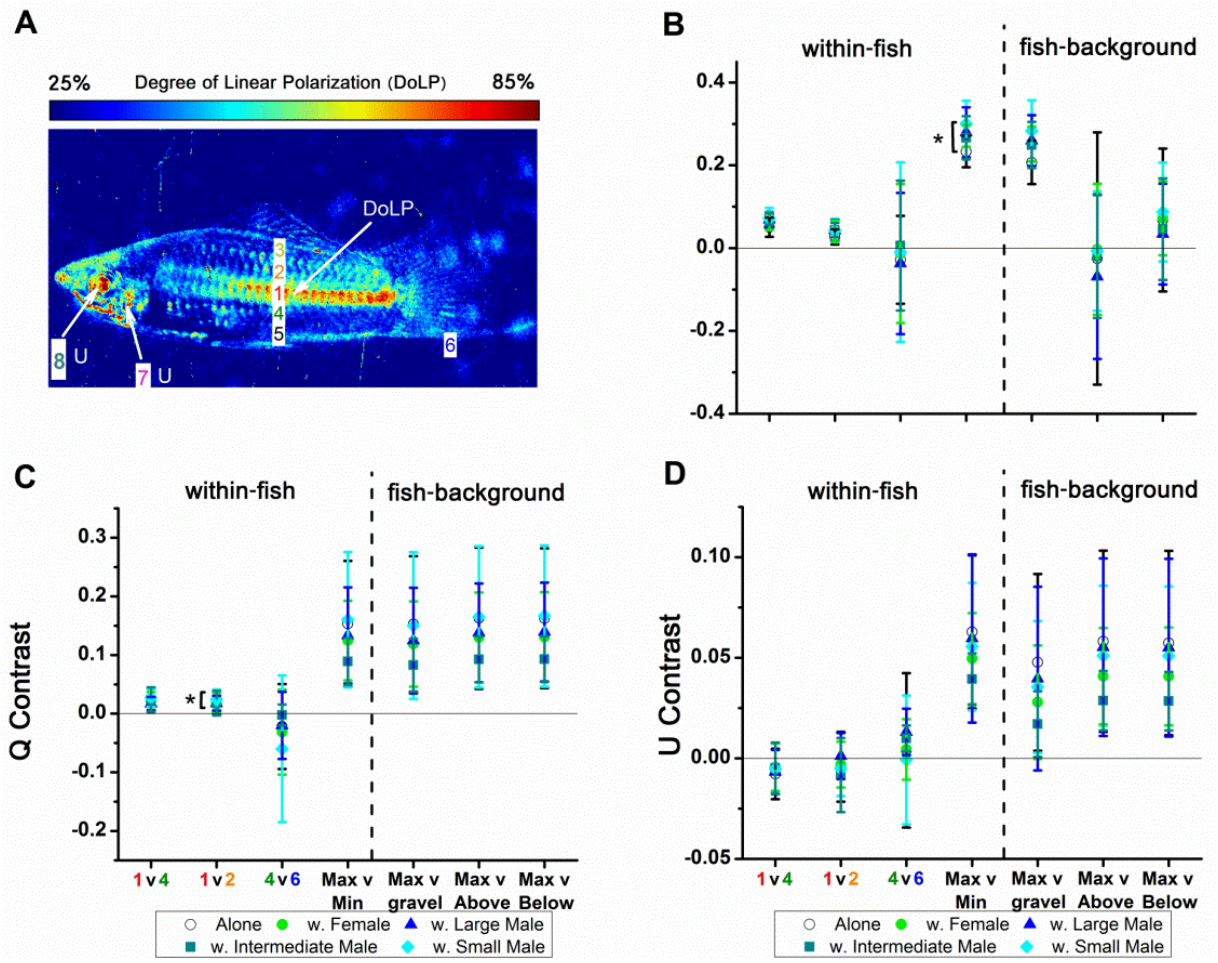


Figure 8: Effect of social partner on large male polarization contrast.

