

SENSORY CUES FOR CONSPECIFIC ASSOCIATIONS IN AQUATIC SAN MARCOS SALAMANDERS

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ABSTRACT: The mode and effectiveness of signals greatly depends on habitat characteristics and the activity patterns of a species. Visual cues frequently are involved in social interactions, although their effectiveness can be reduced with nocturnal species or in habitats with limited visibility. The combination of multiple signals, such as chemical and visual cues, can increase the accuracy and efficacy of communication in these systems. Association preferences of male and female *Eurycea nana* were examined by allowing individuals to choose between members of both sexes based on (1) chemical signals, (2) visual signals, and (3) chemical and visual signals. Both sexes showed stronger associations with the chemical and chemical and visual signals of the opposite sex than with visual cues alone. The simultaneous inclusion of both chemical and visual signals did not increase male or female responses suggesting that chemosensory communication is sufficient for individuals of this aquatic species to distinguish between the sexes at close range. Additionally, the finding that females as well as males exhibit sexual discrimination suggests that both sexes seek out potential mates, a phenomenon rarely seen in salamanders.

Key words: Aquatic salamander; Chemosensory; *Eurycea nana*; Sex identification

ANIMAL communication, although minimally involving only a signaler and a receiver, is often a complicated interaction among different signalers, modes of signals, and the signaling environment (Endler, 1993). Sometimes two signals conveying the same information in different modalities are more effective at eliciting a response than just one signal (Rowe, 1999). When the primary signal is clouded by noise, additional signals can provide a back-up system for the receiver, even though they may not provide as much information as the primary signal (Johnstone, 1996). The effectiveness of signals is greatly affected by the environmental conditions and activity patterns of the signaler and receiver (Endler, 1993). For example, in aquatic environments, turbid water or low light conditions interfere with visual signals, whereas chemical signals could be more effective (Brönmark and Hansson, 2000). Alternatively, significant current can cause chemical cues to disperse rapidly, precluding the receiver's ability to orient toward signalers (Atema, 1996). Both visual and chemical signals might be co-opted in environments where signals are

degraded or masked by environmental noise. Activity patterns similarly are important in determining the type of sensory cues used. Chemical or acoustic cues may be more important for nocturnal organisms as visual cues would be limited in the dark.

There are numerous examples of chemical and visual communication used by salamanders. Chemical signaling has been described in courtship displays (Arnold and Houck, 1982; Houck, 1986), sex recognition (Mathis, 1990), territoriality (Mathis et al., 1995), assessment of familiar and unfamiliar conspecifics (Gillette et al., 2000; Jaeger and Peterson, 2002), as well as in the discrimination between conspecifics and heterospecifics (Houck and Sever, 1994; Secondi et al., 2005; Verrell, 2003). Visual cues also can be important during breeding and aggressive interactions, especially in diurnal, sexually dimorphic species (e.g., Green, 1991; Hosie, 1992). Most studies that report associations based on visual cues do not eliminate the potential effects of chemical signals. The efficacy of sensory cues can vary based on the sexes of signalers and receivers. For example, females of nonbreeding adult long-toed salamanders, *Ambystoma macrodactylum columbianum*, prefer to associate with substrates bearing chemical cues of males, whereas males show no preference for the substrate-borne cues of males or females (Verrell and Davis, 2003).

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The use and relative importance of visual and chemical cues in association behavior are unknown for the threatened San Marcos salamander, *Eurycea nana* (family Plethodontidae). This neotenic and mostly nocturnal species is endemic to the headwaters of the spring-fed San Marcos River, Hays County, Texas (USDI, 1980). Acyclic oviposition and the presence of gravid females and very small larvae year-round suggest that *E. nana* breeds throughout the year (Bogart, 1967; Tupa and Davis, 1976). However, little else is known about the reproductive behavior of these salamanders in captivity or in their natural environment. Although difficult to detect by human observers, *E. nana* is classified as sexually dimorphic with males possessing larger premaxillary teeth (Bishop, 1941) but poorly defined mental and caudal glands (Sever, 1985) compared to females. The presence of these glandular structures suggests the use of courtship pheromones in the mating behavior of these salamanders (Houck, 1986).

Communication in aquatic environments often requires multiple cues because environmental conditions affect the mode and efficacy of signals (Endler, 1993). The habitat of *E. nana* has fast flowing, clear water (Nephelometric Turbidity Units in 2004 = 0.2) that is stable in temperature (21–23 C), chemical properties, and nutrient concentrations year-round (Groeger et al., 1997). We often have observed *E. nana* in small aggregations under rocks and logs throughout the year, suggesting the occurrence of conspecific associations; however, the sexes of the aggregating individuals have not been determined. We predicted that not only would chemical signals be necessary for social communication in *E. nana*, but also that simultaneous chemical and visual information would provide a stronger signal. We expect that in this fast flowing aquatic habitat with high visibility, salamanders could use chemical as well as visual signals to locate and identify conspecifics.

