

# The role of water-borne chemical cues in mediating social interactions of the Texas blind salamander, *Eurycea rathbuni*

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**Abstract.** Association patterns and social interactions of salamanders vary given habitat heterogeneity and behavioural characteristics. Cave ecosystems are generally food limited and encountering mates may be rare. Little is known about the mode of social interactions and preferences in cave-adapted salamanders. We examined social interactions of the cave-adapted, federally endangered, Texas blind salamander, *Eurycea rathbuni*, by examining sex-specific association patterns. We predicted that non-visual (chemical) cues would be an important mode of communication. We found no difference in association patterns between the sexes when presented with a single conspecific and no aggregative behaviours were detected. Interestingly, when given a simultaneous choice between a male or female, females preferred to associate with male conspecifics while males showed no clear preference. The results suggest that females of *E. rathbuni* use chemical cues to either seek males or avoid females and provide valuable insight into the little known social behaviour of this species and possibly other similar species.

**Keywords:** aggregation, captive management, cave, chemical cues, mate selection.

Chemical stimuli provide information important for social interactions in salamanders. Chemical cues are commonly used during courtship (Arnold and Houck, 1982; Houck, 1986; Rollmann, Houck and Feldhoff, 1999), for sex recognition (Mathis, 1990; Page and Jaeger, 2004; Thaker, Gabor and Fries, 2006; Dantzer and Jaeger, 2007), assessment of familiar and unfamiliar conspecifics (Gillette, Jaeger and Peterson, 2000; Jaeger and Peterson, 2002), and territoriality (Mathis et al., 1995; Gautier and Miaud, 2003). The use of chemical cues in social interactions is predicted to be especially important for species in which visual capacity is reduced or lost entirely such as in cave- and aquifer-adapted species. Anthropogenically derived alterations to aquatic environments such as pollution can influence the effectiveness of chemical communication for aquatic species (Fisher, Wong and Rosenthal, 2006). Thus, determining the role of chemical signals in conspecific interactions is essential to managing the

impacts of human encroachment for aquifer- or cave-adapted salamanders.

Association patterns in plethodontid salamanders can be influenced by resource distribution and abundance (Parrish and Edelstein-Keshet, 1999) and may range from territorial spacing to aggregations (Blaustein and Walls, 1995; Gautier et al., 2006). Territoriality or avoidance of conspecifics is not expected when resources are either very limited or very abundant (Carpenter and Macmillen, 1976; reviewed in Maher and Lott, 2000) while aggregation of adult salamanders may occur in situations involving a shared resource such as food, shelter, or mates (Wilson, 1975). The sex of salamanders appears to also influence aggregation patterns as intersexual pairs are more common than intrasexual pairs (Peterson et al., 2000; Jaeger et al., 2001) and females are more likely to aggregate together than are males (Peterson et al., 2000; Verrell and Davis, 2003). In cave ecosystems, resources such as food or mates are typically limited and sparsely distributed (Poulson and White, 1969; Culver, 1982; Barr and Holsinger, 1985), which may result in reduced aggregative tendencies in cave-adapted species. However, encountering mates may be rare and

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individuals may seek conspecifics of the opposite sex while avoiding those of the same sex in cave systems.

The Texas blind salamander, *Eurycea* (formerly *Typhlomolge*) *rathbuni*, is a federally endangered species (U.S. Fish and Wildlife Service, 1967), endemic to underground caves of the Edward's Aquifer, Hays County, Texas (Longley, 1978). The natural habitat of *E. rathbuni* is largely inaccessible to humans so courtship behaviours and feeding habits are known mostly from captivity. The water in the Edward's Aquifer is thermostable at 21–23°C year-round and gravid females and juveniles are observed throughout the year (Longley, 1978) indicating a lack of reproductive seasonality. Because *E. rathbuni* exhibits reduced eyes and complete vision loss, alternative sensory cues are predicted to be used in interactions with conspecifics. Bechler (1988) described three courtship rituals of *E. rathbuni* in captivity and noted that, unlike most plethodontids (Arnold, 1972; Marco et al., 1998), females, as opposed to males, appeared to initiate courtship. Similarly, a study using a closely-related, stream-adapted congener, *Eurycea nana* (Chippindale et al., 2000), found that both males and females actively sought conspecifics of the opposite sex primarily using chemical but not visual cues (Thaker, Gabor and Fries, 2006). In captivity, aggression is rare and aggregations are common (K.E., pers. obs.) but no studies have specifically examined aggregation patterns in *E. rathbuni*.

Although assessing current and past abundances of *E. rathbuni* is difficult, human encroachment and increased water demand on the habitat of *E. rathbuni* has necessitated the maintenance of captive populations for the purposes of species recovery and reintroduction. Unfortunately, breeding in captivity is rare and sporadic (J. Fries, pers. comm.) and studies that elucidate the social behaviours of *E. rathbuni* will aid in management of this species. The goal of this study was to assess association patterns in *E. rathbuni* mediated through non-visual, water-

borne (chemical) cues. Here we test the hypotheses that *E. rathbuni* (1) would prefer to associate with conspecific cues when available and that (2) when given a choice between both sexes, individuals would prefer to associate with the opposite sex.