A) Videopolarimetry image of a large male *X. nigrensis* in false color showing degree of linear polarization (DoLP) reflectance. Colored numbers indicate body regions analyzed: 1, lateral line; 2, upper flank; 3, dorsum; 4, lower flank; 5, ventrum; 6, caudal fin base; 7, operculum; 8, eye. Arrows indicate areas sexually dimorphic in DoLP, Q, or U. **B-D**) Differences in large males' polarization contrast (mean ± 1 SEM) according to social condition for DoLP (**B**), Q (**C**), and U (**D**), plotted for each type of social partner. Contrast values are calculated as the difference between the two regions indicated on the x-axis for body regions labeled in **A** or between the indicated body region and maximum-DoLP (Max) or minimum-DoLP (Min) fish region, gravel substrate, water column DoLP above, or below the fish. Fish were illuminated with horizontally polarized light to mimic midday underwater polarization conditions (You et al. 2011). Asterisks indicate significant differences between the pair of social conditions indicated by brackets in two-tailed *t*-tests: *=$p<.05$ after correction for multiple comparisons using the Benjamini-Hochberg method (see Table 3B).

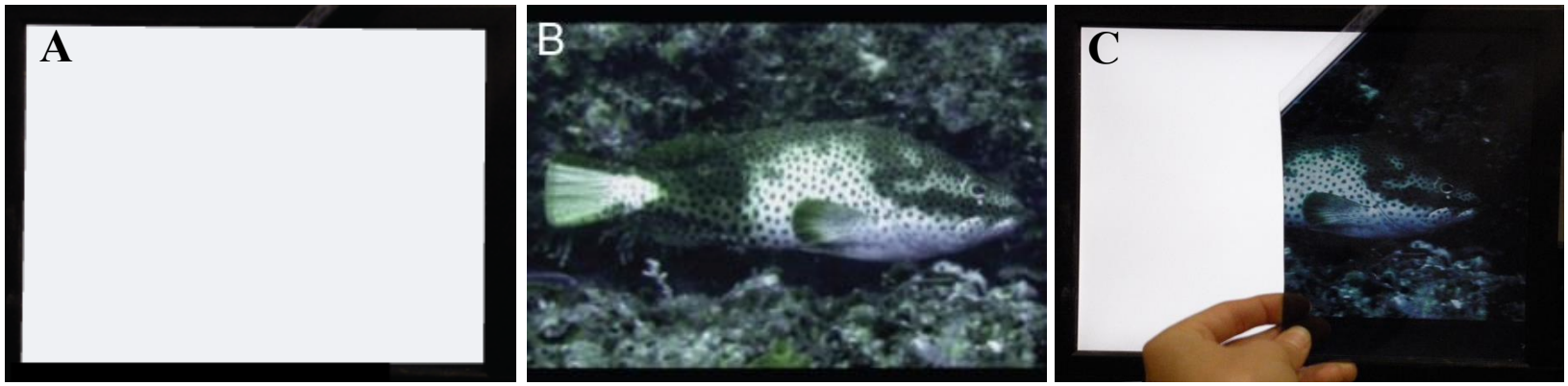


Figure 9: Schematic of assay types (polarization-only and color-brightness).

A) Polarization-only assay. Monitor displaying an image of a territorial rockhind, but with front linear polarizer removed, resulting in an image composed only of polarization contrast (no color or brightness contrast; image invisible to viewers without polarization vision). B) Color-brightness assay. Monitor displaying an image of a territorial rockhind with front linear polarizer intact, resulting in an image composed of color contrast and brightness contrast (visible to viewers with color or luminance vision). C) Demonstration of effect of front linear filter on image display. The front linear filter has been removed from the monitor and is being held in front of the right half of the screen. The monitor displays an image of a territorial rockhind, but the left half of the image is composed only of polarization contrast and thus invisible to humans (as in the polarization-only assay). Replacing the front linear polarizer on the right half of the screen induces color and brightness contrast, allowing viewers without polarization vision to see the image.