We examined the use and efficacy of visual and chemical signals, separate and together, in the social communication of *E. nana*. This experiment was designed to determine if males and females use (1) visual signals, (2) chemical signals, or (3) chemical and visual signals together to differentiate between the sexes.

MATERIALS AND METHODS

Collection and Housing

We conducted laboratory experiments using *E. nana* that were caught approximately 20 m downstream from Spring Lake Dam (San Marcos River, Texas) at least 1 yr prior to the study. Sex was determined by candling (Gillette and Peterson, 2001) and only sexually mature individuals >21 mm in snout-vent length (SVL), following the criteria of Tupa and Davis (1976), were used in the experiments. Gravidity was identified by the visible presence of eggs. Males and females were separated for seven months prior to the study and housed in a tank (L × W × H : 1.8 × 0.6 × 0.5 m) divided by an opaque screen that prevented physical contact between the sexes. During the 7-mo separation, none of the females laid eggs, so gravid females were likely to have unfertilized eggs. The large separating tank had re-circulating well water maintained at 22 ± 1 C with plastic shelters and aquatic moss native to the San Marcos River. Salamanders were maintained on a reversed 12L:12D light cycle, with 40-W fluorescent lights during daylight hours and were fed commercially-raised annelids (*Lumbriculus variegates*) ad libitum. Test salamanders were placed in individual tanks (L × W × H : 33 × 18 × 18 cm) one week prior to conducting behavioral observations. All observations were conducted during “night-hours” with dim illumination from one 25-W incandescent bulb suspended above the testing chamber. This lighting is similar to that used by Gillette et al. (2000), in studies with *Plethodon cinereus* in which visual cues were important.

The Role of Visual and Chemical Signals in Conspecific Associations

We examined the roles of visual and chemical signals in conspecific associations by measuring association behavior of male ($n = 30$) and gravid female ($n = 30$) salamanders when presented with one individual of each sex simultaneously in three sensory treatments: (1) chemical, (2) visual, and (3) chemical and visual signals together. Tests were conducted in a 38-L aquarium (L × W × H : 50 × 20 × 30 cm) with a cylindrical container (8.5 cm diameter) placed at opposite ends of the tank. Each container isolated a size-matched

(± 2 mm SVL) stimulus male or gravid female, measured using digital calipers. The focal male or female could freely move in the aquarium and interact with, but not make physical contact with, stimulus salamanders isolated in the containers. The three treatments were created using isolation containers that were (1) opaque with holes such that potential chemical but not visual signals could be exchanged, (2) clear without holes such that only exchange of visual signals was possible, and (3) clear with holes allowing exchange of both potential chemical and visual signals between the focal and stimulus salamanders. Pilot studies using dyed water confirmed that diffusion from within the isolating containers into the rest of the test aquarium was symmetrical. The mixing of water from isolating containers was minimal and would have biased the results towards no preference for chemical signals, as opposed to a preference for stimuli from one end of the aquarium over the other.

In each trial, we randomly determined the placement of the male or female stimulus individual in its isolation container. We then habituated the focal individual for 10 min in the center of the tank under a clear cylinder (8 cm diameter) with holes. After the habituation cylinder was removed by hand, we started a 10-min observation period when the focal individual began to move. We recorded time (s) spent within 44 mm to each container by the focal individual. The 44 mm radius around each container corresponded to the SVL of the largest individual in the population tested. After the first trial, the tank was rotated 180° at the base, and the test was repeated. Rotating the tank controlled for potential side bias without moving the stimulus individuals, thus preventing the mixing of chemicals within the tank. After each test, the observation tank was drained, scrubbed with 3% hydrogen peroxide solution and rinsed with clean water to remove chemical cues trapped in the sealant (McLennan and Ryan, 1997). Each focal individual and the stimulus male and female were maintained in separate individual tanks until they were tested in all three sensory cue treatments. The treatments were conducted on three consecutive days in a randomized order. After being tested in all three treatments, focal individuals were not retested as

focals, but were re-used once as stimulus individuals in subsequent tests.

