Cohabitation patterns of the San Marcos salamander (*Eurycea nana*)

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Abstract. Social interactions of conspecifics are a function of complex relationships involving resource defense, antipredatory tactics, and mate acquisition. Consequently, individuals often associate non-randomly with conspecifics in their habitats, with spatial distributions of adults ranging from territorial spacing to aggregations. Site tenacity and cohabitation patterns have been well studied in many species of terrestrial salamander; however, less is understood about these behaviors in aquatic species. We examined the cohabitation patterns of intrasexual and intersexual pairs of the federally threatened, paedomorphic San Marcos salamander (*Eurycea nana*) under artificial shelters in a laboratory setting over a 20-day period. We found that intrasexual female pairs and intersexual pairs were found cohabiting more often than intrasexual male pairs. We also assessed site tenacity by examining shelter affinity and found that both males and females inhabited one of the two shelters more often than expected from random habitation, regardless of whether they were in intersexual or intrasexual pairings. Our results indicate that although both sexes of *Eurycea nana* exhibit site affinity, the sex of individuals is an important determinant of cohabitation patterns.

Keywords: aggregation, aquatic salamander, shelter use, site tenacity, territoriality.

Introduction

Individuals of caudate amphibians often associate non-randomly with conspecifics in their habitats, with spatial distributions of adults ranging from territorial spacing to aggregations (see Blaustein and Walls, 1995). Territoriality is demonstrated in salamanders when individuals: (1) exhibit site tenacity, (2) advertise these sites, (3) defend these sites, and (4) successfully resist intrusion into these sites by competitors (Gergits, 1982). Territoriality is not expected when resources are either very limited or very abundant (Maher and Lott, 2000) because the costs of territorial behaviors would outweigh the benefits. Evidence for territoriality in terrestrial salamanders is widespread,

with many species exhibiting at least one of the four tenets above (reviewed in Mathis et al., 1995; Dantzer and Jaeger, 2007). However, less is known about territoriality in aquatic salamanders. Some species of completely aquatic salamanders exhibit only two of the fours tenets of territoriality: site tenacity and agonistic behavior toward intruders (Mathis et al., 1995). Alternatively, at the other end of spatial distribution patterns, aggregations can be facilitated by microhabitat selection (Brown and Orians, 1970) and social factors (Graves and Duvall, 1995) and can reduce the risks of predation and increase mating success (Clark and Dukas, 1994; Morrell and James, 2008; Gascoigne et al., 2009).

Across species and season, the sex of adult salamanders can be an important determinant of aggregation patterns. For example, during the non-breeding season, red-backed salamanders (*Plethodon cinereus*) tend to aggregate in intersexual pairs more than in intrasexual pairs (Jaeger et al., 2001). During the courtship season, although intersexual pairs of *P. cinereus* are found more often than intrasexual pairs, the proportion of female pairs is higher than male pairs (Peterson et al., 2000). Similarly, non-breeding adult female long-toed salaman-

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ders (*Ambystoma macrodactylum*) prefer to cohabit with other females rather than reside alone in burrows, while male-male and intersexual pairs cohabit less often (Verrell and Davis, 2003). In the gregarious salamander *Mertensiella luschani*, adults and juveniles are attracted to shelters that have been chemically marked by conspecifics, although females prefer the scent of other females over that of males (Gautier et al., 2006). In some cases, the same population can exhibit both aggregation and putative "territorial" spacing depending on habitat conditions and the breeding system. For example, several territorial *Ambystoma* spp. (Gehlbach, Kimmel and Weems, 1969; Pough and Wilson, 1970; Nussbaum, Brodie and Storm, 1983) and *Plethodon* spp. (Heatwole, 1960; Wells and Wells, 1976; Jaeger et al., 2001) form aggregations of both sexes during drier conditions because it reduces the rate of desiccation.

The San Marcos salamander (*Eurycea nana*) is a federally threatened (United States Department of the Interior, 1980), aquatic species endemic to the thermostable headwaters of the San Marcos River, Texas. Throughout the year it can be found either singly or in small aggregations of two to four individuals under rocks and logs in their natural habitat (M.T., J.F. and K.E., pers. obs.). *Eurycea nana* interacts non-aggressively in captivity, where it usually aggregates in clumps of aquatic moss and under structures provided for shelter. In previous laboratory experiments, we have shown that *E. nana* associate with the opposite sex using chemical cues alone or chemical & visual cues together (Thaker, Gabor and Fries, 2006). However, no study has systematically examined the cohabitation patterns or site affinity in this species.

