BEHAVIORAL EVIDENCE FOR INTRASPECIFIC SIGNALING WITH ACHROMATIC AND POLARIZED LIGHT BY CUTTLEFISH (MOLLUSCA: CEPHALOPODA)

by

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Summary

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Intraspecific visual communication was studied quantitatively by testing the behavior of *Sepia officinalis*, the common cuttlefish, as senders and receivers of body pattern signals. These signals can be achromatic and/or linearly polarized and are produced by specialized dermal cells. Experiment 1 addressed whether the presence of conspecifics affected the visible, achromatic body patterns of males. These patterns tended to vary among conditions, suggesting limited sensitivity to audience. One set of body patterns varied with the number of conspecifics viewed while an uncorrelated set of body patterns varied with the sex of conspecifics viewed. Males showed high-contrast zebra banding when viewing another male, supporting previous studies of body patterns in male-male agonistic behavior. Experiment 2 addressed the relationship of polarized light patterns with visible body patterns of males and females, and tested whether senders modified their polarization patterns in response to conspecifics. Polarization patterning was only weakly associated with visible body patterns. Females showed more polarized body patterns than did males, but polarized patterns did not differ among conditions; thus, no sensitivity to audience by senders was found. Experiment 3 addressed whether conspecific receivers used information from polarized body patterns. Limited evidence was found for changes in the behavior of female but not male observers, suggesting that female receivers may use polarized patterns as a source of information about conspecifics. The information contained in polarization patterning may complement that contained in zebra patterning such that both males and females advertise their species, sex, location, and size to conspecific receivers.

Keywords: invertebrate, visual communication, marine, audience effect.

Introduction

Communication involves the active sending of a signal to a receiver, with benefits accruing to the sender and possibly to the receiver (Bradbury & Vehrencamp, 1998). Cephalopod communication has been discussed and reviewed (Moynihan, 1985; Hanlon & Messenger, 1996), yet little experimentation has been directed to determine the extent to which body patterns constitute true communication signals. Our experiments address two questions: are visible body pattern signals modulated in response to conspecifics (Experiment 1), and are polarization body patterns also used as signals (Experiments 2 and 3)?

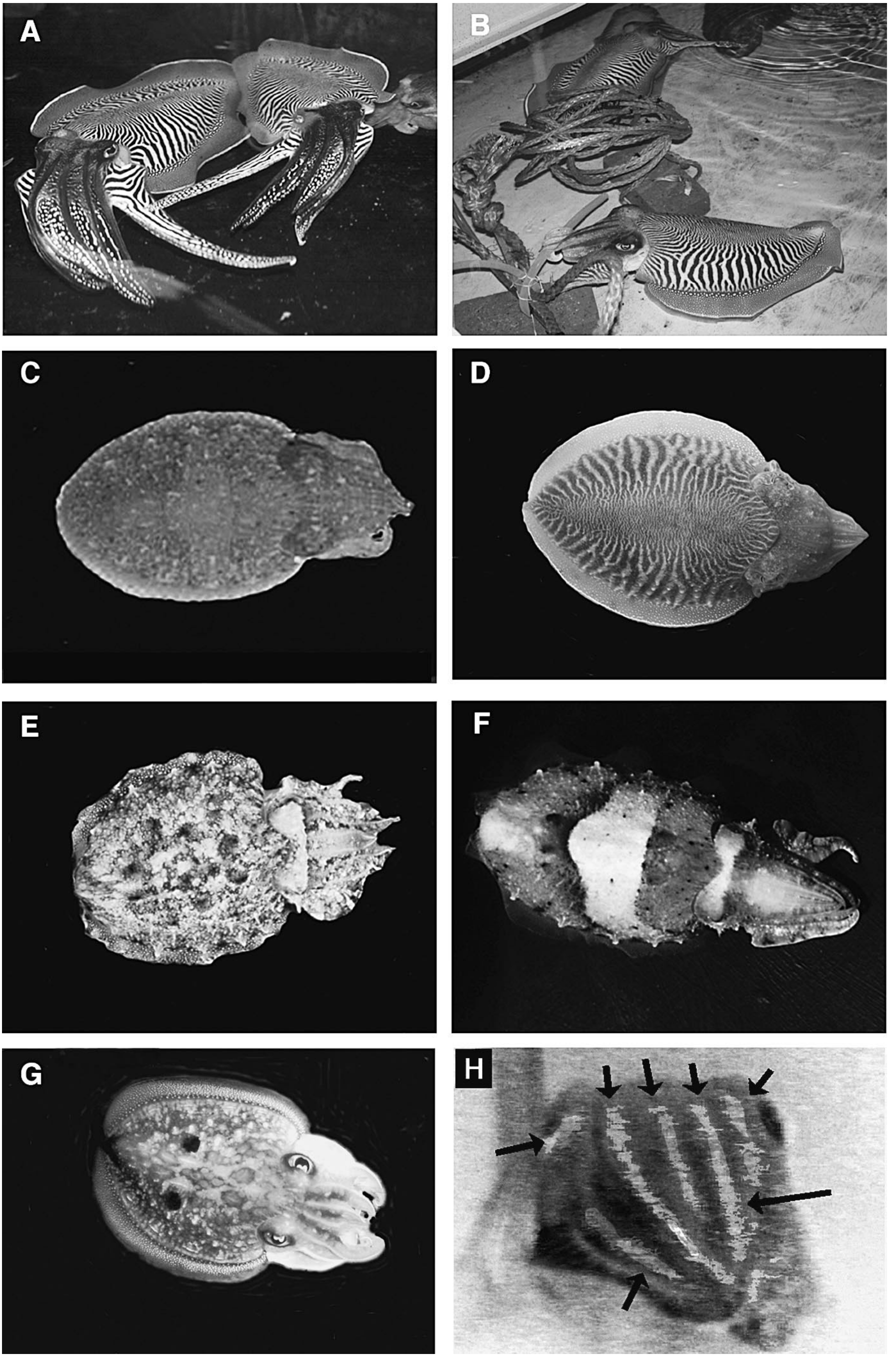
Field data are sparse, but to the best of our knowledge cuttlefish are not social. In captivity, young cuttlefish space themselves out as widely as space permits (Boal *et al.*, 1999) and adult males sometimes kill and cannibalize each other (pers. obs.). In the field, adults of at least one species aggregate to spawn (Hall & Hanlon, 2002), but laboratory experiments with *Sepia officinalis* indicate that even paired, mate-guarding males do not recognize their mate from other females (Boal, 1996). While some authors have speculated that cephalopods' body patterning serves as a visual 'language' (Moynihan & Rodaniche, 1982), it is likely that signals to conspecifics are restricted to indications of agonism or sexual motivation.