We used adult *E. rathbuni* (snout-vent length (SVL) > 40 mm; Brandon, 1971) from a captive population at the San Marcos National Fish Hatchery and Technology Center. These salamanders were offspring of individuals collected by placing nets over spring openings to capture salamanders ascending from the aquifer. They were reared and maintained in aquifer-fed, recirculating tanks at 21–23°C and were fed commercially raised annelids (*Lumbriculus variegatus*), amphipods (*Hyallela azteca*), snails (*Helisoma* sp.), and occasionally zooplankton and freeze-dried crustacean mixtures ad libitum. We conducted the following experiments from January 2008 – March 2009. We removed focal salamanders from the community housing tank and placed them individually into 9.5 l flow-through chambers for at least one week prior to testing. We used adult males ( $n = 16$ ) and females ( $n = 8$ ) of *E. rathbuni* as focal individuals. Sex was determined via candling (Gillette and Peterson, 2001). All males and females were given three tests, comprised of two single choice tests, which allow avoidance behaviour (e.g. Gautier, Lena and Miaud, 2004; Gautier et al., 2006) and one dual choice test to assess preference. Single choice tests consisted of a choice between (1) a female stimulus animal and an empty container or (2) a male stimulus animal and an empty container. In the dual choice test (3), subjects were given a choice between a male and female. Tests were separated by at least two days to maintain independence between tests and the sequence randomized to negate order effects.

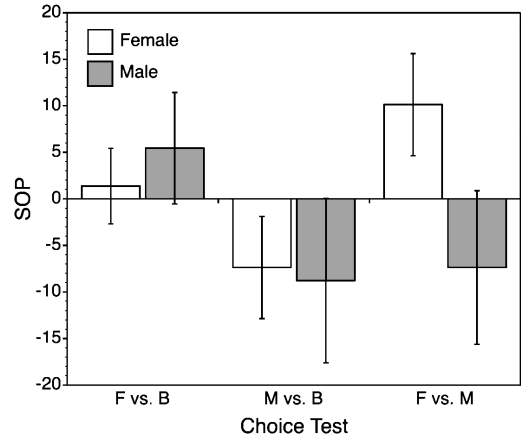
Stimulus animals (or nothing) were placed in perforated containers (diameter = 6 cm) to keep the stimulus individual in one area and to allow the exchange of water-borne chemical stimuli. Stimulus containers were positioned on each end (side chosen randomly) of a 77 cm × 30 cm × 32 cm testing aquaria with 37 l of fresh well water. We drew a circle demarcating one SVL of the largest tested individual (78 mm) on the underside of test aquaria around each stimulus container to indicate the preference zone. The rest of the aquarium was the no preference zone. We placed the stimulus animals into the stimulus containers and the focal salamanders under an acclimation chamber (wire mesh dome) in the center of the testing aquarium and allowed all salamanders to acclimatize for 5 min before the start of the trial. Observations began once the acclimation chamber was removed and we recorded the time (s) that focal individuals spent in each preference zone for 10 min. We rinsed all testing supplies thoroughly with 3% hydrogen peroxide and well water between each test.

#### Statistical analyses

We calculated strength of preference (SOP) for stimulus animals over a blank control in the single choice tests (1 and 2)

by subtracting the time each focal individual spent near an empty container (blank control) from the time spent near the stimulus animal. In the dual choice test (3), we calculated SOP by subtracting time the focal individual spent near the female from time spent near the male. In the single choice tests (1 and 2), positive SOP indicates a preference for a conspecific and negative SOP indicates avoidance. In the dual choice test (3), positive SOP indicates preference for males and negative SOP indicates preference for females. For all tests, SOP scores of 0 indicate no preference. Strength of preference scores for tests 1 and 2 did not meet the assumptions of parametric analyses, but SOP for test 3 did. To test whether focal male and female *E. rathbuni* differed in their preference for a conspecific stimulus of either sex in a single choice test, we compared the SOP of focal males and females using Mann-Whitney *U* tests for test 1 (preference for female) and test 2 (preference for male). To test the hypothesis that males and females would differ in their association preference when choosing simultaneously between a male and a female in the dual choice test (3), we compared the SOP between male and female focal salamanders using a directed *t*-test ( $\gamma = 0.04$  and  $\delta = 0.01$ ; Rice and Gaines, 1994) for unequal variances. We used a directed test because prior studies suggest that salamanders prefer to associate with the opposite sex (Peterson et al., 2000; Jaeger et al., 2001; Thaker, Gabor and Fries, 2006). To examine whether males and females exhibited preference for the opposite sex during the dual choice test, we tested if SOP (SOP of 0 indicates no preference) in test 3 was significantly greater than 0 for females (preference for males) and less than 0 for males (preference for females) using one-sample, one-tailed *t*-tests.

