SENSORY CUES, ASSOCIATION PREFERENCES,

AND SOCIAL INTERACTIONS

OF THE SAN MARCOS SALAMANDER

THESIS

Presented to the Graduate Council of
Texas State University – San Marcos
in Partial Fulfillment of
the Requirements

for the Degree

Master of SCIENCE

by

Maria Thaker, B.S.

San Marcos, Texas
May 2004

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

LIST OF FIGURES & TABLES

ABSTRACT

SENSORY CUES, ASSOCIATION PREFERENCES,

AND SOCIAL INTERACTIONS

OF THE SAN MARCOS SALAMANDER

by

Maria Thaker

Texas State University – San Marcos

May 2004

Supervising Professor: Dr. Caitlin R. Gabor

I studied the sensory communication, association preference, and social interactions of the threatened San Marcos salamander (*Eurycea nana*) in the laboratory. *Eurycea nana* (Plethodontidae) is endemic to the headwaters of the spring-fed San Marcos River (Texas). Within the geographic distribution of this species, temperature, chemical properties, and nutrient concentrations of the water are fairly constant. I expected that this stable habitat and year-round breeding would affect their communication and social behavior.

In chapter II, I examined the additive and multiplicative effectiveness of different sensory cues in the association preference of males and females of *E. nana*. The mode and effectiveness of signals depends on the environmental conditions and activity patterns of animal species. Visual cues frequently are involved in social interactions, although in nocturnal species, chemical and acoustic cues are more important. Multiple cues, however, can increase the accuracy of communication. Therefore, I examined association preferences of male and female salamanders based on conspecific (1) chemical cues, (2) visual cues, and (3) chemical & visual cues when simultaneously exposed to one individual of each sex. Both sexes significantly preferred to associate with the opposite sex when exposed to both chemical, and chemical and visual cues. There was no significant preference for either sex with visual cues alone. The simultaneous inclusion of both chemical and visual cues did not increase male or female preference for the opposite sex, thus chemical cues alone were sufficient to identify sex. I further tested male association preference for the chemical and visual cues of gravid versus non-gravid females. Males did not significantly prefer either type of female. Overall, my results suggest that although chemosensory communication is sufficient for females and males to distinguish between the sexes at close range, the ability of males to discriminate between females of different gravidity may require a different sensory modality.

In chapter III, I examined aspects of social interactions in *E. nana*. Social interactions of conspecifics are a function of the complex relationships among resource defense, anti-predatory tactics, and mate acquisition. Individuals often associate non-randomly with conspecifics in their habitats, where spatial distributions of adults range from territorial spacing to aggregations, depending on the habitat conditions and breeding

status of the interacting individuals. I examined the cohabitation patterns of intrasexual and intersexual pairs of salamanders under artificial shelters across a 20-day period. I also examined individual affinity to the two shelters as a measure of site tenacity. None of the salamanders exhibited significant affinity to a particular shelter. In fact, males in the intrasexual pair treatment affiliated with both shelters equally often, indicating that they move frequently. Female pairs and intersexual pairs were found cohabiting more often than expected from random, whereas pairs of males cohabited in a pattern not significantly different from random. These results demonstrate that females of *E. nana* preferred to cohabit with individuals of both sexes, and males did not cohabit with other males, which could be a non-aggressive tactic to reduce competition or an indication that males move more frequently than females.

CHAPTER I

INTRODUCTION

Communication

Animal communication often involves the interaction of multiple signal components, different signalers, and modes of signals (reviewed in Johnstone, 1997). In mate assessment, for example, signalers have to convey information that elicits a desirable response from receivers. Receivers, on the other hand, must effectively filter through noise in the system and differentiate among the range of signals to determine the best mate. At times, multiple signals conveying the same information in different modalities (i.e., different sensory cues) are more effective at eliciting a response than just one signal (Rowe, 1999). For example, Endler (1993) suggested that visual signals are perceived faster than olfactory signals, although olfactory signals may provide more species specific information.

The use and effectiveness of signals depends on the environmental conditions and activity patterns of the organism (Endler, 1993). In aquatic environments, chemical signals are often more effective, especially since turbid water or low light conditions interferes with the transmission of visual signals (Brönmark and Hannson, 2000). Both visual and chemical signals might be co-opted in complex environments where signals are degraded or masked by environmental noise. Chemical cues may be more important for nocturnal organisms as visual cues would be limited by reduced light. As expected, most caudate amphibians are nocturnal and rely almost entirely on chemical cues to locate prey, predators, and mates (Dawley and Bass, 1989). Therefore, different cues may be emphasized in different contexts (reviewed in Candolin, 2003).