The coleoid cephalopods (cuttlefishes, octopuses and squids) have excellent sensory systems, including vertebrate-like vision (although without color perception; Marshall & Messenger, 1996), a lateral line-like system for mechanoreception, and chemoreceptive abilities (Budelmann, 1994; Budelmann *et al.*, 1997). Are some of these sensory systems used for communication? Cephalopods do not make sounds, and studies of their use of pheromones are still in infancy (Buresch *et al.*, 2003, 2004); however, it has long been assumed that cephalopods use some of their extraordinary body patterning to communicate visually with conspecifics (Wells, 1962).

Cuttlefish, like other coleoid cephalopods, can quickly change the color, brightness, pattern and texture of their skin to produce the overall body pattern (hereafter, referred to simply as 'patterns'; see Hanlon & Messenger, 1996 for details). These changes are a result of activity in the chromatophore system, a network of pigmented organs (chromatophores) that generates black, brown, red, orange or yellow hues, and various types of reflecting cells that generate most wavelengths (including white) by structural reflectance (Hanlon & Messenger, 1996). One type of reflecting cell, the iridophore, also reflects light that is plane polarized (Shashar *et al.*, 1996, 2001). The chromatophores are under direct control of the nervous system (reviewed in Messenger, 2001). The speed (fractions of a second) and diversity (see Fig. 1 for a sample) of the resulting pattern changes are unparalleled in the animal kingdom. This capability has been referred to as 'rapid neural polyphenism' and allows each individual cephalopod to show 15-50 different body patterns (*ibid.*; Hanlon *et al.*, 1999a).

Many of the body patterns of cuttlefish that are visible to humans are clearly useful for crypsis, particularly among benthic juveniles (Hanlon & Messenger, 1988). In cuttlefish, individuals match their body patterns to the overall brightness of the background as well as to the patch size of bright or dark elements in the visual background (Chiao & Hanlon, 2001). Previous research using cuttlefish indicated that uniform, stipple, mottle, and disruptive body patterns serve to camouflage cuttlefish against a wide range of background types (Hanlon & Messenger, 1988).

Body patterns are also used for communication. The best-documented example is the set of body patterns and postures that together constitute the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Intense Zebra Display, an agonistic display shown mostly, but not exclusively, by sexually mature males (Bott, 1938; Tinbergen, 1939; Hanlon & Messenger, 1988; Boal, 1997; Fig. 1a, b). The Intense Zebra Display is an honest signal of fighting intent (Adamo & Hanlon, 1996); pattern components of contest winners are more highly contrasting than those of contest losers. Among males, the Intense Zebra Display is elicited by the sight of a rival male (Messenger, 1970; Shashar *et al.*, 1996). We have no experimental evidence that body patterns other than those of the Intense Zebra Display also serve as signals.

Although dark and light zebra banding (one component of the Intense Zebra Display) is positively correlated to the proximity of another cuttlefish (Boal *et al.*, 1999), some males are much more likely to show an Intense Zebra Display than are other males (Adamo & Hanlon, 1996). It has not been clear, therefore, to what extent body patterns are a response to the presence of conspecifics. Our first experiment was designed to test explicitly whether male cuttlefish modify their body patterns in response to the presence of conspecifics (‘audience effect’; Gyger *et al.*, 1986). We focused particularly on zebra banding because this patterning component of the Intense Zebra Display is easily visible to humans and other predators so is likely associated with the cost of increased predation. We hypothesized that males would

Fig. 1. Examples of body patterns of the cuttlefish *Sepia officinalis*. Each individual can change rapidly from one to another of the patterns. (a) Two males showing Intense Zebra Displays. Note the highly contrasting zebra bands on the mantle, the zebra banded fourth arms extended towards each other, and the dark face of the male on the right (Photo by M. Dobbins). (b) The female (bottom center) is laying an egg while showing an Intense Zebra Display in response to a male that had just swum by (above) (Photo by J.G. Boal). (c) Uniform patterns may be light, such as in this image, very dark, or any uniform shade in between (Photo by R.T. Hanlon). (d) Weak zebra patterning is sometimes used as camouflage and may be combined with other light and dark camouflage patterns (Photo by R.T. Hanlon). (e) Mottles are cryptic patterns typically shown when resting on a variegated gravel substrate. The overall lightness or darkness of the mottle can vary (Photo by R.T. Hanlon). (f) Disruptive patterns are characterized by large regions of the body showing highly contrasting light and dark visual components. Disruptive is used for crypsis on backgrounds with large particle size (*e.g.* cobbles, shells) (Photo by J. Forsythe). (g) Deimatic patterns are characterized by a pale mantle (uniform, mottle or weak zebra) with dark false eye spots and a dark outline of the mantle margin (Photo by J. Forsythe). (h) Polarized patterns on the arms and around the eyes, coded by false brightness for partial polarization >30%, as measured by the polarimeter (Photo by N. Shashar).

modify their body patterns, showing stronger zebra banding when viewing males than when viewing females.

Cephalopods are sensitive to polarized light (Moody & Parris, 1960; Rowell & Wells, 1961; Shashar *et al.*, 2000, 2002). Recent studies using imaging polarimeters have demonstrated that cephalopods show polarized patterns on their skin with dermal iridophore cells that induce partial linear polarization of the reflected light (Shashar *et al.*, 1996, 1997, 2001). Cuttlefish produce these polarization patterns on their face, arms and around their eyes (Fig. 1h). Cephalopods can control some of their iridophores (Cooper *et al.*, 1990), possibly enabling them to control the polarization patterns in their skin (Cronin *et al.*, 1995; Shashar *et al.*, 1996; Shashar & Hanlon, 1997; Hanlon *et al.*, 1999b).

It has been speculated that polarization patterns in marine organisms could be used for communication with conspecifics, perhaps as a channel 'hidden' from predators (Shashar *et al.*, 1996; Marshall *et al.*, 1999). The common cuttlefish, *Sepia officinalis*, ranges in depth from near the surface to the bottom depths of the British Channel and from the turbid coastal waters of the British Isles and northwest Europe to the clear waters of Corsica and Greece (Lythgoe, 1979; Boletzky, 1983). Although the range of transmission of light signals probably changes throughout this range of environmental conditions, the nature of any messages sent with polarization patterns would not be altered. Since many predators of cephalopods, such as marine mammals and some fishes, are apparently not sensitive to the polarization of light (Hawryshyn, 1992), it is conceivable that cuttlefish could communicate with conspecifics using polarization patterns while remaining visually concealed from some of their predators.

In our second experiment, the polarization of body patterns was recorded in the presence and absence of other cuttlefish to determine whether cuttlefish modulate polarization in response to the presence of conspecifics. We hypothesized that cuttlefish control their polarization patterns, and that these patterns differ when they are alone and when they are in sight of another cuttlefish.