Figure 10

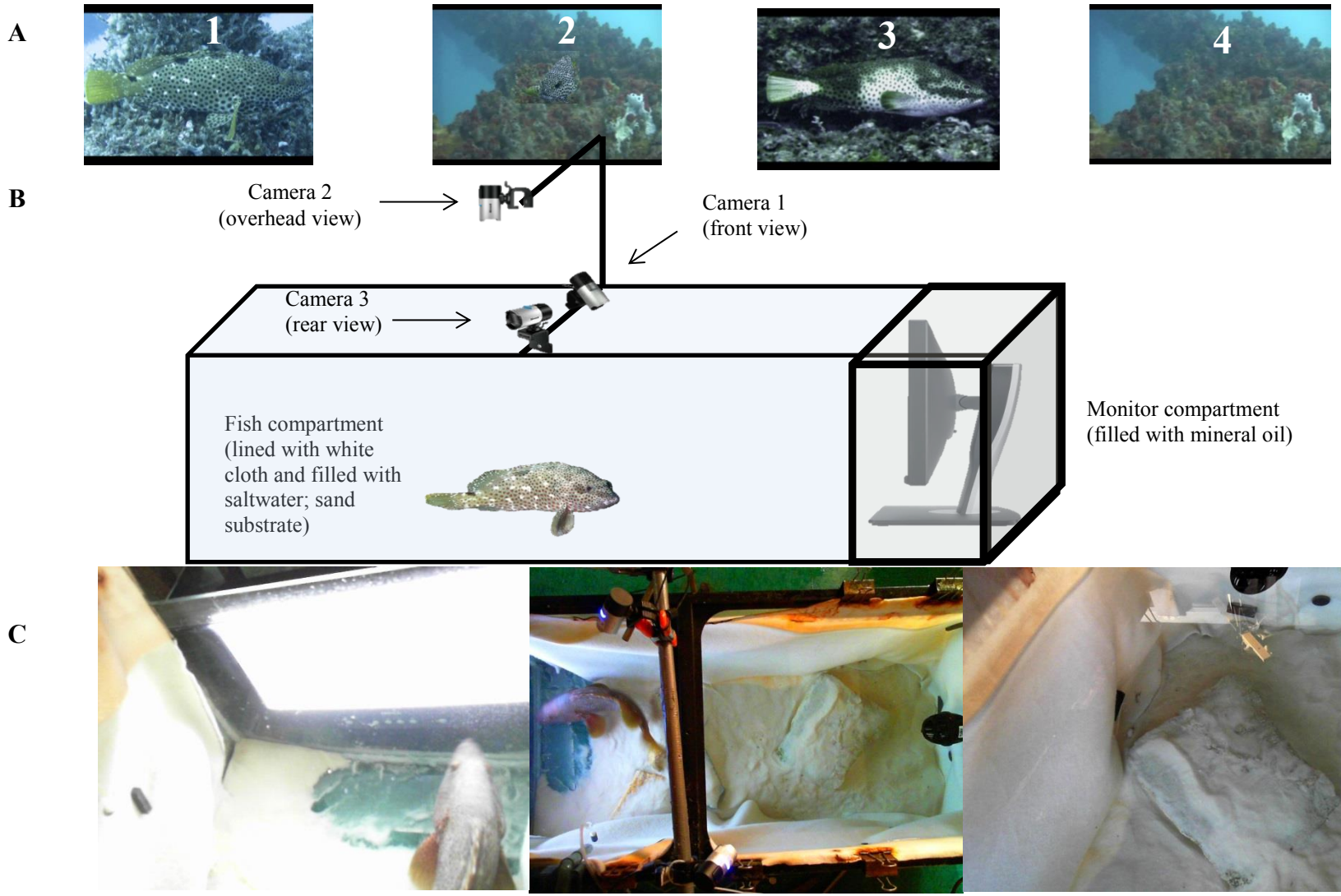

A
1
2
3
4
B
Camera 2
(overhead view)
Camera 1
(front view)
Camera 3
(rear view)
Fish compartment
(lined with white
cloth and filled with
saltwater; sand
substrate)
Monitor compartment
(filled with mineral oil)
C

Figure 10: Rockhind polarization behavioral assay.

