

Polarization signaling in swordtails alters female mate preference

Gina M. Calabrese^a, Parrish C. Brady^a, Viktor Gruev^b, and Molly E. Cummings^{a,1}

^aDepartment of Integrative Biology, University of Texas at Austin, Austin, TX 78712; and ^bAdvanced Sensors Research Laboratory, Department of Computer Science and Engineering, Washington University in St. Louis, Saint Louis, MO 63130

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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 species recognition. However, studies demonstrating that polarization body patterning serves as a communication signal (e.g., with evidence of changes in receiver behavior) are rare among invertebrate taxa and conspicuously absent among vertebrates. Here, we investigate polarization-mediated communication by northern swordtails, *Xiphophorus nigrensis*, using a custom-built videopolarimeter to measure polarization signals and an 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 filter 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 (~5%). Combining videopolarimetry and polarization-manipulated mate choice trials, we found sexually dimorphic polarized reflectance and polarization-dependent female mate choice behavior with no polarization-dependent courtship behavior by males. Male swordtails exhibit greater within-body and body-to-background polarization contrast than females, and females preferentially associate with high-polarization-reflecting males. We also found limited support that males increase polarization contrast in social conditions over asocial conditions. Polarization cues in mate choice contexts may provide aquatic vertebrates with enhanced detection of specific display features (e.g., movements, angular information), as well as a signaling mechanism that may enhance detection by intended viewers while minimizing detection by others.

sensory ecology | animal communication | sexual selection | dynamic signals

When sensory systems evolve to detect environmental properties, the opportunity arises for the evolution of signals that use these features (1–3). The complex interaction of light with atmospheric and underwater particles leads to predictable polarization backgrounds in terrestrial and aquatic environments (4–9). In brief, “polarization” refers to the vibrational behavior of the electromagnetic field, with unpolarized light describing photons vibrating in all possible directions (e.g., sunlight before 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 was the first, to our knowledge, to demonstrate that bees use polarization gradients in the sky as a compass (10). Since 1949, researchers have determined that many invertebrates use polarization cues for celestial orientation (11–13), navigation (14–17), foraging (18), and species recognition (19), and have identified an angular distribution of linear polarized detectors that is responsible for polarization sensitivity in many invertebrate eyes (20). Although polarization body patterning has been measured in a variety of taxa [cephalopods (21–24), stomatopods (25, 26), and butterflies (19)], polarization patterning has only been shown to

affect receiver behavior, and thus function as a signal, in the butterfly *Heliconius cydno* (19).

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 (27–31). This lack of evidence for polarization-mediated behavior in vertebrates may be due, in part, to the current lack of a mechanistic understanding of vertebrate polarization sensitivity or to the fact 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 (32)]; hence, vertebrate polarization sensitivity likely employs a different mechanism for detection than do invertebrates (current hypotheses reviewed in refs. 33, 34). Despite differences in mechanisms, there is ample behavioral evidence from behavioral and physiological training experiments (28, 35–40), as well as direct cellular recordings in the retina (41, 42), optic nerve (29, 41, 42), and optic tectum (43, 44), that many nonmammalian vertebrates, particularly fish, have polarization sensitivity. 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. In the aquatic environment of the northern swordtail, *Xiphophorus nigrensis*, scattering interactions between light and water molecules produce a polarization

Significance

Polarization, the alignment of light waves in a plane, is a property that many nonhuman animals can detect. Polarized body patterning on some animals has prompted research into polarization as a signaling modality, but experimental evidence is lacking. We found evidence for sexual selection on polarization ornamentation in a swordtail fish: sexually dimorphic polarization patterning with higher polarization contrast in males and females exhibiting a mate preference for this ornamentation. We manipulated male polarization properties in a mate choice experiment and found that females prefer males with polarized over depolarized ornamentation and that males may increase polarization contrast in social environments. Polarization signaling may provide enhanced display detection, along with privatized communication, due to the directional component of polarized light.

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¹To whom correspondence should be addressed. Email: mcumings@austin.utexas.edu.

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gradient that is dependent on the location of the sun (45). The near-surface underwater environment is characterized by a high degree of linear polarization [DoLP; the fraction of light that is polarized (46)], which varies in terms of its plane of orientation (9). 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 on 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 (41). 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 (41).