If cuttlefish do not modulate their polarization patterns, it is still possible that the unmodulated polarization patterns contain information (*e.g.* presence, size or sex) that could be useful to conspecifics. Thus, in our third experiment, we distorted polarization using filters between adjacent cuttlefish to determine if the behavior of the receivers depended upon the polarization of skin patterns of conspecific senders. We hypothesized that cuttlefish would be more visible to conspecifics if polarization were not distorted.

General methods

All subjects were sexually mature *Sepia officinalis* L. obtained from the National Resource Center for Cephalopods (NRCC), located at the University of Texas Medical Branch (UTMB) in Galveston, Texas. The cuttlefish had been reared from eggs collected from the coast of Normandy, France. Rearing and maintenance techniques have been described elsewhere (Forsythe *et al.*, 1991, 1994; Hanley *et al.*, 1998). Experiment 1 was conducted at the NRCC; experiments 2 and 3 were conducted at the Marine Biological Laboratory (MBL) in Woods Hole, Massachusetts. Unique zebra banding patterns on each adult cuttlefish allowed us to identify individuals.

At the UTMB, housing (2.4 × 5.5 × 0.9 m deep) and experimental tanks (described below) were on separate recirculating systems of approximately 4,000 l each. Seawater was obtained from the Gulf of Mexico. Water temperatures ranged from 18-22°C and salinity ranged from 33-35 ppt. Natural lighting was supplied by large east- (experimental tanks) or west- (housing tank) facing windows and was supplemented during the day by florescent overhead lights and during videotaping by two 500-watt halogen lamps. The housing tank was divided with a net to separate males and females. The cuttlefish were fed frozen shrimp twice per day *ad libitum*.

At the MBL, housing (a variety of tanks, range 215 to 7430 l) and experimental tanks (described below) were on recirculating systems of seawater obtained from Great Harbor. Water temperatures ranged from 15-22°C and salinity ranged from 32-35 ppt. Natural lighting was supplied by windows and was supplemented during the day by florescent overhead lights and during Experiment 2, by two 500-watt halogen lamps. The cuttlefish were fed a mixture of frozen and live fish and shrimp twice per day *ad libitum*.

Light in nature is almost always partially polarized with both linear and circular components (Waterman, 1984). As of yet, demonstrations of polarization sensitivity in animals have been limited to the linear component of polarization, with no animal known to be sensitive to circular or elliptically polarized light. Therefore, in this paper, we limit ourselves to linear polarization and refer to sensitivity to the characteristics of linear polarization as polarization sensitivity, and to visual patterns containing a linearly polarized component (larger than 0) as polarized patterns or polarization patterns. Non-polarized patterns that are visible to the unaided eye of humans will be referred to as visible patterns.

Statistics for non-significant results were omitted for the sake of brevity, unless a lack of significant difference was of particular interest. Statistical analyses were performed using StatSoft software (2000), in consultation with Zar (1999), Sokal & Rohlf (1995) and Siegel & Castellan (1988).

Experiment 1

Do male cuttlefish modify their body patterns in response to the presence of male and female conspecifics? In *Sepia officinalis*, the Intense Zebra Display comprises strongly contrasting zebra bands, a dark eye ring, a dark face, and a white fin line (Fig. 1a, b); in addition, the fourth arm is commonly extended towards the other cuttlefish. For males, this arm has particularly strongly contrasting zebra bands and white arm spots (Fig. 1a). We chose to grade body patterns rather than displays, such as the Intense Zebra Display, and supplement human scoring with variables coded using image analysis software to minimize inadvertent human subjectivity and potential bias in this experiment. Males were studied because they are the primary users of the Intense Zebra Display.

Methods for Experiment 1

Four experimental tanks (1.2 × 1.8 × 0.8 m deep) were placed side-by-side and divided into thirds by clear and opaque movable partitions. Subjects ($N = 26$ males; 15.0-23.0 cm mantle length, ML) were placed individually into the center section of an experimental tank and allowed to acclimate. In each trial, one other cuttlefish could be placed in each end section ('viewed cuttlefish'). After a minimum of 10 min, the opaque partitions were raised, allowing visual contact between the subject and the viewed cuttlefish.

A standard Hi-8 video camera was suspended from a track on the ceiling, allowing the camera to be moved from one experimental tank to the next by means of a string-and-pulley system. During each trial, body patterns were videotaped for 10 min starting 4 min after the opaque barrier was lifted. At the end of the trial, the opaque partitions were replaced and the viewed cuttlefish were returned to the housing tank.

Each subject was videotaped in six conditions, presented in random order. Subjects viewed an empty tank, one female cuttlefish, one male cuttlefish, two female cuttlefish, two male cuttlefish, or one female and one male cuttlefish. Male cuttlefish were used as both subjects and as viewed cuttlefish; viewed cuttlefish were assigned haphazardly.

The first three minutes of videotape of each sequence were excluded from scoring; thereafter, frames were captured every 20 seconds until 10 frames were obtained (occasionally one or two frames were discarded because of glare). Cuttlefish respond quite rapidly to changes in their environment but body patterns and behavior in any one environment typically appear quite stable for protracted periods of time (hours to days, Boal, 1996); thus, our sampling regime appeared reasonable. Each frame was analyzed using the public domain NIH Image program (U.S. National Institutes of Health; http://rsb.info.nih.gov/nih-image/) and then by two observers, one experienced human observer (RTH) and one trained student. Both were blind to the sex of all cuttlefish. The human observers scored all tapes and then went back and reviewed them all a second time to confirm their scores.

Variables scored by computer (using NIH Image) included the following mantle characteristics: mantle darkness (maximum pixel density over the entire mantle, range 0 [white]-255 [black]), mantle lightness (minimum pixel density over the entire mantle), distance to the nearest comparison cuttlefish (nearest point to nearest point), and mantle darkness and lightness of the nearest viewed cuttlefish. The variable contrast, computed as the difference between maximum and minimum mantle pixel density divided by the sum of maximum and minimum pixel density, provided a numerical measure of how strongly contrasting the body patterns of the cuttlefish appeared.

Variables scored by the human observers included the body patterns of zebra (moderately intense or strongly intense; Fig. 1a, b), uniform (light, moderately dark or very dark; Fig. 1c), weak zebra (light or dark; Fig. 1d), mottle (light or dark; Fig. 1e) and disruptive (weak or strong; Fig. 1f). Also recorded were deimatic spots ('false eye spots'; unilateral or bilateral; Fig. 1g), first pair of arms held up, fourth arm extended out laterally (left, right or both; Fig. 1a) and eye ring (left, right or both; Fig. 1a, b). A complete description of body patterns can be found elsewhere (Hanlon & Messenger, 1988).