A) Four images were displayed to rockhinds with a 90 second duration/image in a randomized order during a 24-minute treatment period. This assay was conducted twice for each fish, once in which images were displayed from an LCD monitor with the front linear polarizer intact, such that color and brightness contrast was unaltered (color-brightness assay: images visible to viewers without polarization vision), and once in which images were displayed from an LCD monitor with the front linear polarizer removed, such that color and brightness contrast was removed and polarization contrast was intact (polarization assay: images invisible to viewers without polarization vision). Rockhinds rested for at least 48 hours between polarization and color-brightness assays, and the order of these assays was randomized. **B)** Rockhinds were filmed during the behavioral assays in an experimental tank. Trials consisted of a 10-minute acclimation, followed by a 24-minute control in which the monitor displayed a white background, followed by a 24-minute treatment of either the color-brightness assay or the polarization assay. The control and treatment were filmed from three camera angles. The display monitor was held in a separate compartment of the tank filled with mineral oil to prevent Fresnel reflections. White cloth lined the rear and side panels of the fish compartment to further prevent Fresnel reflections. **C)** Fish were filmed during control and treatment from three camera angles (front view: left panel; overhead view: center panel; rear view: right panel). Video from the overhead view was used for motion tracking, and video from all three views was used to score behaviors. All behavioral analysis and motion tracking was done post-trial.

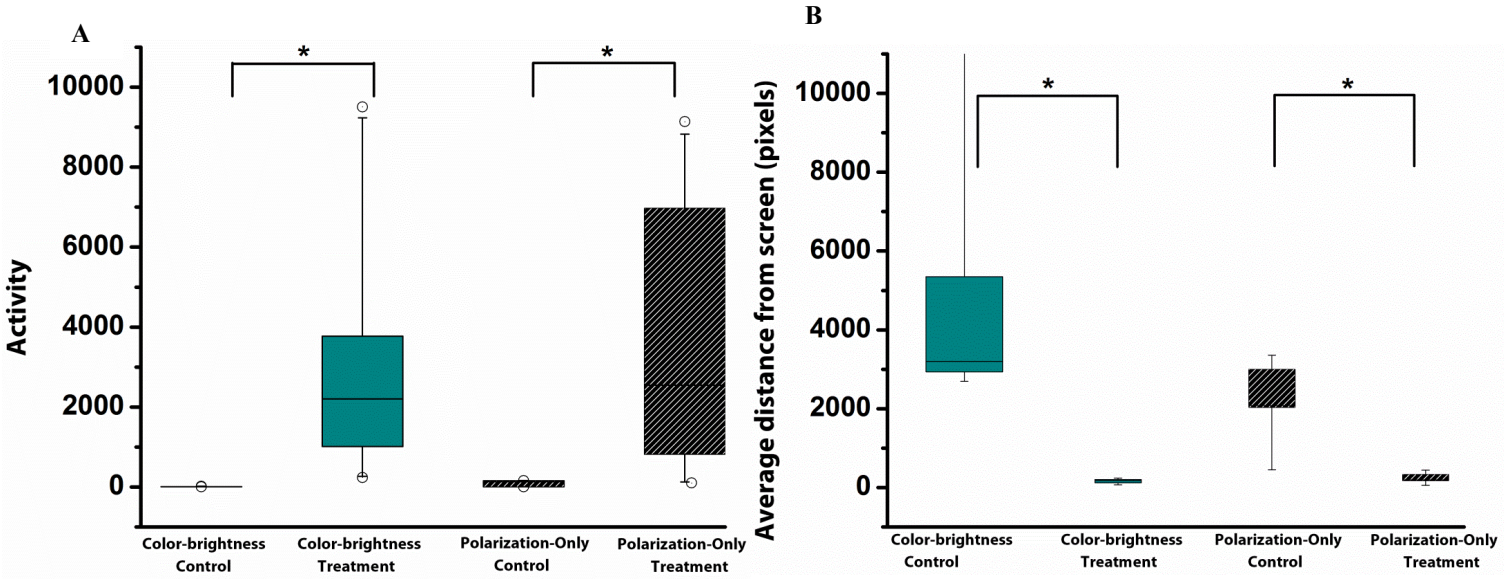


Figure 11: Effect of image display assay on motion tracking metrics.

A) Rockhind total activity and B) average distance from screen during control (no image displayed) and treatment (images of conspecifics and coral displayed), for the two assay types: color-brightness assay and polarization-only assay. Control and treatment differed significantly for each assay type, but there were no significant effect pf assay type (see Table 5). Activity was calculated as total path-length of the fish in pixels.

