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]

[a]Department of Integrative Biology, University of Texas at Austin, Austin, TX 78712; and [b]Advanced Sensors Research Laboratory, Department of Computer Science and Engineering, Washington University in St. Louis, Saint Louis, MO 63130

Edited by Gene E. Robinson, University of Illinois at Urbana–Champaign, Urbana, IL, and approved July 30, 2014 (received for review November 13, 2013)

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.

Author contributions: G.M.C., P.C.B., and M.E.C. designed research; G.M.C. performed research; P.C.B. and V.G. contributed new reagents/analytic tools; G.M.C. analyzed data; and G.M.C., P.C.B., V.G., and M.E.C. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

[1]To whom correspondence should be addressed. Email: mcummings@austin.utexas.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1321368111/-/DCSupplemental.

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

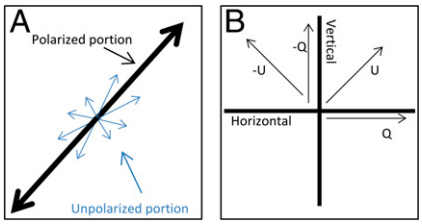

Fig. 1. Schematic of polarization of light. (*A*) Graphical description of the degree of polarized light (i.e., the amount that is polarized). Arrows indicate the direction of the electric field of light, and thickness indicates the amount of light with the electric field aligned in that direction. Unpolarized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

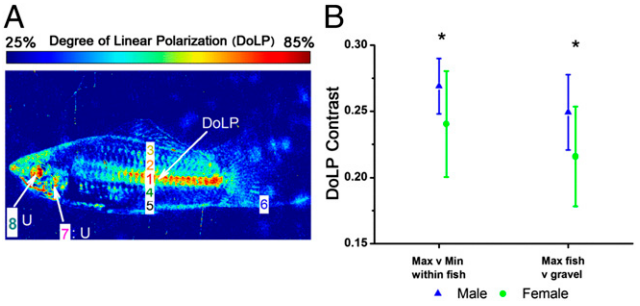

Fig. 2. *X. nigrensis* sexual dimorphism in polarization reflectance and contrast. (*A*) Videopolarimetry image of a large male *X. nigrensis* in false color showing the DoLP reflectance. Videopolarimetry measurements were collected from free-swimming large males ($n = 12$) and females ($n = 17$) under 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. 3*A*), 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. 2*A*). 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. 2*A*, Fig. S1 *B* and *D*, and Table S2; both $P < 0.05$ after Benjamini–Hochberg correction). Sexually

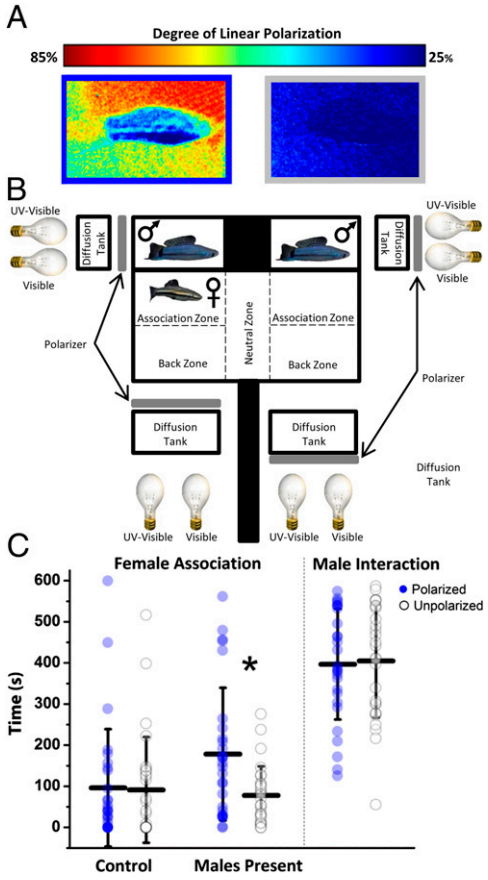

Fig. 3. Female mate preference for male polarization ornamentation. (*A*) Videopolarimetry image showing the DoLP reflectance of a courter male in the high-DoLP experimental condition (*Left*) and the same male in the low-DoLP experimental condition (*Right*). (*B*) Schematic of female mate preference 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 ± 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. 2*B*, 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. 3 *A* 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. 3 *A* and *B*). The two experimental conditions significantly altered male DoLP reflectance by a factor of 2 (P << 0.01; Fig. 3*A* 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–Wilkes 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 neurogenetic 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. 3*C*; 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. 3*C*; 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. 3*C*; 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, $F = 7.83$; $df_{polarization\_treatment} = 1$, $df_{error} = 16$, $P = 0.01$), 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 ($F = 2.16$, $df_{interaction} = 5$, $df_{error} = 16$, $P = 0.11$) 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. 3*C*) 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. 2*B*), 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

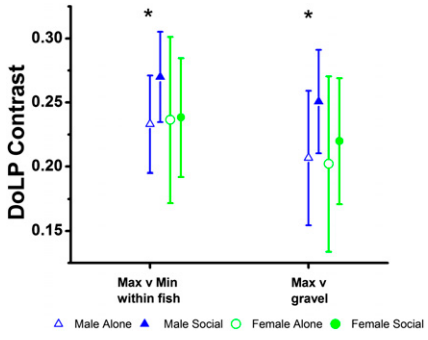

Fig. 4. Social modulation of DoLP contrast. DoLP contrast values [mean contrast ± 1 SE of males ($n = 12$) and females ($n = 17$)] are plotted as the difference between the maximum-DoLP (Max) and minimum-DoLP (Min) fish regions and the difference between the Max-DoLP fish region and gravel substrate. 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 $\left(\frac{\sqrt{Q^2+U^2}}{I}\right.$, where I = total intensity$\left.\right)$, 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 asocial 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. 3*B*). 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. 3*B*, *Left* side; polarization standard mean DoLP = 23.5 ± 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. 3*B*, *Right* side; polarization standard mean DoLP = 5.38 ± 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. 3*A*); 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. 3*B*], blinded to polarization condition.