We did not expect all body patterns to be used independently of each other. Principal component analyses (PCA) were used to objectively group correlated patterns together, first with just computer-scored variables (CS-F1 and CS-F2) and then with just human-scored variables (HS-F1 and HS-F2). Because factors from these two PCAs were also significantly correlated with each other, a third PCA was performed using all variables together (AV-F1 and AV-F2). Finally, a fourth PCA was used specifically with variables that were highly intercorrelated and that were expected to describe Intense Zebra Displays (IZD-F1 and IZD-F2).

To determine if body patterns differed among conditions, repeated measures analyses of variance were performed (six conditions with ten frames per condition) using key variables and principal component factors as dependent variables and condition as the specific effect. Where results yielded trends but not significant differences, further analyses of variance were used for exploratory purposes only. Differences between individual cuttlefish are apparent to even casual observers; therefore, these differences were not examined explicitly here.

Results for Experiment 1

Coefficients of concordance between the two human scorers were highly significant for all variables (Kendall's coefficients of concordance, $13.23 \leqslant \chi^2 \leqslant 72.00$, $p < 0.0001$ all); data from the experienced observer only were used in statistical analyses.

Body patterning components were grouped into correlated, meaningful combinations using Principal component analyses. In each case, two factors were obtained that had eigen values greater than 1.0 and that explained at least 15% of the variability in the data set (Table 1). Contrast in mantle patterning was captured by computer-scored variables (CS-F1, AV-F1, IZD-F1), as was arm position (CS-F2). The distinction between the contrast of zebra patterns (Fig. 1a, b) and the contrast of other patterns (*e.g.* disruptive or mottle, Fig. 1e, f) was captured by human-scored variables (HS-F1, IZD-F2), as was the presence of deimatic spots (HS-F2; Fig. 1g).

The eight factors (Table 1) and contrast (a computer-scored variable) were used to test for differences in body patterning in response to condition. No single factor or variable differed significantly between conditions ($F_{5,25} \leqslant 2.37$, $p \geqslant 0.05$). Thus, we have no strong evidence that cuttlefish body patterns differ depending upon the particular conspecific(s) viewed.

TABLE 1. *Experiment 1: Results of principle component analyses of male body patterning variables*

PCA	Variables included	Factor	% Variance explained	Key variables (+)	Key variables (−)	Interpretation	Sensitivity to context
1	Scored by computer (image analysis software) ($N = 5$)	CS-F1	62%	contrast	mantle lightness, face darkness, mantle darkness	Contrast in body pattern (Fig. 1a, e, f)	N.S.
		CS-F2	20%	–	arms	Arm position (Fig. 1a, d, f)	N.S.
2	Scored by experienced human ($N = 13$)	HS-F1	24%	zebra	mottle, uniform, disruptive	Zebra *vs* cryptic (Fig. 1a, d *vs* Fig. 1c, e, f)	$F_{5,25} = 2.37$, $0.05 < p < 0.10$ Female *vs* male conspecifics: $F_{1,25} = 4.51$, $p < 0.05$
		HS-F2	15%	diematic spots	–	Presence of diematic spots (Fig. 1g)	$F_{5,25} = 2.31$, $0.05 < p < 0.10$ One *vs* two conspecifics: $F_{1,25} = 5.125$, $p < 0.05$
3	All variables combined ($N = 18$)	AV-F1	21%	mantle lightness	contrast	Uniformity in body pattern (Fig. 1a, e, f)	$F_{5,25} = 2.16$, $0.5 < p < 0.10$
		AV-F2	16%	zebra, 4th arm position, mantle darkness, mantle lightness	mottle, uniform, disruptive, contrast	Intense Zebra Display *vs* cryptic (Fig. 1a, d *vs* Fig. 1c, e, f)	N.S.
4	Variables expected to relate to Intense Zebra Displays ($N = 7$)	IZD-F1	45%	mantle lightness, mantle darkness, face darkness	–	High-contrast patterns other than Intense Zebra Display (Fig. 1c, e, f)	N.S.
		IZD-F2	26%	zebra, 4th arm out, eye ring	–	Intense Zebra Display (Fig. 1a)	$F_{5,25} = 2.05$, $0.05 < p < 0.10$ Female *vs* male conspecifics: $F_{1,25} = 8.34$, $p < 0.01$

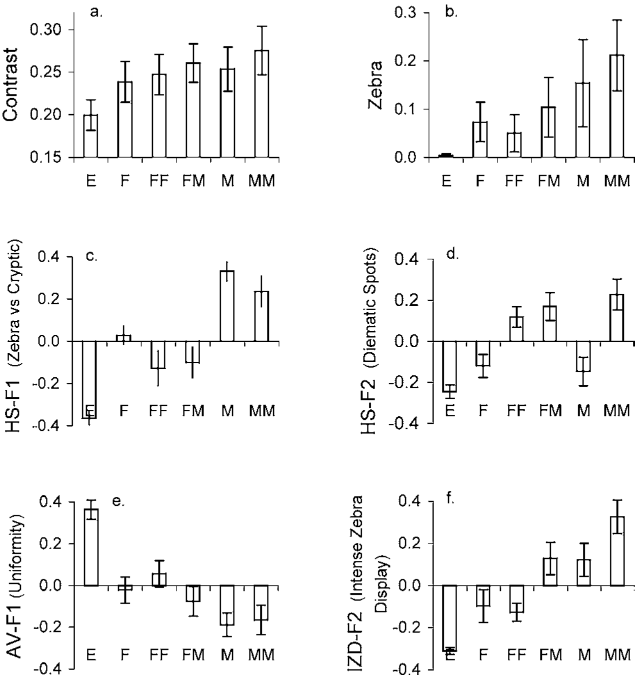

Fig. 2. Experiment 1: Body patterns of male cuttlefish varied in response to the sight of conspecifics. Body patterns (mean ± sem; $N = 26$) when viewing an empty tank (E) or conspecifics: a single female (F), two females (FF), a female and a male (FM), a single male (M), or two males (MM). (a) Contrast was computed as the difference between mantle darkness and mantle lightness (measured using image analysis software) divided by the sum of mantle darkness and mantle lightness. (b) Zebra body pattern was scored directly by human observers. (c-f) HS-F1, HS-F2, AV-F1, and IZD-F2 are factors that result from principal component analyses (Table 1); an interpretation of each is given in parentheses on the y-axis.