Statistical Analyses

We measured the responsiveness of focal individuals by dividing the total time each focal individual spent associating with both stimulus individuals over the total time of observation (similar to apathy measurement in Ptacek and Travis, 1997). To determine the roles of visual and chemical signals in conspecific associations, we defined the strength of association for the opposite sex as the total time spent near the opposite sex minus the total time spent near the same sex stimulus. Positive strength-of-association values indicate greater association with the opposite sex compared to same sex conspecifics. We compared the strength of association of both sexes among the sensory treatments (chemical, visual, chemical and visual) using a repeated measures ANOVA. Data met the assumptions of normality. Alpha was Bonferroni reduced to 0.0167 for post-hoc comparisons.

RESULTS

Focal males and females were similarly responsive, spending a mean of 26% and 21% respectively of the total time associating with the stimulus individuals (range: 2–95%). Both males and females were more responsive when only chemical signals were available (34%) and when chemical and visual signals together were available (29.7%), compared to when only visual signals were available (7%).

Females and males had similar strength of association for the opposite sex in all the sensory treatments ($df = 1$, $F = 2.776$, $P = 0.0974$). Strength of association did, however, greatly differ depending on the sensory modality available ($df = 2$, $F = 36.341$, $P < 0.001$, Fig. 1). Compared to when only visual signals were available, both males and females had greater strength of association for the opposite sex when chemical signals alone were available (Bonferroni comparison: $P < 0.001$) and when chemical and visual signals together were available (Bonferroni comparison: $P < 0.001$). The combination of chemical and visual signals did not increase the strength of association for the opposite sex compared to

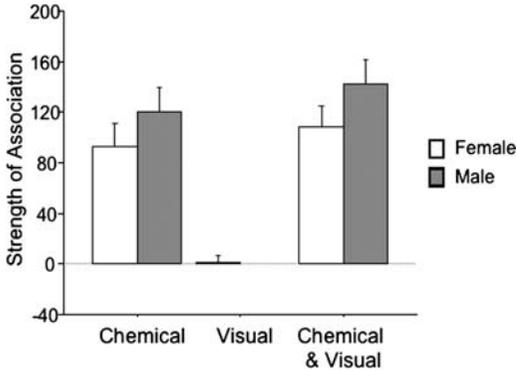


FIG. 1.—Strength of association for the opposite sex (time with opposite sex minus time with same sex, in seconds) by females ($n = 30$) and males ($n = 30$) when only chemical signals, only visual signals, and both chemical and visual signals were available. Bars represent means and 1 SE.

when only chemical signals were available (Bonferroni comparison: $P < 0.220$).

DISCUSSION

In *E. nana*, both males and females with access to chemical signals alone and both visual and chemical signals associated more with individuals of the opposite sex than with same-sex individuals. Individuals of both sexes did not exhibit association preferences based on visual signals alone, but did so on the basis of visual plus chemical signals, thus supporting the hypothesis that chemical signals are the means of intersexual communication in this species. According to Rowe (1999), multimodal signals provide more reliable information, and are more effective at eliciting responses from receivers. Our results suggest that chemical cues can be sufficient for sex identification.

Chemical signaling via sex attractors is important for numerous other aquatic breeding salamanders and newts (e.g., Arnold, 1972; Houck and Verrell, 1993; Verrell, 1985). Adult male red-spotted newts, *Notophthalmus viridescens*, (Verrell, 1985), and male smooth newts, *Triturus vulgaris* (Verrell, 1986) during the breeding season, not only preferred the odors of females but preferred larger over smaller females using either olfactory or visual cues. Males and females of both of these diurnal species of newts are sexually dimorphic, and there is obvious visible variation

(e.g., body size) among individuals of the same sex. Hence, the use of visual cues in addition to chemical cues is not surprising in sex discrimination and mate choice for these species. We observed individuals of *E. nana* directly approaching isolated salamanders when only a visual stimulus was provided, thus suggesting that visual cues were detected under the test conditions. The predominant use of chemosensory communication in close range, however, is expected in *E. nana* given that these salamanders are nocturnal, completely aquatic, and have little obvious visible intersexual morphological variation. The finding that both females and males associated with chemical signals of the opposite sex suggests that not only males, but also females in reproductive condition may seek potential mates. Rarely has this behavior been observed for salamanders (Marco et al., 1998). For example, during the breeding season, adult female palmate newts, *Triturus helveticus*, were attracted to chemical cues of conspecifics regardless of sex (Secondi et al., 2005). Whether responses of male and female *E. nana* indicate preference for the opposite sex or avoidance of the same sex is not known. However, our results clearly demonstrate that both male and female salamanders emphasize the use of chemosensory communication to distinguish between the sexes.

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