Materials and methods

Subjects

Eurycea nana (Caudata: Plethodontidae) is a completely aquatic, obligatorily neotenic salamander species. In the natural habitat (Tupa and Davis, 1976) and in captivity (M.T., J.F. and K.E., pers. obs.), juveniles and gravid females of *E. nana* are present year-round, indicating a lack of reproductive seasonality in this species. All individuals used in the experiment were first-generation, laboratory-reared offspring of wild-caught *E. nana*. Experimental salamanders originated from 3 large, mixed-sex breeding tanks containing 20-40 adults/tank and thus, were unlikely to be matched with siblings during trials. We used digital calipers to measure snout-vent length (SVL) from the tip of the snout to the posterior end of the vent. We used the candling method (Gillette and Peterson, 2001) to sex *E. nana* and used only adult males and non-gravid adult females as test subjects (SVL > 25 mm; Tupa and Davis, 1976).

We uniquely marked every test individual with Visible Implant Flourescent Elastomers (VIE) (Northwest Marine Technology), following procedures from Bailey (2004). Elastomers were injected within the dermal layer in up to five body locations: one on the tail, one below each foreleg, and one above each hindleg. These VIE are faintly visible through the dark skin pigmentation but fluoresce under ultra-violet light. We conducted experiments 3 weeks after the marking procedure, between October 2003 and January 2004 at the National Fish Hatchery and Technology Center, San Marcos, Texas.

Procedure

Cohabitation experiments were conducted in testing tanks that were flow-through aquaria ($24 \times 12.5 \times 13$ cm), each equipped with a screened stand-pipe. Each testing tank had 10-L of well water that was kept fresh and thermostable ($22°$C) during the observation period with re-circulating well water. Testing tanks were set-up in a semi-outdoor structure with natural light supplemented by a 40-W full spectrum, fluorescent light. We placed one artificial shelter, labeled A or B, at two diagonal corners of each tank. Shelters were made from a 10-cm long PVC pipe (2.5 cm diameter) cut in half along its longitudinal axis. We fed salamanders every 5 days by placing a small amount of commercially raised annelids (*Lumbriculus variegatus*) in the center of each tank, equidistant from both shelters.

To examine cohabitation patterns, we assigned pairs of salamanders to one of three treatments ($n = 10$ pairs per treatment): (1) male-male pair, (2) female-female pair, or (3) male-female pair. We size-matched paired individuals within ± 3 mm SVL (mean difference $= 0.37$ mm), and each salamander was used only once in the study. Salamanders were previously housed in sex-specific group tanks, and individuals were separated from each other for at least 1 week prior to testing. We simultaneously placed each pair in a testing tank and allowed 24 h for habituation. Following habituation, we examined the position of each salamander once daily from 1100-1200 for 20 consecutive days. We recorded the position of each individual, without disturbing them or the tank environment, by identifying the salamanders based on their VIE markers using a portable ultra-violet light. The position of each salamander was recorded as under shelter A, shelter B, or not under shelter. On day 20, after the last position was recorded, we removed all individuals and cleaned the tanks with 3% hydrogen peroxide solution to remove chemical cues trapped in the sealant (McLennan and Ryan, 1997). We then rinsed the tanks thoroughly with clean water prior to their use in subsequent trials.

Analyses

To test for shelter affinity, we randomly selected one individual from each intrasexual pair to maintain independence between individuals within each analysis and used both males and females from the intersexual pair. We compared the average number of times individuals of each sex in each treatment were found under each shelter with an expectation of random residency (mean total times found under a shelter/2) using a χ^2 test. We next assessed whether individuals were choosing to cohabit as opposed to residing alone by comparing the number of times members of a pair were found cohabiting when using a shelter with the null expectation of random cohabitation (mean total days/2 = 10). To test for differences in cohabitation patterns among male-male, female-female, or male-female pairs, we compared the mean number of days pairs were found cohabiting under a shelter using a Kruskal-Wallis Test with subsequent multiple comparison procedures ($\alpha = 0.05$) (Siegel and Castellan, 1988).

Results

Salamanders were under one of the two shelters in at least 75% of the observations. Males and females in both intersexual and intrasexual pairs exhibited site affinity because they inhabited one of the two shelters significantly more than would be expected by random chance (table 1). During the 20 days of observation, female-female ($Z = -2.554, p = 0.011$) and intersexual pairs ($Z = -2.609, p = 0.009$) were found cohabiting more often than expected from random while male-male pairs were not ($Z = -0.491, p = 0.623$). Further, cohabitation occurrence by female-female pairs and female-male pairs was significantly greater than that of male-male pairs, but did not differ from each other ($H_2 = 6.18, p = 0.045$; fig. 1).