Cuttlefish may distinguish between these conditions, however. Five factors or variables showed trends towards differentiation in responses to condition (HS-F1, HS-F2, AV-F1, IZD-F2, contrast; $F_{5,25} = 2.02$-2.37, $p < 0.10$ all; Table 1 and Fig. 2). Body patterns when cuttlefish viewed an empty tank were noticeably different from when the subjects viewed another cuttlefish (Fig. 2). But were there differences in body patterns among the conditions that involved the sight other cuttlefish? To test for differences in responding based on the sex of conspecifics, responses to females were compared to responses to males (one or two females versus one or two males). Cuttlefish showed significantly more of the behaviors associated with Intense Zebra Displays to other males than they did to females (HS-F1: $F_{1,25} = 4.51$,

$p < 0.05$; IZD-F2: $F_{1,25} = 8.34$, $p < 0.01$) (Fig. 2c, f). To test for differences in responding based on the number of other cuttlefish in view, responses to one conspecific were compared to responses to two conspecifics (one male or female versus two males or females). Cuttlefish were significantly more likely to show deimatic spots when they viewed two cuttlefish than when they viewed just one other (HS-F2: $F_{1,25} = 5.125$, $p < 0.05$) (Fig. 2d). Thus, our data suggest that these male cuttlefish did make some condition differentiation, particularly between male and female conspecifics, and this differentiation was reflected in their body patterning.

It is reasonable to assume that the behavior of the viewed cuttlefish influenced the subjects' behavior. The contrasts of the subject and the other cuttlefish were indeed positively correlated ($r^2 = 0.12$).

Discussion for Experiment 1

Cuttlefish body patterns were marginally sensitive to the presence of viewed conspecifics. Suggestive differences in body patterns were found in response to (1) the sight of an empty tank as compared to other cuttlefish (all variables, Fig. 2), (2) the sight of females as compared to males (zebra patterning, Fig. 2c, f), and (3) the sight of one as compared to two individuals of the same sex (deimatic spots, Fig. 2d). We expect that a larger sample size would more clearly distinguish responses to this graded series of stimuli.

The differences in behavior between conditions rested primarily on differences in behaviors related to Intense Zebra Displays. Our results are therefore consistent with previous research indicating that zebra bands, eye ring and arm position are used for communication (Bott, 1938; Tinbergen, 1939). Diematic behaviors, such as deimatic spots ('false eye' spots; Fig. 1g) are used to threaten, startle, or frighten (Hanlon & Messenger, 1996); our results do not refute this interpretation. No surprising variable combinations appeared in any principal component factor, suggesting that important component combinations have not been missed in earlier, qualitative studies (*e.g.* Boal *et al.*, 1999). In sum, we find no evidence that visible body patterns other than those constituting Intense Zebra Display and deimatic behavior are important in communication.

Differences in body patterns among conditions were found primarily for variables scored directly by humans (*e.g.* HS-F1, HS-F2; IZD-F2 as compared with IZD-F1; Table 1). Does this result mean that the differences in body patterning that we found were simply the result of unintended human bias? We think not. First, the concordances between the scores of the two human observers were highly significant. The student was unaware of the goals of the study and neither scorer knew the sex of the viewed, other cuttlefish. Second, positive correlations were found between computer-scored and human-scored variables (*e.g.* AV-F2; Table 1). Third, the computer measures of mantle darkness and lightness (*i.e.* contrast) confounded several very different and differently-used body patterns (zebra, mottle and disruptive, Fig. 1a, b, e, f) that are easily distinguished by humans. Although the software did provide objective measures of the intensity of the darkness and lightness of body patterns, which are difficult for humans to score directly, it did not differentiate between patterns with similar contrast levels.

Experiment 2

Is polarization actively used to signal to conspecifics? Cuttlefish produce polarization patterns on their face, arms and around their eyes (Fig. 1h) with dermal iridophore cells. Cuttlefish can control some of their iridophores (Cooper *et al.*, 1990); therefore, it is possible that they can control polarization patterns on their skin independently of their control of visible patterns. What is the relationship between the body patterns visible to our eyes and polarization display characteristics? Do cuttlefish modify polarization patterns in response to the presence of conspecifics?

Methods for Experiment 2

Two cohorts of adult cuttlefish were tested separately. The first cohort ($N = 8$, 4 females and 4 males) was reared from eggs at the NRCC. They were shipped, fully-grown and sexually mature (mantle lengths 17.0-19.5 cm), to the MBL two months prior to experiments. Water temperature was 15°C throughout the experiments. The second cohort of cuttlefish ($N = 5$, 3 females and 2 males) was shipped from the NRCC to the MBL as eggs 10 months before experiments began, where they were raised to maturity (mantle lengths 16.5-19.5 cm). Water temperature was 20°C throughout the experiments.

Experiments were conducted in a single glass tank ($120 \times 44 \times 57$ cm deep) filled with 225 l of recirculating seawater. Two glass partitions, placed side-by-side, divided the tank in half. An opaque Plexiglas barrier could be placed between the partitions to prevent the cuttlefish on either side from seeing each other. Water could pass around the edges of the barrier and partitions. Two 500-watt halogen lamps were placed, one at each end of the tank, to improve lighting for the video camera.

An imaging polarimeter was placed approximately 1 m in front of the tank. A description of this polarimeter can be found elsewhere (Hanlon *et al.*, 1999b). In short, a neutral prismatic splitter replaced the dichroic prism in a 3- tube ENG video camera. Light passing through the camera's aperture and lens was split by this prismatic filter into three equal broad-spectrum images. These images were then transmitted to the camera's original three light-sensitive tubes through linearly polarized filters (Polaroid HNP'B) oriented at 0°, 45° and 90°, one for each imaging tube. In this way, the angle of polarization became coded similar to the way that hue (color) information was originally coded (*e.g.* 0° for the red channel, 45° for the blue channel and 90° for the green channel), such that zero polarization was mapped along the black-white axis (*i.e.* gray scale) while hue and saturation could be correlated with polarization angle and percent polarization in the image (false color). This polarimeter allowed for continuous video recording of moving animals; however, it could not be placed directly in the water, and lighting conditions (which could be controlled) and the orientation of the animal in relationship to the camera and the light source (which could not) greatly influenced the recording, and especially the orientation of polarization (false color). A microphone was used to simultaneously record experimenters' descriptions of cuttlefish behavior during trials.

A single cuttlefish was placed in one side of the experimental tank and allowed to acclimate for at least one hour with the video lights off. The video camera was set up during this acclimation time. The video lights were then turned on and the cuttlefish was filmed, alone, for 5-10 minutes. Next, the opaque barrier was inserted between the two glass partitions and a second cuttlefish was added to the empty side of the tank. Both cuttlefish were filmed while the opaque barrier still separated them. The opaque barrier was then removed, and the two cuttlefish were filmed alternately while they could see and react to each other. After several minutes of filming the two cuttlefish, the opaque barrier was re-inserted. This sequence of events was then repeated, allowing the cuttlefishes' reactions to each other to be filmed a second time. The time intervals for the filming of each animal were not standardized, and filming was done *ad libitum*. Our intent was to test each cuttlefish alone, with a female, and with a male, and to film long enough that a full repertoire of behaviors would be recorded. Unfortunately, one of the females was not tested with another female and another female was not tested alone. The total time videotaped and scored was 7:46 h and 52 distinct cuttlefish combinations were recorded.