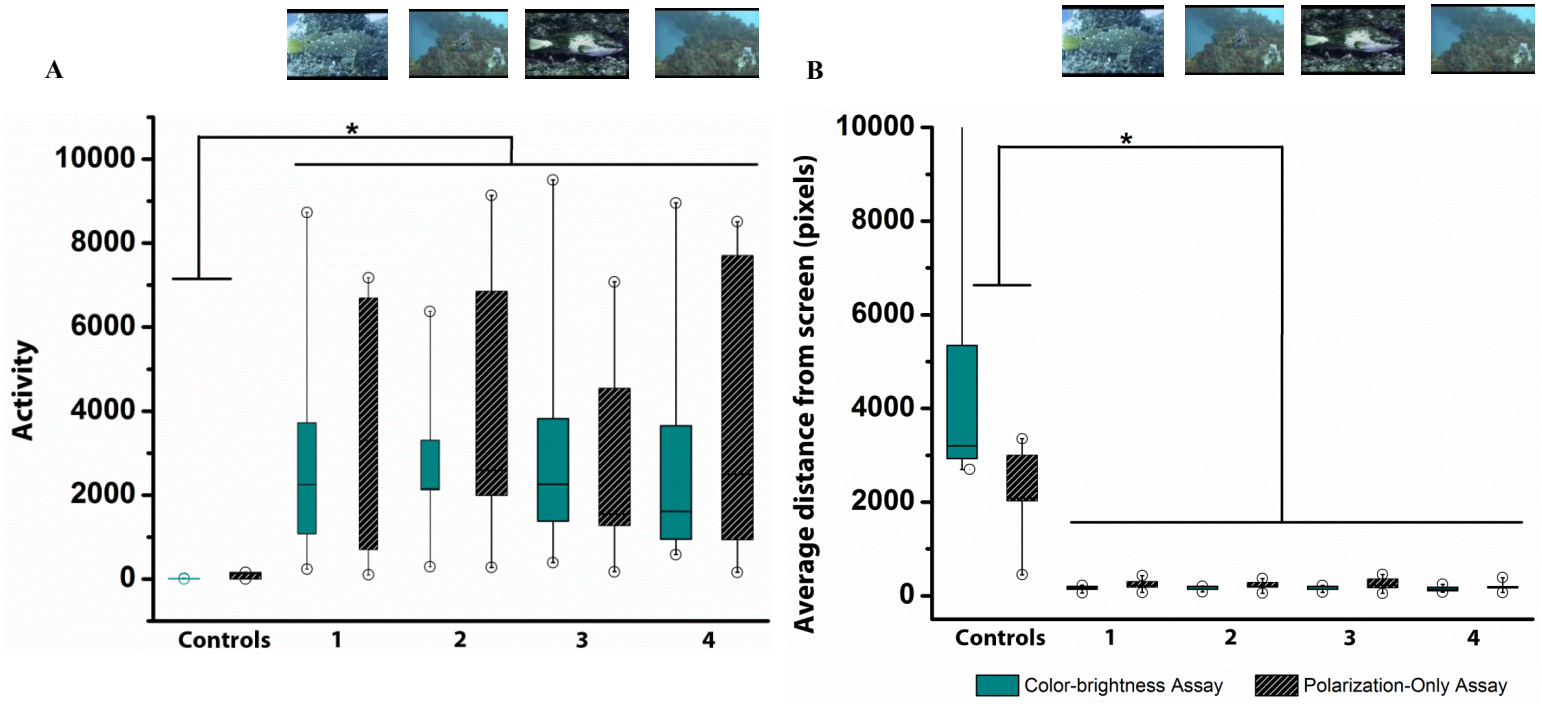
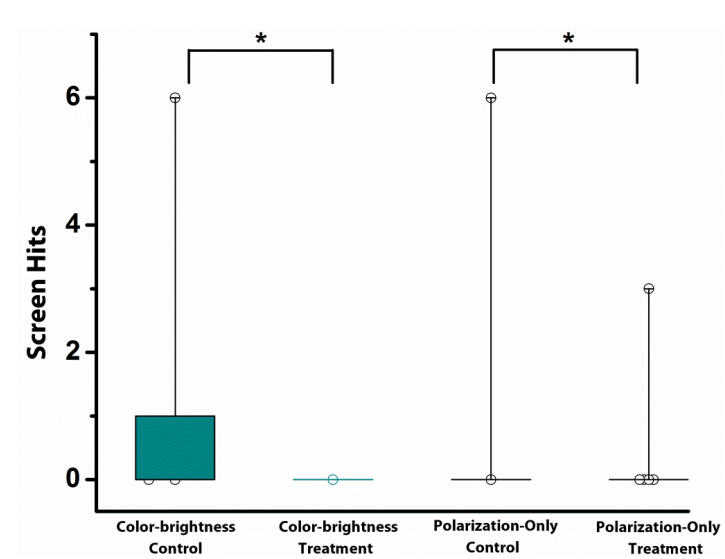
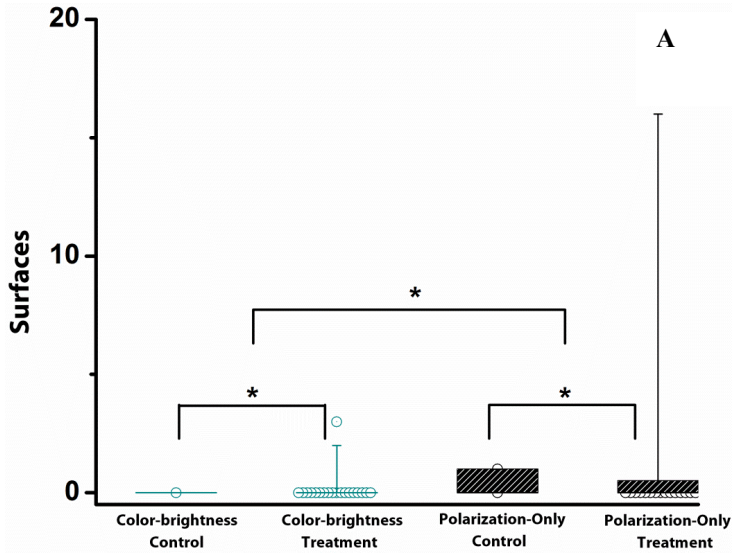