ACKNOWLEDGMENTS. We thank Michael Ryan and Alex Jordan for supplying additional large male swordtails. We thank members of the M.E.C. laboratory for comments; Chad Brock for comments and assistance in experimental design; and Kat Ruddick, Julia Cosgrove, Emily Powell, James Macmillan, and Lynette Strickland for assistance in data collection. We also thank the Brackenridge Field Laboratory (University of Texas) for animal care facilities and the Mexican government for fish-collecting permits. This research was funded by Office of Naval Research (www.onr.navy.mil/) Multi-University Research Initiative Grant N000140911054 (to M.E.C.), Air Force Office of Scientific Research (www.wpafb.af.mil/afrl/afosr/) Grants FA9550-12-1-0321 and FA9550-10-1-0121 (to V.G.), and National Science Foundation (www.nsf.gov) Division of Ocean Sciences Grant 1130793 (to M.E.C. and V.G.).

1. Endler JA (1992) Signals, signal conditions, and the direction of evolution. *Am Nat* 139 (Suppl):S125–S153.
2. Endler JA, Basolo AL (1998) Sensory ecology, receiver biases and sexual selection. *Trends Ecol Evol* 13(10):415–420.
3. Ryan MJ, Cummings ME (2013) Perceptual biases and mate choice. *Annu Rev Ecol Evol Syst* 44:437–459.
4. Waterman TH (1954) Polarization patterns in submarine illumination. *Science* 120(3127): 927–932.
5. Ivanoff A, Waterman TH (1958) Factors, mainly depth and wavelength, affecting the degree of underwater light polarization. *J Mar Res* 16(255):283–307.
6. Shashar N, Cronin TW, Wolff LB, Condon MA (1998) The polarization of light in a tropical rain forest. *Biotropica* 30(2):275–285.
7. Cronin TW, Shashar N (2001) The linearly polarized light field in clear, tropical marine waters: Spatial and temporal variation of light intensity, degree of polarization and e-vector angle. *J Exp Biol* 204(Pt 14):2461–2467.
8. Treibitz T, Schechner YY (2009) Active polarization descattering. *IEEE Trans Pattern Anal Mach Intell* 31(3):385–399.
9. You Y, et al. (2011) Measurements and simulations of polarization states of underwater light in clear oceanic waters. *Appl Opt* 50(24):4873–4893.
10. von Frisch K (1949) Die Polarisation des Himmelslichtes als orientierender Faktor bei den Tanzen der Bienen. *Experientia (Basel)* 5(4):142–148, German.
11. Wehner R (1989) Neurobiology of polarization vision. *Trends Neurosci* 12(9):353–359.
12. Dacke M, Nilsson DE, Scholtz CH, Byrne M, Warrant EJ (2003) Animal behaviour: Insect orientation to polarized moonlight. *Nature* 424(6944):33.
13. Horvath G, Varju D (2004) *Polarized Light in Animal Vision: Polarization Patterns in Nature* (Springer, Berlin).
14. Goddard SM, Forward RB (1991) The role of underwater polarized light pattern in sun compass navigation of the grass shrimp, *Palaemonetes vulgaris*. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* 169(4):479–491.
15. Ritz DA (1991) Polarized light responses in the shrimp *Palaemonetes vulgaris* (Say). *J Exp Mar Biol Ecol* 154(2):245–250.