McLennan and Ryan (1997) have suggested that mate recognition is not a simple process and likely is based on complex responses to information from both visual and

chemical cues. In mate choice studies, visual cues usually are studied from the female perspective because males often are visually distinct with bright conspicuous colors, exaggerated physical traits, and elaborate behaviors (e.g., Basolo, 1990; Rosenthal et al., 1996; Basolo and Trainor, 2002). For example, female smooth newts, *Triturus vulgaris*, accept more spermatophores from males with greater tail height than those with smaller tail height (Green, 1991; Hosie, 1992). Chemical cues also are likely to be important in mate selection because they might indicate the sex (Dawley, 1984a, b) and reproductive status (Farr and Travis, 1986) of an individual. In many terrestrial and aquatic salamanders, females usually produce chemical cues that function as sex attractors while males usually produce courtship hormones that increase female receptivity (Arnold and Houck, 1982; Houck, 1986; Houck and Reagan, 1990; Houck and Verrell, 1993). In some species, chemical cues can even stimulate reproduction during the early stages of courtship (Duellman and Trueb, 1986). In the cichlid fish *Haplochromis burtoni*, chemical cues from gravid females provide sufficient stimulus to elicit reproductive activity in males but visual cues are not enough (Crapon de Crapona, 1980). While visual or chemical signals may be sufficient to elicit a response, the simultaneous use of both signals may increase the response of the receiver. In big-clawed snapping shrimp, *Alpheus heterochaelis*, concurrent visual and chemical cues provide the most information about sex and quality (Hughes, 1996). Female freshwater angelfish, *Pterophyllum scalere*, not only spawn at higher rates in the presence of visual or chemical cues from males, they spawn at even higher rates when exposed to both signals simultaneously (Chien, 1973).

Social Interactions

Social interactions between and within species can be a complex relationship between resource defense, anti-predatory tactics, and mate acquisition (Hixon, 1987). These behaviors will in turn affect the distribution as well as associations among conspecifics in their habitat. For example, the way territories are arranged greatly influences an individual's access to potential mates. Thus, studies of social interactions can range from determining distributions of conspecifics in their environment to studying individual interactions.

Distributions of adults vary from territorial spacing to large conspecific aggregations. A territory was defined by Noble (1939) as a "defended space" where individuals secure a patchy or limited resource, mainly food or mates. Conspecifics that defend these limited resources experience higher net fitness, despite the energetic costs, and potential risks of predation and injury (Brown, 1964). Distributions of territorial and non-territorial species overlap closely with local variation in resource densities (Wiens, 1976; Davies and Houston, 1984). However, individuals may settle in clusters because they are attracted to conspecifics (see review by Stamps, 1988).

As with territoriality, there are several benefits and costs to aggregating. Some of the major advantages of group living or forming aggregations are the (1) reduction in predator pressure through increased detection of predators, group defense, or by the dilution effect, (2) improved efficiency in foraging, (3) improved defense of food resources against other groups, and (4) breeding success (reviewed in Alexander, 1974). On the other hand, group living among adults increases (1) the competition for food and mates, and (2) the spread of diseases and parasites (reviewed in Alexander, 1974).

Comparisons within and between animal species suggest that food availability and predator avoidance are the two main environmental influences on the occurrence and size of conspecific clusters (Krebs and Davies, 1993). There is a notable trade-off between the benefits of reduced predator pressure gained by group living and the cost of increased competition for food (e.g., Elgar, 1986). Alternatively, social influences of clustering are primarily facilitated by breeding, communal egg-laying, and group parental care (see reviews in Bradbury and Gibson, 1983; Blaustein and Walls, 1995)