Figure 12: Effect of individual display image on motion tracking metrics.

A) Rockhind total activity and B) average distance from screen during control (no image displayed) and each of the treatment images (shown above panel and in figure 10A), for the two assay types: color-brightness assay and polarization-only assay. There was no significant effect of assay type on either metric (Table 6), but activity and average distance from screen differed significantly between control and each treatment image, for both assay types (Table 7). Activity was calculated as total path-length of the fish in pixels.





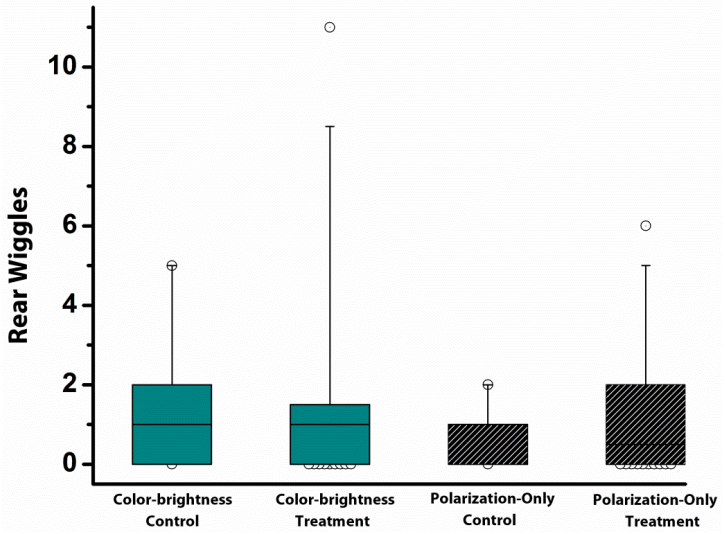


Figure 13: Effect of image display assay on frequencies of discrete behaviors.

Frequencies of A) screen hits, B) surfaces, and C) rear wiggles during control (no image displayed) and treatment (images of conspecifics and coral displayed), for the two assay types: color-brightness assay and polarization-only assay. Behaviors are described in table 4. Frequencies of screen hits and surfaces differed significantly between control and treatment, but only surfaces differed across assay types (see Table 8).