16. Schwind R (1999) Daphnia pulex swims towards the most strongly polarized light—A response that leads to 'shore flight'. *J Exp Biol* 202(Pt 24):3631–3635.
17. Shashar N, Sabbah S, Aharoni N (2005) Migrating locusts can detect polarized reflections to avoid flying over the sea. *Biol Lett* 1(4):472–475.
18. Shashar N, Hagan R, Boal JG, Hanlon RT (2000) Cuttlefish use polarization sensitivity in predation on silvery fish. *Vision Res* 40(1):71–75.
19. Sweeney A, Jiggins C, Johnsen S (2003) Insect communication: Polarized light as a butterfly mating signal. *Nature* 423(6935):31–32.
20. Labhart T, Meyer EP (1999) Detectors for polarized skylight in insects: A survey of ommatidial specializations in the dorsal rim area of the compound eye. *Microsc Res Tech* 47(6):368–379.
21. Shashar N, Rutledge P, Cronin T (1996) Polarization vision in cuttlefish in a concealed communication channel? *J Exp Biol* 199(Pt 9):2077–2084.
22. Shashar N, Hanlon RT (1997) Squids (*Loligo pealii* and *Euprymna scolopes*) can exhibit polarized light patterns produced by their skin. *Biol Bull* 193(2):207–208.
23. Boal JG, et al. (2004) Behavioral evidence for intraspecific signaling with achromatic and polarized light by cuttlefish (Mollusca: Cephalopoda). *Behaviour* 141(7):837–861.
24. Mäthger LM, Hanlon RT (2006) Anatomical basis for camouflaged polarized light communication in squid. *Biol Lett* 2(4):494–496.
25. Marshall J, Cronin TW, Shashar N, Land M (1999) Behavioural evidence for polarisation vision in stomatopods reveals a potential channel for communication. *Curr Biol* 9(14):755–758.
26. Chiou TH, et al. (2008) Circular polarization vision in a stomatopod crustacean. *Curr Biol* 18(6):429–434.
27. Taylor DH, Adler K (1973) Spatial orientation by Salamanders using plane-polarized light. *Science* 181(4096):285–287.
28. Cameron DA, Pugh EN, Jr (1991) Double cones as a basis for a new type of polarization vision in vertebrates. *Nature* 353(6340):161–164.
29. Parkyn DC, Hawryshyn CW (1993) Polarized-light sensitivity in rainbow-trout (*Oncorhynchus mykiss*)—Characterization from multiunit responses in the optic nerve. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* 172(4):493–500.
30. Flamarique IN, Browman HI (2001) Foraging and prey-search behaviour of small juvenile rainbow trout (*Oncorhynchus mykiss*) under polarized light. *J Exp Biol* 204(Pt 14):2415–2422.
31. Muheim R, Phillips JB, Akesson S (2006) Polarized light cues underlie compass calibration in migratory songbirds. *Science* 313(5788):837–839.
32. Flamarique IN, Hárosi FI (2002) Visual pigments and dichroism of anchovy cones: A model system for polarization detection. *Vis Neurosci* 19(4):467–473.
33. Kamermans M, Hawryshyn C (2011) Teleost polarization vision: How it might work and what it might be good for. *Philos Trans R Soc Lond B Biol Sci* 366(1565):742–756.
34. Roberts NW, Porter ML, Cronin TW (2011) The molecular basis of mechanisms underlying polarization vision. *Philos Trans R Soc Lond B Biol Sci* 366(1565):627–637.
35. Davitz MA, McKaye KR (1978) Discrimination between horizontally and vertically polarized light by the cichlid fish *Pseudotropheus macrophthalmus*. *Copeia* (2): 333–334.
36. Hawryshyn CW, McFarland WN (1987) Cone photoreceptor mechanisms and the detection of polarized-light in fish. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* 160(4):459–465.
37. Hawryshyn CW, Arnold MG, Bowering E, Cole RL (1990) Spatial orientation of rainbow-trout to plane polarized light—The ontogeny of e-vector discrimination and spectral sensitivity characteristics. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* 166(4):565–574.
38. Hawryshyn CW, Bolger AE (1990) Spatial orientation of trout to partially polarized-light. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* 167(5):691–697.
39. Parkyn DC, Austin JD, Hawryshyn CW (2003) Acquisition of polarized-light orientation in salmonids under laboratory conditions. *Anim Behav* 65(5):893–904.
40. Mussi M, Haimberger TJ, Hawryshyn CW (2005) Behavioural discrimination of polarized light in the damselfish Chromis viridis (family Pomacentridae). *J Exp Biol* 208(Pt 16):3037–3046.
41. Ramsden SD, Anderson L, Mussi M, Kamermans M, Hawryshyn CW (2008) Retinal processing and opponent mechanisms mediating ultraviolet polarization sensitivity in rainbow trout (*Oncorhynchus mykiss*). *J Exp Biol* 211(Pt 9):1376–1385.
42. Hawryshyn CW, Ramsden SD, Betke KM, Sabbah S (2010) Spectral and polarization sensitivity of juvenile Atlantic salmon (*Salmo salar*): Phylogenetic considerations. *J Exp Biol* 213(Pt 18):3187–3197.
43. Waterman TH, Hashimoto H (1974) E-vector discrimination by goldfish optic tectum. *J Comp Physiol* 95(1):1–12.
44. Waterman TH, Aoki K (1974) E-vector sensitivity patterns in the goldfish optic tectum. *J Comp Physiol* 95(1):13–45.
45. Waterman TH (2006) Reviving a neglected celestial underwater polarization compass for aquatic animals. *Biol Rev Camb Philos Soc* 81(1):111–115.
46. Shashar N, Sabbah S, Cronin TW (2004) Transmission of linearly polarized light in seawater: Implications for polarization signaling. *J Exp Biol* 207(Pt 20):3619–3628.
47. Cummings ME (2012) Looking for sexual selection in the female brain. *Philos Trans R Soc Lond B Biol Sci* 367(1600):2348–2356.
48. Ryan MJ, Wagner WE, Jr (1987) Asymmetries in mating preferences between species: Female swordtails prefer heterospecific males. *Science* 236(4801):595–597.
49. Basolo AL (1990) Female preference predates the evolution of the sword in swordtail fish. *Science* 250(4982):808–810.
50. Rosenthal GG, Evans CS (1998) Female preference for swords in *Xiphophorus helleri* reflects a bias for large apparent size. *Proc Natl Acad Sci USA* 95(8):4431–4436.
51. Cummings ME, Rosenthal GG, Ryan MJ (2003) A private ultraviolet channel in visual communication. *Proc Biol Sci* 270(1518):897–904.