A single human observer (KHV) scored all videotapes. Variables scored included presence or absence of polarization reflections from anywhere on the cuttlefish (0/1), orientation of polarization (as indicated by false color), and location of the polarization reflection (arms, eyes, face). A saturation value (whole integer: 1-weak to 3-bright) was assigned to each polarization reflection. Visible body patterning was also recorded, including arm position, body position, face darkness, zebra banding, and other (uniform, mottle and disruptive; see Experiment 1). For purposes of analysis, mantle patterns were categorized as either cryptic (weak zebra and other) or not cryptic (zebra and strong zebra). The presence or absence of the opaque barrier was noted as well as the cuttlefish's activity (resting on the bottom, hovering or swimming) and orientation relative to the camera.

A full data set was created as follows. Each time a change in any one of the above variables was noticed, a new record was created. This record included the start and stop times, and hence duration of the set of body patterns. This data set was used for calculating basic statistics such as the average duration of types of body patterns and the correlations between polarization and nonpolarization body patterns (Spearman's rank order correlations).

In this full data set, each cuttlefish was represented a different number of times. In addition, each cuttlefish experienced a different total number of interactions. Consequently, the data were condensed such that each subject-situation combination (subject alone, subject-particular other cuttlefish) comprised a single record. In this condensed data set, three sets of variables were computed: (1) % of time showing a polarization reflection (arms, eyes, face, anywhere), (2) number of switches per minute from polarized to unpolarized, or vice versa, on any part of the body, and (3) percent of time showing each type of visible body pattern. Percentages were arcsine transformed because their values were often low (Zar, 1999). Principal component analyses were performed using this condensed data set.

Because some cuttlefish were tested more times than others in a particular condition (alone, or viewing a female or male through a clear or opaque barrier), the behavior scores of subjects used multiple times in a single condition type were averaged. This highly condensed data set was used for all repeated measures analyses of variance.

Results for Experiment 2

The length of time that the cuttlefish showed any particular configuration of recorded patterning variables varied widely (mean ± standard error: all variables 9.9 ± 9.5 sec, $N = 2825$; visible body patterns 28.9 ± 43.1 sec, $N = 966$; polarized patterns excluding false color, 16.8 ± 21.7 sec, $N = 1127$).

The presence or absence of polarization was not related to body orientation (center, right or left; $\chi^2_2 = 1.75$, $p > 0.10$). False color, an indication of the orientation of polarization, depended on the orientation of the cuttlefish relative to the camera ($r \times c$ contingency tables, false color (red, green, blue) × body orientation (center, right, left): both eyes and arms $\chi^2_4 > 71.00$, $p < 0.001$; face $\chi^2_4 = 8.32$, $p < 0.10$). False color was not analyzed further, therefore, and was not used to compute changes in polarization displays (switches).

Polarization of body patterning was only weakly related to other body patterns visible to human eyes. Polarization of the face was most common when the cuttlefish were showing cryptic body patterns (Fig. 1c, d, e, f; $r = 0.20$), and particularly when they were showing an anterior head bar (dark band across the face, $r = 0.39$). Polarization of the eyes was negatively associated with the first pair of arms raised to form a V ($r = -0.17$), and when the arms were together and extended away from the head ($r = -0.17$). Polarization of the arms was negatively associated with strongly banded fourth arms, typical of male Intense Zebra Displays (Fig. 1a; $r = -0.21$).

A principal component analysis of all body patterning variables yielded two factors that explained at least 10% of the variance. Key variables for the first factor were all visible body patterns (especially cryptic patterning and eye ring) while key variables for the second factor were all polarization

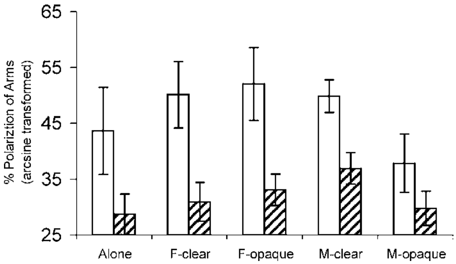

Fig. 3. Experiment 2: Females (open bars; $N = 7$) show more polarization patterning than males (hatched bars; $N = 6$). The polarization patterns of cuttlefish (mean ± sem) did not change significantly with condition (alone or separated by a clear or opaque barrier from a female or male conspecific).

patterns (especially polarization of the arms and eyes). Since factors are uncorrelated, by definition, visible body patterns were only weakly indicative of polarization patterns.

We found no evidence that cuttlefish modified the polarization of their body patterning in response to conspecifics, for any condition (alone, viewing a female or male through a clear or opaque partition), or any of our polarization variables (presence of polarization, and polarization of the arms, eyes or face; $F_{4,9} \leqslant 2.46$, $p \geqslant 0.10$). Females were more likely to show polarization of the arms than were males ($F_{1,9} = 7.37$, $p < 0.05$; Fig. 3); no other polarization variables differed between male and female subjects.

Discussion for Experiment 2

Polarized body patterns were only weakly related to the body patterns visible to human eyes, with more polarized reflections (here termed polarization) shown in conjunction with cryptic body patterns. Females showed more polarization than did males; however, males showed more zebra banding, especially on the arms, and zebra banding was negatively correlated with polarization.

It is possible that there exists a functional incompatibility between showing strong zebra bands and showing polarization patterns due to the anatomy of the skin. To produce strong zebra bands, the pigmented chromatophores must be expanded maximally in the dark bands while in the white bands the reflective leucophores broadcast all wavelengths maximally (see Hanlon & Messenger, 1988; Figs 14-17). Both entities would dominate optical properties of the iridophores, from which we believe the polarization emanates

(Shashar *et al.*, 2001). If such a functional incompatibility exists, we would expect males that were alone, and hence not showing much zebra (Fig. 2b — viewing empty tank), to show more polarization than males viewing other males; this was clearly not the case (Fig. 3). Further experimentation is required to understand the mechanisms and function of the polarization reflectance of cuttlefish skin.

No effect of the presence of conspecifics on polarization of body patterns was found; consequently, we have no evidence to suggest that polarization is modulated in response to the presence of conspecifics.

Experiment 3

Although we have no evidence that senders modify the polarization of their body patterning in response to the presence of conspecifics, it remains possible that conspecific receivers use polarization to detect the presence of conspecifics. If polarization serves as a simple signal in this way, then distortion of polarization should change the signal and perhaps change the behavior of the receiver. Is the behavior of male and female cuttlefish different when they view each other through film that distorts polarization as compared to viewing each other through glass that transmits normal polarization?