One of the most direct methods to evaluate social interactions is to examine cohabitation patterns. In the cave-dwelling salamander, *Proteus anguinus*, mating occurs under defended shelters, and then females remain alone with their eggs until they hatch (Guillaume, 2000). When non-sexually active, individuals of this species exhibit gregarious behavior in the laboratory by preferring to cohabit rather than reside alone (Guillaume, 2000). For the terrestrial red-backed salamander, *Plethodon cinereus*, cover objects provide patches of moisture and foraging refuges when the surrounding leaf litter is dry (Jaeger, 1980). Cover objects also provide a location for courtship and mate selection (Mathis, 1991; Hom et al., 1997). Daily sampling of red-backed salamanders showed that significantly greater than random intersexual pairs were found together under cover objects during both the breeding and non-breeding seasons (Jaeger et al., 2001). Long-toed salamanders, *Ambystoma macrodactylum*, court, mate and lay eggs in stationary water in the spring; but when not breeding, these salamanders reside in subterranean burrows on land (Verrell et al., 2001). Conspecific associations in burrows likely lower energy expenditure, reduce desiccation, and lessen predation risk (Blaustein and Walls, 1995). Alternatively, associations may increase competition for prey

(Blaustein and Walls, 1995). When cohabitation patterns were examined, non-breeding females of this species cohabited with other females more frequently (Verrell and Davis, 2003). Thus, the pattern of social interactions in salamanders depends on environmental conditions, and hence, varies considerably.

Study Species: San Marcos Salamander (*Eurycea nana*)

Eurycea nana (family Plethodontidae) is endemic to the headwaters of the San Marcos River, Hays County, Texas (Bishop, 1941; Baker, 1961). Based on phylogenetic reconstruction, Chippindale et al. (1998) reconfirmed the systematic status of *E. nana* as a unique species from the other *Eurycea* species in the region. *Eurycea nana* is lungless, neotenic and does not co-exist with any other salamanders, as the geographically overlapping Texas blind salamander, *Eurycea rathbuni*, is subterranean.

The San Marcos Springs that discharge from the Edwards Aquifer through limestone faults along the Balcones Fault Zone fill Spring Lake at the headwaters of the San Marcos River (Brune, 1981). The range of *E. nana* extends from Spring Lake to 150 m downstream (Nelson, 1993), where the substrate consists of sand, gravel, and rocks. Some of these areas also have vegetative cover, predominantly aquatic moss (*Amblystegium riparium*) and filamentous algae (*Lyngbya spp.*). This limited distribution, and the dependence of the salamanders on spring flow, resulted in the federal listing of *E. nana* as a threatened species (USDI, 1980). The springs are slightly alkaline (250-270.5 mg $CaCO_3$/L), with temperatures ranging from 21.1 to 22.5°C (Groeger et al., 1997). Dissolved oxygen levels are approximately 4 mg/L and pH levels range from 6.9 to 7.8

(USFWS, 1984). The average recorded flow rate from the springs between 1957 and 1991 was 4.6 m³/s (range: 1.3-12.1 m³/s) (Buckner and Shelby, 1991).

The diet of *E. nana* is composed of amphipods, small aquatic snails, and insect larvae and pupae (Tupa and Davis, 1976). Based on testicular development in males and egg maturation in females, Tupa and Davis (1976) reported that wild males of *E. nana* reach sexual maturity at snout-vent length (SVL) of 19 mm and females at 21 mm. The acyclic oviposition, and the presence of gravid females and very small larvae every month of the year, suggest that *E. nana* breeds all year round (Bogart, 1967; Tupa and Davis, 1976). No eggs have been found in nature, but from observations in captivity, eggs typically are laid singly in aquatic moss and on rocks. The average number of eggs per female laying event is 34.7 with an average of 10.3 hatchlings per clutch, and the time between oviposition and hatching ranges from 12 to 23 days (Najvar, 2001).

Although activity patterns have not been recorded in the wild, *E. nana* appears to be nocturnal, with more activity in laboratory tanks at night than during the day (Fries, 2002). This pattern is similar to many other plethodontid salamanders of the genus *Eurycea* observed in the wild (Bishop, 1943; Gordon, 1953; Hutchison, 1958; Smith, 1961; MacCulloch and Bider, 1975). The spatial distribution of *E. nana* in their natural habitat is unrecorded, but these salamanders have been found alone or in small aggregations of up to four individuals under rocks and logs (J. N. Fries, pers. comm.; pers. obs.).

Aggression in *E. nana* also is undocumented but studies in other *Eurycea* species have reported varied results. Jaeger (1988) found that males of *E. longicauda* did not exhibit aggressive behavior towards other males in the laboratory, while Grant (1955)

and Arnold (1977) found that males of *E. bislineata* assaulted other males who entered their territories. From laboratory observations of *E. nana*, males and females are unlikely to be aggressive over food because excess food is always present (J. N. Fries, pers. comm.; pers. obs.). Aggression over food or mates in the wild is possible. These salamanders, however, have not been observed defending territories in the laboratory tanks, and have been seen aggregated together in the clumps of aquatic moss and under the plastic structures provided for shelter (J. N. Fries, pers. comm.; pers. obs.).