52. Walling CA, Royle NJ, Lindstrom J, Metcalfe NB (2010) Do female association preferences predict the likelihood of reproduction? *Behav Ecol Sociobiol* 64(4):541–548.
53. Ryan MJ, Causey BA (1989) "Alternative" mating behaviour in the swordtails *Xiphophorus nigrensis* and *Xiphophorus pygmaeus* (Pices: Poeciliidae). *Behav Ecol Sociobiol* 24(6):341–348.
54. Ramsey ME, Wong RY, Cummings ME (2011) Estradiol, reproductive cycle and preference behavior in a northern swordtail. *Gen Comp Endocrinol* 170(2):381–390.
55. Cummings ME, Mollaghan DM (2006) Repeatability and consistency of female preference behaviours in a northern swordtail, *Xiphophorus nigrensis*. *Anim Behav* 72(1): 217–224.
56. Mäthger LM, Shashar N, Hanlon RT (2009) Do cephalopods communicate using polarized light reflections from their skin? *J Exp Biol* 212(Pt 14):2133–2140.
57. Douglas JM, Cronin TW, Chiou TH, Dominy NJ (2007) Light habitats and the role of polarized iridescence in the sensory ecology of neotropical nymphalid butterflies (Lepidoptera: Nymphalidae). *J Exp Biol* 210(Pt 5):788–799.
58. Gruev V, Perkins R, York T (2010) CCD polarization imaging sensor with aluminum nanowire optical filters. *Opt Express* 18(18):19087–19094.
59. Hawryshyn CW (1991) Light-adaptation properties of the ultraviolet-sensitive cone mechanism in comparison to the other receptor mechanisms of goldfish. *Vis Neurosci* 6(4):293–301.
60. Wong RY, So P, Cummings ME (2011) How female size and male displays influence mate preference in a swordtail. *Anim Behav* 82(4):691–697.
61. Matz MV, Marshall NJ, Vorobyev M (2006) Are corals colorful? *Photochem Photobiol* 82(2):345–350.
62. Cheney KL, Marshall JN (2009) Mimicry in coral reef fish: How accurate is this deception in terms of color and luminance? *Behav Ecol* 20(3):459–468.
63. Chiao C-C, Wickiser JK, Allen JJ, Genter B, Hanlon RT (2011) Hyperspectral imaging of cuttlefish camouflage indicates good color match in the eyes of fish predators. *Proc Natl Acad Sci USA* 108(22):9148–9153.
64. Rick IP, Bloemker D, Bakker TCM (2012) Spectral composition and visual foraging in the three-spined stickleback (Gasterosteidae: *Gasterosteus aculatus* L.): Elucidating the role of ultraviolet wavelengths. *Biol J Linn Soc Lond* 105(2):359–368.
65. Hankinson SJ, Morris MR (2002) Sexual selection and species recognition in the pygmy swordtail, *Xiphophorus pygmaeus*: Conflicting preferences. *Behav Ecol Sociobiol* 51(2):140–145.
66. Rosenthal GG, Wagner WE, Ryan MJ (2002) Secondary reduction of preference for the sword ornament in the pygmy swordtail *Xiphophorus nigrensis* (Pices: Poeciliidae). *Anim Behav* 63(1):37–45.
67. Rosenthal GG, Ryan MJ (2011) Conflicting preferences within females: Sexual selection versus species recognition. *Biol Lett* 7(4):525–527.
68. Gabor C (1999) Association patterns of sailfin mollies (*Poecilia latipinna*): Alternative hypotheses. *Behav Ecol Sociobiol* 46(5):333–340.
69. Ryan MJ, Hews DK, Wagner WE (1990) Sexual selection on alleles that determine body size in the swordtail *Xiphophorus nigrensis*. *Behav Ecol Sociobiol* 26(4):231–237.
70. Cummings ME, et al. (2008) Sexual and social stimuli elicit rapid and contrasting genomic responses. *Proc Biol Sci* 275(1633):393–402.
71. Ramsey ME, Maginnis TL, Wong RY, Brock C, Cummings ME (2012) Identifying context-specific gene profiles of social, reproductive, and mate preference behavior in a fish species with female mate choice. *Front Neurosci* 6:62.
72. Lettvin JY, Maturana HR, McCulloch WS, Pitts WH (1959) What the frog's eye tells the frog's brain. *Proceedings of the Institute of Radio Engineers* 47(11):1940–1951.
73. Hubel DH, Wiesel TN (1959) Receptive fields of single neurones in the cat's striate cortex. *J Physiol* 148(3):574–591.
74. Hubel DH, Wiesel TN (1962) Receptive fields, binocular interaction and functional architecture in the cat's visual cortex. *J Physiol* 160(1):106–154.
75. Endler JA (1983) Natural and sexual selection on color patterns in poeciliid fishes. *Environmental Biology of Fishes* 9(2):173–190.
76. Endler JA, Thery M (1996) Interacting effects of lek placement, display behaviour, ambient light, and color patterns in three neotropical forest-dwelling birds. *Am Nat* 148(1):421–452.
77. Andersson S, Örnborg J, Andersson M (1998) Ultraviolet sexual dimorphism and assortative mating in blue tits. *Proc Biol Sci* 265(1395):445–450.
78. Boughman JW (2001) Divergent sexual selection enhances reproductive isolation in sticklebacks. *Nature* 411(6840):944–948.
79. Gomez D (2004) Influence of ambient light on the evolution of colour signals: Comparative analysis of a Neotropical rainforest bird community. *Ecol Lett* 7(4):279–284.
80. Pauers MJ, McKinnon JS, Ehlinger TJ (2004) Directional sexual selection on chroma and within-pattern colour contrast in *Labeotropheus fuelleborni*. *Proc Biol Sci* 271(Suppl 6):S444–S447.
81. Brooks R, Endler JA (2001) Direct and indirect sexual selection and quantitative genetics of male traits in guppies (*Poecilia reticulata*). *Evolution* 55(5):1002–1015.
82. Cummings ME (2007) Sensory trade-offs predict signal divergence in Surfperch. *Evolution* 61(3):530–545.
83. Stuart-Fox D, Moussalli A (2008) Selection for social signalling drives the evolution of chameleon colour change. *PLoS Biol* 6(1):e25.
84. Cummings ME, Crothers LR (2013) Interacting selection diversifies warning signals in a polytypic frog: An examination with the strawberry poison frog. *Evol Ecol* 27(4): 693–710.
85. Brady PC, Travis KA, Maginnis T, Cummings ME (2013) Polaro-cryptic mirror of the lookdown as a biological model for open ocean camouflage. *Proc Natl Acad Sci USA* 110(24):9764–9769.
86. Rutowski RL, Nahm AC, Macedonia JM (2010) Iridescent hindwing patches in the Pipevine Swallowtail: Differences in dorsal and ventral surfaces relate to signal function and context. *Funct Ecol* 24(4):767–775.
87. Cronin TW, Marshall J (2011) Patterns and properties of polarized light in air and water. *Philos Trans R Soc Lond B Biol Sci* 366(1565):619–626.