Swordtails are a highly tractable system for studying visual mate preference behavior in the laboratory (47–51) using a simple measure of association time that significantly predicts female mating intent (52). In *X. nigrensis*, females prefer the large courting males over the small nonornamented male class that relies on force copulation for mating success (53). In this species, female preference or receptivity behaviors do not vary across the reproductive cycle (54), and preference behaviors, such as association time, are repeatable and consistent (55). 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 three levels of evidence (56): (i) polarization signal production by a sender, (ii) detection of the signal by a receiver, and (iii) change in receiver behavior that is adaptive to the sender or receiver. Although polarization body patterning (step 1) has been described in several invertebrate species (reviewed in refs. 56, 57), evidence for adaptive behavioral responses by receivers (steps 2 and 3) has been limited in invertebrates (19), 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* uses polarization cues for communication, we first compared polarization patterning between males and females with a custom-built videopolarimeter (58), calculating polarization contrast for DoLP, Q, and U (Fig. 2). We then quantified female mate preference response to large

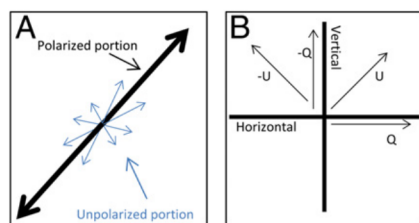


Fig. 1. Schematic of polarization of light. (A) Graphical description of the degree of polarized light (i.e., the amount that is polarized). Arrows indicate the direction of the electric field of light, and thickness indicates the amount of light with the electric field aligned in that direction. Unpolarized light is equivalent to light with a randomized electric field direction (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.

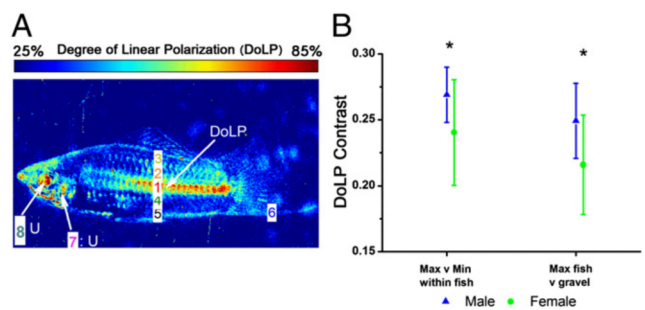


Fig. 2. *X. nigrensis* sexual dimorphism in polarization reflectance and contrast. (A) Videopolarimetry image of a large male *X. nigrensis* in false color showing the DoLP reflectance. Videopolarimetry measurements were collected from free-swimming large males ($n = 12$) and females ($n = 17$) under identical polarization conditions and body positions. Colored numbers indicate measured body regions: 1, lateral line; 2, upper flank; 3, dorsum; 4, lower flank; 5, ventrum; 6, caudal fin base; 7, operculum; and 8, eye. Arrows indicate body regions with significant sexual dimorphism in DoLP, Q, or U. (B) Mean and SE of within-body [maximum (Max) - minimum (Min)] and body-to-background (fish Max - gravel) DoLP contrast measures for the 12 males and 17 females measured in A. Arrows in A and asterisks in B indicate significant differences ($P < 0.05$) before Benjamini-Hochberg corrections for multiple comparisons (statistics are provided in Tables S2 and S3).

males with altered polarization-reflecting ornamentation using a mate choice assay that predicts female mating intent (52). 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 a male's light environment. The high-polarization (high-DoLP) treatment significantly increases the polarization of males by 214–289% relative to the low-polarization (low-DoLP) treatment (Fig. 3A), while altering signal color and intensity below fish detection thresholds (Table S1). By significantly altering the polarization of signaling males while keeping variation in signal color and intensity below visual detection thresholds (59), we can isolate the female mating response to differences in the polarization features of the male. Finally, we measured the polarization contrast features of males and females swimming alone relative to social conditions to determine whether polarization features differed by social context. We found evidence that *X. nigrensis* uses polarization cues for communication with our measurements of sexually dimorphic polarized ornamentation and differential female response toward males with high-polarization contrast.