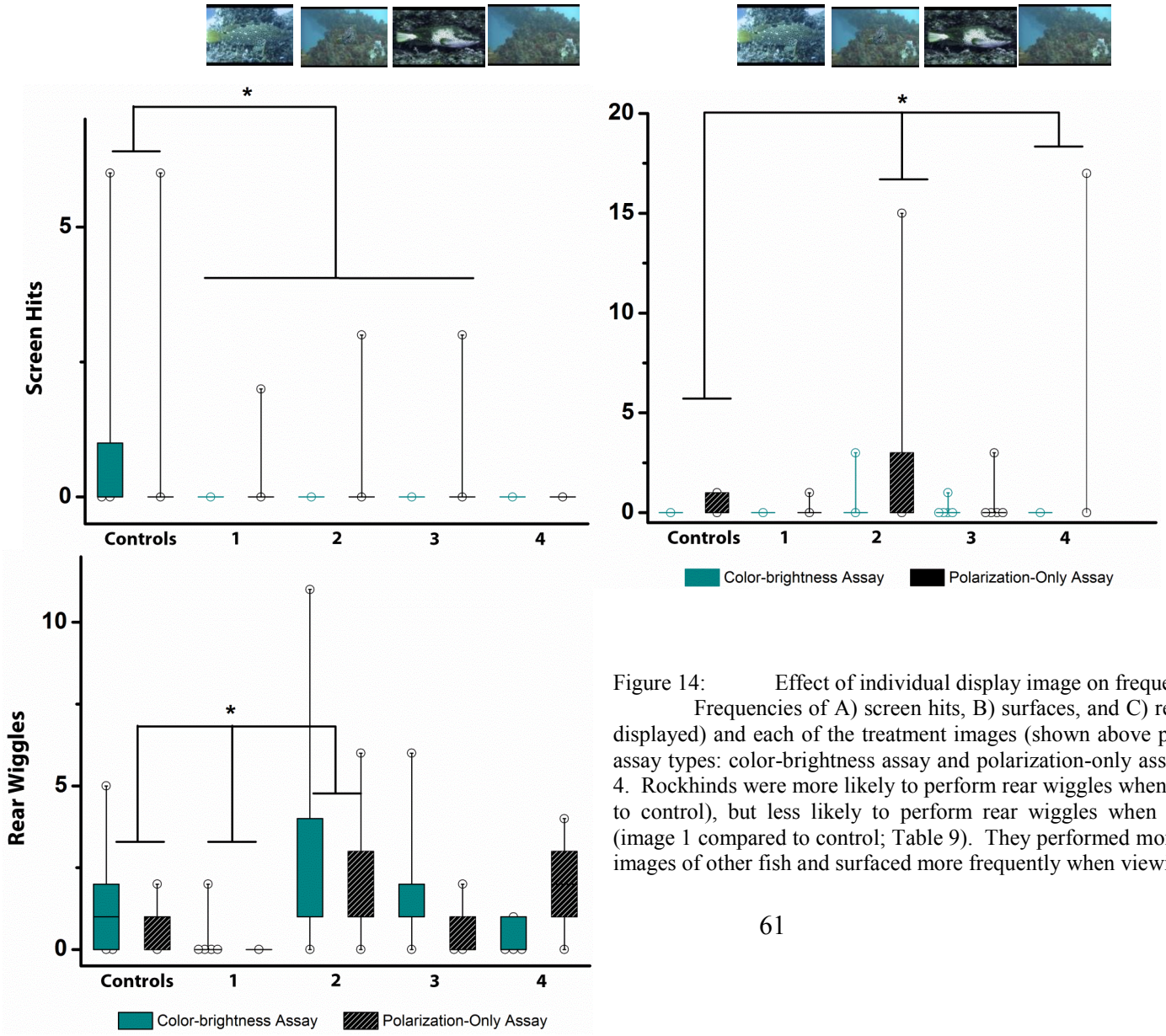


Figure 14: Effect of individual display image on frequencies of discrete behaviors.

Frequencies of A) screen hits, B) surfaces, and C) rear wiggles during control (no image displayed) and each of the treatment images (shown above panel and in figure 10A), for the two assay types: color-brightness assay and polarization-only assay. Behaviors are described in table 4. Rockhinds were more likely to perform rear wiggles when viewing females (image 2 compared to control), but less likely to perform rear wiggles when viewing cryptically-patterned males (image 1 compared to control; Table 9). They performed more tank fewer tank hits when viewing images of other fish and surfaced more frequently when viewing females and coral (Table 9).

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