Supporting Information

Calabrese et al. 10.1073/pnas.1321368111

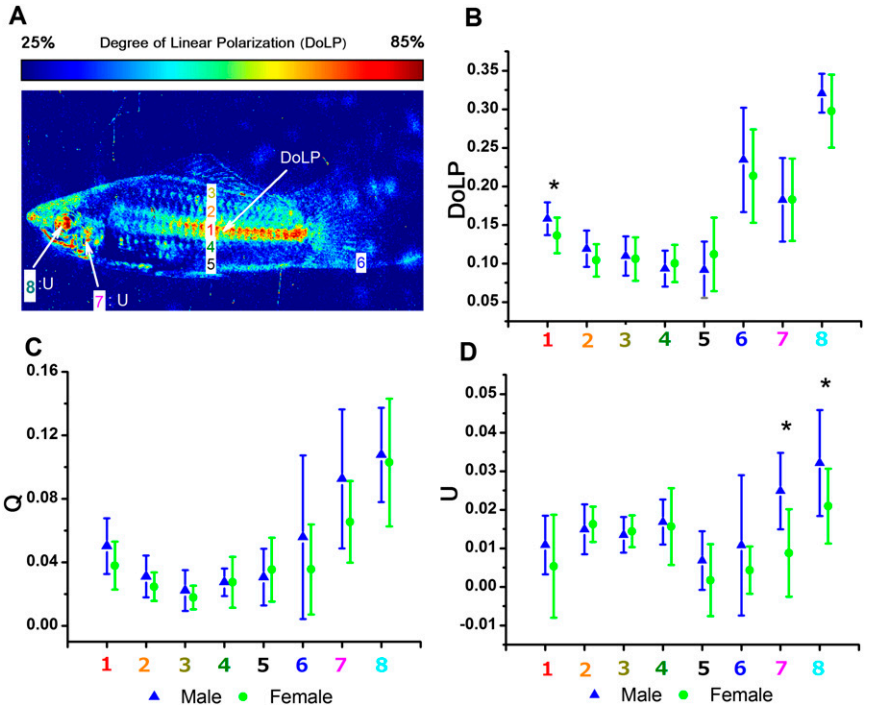

Fig. S1. Differences in male and female *Xiphophorus nigrensis* polarization reflectance. (*A*) Videopolarimetry image of a large male *X. nigrensis* in false color showing the degree of linear polarization (DoLP) reflectance. Colored numbers indicate body regions analyzed: 1, lateral line; 2, upper flank; 3, dorsum; 4, lower flank; 5, ventrum; 6, caudal fin base; 7, operculum; and 8, eye. Arrows indicate body regions with statistically significant ($P < 0.05$) sexual dimorphism in DoLP, Q, or 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. DoLP (*B*), Q (*C*), and U (*D*) of the body region given are on the *x* axis (numbers refer to body regions in *A*), plotted as mean ± 1 SE for large males and females. Fish were illuminated with horizontally polarized light to mimic midday underwater polarization conditions (1). Arrows in *A* and asterisks in *B* and *D* indicate significant differences ($P < 0.05$) before Benjamini–Hochberg corrections for multiple comparisons (statistics are provided in Tables S2 and S3).

1. You Y, et al. (2011) Measurements and simulations of polarization states of underwater light in clear oceanic waters. *Appl Opt* 50(24):4873–4893.

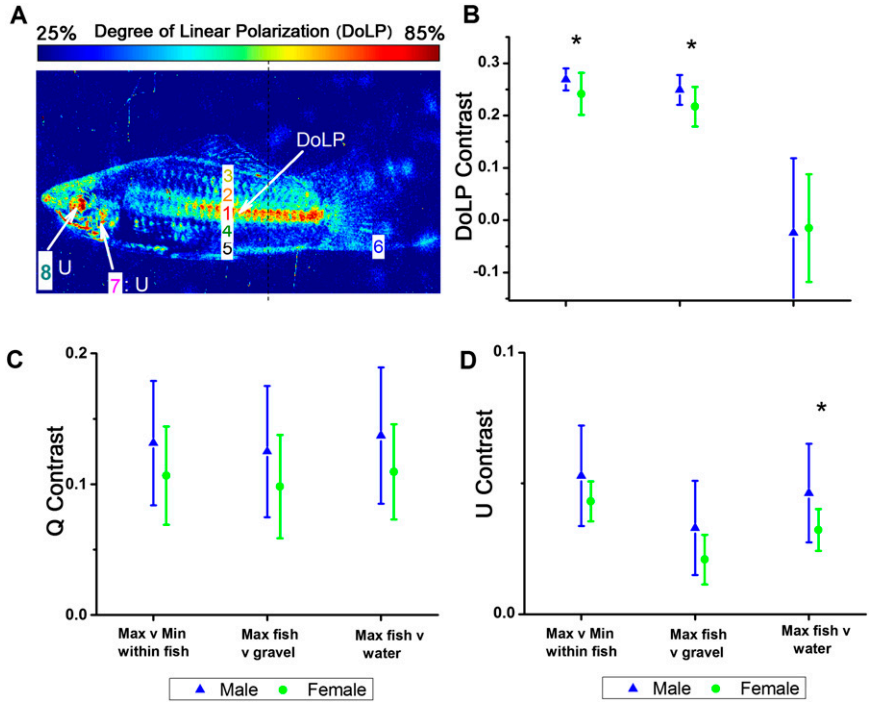

Fig. S2. Differences in male and female *X. nigrensis* polarization contrast. (*A*) Videopolarimetry image of a large male *X. nigrensis* in false color showing the DoLP reflectance. Colored numbers indicate measured body regions: 1, lateral line; 2, upper flank; 3, dorsum; 4, lower flank; 5, ventrum; 6, caudal fin base; 7, operculum; and 8, eye. Arrows indicate body regions with statistically significant ($P < 0.05$) sexual dimorphism in DoLP, Q, or U. Differences in male and female polarization contrast (mean $\pm$ 1 SE) for DoLP (*B*), Q (*C*), and U (*D*) are given. Contrast values are calculated as the difference between the two regions indicated on the *x* axis [e.g., maximum (Max)-DoLP fish region − minimum (Min)-DoLP fish region]. Fish were illuminated with horizontally polarized light to mimic midday underwater polarization conditions (1). Arrows in *A* and asterisks in *B* and *D* indicate significant differences ($P < 0.05$) before Benjamini–Hochberg corrections for multiple comparisons (statistics are provided in Tables S2 and S3).