Discussion

We studied the social interactions of *E. nana* by examining patterns of shelter affinity and cohabitation of intrasexual and intersexual pairs, when the pairs were provided with two shelters and observed once daily for a 20-day period. Shelter affinity or site tenacity has been found in numerous terrestrial salamanders (Jaeger et al., 1993; Ribéron and Miaud, 2000; Gautier and Miaud, 2003) and, according to Gergits (1983), is one of the four tenets of territoriality in caudate amphibians. We found that both males and females of the aquatic *E. nana* showed site tenacity, regardless of pairing treatment. Additionally, female pairs and intersexual pairs were found cohabiting more often than male-male pairs, suggesting selective aggregative behavior.
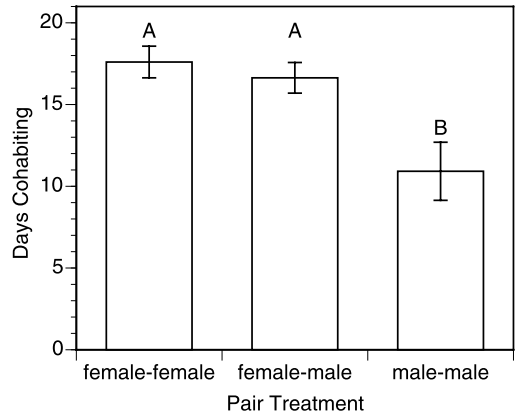

Figure 1. Mean ($\pm SE$) days spent cohabiting by *Eurycea nana* in intrasexual and intersexual pairs. Each pair was observed once daily for 20 days and recorded as cohabiting under the same shelter or not. Letters denote significant difference among pair treatments in the number of days they cohabited over the 20-day period (Kruskal-Wallis multiple comparisons, $\alpha = 0.05$).

Table 1. Patterns of shelter affinity for males and females of *Eurycea nana* in intrasexual and intersexual pairs. Shelter affinity is the mean ($\pm SD$) number of days individuals were found under the most used of two available shelters over 20 days. Shelter affinity was detected in both males and females in both intersexual and intrasexual pairs because individuals inhabited one of the two shelters significantly more than predicted from random choice.

Treatment	Sex	n	Shelter affinity mean $\pm SD$	χ^2	p
Female-female	Female	10	16.8 ± 4.38	6.76	<0.01
Male-male	Male	10	16.1 ± 2.81	7.95	<0.01
Female-male	Female	10	17.1 ± 3.35	11.72	<0.01
	Male	10	17.0 ± 2.79	10.18	<0.01

Cohabitation patterns can reflect many aspects of social behavior such as mate choice, anti-predatory tactics, or resource use. For terrestrial red-backed salamanders, cover objects not only provide patches of moisture and foraging refuges when the surrounding leaf litter is dry (Jaeger, 1980), but also provide locations for courtship and mating (Mathis, 1991; Hom, Jaeger and Willits, 1997). *Eurycea nana* lives in a habitat that is thermostable at 21.1-22.5°C and fairly constant in terms of chemical properties and nutrient concentrations across the seasons (Groeger et al., 1997) so limitations in food resources or suitable microhabitat are unlikely. Thus, aggregation and shelter use patterns by *E. nana* might instead be reflective of anti-predatory tactics in this species (Wood and Ackland, 2007). If so, aggregating under natural shelters (rocks and logs) could benefit individuals by reducing search time for adequate shelters (Gautier et al., 2006; Briones-Fourzán, Ramírez-Zaldívar and Lonzano-Álvarez, 2008), reducing individual risk via the dilution effect (Watt, Nottingham and Young, 1997), and by increased probability of predator detection (Lima, 1995). Further, site tenacity in this species might result from selection favoring individuals who stay in suitable shelters because searching for new shelters can substantially increase the risk of predation (Eklöv and Persson, 1995). Despite the anti-predator benefits of aggregation, these alone cannot explain the cohabitation patterns of *E. nana* because male pairs cohabited randomly and not selectively with other males.

The cohabitation patterns we observed in the laboratory for intersexual pairs also might result from mate-seeking behavior, although this does not explain patterns observed for female pairs. Females preferred to cohabit with conspecifics in both intersexual and intrasexual pairings and these patterns did not differ between pair treatments. Female preference for cohabitation with other females and males has been seen in other salamanders (Gautier et al., 2006; Malmgren, Andersson and Simon, 2007). The intersexual cohabitation patterns we observed might reflect these aggregative tendencies of females or mate seeking behavior by males, although these should not be considered mutually exclusive. Male-female pairs cohabited significantly more than expected from random chance while cohabitation patterns of male-male pairs did not differ from random indicating that males preferred to cohabit with females, but were neither seeking nor avoiding other males. These cohabitation patterns of males suggest that male-male aggression is unlikely in this species, but males that preferentially cohabit with females might gain mating opportunities.

Future experiments should address possible ecological drivers of these cohabitation patterns such as food availability, predation pressures, familiarity of conspecifics, and group size. For example, studies using more than two individuals in different sex ratios with several shelter options would be informative as would studies examining shelter use and cohabitation patterns under the risk of predation. Such designs would elucidate whether these salamanders are aggregating due to predation pressure and if cohabitations are a function of mate choice. The results of this study and future experiments further examining social interactions of *E. nana* also will be useful for designing better refugia for captive management of this threatened species.

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