Results

Sexual Dimorphism in Polarization Patterning. To quantify DoLP, Q, and U reflectance from swordtails, we filmed free-swimming females ($n = 17$) and large courter males ($n = 12$) with a videopolarimeter under horizontally polarized illumination mimicking natural shallow-water polarization conditions (9), with background water DoLP measuring 34%. Because polarization properties are strongly influenced by the position of sender and receiver, we controlled for fish position by selecting frames for analysis in which the fish was perpendicular to the camera (e.g., full lateral flank was visible) and the fish's long axis was within 15° of horizontal (an example is provided in Fig. 2A). These positioning criteria mimic the most behaviorally relevant position for courtship display (53, 60), 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 males reflected significantly higher DoLP than females on the lateral line ($P = 0.02$) and significantly higher U components ($P = 0.0004$) from the operculum (Fig. 2A, Fig. S1 B and D, and Table S2; both $P < 0.05$ after Benjamini-Hochberg correction). Sexually

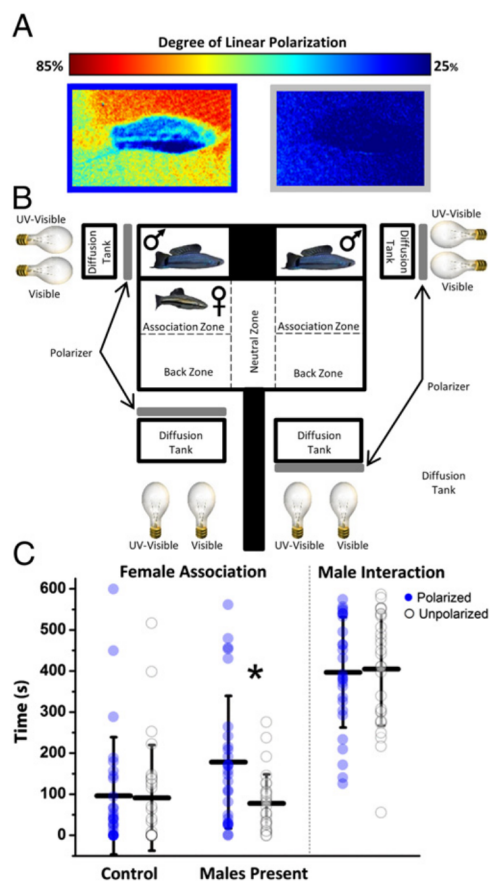


Fig. 3. Female mate preference for male polarization ornamentation. (A) Videopolarimetry image showing the DoLP reflectance of a courter male in the high-DoLP experimental condition (Left) and the same male in the low-DoLP experimental condition (Right). (B) Schematic of female mate preference experiment. Females were presented with two side-by-side male chambers and presented with a pair of size-matched large males. Each male was illuminated from the front and side by visible-range and UV-visible bulbs. Diffusion tanks depolarized source light, and 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), with minimal differences in male hue, saturation, and luminance values between polarization conditions (measurements and statistics are provided in Tables S1 and S4). 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) *X. nigrensis* female preference for polarized males. Data points indicate time in polarized (blue) and unpolarized (gray) conditions, with the overlaid black lines and error bars showing mean \pm 1 SE. Females ($n = 28$) spent significantly more time associating with males in the polarized condition relative to the unpolarized condition ($*P = 0.01$, paired $t_{df=27} = 2.76$), although showing no difference in male-absent controls ($P = 0.90$, paired $t_{df=27} = 0.12$). Male interaction time with females did not differ between polarized and unpolarized conditions ($P = 0.85$, paired $t_{df=27} = -0.19$).

dimorphic differences in polarization reflectance resulted in greater polarization contrast for males relative to females in both DoLP within-body ($P = 0.02$) and body-to-background ($P = 0.01$) contrast measures, as well as higher U body-to-background ($P = 0.03$) contrast (Fig. 2B, Fig. S2, and Table S3), with DoLP background contrast measures surviving ($P = 0.03$), within-body DoLP exhibiting marginal significance in ($P = 0.053$), and U contrast not surviving ($P = 0.096$) Benjamini–Hochberg multiple hypothesis corrections.