Little is known about the mating behavior of these salamanders in captivity or in their natural environment. *Eurycea nana* is described as being sexually dimorphic with males possessing poorly defined mental and caudal glands (Sever, 1985) and larger premaxillary teeth than females (Bishop, 1941). The presence of these structures suggests the importance of courtship hormones in the sexual behavior of these salamanders (Houck, 1986). The mental glands, located on the chins of male plethodontid salamanders, produce courtship pheromones that are conveyed to females via direct rubbing on the nares or through the scraping of premaxillary teeth on the dorsa of females (Houck and Reagan, 1990). In plethodontid salamanders, courtship also involves tail-straddle walk as a prerequisite for spermatophore deposition (Arnold, 1977). As described by Arnold (1977), tail-straddle walk proceeds as follows: the female positions herself astride the male's tail, with her head resting on the proximal portion. The male moves forward while undulating the whole length of his tail laterally and the female, remaining astride his tail, follows. The male pauses, deposits a spermatophore on the substrate and continues walking forward. The female follows until the male removes his tail from beneath her and begins repeatedly to raise and lower his pelvis and proximal

part of his tail. This in turn raises and lowers the forebody of the female, which assists in the lodging of the spermatophore into her cloaca. Courtship behavior in *E. nana* was observed and recorded once, where the pair engaged in tail-straddle walk for 54 min but did not follow with sperm deposition (pers. obs.).

I explored aspects of social behavior of *E. nana* in the laboratory in two papers that are to be submitted for publication. In Chapter II, I examined sensory communication and association preferences, with additional results reported in Appendix 1. In Chapter III, I examined aggregation patterns and territoriality.

REFERENCES

Alexander RD, 1974. The evolution of social behavior. Ann. Rev. Ecol. Syst. 5:325-383.

Arnold SJ, 1977. The courtship behavior of North American salamanders with some comments on Old World salamandrids. p. 141-183 In: The Reproductive Biology of Amphibians (Taylor DH, Guttman SI, eds.). New York: Plenum Press.

Arnold SJ, Houck LD, 1982. Courtship pheromones: evolution by natural and sexual selection. pp. 173-211 In: Biochemical Aspects of Evolutionary Biology (Nitecki M ed.). Chicago, IL: Univ. Chicago Press.

Baker JK, 1961. Distribution of and key to the neotenic *Eurycea* of Texas. The Southwest. Nat. 6:27-32.

Basolo AL, 1990. Female preference for male sword length in the green swordtail, *Xiphophorus helleri* (Pisces: Poeciliidae). Anim. Behav. 40:332-338.

Basolo AL, Trainor BC, 2002. Multi-component male trait evolution via a preexisting bias. Anim. Behav. 63:469-474.

Bishop SC, 1941. Notes on the salamanders with descriptions of several new forms. Occa. Papers Mus. Zool., Univ. Michigan 451:1-21.

Bishop SC, 1943. A Handbook of Salamanders. Ithaca, New York: Comstock Publishing.

Blaustein AR, Walls SC, 1995. Aggregation and kin recognition. p. 568-602. In: Amphibian Biology, Vol. 2 (Heatwole H, Sullivan BK, eds.). New South Wales, Australia: Surrey Beatty and Sons.

Bogart JP, 1967. Life history and chromosomes of the neotenic salamanders of the Edwards Plateau (MS Thesis). Austin, Texas: University of Texas.

Bradbury JW, Gibson RM, 1983. Leks and mate choice. p. 109-138. In: Mate Choice (Bateson P, ed.). Cambridge: Cambridge Univ. Press.

Brönmark C, Hannson L-A, 2000. Chemical communication in aquatic systems: an introduction. Oikos 88:103-109.

Brown JL, 1964. The evolution of diversity in avian territorial systems. Wilson Bull. 76:160-169.

Brune G, 1981. Springs of Texas. Fort Worth: Branch-Smith Inc.

Buckner HD, Shelby WJ, 1991. Water resources data Texas water year 1991. Vol. 3. U.S. Geological Survey Water - Data report TX-91-3.

Candolin U, 2003. The use of multiple cues in mate choice. Biol. Rev. 78:575-595.