Methods for Experiment 3

Sets of 38 l, flow-through tanks were placed adjacent to each other in a 2 × 2 or 2 × 3 array and movable partitions were constructed to fit between adjacent pairs. Partitions were constructed of clear glass, opaque materials, or polarization-distorting film. A standard Hi-8 video camera was suspended over the tanks to record trials. All cuttlefish were given 1 hr to acclimate to the testing tanks before experiments began.

The first set of trials was a preliminary attempt to determine whether behavior would change if polarization were distorted. Each cuttlefish ($N = 24$, 12 males and 12 females; 14.0-17.5 cm ML) was videotaped when in sight of one other cuttlefish through either a clear, polarization-preserving barrier or a clear but polarization-distorting filter (Shashar *et al.*, 2000). This filter maintains directional, intensity, and nearly all chromatic characteristics of light passing through but distorts the linear polarization characteristics to produce a depolarized image. For each set of four cuttlefish (2 × 2 tank array) trials were run consecutively. Our intention was to test each individual once in each condition (see below), and three times alone. As a result of experimenter error, four males and four females were tested twice with the opposite sex and not at all with the same sex.

In this first set of trials, test conditions included alone, viewing a female through a clear barrier or through a polarization distorting barrier, and viewing a male through a clear barrier or through a polarization-distorting barrier. The cuttlefish were alone in trials 1, 4 and 7; the order of other types of trials was randomized. Activity level was used as a simple indicator of behavioral response with 0 indicating no activity, 1 indicating low activity (1-3 minor movements or pattern changes), 2 indicating intermediate activity (4-6 movements or pattern changes), and 3 indicating high activity (more than 6 movements or pattern changes). Movements included fin undulations, arm movements, and body orientation changes or translocations; pattern changes included any visible change in body pattern, from lightening or darkening a single visible body pattern to switching between very different body patterns. Scores for individuals tested more than once in the same condition were averaged.

The second set of trials was designed to gain more information about the importance of polarization to female receivers viewing other females. Females ($N = 10$) were videotaped in a total of four test conditions and multiple behaviors were scored. The test conditions included viewing an empty tank through a clear barrier or through a polarization-distorting filter, and viewing another female through a clear barrier or through a polarization-distorting filter.

In this second set of trials, all cuttlefish were used both as subjects and as viewed other cuttlefish; behavior was scored only when the cuttlefish was acting as a subject. Each female was exposed to each test condition for 15 min with 15 min isolation between conditions. After trials, videotapes were scored for behavior every 30 sec for 10 min. Variables scored included movement (extended fourth arm, fin movement, burying, swimming, jetting, rotating in place, still), body pattern (mottle, uniform, disruptive, false eyespots, passing cloud, strongly contrasting zebra bands), location (top, bottom or middle of water column), and body orientation relative to the viewed tank (body orientation in aggressive interactions such as the Intense Zebra Display is parallel to the opponent; Fig. 1a). Behaviors were recorded as frequencies only; the length of time the behavior was shown was not recorded. Composite variables computed included total movement, number of body pattern changes, number of orientation changes, percent of orientations parallel to the adjacent tank, and percent of body patterns that were potential signals (false eyespots, passing cloud, strongly contrasting zebra bands) as compared to cryptic (mottle, uniform, disruptive). A principal component analysis was performed to combine variables into meaningful, correlated groupings before comparisons were made between conditions.

The third set of trials was designed to focus on the importance of the polarized patterns of males to male and female receivers. Subjects were 10 female and 13 male cuttlefish. Only two conditions were tested: viewing males through clear barriers and viewing males through distorting barriers. Videotapes were scored in the same way as in the second set of trials, above, except that duration of behaviors was also recorded.

The three sets of trials appeared to suffer from small sample size, high individual variability, and consequent lack of statistical power. Data from the three sets of trials were pooled, therefore, to try to improve resolution. Each of the three sets of trials included measurements of activity; trial sets two and three had broken activity into its component behaviors, but the data could be simplified to match that of the first set of trials. Thus, we compared the activity of cuttlefish (according to any variable) when viewing another cuttlefish through a clear barrier to that when viewing the other cuttlefish through a polarization-distorting filter. Our goal was simply to evaluate whether activity was different between trials with clear and distorting filters. No cuttlefish was equally active in the two conditions. Because this analysis was performed posthoc, analyzing data a second time, we reduced our threshold for statistical significance to $p < 0.01$.

Results for Experiment 3

In the first set of trials, males were more active in all conditions than were females (Fig. 4a). Distorting polarization did not appear to have any effect on male behavior (Fig. 4a). The distorting filter had no effect on females' responses to males; curiously, females viewing other females through a distorting filter showed no activity whatsoever ($N = 8$ females; Fig. 4a).

In the second set of trials, the principal component analysis yielded two factors. The first factor explained 45 percent of the variance; key variables were (+) movement, (+) orientation changing and (+) pattern changing. This factor can be thought of as general activity and it did not differ significantly between conditions ($F_{3,9} = 1.68$, $p < 0.25$; Fig. 4b). The second factor explained 24 percent of the variance; key variables were (+) percent of patterns that can be considered signals (as compared with crypsis) and (+) percent of time oriented parallel to the other cuttlefish. This component can be thought of as interacting; this factor also did not differ between conditions.

In the third set of trials, most of the subjects did not show any behavioral changes. Among the females that responded, there was a non-significant trend towards reacting more strongly when viewing a male through a clear barrier than through a polarization-distorting filter. Females oriented in parallel to the males more often (events, 2 of 2; seconds, 3 of 3) and showed more zebra banding (events, 5 of 6; seconds, 4 of 6) when viewing males through the clear barrier than the distorting filter (Fig. 4c). No such trend was found among the male subjects that responded. The number of males that oriented in parallel to the viewed males (events, 2 of 7; seconds, 4 of 8), showed more zebra banding (events, 6 of 9; seconds, 8 of 13), and more Intense Zebra Displays (events, 4 of 6; seconds, 3 of 7) with the clear barrier was not greater than the number showing such behaviors with the distorting filter (Fig. 4c).

In all three sets of trials taken together, females were more active when viewing conspecifics through clear barriers than when they viewed conspecifics through distorting barriers ($\chi_1^2 = 8.33$, $p < 0.01$); males showed no such difference ($\chi_1^2 = 0.03$, $p > 0.50$; Fig. 4d).