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. 3A and B and Tables S1 and S4). 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. 3A and B). The two experimental conditions significantly altered male DoLP reflectance by a factor of 2 ($P < 0.01$; Fig. 3A and Table S1). The gain in male DoLP reflectance in high-DoLP conditions relative to low-DoLP conditions varied by body region, with a 214% increase in the dorsum region to a >280% increase in flank and operculum regions and a 225% gain in within-body contrast (maximum – minimum contrast; Table S1). To ensure that the experimental manipulation did not significantly alter nonpolarization features of the male display, such as the luminance or color (hue and saturation) features, we directly measured the change in male swordtail polarization, luminance, and color features between the high- and low-DoLP conditions. Although direct measurements of teleost photoreceptor detection limits are limited to goldfish (59), most researchers assume a conservative detection limit of 5% across a range of freshwater and saltwater species (61–64). Here, we also apply a conservative assumption that differences (<5%) in measured color and luminance properties of male swordtails across polarization treatments are most likely not detectable to the swordtail visual system. We found no statistically significant, visually detectable change in swordtail color features (hue, saturation, and luminance) across all eight body regions between males illuminated in the high-DoLP condition relative to the low-DoLP condition (Table S1), with one exception [dorsum region exhibiting a marginally detectable change in luminance (6%); Table S1].

All measurements of association time passed Shapiro–Wilks tests for normality, allowing us to use parametric statistics to investigate female preference behavior. Association time is a standard and well-supported metric of female mate preference in swordtails (48–52, 65–67). Although it has been suggested that female association may reflect shoaling preferences when stimuli differ in size in the sailfin molly, *Poecilia latipinna* (68), the use of association time to assess *X. nigrensis* female preference for polarization ornamentation is well supported for the following reasons: (i) males in this experiment were size-matched (eliminating the possibility of size-directed shoaling preference); (ii) association time has been shown to reflect *X. nigrensis* female mating preference accurately in the wild (69), is consistent with sexual receptivity behaviors exhibited in barrier-free trials (55), and does not vary over a female’s reproductive cycle (54); and (iii) whole-brain gene expression profiling comparisons in *X. nigrensis* between females associating with males vs. females associating with females are significantly different, suggesting that from a neuro-genetic perspective, associating with males is an entirely different behavioral process than shoaling with females (70, 71). Furthermore, in a congeneric swordtail species, *Xiphophorus helleri*, female association preferences for particular males predict the probability of female reproduction with those males in free access trials (52).

In our control trials (males absent), *X. nigrensis* females exhibited no significant difference in time spent in high-DoLP vs. low-DoLP association zones (Fig. 3C; paired $t_{df=27} = 0.12$, $P = 0.90$) or in time spent in all high-DoLP vs. all low-DoLP zones (high-DoLP association + back mean = 247 s; low-DoLP association + back mean = 244 s; paired $t_{df=27} = 0.04$, $P = 0.97$). However, when males were present, females spent significantly more time in the high-DoLP association zone than in the low-DoLP association zone (Fig. 3C; paired $t_{df=27} = 2.76$, $P = 0.01$) but did not differ in time spent in high-DoLP and low-DoLP back zones (paired $t_{df=27} = 0.61$, $P = 0.55$). These results indicate that females have no preference for the polarization state of the environment but do have 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. 3C; paired $t_{df=27} = -0.19$, $P = 0.85$), 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, $F = 7.83$; $df_{\text{polarization treatment}} = 1$, $df_{\text{error}} = 16$, $P = 0.01$), but neither male pair ($F = 1.01$, $df_{\text{pair identity}} = 5$, $df_{\text{error}} = 16$, $P = 0.44$) nor the interaction of polarization treatment * male pair ($F = 2.16$, $df_{\text{interaction}} = 5$, $df_{\text{error}} = 16$, $P = 0.11$) was a significant explanatory variable.