1. You Y, et al. (2011) Measurements and simulations of polarization states of underwater light in clear oceanic waters. *Appl Opt* 50(24):4873–4893.

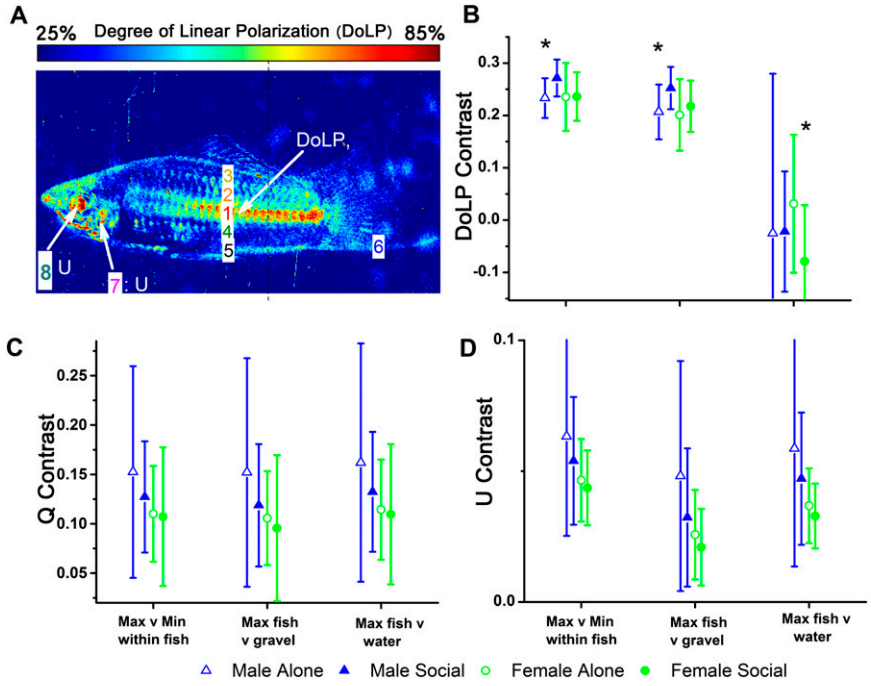

Fig. S3. Effect of social condition on male and female polarization contrast. (*A*) Videopolarimetry image of a large male *X. nigrensis* in false color showing the DoLP reflectance. Colored numbers indicate body regions analyzed: 1, lateral line; 2, upper flank; 3, dorsum; 4, lower flank; 5, ventrum; 6, caudal fin base; 7, operculum; and 8, eye. Arrows indicate body regions with statistically significant ($P < 0.05$) sexual dimorphism in DoLP, Q, or U. Contrast in DoLP (*B*), Q (*C*), and U (*D*), plotted as mean ± 1 SE for males ($n = 12$) and females ($n = 17$) alone and in social conditions (trials with males and with females combined), are given. Contrast values are calculated as the difference between the two regions indicated on the *x* axis (e.g., Max-DoLP fish region − Min-DoLP fish region). Fish were illuminated with horizontally polarized light to mimic midday underwater polarization conditions (1). Arrows in *A* and asterisks in *B* indicate significant differences ($P < 0.05$) before Benjamini–Hochberg corrections for multiple comparisons (statistics are provided in Table S5).

Table S1. Comparison of degree of linear polarization (DoLP), luminance, and color features of male swordtails (n = 10) across high-DoLP and low-DoLP experimental conditions

Measure	Statistic	Lateral line	Upper flank	Dorsum	Lower flank	Ventrum	Fin base	Operculum	Eye	Contrast (maximum − minimum)
Luminance	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
	P value	0.15	0.25	**0.01**	0.64	0.11	0.16	0.25	0.15	0.24
	Relative difference	0.06	0.03	0.06	0.01	0.03	0.04	−0.02	0.05	−0.04
Hue	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
	P value	0.63	0.35	0.51	0.70	0.71	0.01	0.83	0.37	0.37
	Relative difference	−0.01	−0.02	−0.01	−0.01	<0.01	−0.02	<0.01	−0.03	−0.11
Saturation	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
	P value	0.64	0.44	0.89	0.33	0.32	0.55	0.07	0.72	0.10
	Relative difference	0.01	0.01	<0.01	0.02	0.01	0.01	0.04	<0.01	−0.08
DoLP	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
	P value	**0.0001**	**0.0002**	**0.0013**	**0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
	Relative difference	2.56	2.86	2.14	2.83	2.50	2.36	2.89	2.33	2.25