Discussion for Experiment 3

We found no evidence that polarized signals affected male behavior. In the first set of trials, males responded primarily to the sex of the other cuttlefish

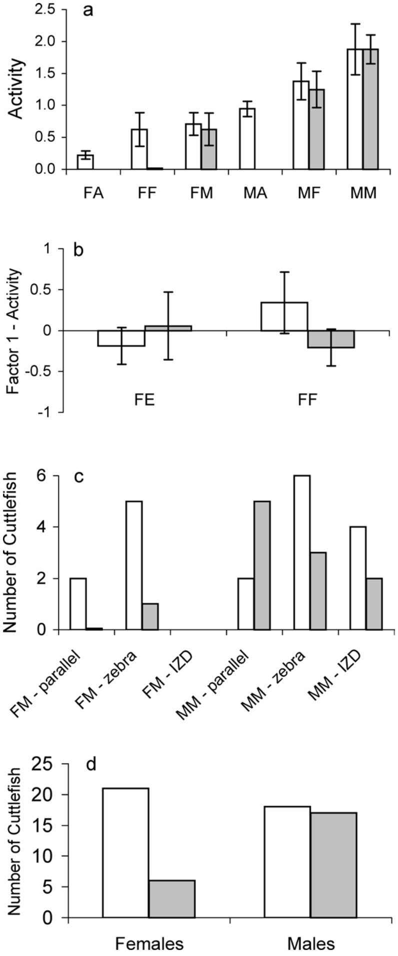

Fig. 4. Experiment 3: Females behave differently when polarization is distorted. Responses of cuttlefish (mean ± sem) when viewing another cuttlefish in an adjacent tank through a clear, polarization-preserving barrier (open bars) or a polarization distorting filter (filled bars). (a) The gross activity of females viewing females was higher with the clear barrier than with the distorting filter ($N = 12$ females, $N = 12$ males; first set of trials): female alone (FA), female viewing another female (FF), female viewing a male (FM), male alone (MA), male viewing a female (MF), and male viewing another male (MM). (b) Females ($N = 10$; second set of trials) tended to be more active, as measured by factor 1 of a principal components analysis, when viewing other females (FF) through a clear barrier rather than a distorting filter. This tendency was not evident when females viewed an empty tank (FE). (c) Number of cuttlefish ($N = 10$ females, $N = 13$ males; third set of trials) that showed the behaviors of parallel, zebra and Intense Zebra Display (IZD) when viewing male cuttlefish: females viewing a male (FM), males viewing a male (MM). Females responded more strongly to males viewed through

rather than to whether or not polarization was distorted (Fig. 4a). In the third set of trials, the three variables of parallel, zebra and Intense Zebra Display would be expected to vary similarly if a real, yet undetected difference existed in how they perceived conspecifics through clear and distorting filters, yet this was not the case (Fig. 4c). When all trials were combined, 18 males were more active while 17 males were less active ($N = 35$) when viewing a conspecific through a clear barrier than through a polarization-distorting filter (Fig. 4d). These initial data suggest that polarization is not important to male receivers, at least under the experimental conditions we provided.

Polarization distortion could be important to female behavior. In the first set of trials, females were more active when viewing another female through a clear barrier that transmitted normal polarization than through a filter that distorted polarization (Fig. 4a). In the second set of trials, this trend appeared again in factor 2, corresponding to interactions (Fig. 4b). In the third set of trials, females oriented in parallel more often and showed more zebra banding to males viewed through a clear barrier than through a polarization-distorting filter (Fig. 4c). Although none of these data were significant alone, when taken together they suggest that polarization information could be important to female receivers. Twenty-one of 27 females were more active when viewing other females through a clear barrier than when viewing them through a polarization-distorting barrier (Fig. 4d). These data indicate that polarization information could be important to female receivers. Further study is required to confirm this tentative finding; we suggest that future studies include more trials in which the cuttlefish views an empty tank through a distorting filter to be sure that cuttlefish are responding to the distorted signals of conspecifics rather than the distortion of the environment.

If polarized body patterns contain signals for intraspecific communication, there must be an evolutionary advantage to signalers for sending these signals. What kind of information could the cuttlefish be transmitting? Cuttlefish normally do not aggregate closely unless spawning (Mangold, 1989;

Fig. 4. (Continued) a clear barrier than to males viewed through a distorting filter. The pattern of responses of males was less clear. (d) Number of cuttlefish in all three sets of trials ($N = 27$ females, $N = 35$ males) that responded with more activity to the sight of another cuttlefish through a clear, polarization-preserving barrier (open bars) or a polarization-distorting filter (filled bars). Females were significantly more active when viewing conspecifics through a clear barrier than through a distorting filter; no such difference was found for males.

Boletzky, 1983; Boal *et al.*, 1999; Hall & Hanlon, 2002); thus, polarization of body patterns could be a signal to conspecifics that means 'I am here,' or 'stay away.' The sender could transmit this visual signal without breaking crypsis to potential predators. Adult males often show non-cryptic, agonistic zebra bands in interactions; polarized patterns would not be needed by these males to advertise their presence. Consistent with this interpretation, polarized body patterns were significantly more likely to be shown in conjunction with cryptic visible body patterns than with highly visible patterns, females were more likely than males to show cryptic patterning (Boal *et al.*, 1999) and polarization patterning (Experiment 2), and female activity was lower when viewing other females through polarization-distorting filters than when viewing them through clear filters. (Curiously, mating cuttlefish do not show polarization (Shashar *et al.*, 1996).) The potential information contained in polarized body patterns may complement that of strongly contrasting zebra banding and might be useful in turbid water or at crepuscular periods to communicate the species, sex, location, and size of the signaler.

Shashar *et al.* (1996) reported that both males and females were less responsive to mirror images of themselves when the polarization component was distorted than when it was preserved. Our results with females are consistent with these previous results but our results with males are not. We speculate that the confinement of our experimental tanks prevented the avoidance behavior that was common in experiments by Shashar *et al.* (1996). As imaging polarimeters are refined, there may be improvements in the quality and quantity of polarized light signals that can be detected and videotaped for these types of difficult behavioral analyses.

General discussion

We found only limited evidence that achromatic or polarized patterns varied in response to the presence of conspecifics. Achromatic patterns (deimatic spots and components of Intense Zebra Displays) varied depending on the presence, number and sex of viewed conspecifics (Experiment 1). Females were more likely to show polarization and less likely to show strongly contrasting zebra bands than were males, but we found no evidence that senders modified their polarized patterns in response to the sight of conspecifics (Experiment 2). From the receiver's point of view, signals that are not actively modified could still provide useful information about the sender. Our data suggest that female receivers did modify their behavior according to the polarization information they received (Experiment 3). The informational nature of such signals remains unknown.

This study is a crude first attempt to quantitatively assess signaling. We used random time samples, thus missing sequential information and signals of very short durations; we also cataloged only gross behaviors, thus missing subtle signals. We knew nothing about the history of our subjects; it is possible that dominance plays a role in the behavior of both signalers and receivers. Further details about visual communication in cuttlefish could be revealed through detailed analyses of interactions between known individuals in a stable environment.

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