Social Modulation of Polarization Patterning. We used videopolarimetry of males ($n = 12$) and females ($n = 17$) in social and asocial contexts to measure within-body and body-background DoLP, Q, and U contrasts (Fig. 4, Fig. S3, and Table S5). We found limited evidence that males, but not females, exhibit an increase in within-body ($P = 0.02$) DoLP contrast in social conditions relative to asocial conditions (Fig. 4). For body-to-background contrast changes between social and asocial conditions, males exhibited a trend toward increasing fish against gravel background contrasts ($P = 0.03$; Fig. 4) and females exhibited a trend toward decreasing fish against water background contrasts ($P = 0.02$; Fig. S3). However, none of these social modulation trends survived Benjamini–Hochberg correction (Table S5).

Discussion

X. nigrensis swordtails meet the evidential criteria for polarization signaling outlined by Mäthger et al. (56). Male swordtails produce a polarization signal (sexually dimorphic polarization patterns; Fig. 2) that is sensed by receivers (females increase association time in the presence of the signal; Fig. 3C) and alters female behavior in an adaptive manner for males [female association time is predictive of reproductive success in swordtails (52, 69)]. Sexually dimorphic polarization reflectance resulted in higher male within-body and body-to-background contrast measurements (Fig. 2B), suggesting that males are easier to detect than females for a polarization-sensitive viewer. This study is the first demonstration, to our knowledge, of polarization communication in a vertebrate.

Our results suggest that variation in polarization ornamentation may be under sexual selection in this species. *X. nigrensis* males and females are significantly dimorphic in both degree and angular components of polarization reflectance, and females prefer polarization-reflecting males (Figs. 2 and 3). Female preference for

males exhibiting higher polarization contrast ornamentation may be due to the increased conspicuousness that polarization ornamentation provides, because contrast between patches within an animal, or between an animal and background, increases the detectability of signals (1). Contrast detection is a key feature of processing visual stimuli in the brain (72–74), and contrast measures from other visual signal components (e.g., color, luminance contrast) are important features in sexual selection across taxa (75–84). 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 (85). 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 (86). Second, the aquatic polarization environment is not axially symmetrical at low solar angles (4, 9, 85, 87). The position of the sun relative to the signaler and receiver affects the background polarization features (DoLP, Q, and U) observed by the receiver; thus, 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 change polarization features facultatively and contrast with background through rotation or repositioning (85), allowing swordtails to customize signaling features to accommodate signaling microenvironments and multiple viewers. Future studies should investigate whether polarization signalers strategically vary their display position in their environment.

The sexual dimorphism in polarization contrast observed in this species may be context-dependent. Comparison of polarization attributes of males and females in social and asocial contexts reveals that males show a limited trend toward increasing their within-body and body-to-background polarization contrast in social conditions relative to asocial conditions (Fig. 4). Although these results do not survive correction for multiple hypotheses testing, they are consistent with the concept of polarization ornamentation as a sexually selected communication trait. If males are using polarization signals to court females and to mediate male–male interactions, increasing the expression of these signals during social interactions is expected, particularly if they are costly to produce [energetically or in terms of predator detection (3)]. Our strict positioning criteria for analysis controlled for the effects of body position on polarization features, preventing detection of any effect of movement and positioning differences across social contexts on polarization signaling. Hence, future studies may examine the difference in polarization contrast across all body positions in social conditions relative to asocial conditions. Receiver-dependent differences in polarization signaling could be achieved by alterations in the male's body position during social interactions. 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, with minimal differences in luminance or color between high-DoLP and low-DoLP conditions (Fig. 3). The visual system is capable of simultaneously processing different visual cues (e.g., hue, saturation, intensity); thus, it is essential that behavioral studies isolate the component of interest without manipulating other important features of the stimuli. The ability to alter polarization properties of male stimuli significantly, while minimally altering the intensity and color attributes of males below visual detection

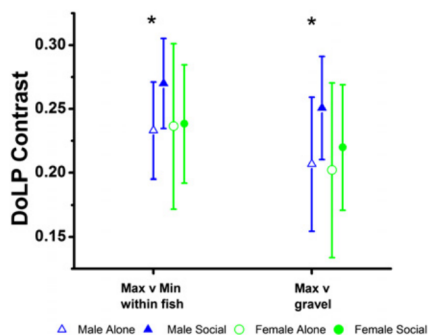


Fig. 4. Social modulation of DoLP contrast. DoLP contrast values [mean contrast ± 1 SE of males ($n = 12$) and females ($n = 17$)] are plotted as the difference between the maximum-DoLP (Max) and minimum-DoLP (Min) fish regions and the difference between the Max-DoLP fish region and gravel substrate. Asterisks indicate significant differences ($P < 0.05$) before Benjamini–Hochberg corrections for multiple comparisons (statistics are provided in Table S5).

thresholds (Table S1), is a critically important advancement for behavioral studies in polarization. Quantifying the effects of polarization treatments on the color and luminance properties of the stimulus organism is an important control that should be routinely incorporated into polarization communication studies.