Chien AK, 1973. Reproductive behaviour of the angelfish *Pterophyllum scalare* (Pisces: Cichilidae) II. Influence of male stimuli upon the spawning rates of females. Anim. Behav. 21:457-463.

Chippindale PT, Price AH, Hillis DM, 1998. Systematic status of the San Marcos salamander, *Eurycea nana* (Caudata: Plethodontidae). Copeia 1998:1046-1049.

Crapon de Crapona MD, 1980. Olfactory communication in a cichlid fish, *Haplochromis burtoni*. Z. Tierpsychol. 52:113-134.

Davies NB, Houston AI, 1984. Territory economics. p. 148-169. In: Behavioral Ecology: An Evolutionary Approach, 2nd ed. (Krebs JR, Davies NB, eds.). Sunderland, MA: Sinauer Associates.

Dawley EM, 1984a. Identification of sex through odors by male red-spotted newts, *Notophthalmus viridescens*. Herpetologica 40:101-105.

Dawley EM, 1984b. Recognition of individual, sex and species odours by salamanders of the *Plethodon glutinosus - P. jordani* complex. Anim. Behav. 32:353-361.

Dawley EM, Bass AH, 1989. Chemical access to the vomeronasal organs of a plethodontid salamander. J Morphol. 200:163-174.

Duellman WE, Trueb L, 1986. The Biology of Amphibians. Baltimore, MD: John Hopkins Univ. Press.

Elgar MA, 1986. The establishment of foraging flocks of house sparrows: Risk of predation and daily temperature. Behav. Ecol. Sociobiol. 19:433-438.

Endler JA, 1993. Some general comments on the evolution and design of animal communication systems. Phil. Trans. R. Soc. Lond. B. 240:215-225.

Farr JA, Travis J, 1986. Fertility advertisement by female sailfin mollies, *Poecilia latipinna* (Pisces: Poeciliidae). Copeia 1986:467-472.

Fries JN, 2002. Upwelling flow velocity preferences of captive adult San Marcos salamanders. N. Am. J. Aqua. 64:113-116.

Gordan RE, 1953. A population of Holbrook's salamander, *Eurycea longicauda guttolineata* (Holbrook). Tulane Stud. Zool. 1:55-60.

Grant WC, 1955. Territorialism in two species of salamanders. Science 121:137-138.

Green AJ, 1991. Large male crests, an honest indicator of condition, are preferred by female smooth newts, *Triturus vulgaris* (Salamandridae) at the spermatophore transfer stage. Anim. Behav. 41:67-369.

Groeger AW, Brown PF, Tietjen TE, Kelsey TC, 1997. Water quality of the San Marcos River. Texas J. Sci. 49:279-294.

Guillaume O, 2000. Role of chemical communication and behavioral interactions among conspecifics in the choice of shelters by the cave-dwelling salamander *Proteus anguinus* (Caudata, Proteidae). Can. J. Zool. 78:167-173.

Hixon MA, 1987. Territory area as a determinant of mating systems. Am. Zool. 27:229-247.

Hom CL, Jaeger RG, Willits NH, 1997. Courtship behaviour in male red-backed salamanders: the ESS dating game. Anim. Behav. 54:715-724.

Hosie C, 1992. Sexual motivation and mate choice in the female smooth newt, *Triturus vulgaris*. Ph.D. Thesis, The Open University, Milton Keynes, United Kingdom.

Houck LD, 1986. The evolution of salamander courtship pheromones. p. 173-190. In: Chemical Signals in Invertebrates, Vol. 4 (Müller-Schwartze D, Silverstein RM, eds.) New York: Plenum Press.

Houck LD, Reagan NL, 1990. Male courtship pheromones increase female receptivity in a plethodontid salamander. Anim. Behav. 39:729-734.

Houck LD, Verrell PA, 1993. Courtship behavior in plethodontid salamanders: A review. Herpetologica 49:175-184.

Hughes M, 1996. The function of concurrent signals: visual and chemical communication in snapping shrimp. Anim. Behav. 52:247-257.

Hutchison VH, 1958. The distribution and ecology of the cave salamander, *Eurycea lucifuga*. Ecol. Monogr. 28:1-20.

Jaeger RG, 1980. Microhabitats of a terrestrial forest salamander. Copeia 1980:265-268.

Jaeger RG, 1988. A comparison of territorial and non-territorial behaviour in two species of salamanders. Anim. Behav. 36:307-310.