For both focal males and females in the single choice tests (1 and 2), mean SOP for stimulus females was positive and mean SOP for stimulus males was negative (fig. 1). When given the choice between a female and a blank, there was no difference between the sexes in their SOP (Mann-Whitney *U*,  $z_1 = 0.056$ ,  $P = 0.96$ ; fig. 1). Similarly, when given the choice between a male and a blank, the sexes did not differ in their SOP for males ( $z_1 = -0.696$ ,  $P = 0.49$ ; fig. 1). When given the choice between a male and a female, focal females showed a preference for males (positive SOP) while focal males showed a preference for females (negative SOP) (fig. 1). Consequently, focal males and females significantly differed in their SOP for stimulus males (directed *t*-test,  $t_{22} = -1.766$ ,  $P = 0.04$ ; fig. 1). The SOP for males showed by females was significantly greater than 0 (one-sample, one-tailed *t*-test,



**Figure 1.** Mean ( $\pm$ SE) strength of preference (SOP) for male and female *Eurycea rathbuni* in three tests between conspecific males (M), females (F), or blank controls (B). Positive SOP values for F vs. B and M vs. B tests indicate preference for a conspecific, and positive SOP for F vs. M tests indicate preference for a male.

$t_7 = 1.844$ ,  $P = 0.05$ ), but the SOP for females showed by males did not differ from zero ( $t_{15} = -0.894$ ,  $P = 0.19$ ).

## Discussion

We predicted that cave dwelling organisms would be dependent on non-visual cues for association preference yet we found little evidence of aggregative behaviors based on waterborne chemical cues in *E. rathbuni*. We did find that females preferred to associate with males over females when presented with each sex simultaneously. As average female SOP for males was positive (fig. 1) and significantly greater than zero in this dual choice test, this suggests that female *E. rathbuni* either seek out males or avoid other females, although these possibilities are not necessarily mutually exclusive. Males, however, showed no significant association preference in any tests.

Females being the more active sex in seeking mates is uncommon in most salamanders as males typically initiate courtship (Marco et al., 1998). However, our results compliment the finding that females appeared to take the more active role in initiating courtship in *E. rath-*

*buni* (Bechler, 1988). Additionally, our results are consistent with Thaker, Gabor and Fries (2006) who found that both males and females of the closely related, stream-adapted congener, *E. nana* (Chippindale et al., 2000), actively sought conspecifics of the opposite sex. Unlike for *E. nana* (Thaker, Gabor and Fries, 2006), we did not detect significant association preferences from males of *E. rathbuni*; however, this may simply be a result of lowered aggregative tendencies in *E. rathbuni* as compared to *E. nana* or may be due to the smaller sample size in this study which precluded us from detecting male preferences.

Unexpectedly, females of *E. rathbuni* preferred males during the dual choice test (3), but exhibited avoidance of males (negative SOP) during the single choice test (2). This suggests that females show preferences for males only in the presence of another female. Agonistic behaviors are common in plethodontid salamanders and are expected when resources such as food or mates are limited (Mathis et al., 1995). In cave ecosystems, encountering mates may be rare, and females may exhibit agonistic behaviors towards other females in the presence of potential mates. For example, female red-backed salamanders in intersexual pairings respond more aggressively to female than to male intruders, even during the non-breeding season (Lang and Jaeger, 2000). Thus, it is possible that focal females in the dual choice trial exhibited preference for males as this would ensure their own mating success or to avoid potentially costly agonistic encounters with stimulus females. Females exhibiting agonistic behaviours toward other females in the presence of males is consistent with the hypothesis that females are also the more active sex in seeking mates. Thus, these two complimentary hypotheses provide a potential explanation for the results of our study.

Resource availability is expected to be a primary predictor of social interactions in salamanders (Mathis et al., 1995). In cave ecosystems, resources are typically limited and sparsely dis-

tributed (Culver, 1982); however, given the difficulty in accessing the habitat of *E. rathbuni*, the distribution and abundance of resources such as food and mates in the aquifer is unknown. *Eurycea rathbuni* used in this study had been reared in captivity with abundant food resources and potential mates and few were available for testing. It is possible that salamanders recently collected from the natural habitat may exhibit different social behaviours stemming from differences in resource availability between captivity and the natural habitat. In this species, adults are rarely collected from the aquifer so it was not possible to examine if this is occurring. Our results do indicate that chemical cues are important for social interactions in *E. rathbuni*. This suggests water quality may be an important component to the maintenance of this species. Fisher, Wong and Rosenthal (2006) found that alterations to the chemical environment (via anthropogenic effects) disrupt chemical communication in freshwater fish. The same may affect these salamanders. Increasing human demands on water, human encroachment, and pollution in many of these habitats exacerbate the need for research aimed at elucidating the behavioural interactions of these species. This is especially true for successful management and captive breeding of protected species such as *E. rathbuni*.

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