Four replicate measures of male *Xiphophorus nigrensis* (n = 10) and a color standard (X-rite Colorchecker MSCCPPCC0113) were collected in a stationary position in both high-DoLP and low-DoLP experimental conditions. For each replicate, a male was filmed for 10 s with a standard color video camera (Olympus Stylus Tough TG-830 underwater camera) for color (hue and saturation) and luminance (intensity) values, as well as with the videopolarimeter to evaluate changes in DoLP (Fig. 3*A*). We calculated the average of each male's high-DoLP and low-DoLP replicate measurements, tested these averages for normality with the Shapiro–Wilkes test (all $P > 0.05$), and then used these averages to perform paired t tests (n = 10, df = 9) to evaluate male (Fig. S1*A*) and color-chip (Fig. S1*B*) differences between measurements in high-DoLP and low-DoLP conditions. Because all significant P values survived Benjamini–Hochberg correction for multiple comparisons, precorrection P values are reported. To evaluate whether differences in parameter measures were visually detectable, we calculated the "relative difference" of a male's average luminance, hue, saturation, and DoLP features between the high-DoLP and low-DoLP conditions as the quantity (high-DoLP illumination mean − low-DoLP illumination mean)/low-DoLP illumination mean). These relative difference measures were then compared with luminance contrast thresholds as measured in goldfish photoreceptors (1). Luminance contrast thresholds, or Weber fractions, set the lower limit of visual detection. Luminance contrast thresholds vary by photoreceptor; in goldfish, UV-sensitive cone photoreceptors exhibit the highest thresholds (0.32), whereas the other photoreceptors have detection limits near 0.05 (blue-sensitive = 0.045, red-sensitive = 0.056, green-sensitive = 0.032; data from ref. 1). We assume that swordtail visual detection limits are similar to goldfish visual detection limits, and therefore use 0.05 as a conservative detection limit. Parameter differences that were both statistically significant ($P < 0.05$) and exceeded the visual detection threshold (relative difference >0.05) are shown in bold. The swordtail male DoLP is significantly higher for all fish regions under high-DoLP illumination ($P << 0.001$) and at differences greater than 200% between high-DoLP and low-DoLP treatments. Swordtail male hue and saturation showed no significant or visually detectable change across all eight body regions, and only one body region (dorsum) showed a luminance difference marginally above the threshold of detection (6%). Measurements of the color standard (X-rite Colorchecker MSCCPPCC0113; Table S4) reveal only one significant difference that is marginally above the 5% threshold of detection between high-DoLP and low-DoLP illumination (8% relative difference for pink).

1. You Y, et al. (2011) Measurements and simulations of polarization states of underwater light in clear oceanic waters. *Appl Opt* 50(24):4873–4893.

Table S2. Comparison of polarization features between males (n = 12) and females (n = 17)

Measure	Sex	Statistic	Lateral line	Upper flank	Dorsum	Lower flank	Ventrum	Fin base	Operculum	Eye
DoLP	M	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	0.18 ± 0.05	0.32 ± 0.03
	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	0.18 ± 0.05	0.30 ± 0.05
	M. vs. F	*P* value	**0.02 (0.046)**	0.09	0.71	0.45	0.21	0.40	0.13	0.12
Q	M	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
	M. vs. F	*P* value	0.06	0.16	0.30	0.99	0.51	0.23	0.07	0.72
U	M	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
	M. vs. F	*P* value	0.17	0.55	0.59	0.69	0.12	0.26	**0.0004 (0.001)**	**0.03** (0.077)

Comparison of *X. nigrensis* male and female polarization reflectance. 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. Mean and SE of (DoLP), Q, and U measurements were collected on 12 free-swimming large male (M) and 17 female (F) swordtails from eight body regions. Mean ± SE of the gravel substrate is as follows: DoLP: 0.092 ± 0.003, Q: 0.016 ± 0.002, and U: 0.012 ± 0.001. Mean ± SE of the water column is as follows: DoLP: 0.344 ± 0.027, Q: 0.008 ± 3.25E-4, and U: 8.75E-4 ± 9.600E-5. *P* values indicate results of Welch's two-sample *t* tests for differences between males and females before corrections for multiple comparisons (with *P* values after Benjamini–Hochberg corrections for multiple comparisons given in parentheses). All *P* values <0.05 are indicated in bold.

Table S3. Comparison of polarization contrast between males (n = 12) and females (n = 17)

Measure	Sex	Statistic	Max vs. min within fish	Max fish vs. gravel	Max fish vs. water
DoLP	M	Mean ± SE	0.27 ± 0.02	0.25 ± 0.03	−0.02 ± 0.14
	F	Mean ± SE	0.24 ± 0.04	0.22 ± 0.04	−0.02 ± 0.10
	M. vs. F	*P* value	**0.02** (0.053)	**0.01 (0.03)**	0.88
Q	M	Mean ± SE	0.13 ± 0.05	0.12 ± 0.05	0.14 ± 0.05
	F	Mean ± SE	0.11 ± 0.04	0.10 ± 0.04	0.11 ± 0.04
	M. vs. F	*P* value	0.15	0.14	0.13
U	M	Mean ± SE	0.05 ± 0.02	0.03 ± 0.02	0.05 ± 0.02
	F	Mean ± SE	0.04 ± 0.01	0.02 ± 0.01	0.03 ± 0.01
	M. vs. F	*P* value	0.13	0.06	**0.03** (0.096)

Comparison of *X. nigrensis* male and female polarization contrast. Mean and SE of within-body contrast [body region with maximum value (Max) − body region with minimum value (Min)] and body-to-background contrast measures [Max fish − gravel and Max fish − background water) across the same individuals in Table S2. Mean ± SE of gravel substrate is as follows: DoLP: 0.092 ± 0.003, Q: 0.016 ± 0.002, and U: 0.012 ± 0.001. Mean ± SE of the water column is as follows: DoLP: 0.344 ± 0.027, Q: 0.008 ± 3.25E-4, and U: 8.75E-4 ± 9.600E-5. *P* values indicate results of Welch's two-sample *t* tests for differences between males and females before corrections for multiple comparisons (with *P* values after Benjamini-Hochberg corrections for multiple comparisons given in parentheses). All *P* values <0.05 are indicated in bold.