Although evidence for vertebrate polarization sensitivity has been well documented for decades (36), 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. Although 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.

Materials and Methods

Swordtails were collected from Brackenridge Field Laboratory populations stocked with *X. nigrensis* from Nacimiento del Rio Choy, San Luis Potosí, Mexico. Courter males and females were selected at random from the sample obtained by seining the stock populations, and all measurements and behavioral trials were conducted following approved University of Texas Institutional Animal Care and Use Committee protocols (AUP-2012-00033).

Reflectance Measurements. Large males ($n = 12$) 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) (9). 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 male and female was filmed alone, and with

a stimulus fish of each sex. 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 (WaveMetrics) programs from selected frames (averaging up to five frames per video) that met positional criteria (fish's long axis perpendicular to the camera and within 15° of horizontal). For sexual dimorphism comparisons, we calculated an average for each body region across all conditions (alone, with male, and with female) for each individual ($n = 12$ males, $n = 17$ females). For social modulation comparisons, we calculated the average for each individual's body region within social conditions (alone trials) and compared that with the average across social conditions (with male and with female). Because all data passed Shapiro-Wilkes tests for normality, Welch two-sample t tests were used for all comparisons, and Benjamini-Hochberg corrections were applied to correct for multiple comparisons.

Behavioral Experiment. Swordtails ($n = 28$ female subjects, $n = 9$ large males as stimuli) were isolated for at least 1 wk before preference testing to ensure motivation to mate. Males were size-matched to form six pairs, each of which was used to test three to seven females.

Females were presented with two side-by-side male chambers (Fig. 3B). Each male was illuminated from the front and side by a visible-

range bulb (Capsylite 120 W/120 V Spot; Sylvania) and a UV visible-range bulb (Reptile-UV 160 W/120 V; MegaRay Zoologist). Diffusion tanks [2-gallon tank of an aqueous dispersion of magnesium hydroxide, a 1:277 dilution of Maalox (Novartis)] depolarized source light, and UV-transmissive horizontal polarizers (Bolder Vision Optik) 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. 3B, Left side; polarization standard mean DoLP = $23.5 \pm 11.2\%$ and maximum 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 such that light was polarized but subsequently depolarized before reaching the experimental tank (Fig. 3B, Right side; polarization standard mean DoLP = $5.38 \pm 2.43\%$ and maximum DoLP = 11.2%). Intensity measurements between the high- and low-DoLP conditions did not significantly differ [polarization standard orthogonal to male (90°): paired $t_{df=7} = 2.11$, $P = 0.08$; polarization standard 45° to male: paired $t_{df=7} = 0.35$, $P = 0.74$; and polarization standard -45° to male: paired $t_{df=7} = 1.47$, $P = 0.18$].

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 10 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 (National Institutes of Health)]. Males were positioned to mimic their "lateral display" during courtship bouts, with the fish's long axis perpendicular to a potential viewer (Fig. 3A); hence, these measurements should represent one of the most biologically relevant views to females. Each male was recorded in the high-DoLP and low-DoLP conditions with four replicate measures (alternating the order) for up to 1 min.

During the behavior trials, DoLP conditions (high vs. low) were alternated between sides for each trial, as were the individual males of each size-matched pair. Males could not see one another, but 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 the absence of male stimuli, followed by a 10-min preference test (males in front of the barriers and visible to females). Trials were filmed, and videotapes were scored for the time females spent in each zone and male interaction time [time spent moving within the front portion of a male's chamber (i.e., the 8-cm portion directly adjacent to the female chamber in Fig. 3B)], blinded to polarization condition.

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