Table S4. Comparison of color standards across high-DoLP and low-DoLP experimental conditions (n = 10 measures)

Measure	Statistic	Blue	Gold	Rose	Green	Orange	Pink	Red	White	Yellow
Luminance	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
	P value	0.80	0.22	0.24	0.50	0.01	0.09	0.42	0.02	0.02
	Relative difference	−0.01	−0.02	−0.02	−0.19	−0.04	−0.03	−0.02	−0.03	−0.03
Hue	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
	P value	0.01	0.77	0.80	0.31	0.01	**0.004**	0.91	0.002	0.02
	Relative difference	−0.01	<0.01	<0.01	0.11	−0.01	0.08	<0.01	0.03	0.01
Saturation	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
	P value	0.10	0.17	0.19	0.39	0.29	0.20	0.24	0.03	0.02
	Relative difference	−0.07	−0.01	<0.01	−0.01	−0.01	0.01	<0.01	0.04	0.01

Four replicate measures of male *X. nigrensis* (n = 10) and a color standard (X-rite Colorchecker MSCCPPCC0113) were collected in a stationary position in both high-DoLP and low-DoLP experimental conditions. For each replicate, a male was filmed for 10 s with a standard color video camera (Olympus Stylus Tough TG-830 underwater camera) for color (hue and saturation) and luminance (intensity) values, as well as with the videopolarimeter to evaluate changes in DoLP (Fig. 3*A*). We calculated the average of each male's high-DoLP and low-DoLP replicate measurements, tested these averages for normality with the Shapiro–Wilkes test (all $P > 0.05$), and then used these averages to perform paired *t* tests (n = 10, df = 9) to evaluate male (Fig. S1*A*) and color-chip (Fig. S1*B*) differences between measurements in high-DoLP and low-DoLP conditions. Because all significant *P* values survived Benjamini–Hochberg correction for multiple comparisons, precorrection *P* values are reported. To evaluate whether differences in parameter measures were visually detectable, we calculated the relative difference of a male's average luminance, hue, saturation, and DoLP features between the high-DoLP and low-DoLP conditions as the quantity (high-DoLP illumination mean − low-DoLP illumination mean)/low-DoLP illumination mean). These relative difference measures were then compared with luminance contrast thresholds as measured in goldfish photoreceptors (1). Luminance contrast thresholds, or Weber fractions, set the lower limit of visual detection. Luminance contrast thresholds vary by photoreceptor; in goldfish UV-sensitive cone photoreceptors exhibit the highest thresholds (0.32), whereas the other photoreceptors have detection limits near 0.05 (blue-sensitive = 0.045, red-sensitive = 0.056, green-sensitive = 0.032; data from ref. 1). We assume that swordtail visual detection limits are similar to goldfish visual detection limits, and therefore use 0.05 as a conservative detection limit. Parameter differences that were both statistically significant ($P < 0.05$) and exceed the threshold of visual detection (relative difference > 0.05) are shown in bold. The swordtail male DoLP is significantly higher for all fish regions under high-DoLP illumination ($P << 0.001$) and at differences greater than 200% between high-DoLP and low-DoLP treatments. Swordtail male hue and saturation showed no significant or visually detectable change across all eight body regions, and only one body region (dorsum) showed a luminance difference marginally above the threshold of detection (6%). Measurements of the color standard (X-rite Colorchecker MSCCPPCC0113) reveal only one significant difference that is marginally above the 5% threshold between high-DoLP and low-DoLP illumination (8% relative difference for pink).

1. You Y, et al. (2011) Measurements and simulations of polarization states of underwater light in clear oceanic waters. *Appl Opt* 50(24):4873–4893.

Table S5. Comparison of male (n = 12) and female (n = 17) *X. nigrensis* polarization contrast across social conditions

Measure	Social partner	Statistic	Max vs. min within fish	Max fish vs. gravel	Max fish vs. water
DoLP	M alone	Mean ± SE	0.23 ± 0.04	0.21 ± 0.05	−0.03 ± 0.30
	F alone	Mean ± SE	0.24 ± 0.06	0.20 ± 0.07	0.03 ± 0.13
	M social	Mean ± SE	0.27 ± 0.04	0.25 ± 0.04	−0.02 ± 0.12
	F social	Mean ± SE	0.24 ± 0.05	0.22 ± 0.05	−0.08 ± 0.11
	M vs. F alone	*P* value	0.91	0.81	0.56
	M vs. F social	*P* value	0.05	0.08	0.23
	M alone vs. social	*P* value	**0.02** (0.07)	**0.03** (0.11)	0.97
	F alone vs. social	*P* value	0.89	0.41	**0.02** (0.098)
Q	M alone	Mean ± SE	0.15 ± 0.11	0.15 ± 0.12	0.16 ± 0.12
	F alone	Mean ± SE	0.11 ± 0.05	0.11 ± 0.05	0.11 ± 0.05
	M social	Mean ± SE	0.13 ± 0.06	0.12 ± 0.06	0.13 ± 0.06
	F social	Mean ± SE	0.11 ± 0.07	0.10 ± 0.07	0.12 ± 0.07
	M vs. F alone	Mean ± SE	0.22	0.21	0.21
	M vs. F social	*P* value	0.62	0.56	0.56
	M alone vs. social	*P* value	0.47	0.38	0.45
	F alone vs. social	*P* value	0.86	0.90	0.92
U	M alone	Mean ± SE	0.06 ± 0.04	0.05 ± 0.04	0.06 ± 0.05
	F alone	Mean ± SE	0.05 ± 0.02	0.03 ± 0.02	0.04 ± 0.01
	M social	Mean ± SE	0.05 ± 0.02	0.03 ± 0.03	0.05 ± 0.03
	F social	Mean ± SE	0.04 ± 0.01	0.02 ± 0.01	0.03 ± 0.01
	M vs. F alone	Mean ± SE	0.19	0.12	0.14
	M vs. F social	*P* value	0.23	0.22	0.10
	M alone vs. social	*P* value	0.49	0.30	0.46
	F alone vs. social	*P* value	0.62	0.44	0.45

Mean and SE of DoLP, Q, and U contrast values were calculated in both asocial and social conditions as in Table S3. Pre-Benjamini–Hochberg correction *P* values indicate the results of Welch's two-sample *t* tests for differences within social conditions between sexes [e.g., male (n = 12) vs. female (n = 17) alone] and within sex between social conditions [e.g., male alone (n = 12) vs. social (n = 12)]. (*P* values after Benjamini–Hochberg corrections for multiple comparisons are given in parentheses.). All *P* values <0.05 are indicated in bold.