Jaeger RG, Peterson MG, Gollmann G, Gollman B, Townsend VR, 2001. Salamander social strategies: living together in female-male pairs. J. Herpetol. 35:335-338.

Johnstone RA, 1996. Multiple displays in animal communication: 'backup signals' and 'multiple messages'. Phil. Trans. R. Soc. Lond. B-Biol. Sci. 351:329-338.

Johnstone RA, 1997. The evolution of animal signals. p. 155-178. In: Behavioral Ecology: An Evolutionary Approach, 4th ed. (Krebs JR, Davies NB, eds.) Oxford: Blackwell Science.

Krebs JR, Davies NB, 1993. An Introduction to Behavioural Ecology, 3rd ed. Oxford: Blackwell Science.

MacCulloch RD, Bider JR, 1975. Phenology, migrations, circadian rhythm and the effect of precipitation on the activity of *Eurycea b. bislineata* in Quebec. Herpetologica 31:433-439.

Mathis A, 1991. Territories of male and female terrestrial salamanders: costs, benefits and intersexual spatial associations. Oecologia 86:443-440.

McLennan DA, Ryan MJ, 1997. Response to conspecific and heterospecific olfactory cues in the swordtail *Xiphophorus cortezi*. Anim. Behav. 54:1077-1088.

Najvar PA, 2001. The effects of diel water quality fluctuations on reproduction and growth in the San Marcos salamander (M. S. Thesis). San Marcos, Texas: Southwest Texas State University.

Nelson JM, 1993. Population size, distribution, and life history of *Eurycea nana* in the San Marcos River (M. S. Thesis). San Marcos, Texas: Southwest Texas State University.

Noble GK, 1939. The role of dominance in the social life of birds. Auk 56:263-273.

Rosenthal GG, Evans CS, Miller WL, 1996. Female preference for dynamic traits in green swordtail, *Xiphophorus helleri*. Anim. Behav. 51: 811-820.

Rowe C, 1999. Receiver psychology and the evolution of multicomponent signals. Anim. Behav. 58:921-931.

Sever DM, 1985. Sexual dimorphic glands of the *Eurycea nana, Eurycea neotenes*, and *Typhlomolge rathbuni* (Amphibia: Plethodontidae). Herpetologica 41:71-84.

Smith PW, 1961. The amphibians and reptiles of Illinois. Bull. Illinois Nat. Hist. Sur. 28:1-298.

Stamps JA, 1988. Conspecific attraction and aggregation in territorial species. Am. Nat. 131:329-347.

Tupa DD, Davis WK, 1976. Population dynamics of the San Marcos salamander, *Eurycea nana* Bishop. Texas J. Sci. 27:179-195.

(USDI) United Stated Department of the Interior, 1980. Endangered and threatened wildlife and plants: listing of San Marcos salamander as threatened, the San Marcos gambusia as endangered, and the listing of critical habitat for Texas wild

rice, San Marcos salamander, San Marcos gambusia, and fountain darter. Federal

Register 45:47355-47364.

(USFWS) United States Fish and Wildlife Service, 1984. San Marcos River Recovery

Plan. U.S. Fish and Wildlife Service, Albuquerque, New Mexico; v - 109.

Verrell P, Davis K, 2003. Do non-breeding, adult long-toed salamanders respond to

conspecifics as friends or as foes? Herpetologica 59:1-7.

Verrell P, Strand PN, Hanson E, 2001. Sexual dimorphism in mate location in the long-

toed salamander, *Ambystoma macrodactylum columbianum*. Ethology 107:1-7.

Wiens JA, 1976. Population responses to patchy environments. Ann. Rev. Ecol. Syst.

7:81-120.

CHAPTER II

SENSORY COMMUNICATION AND ASSOCIATION PREFERENCES

Animal communication, although minimally involving only a signaler and a receiver, is often a complicated interaction among different signalers and modes of signals. 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, these 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 depends greatly on the environmental conditions and activity patterns of the organism (Endler 1993). In aquatic environments, turbid water or low light conditions interfere with visual signals whereas chemical signals could be more effective (Brönmark & Hannson 2000). Additionally, high flow conditions can cause chemical cues to disperse rapidly, precluding the ability for a receiver to orient towards the signaler (Atema 1996). Both visual and chemical signals might be co-opted in environments where signals are degraded or masked by environmental noise. Activity patterns are equally 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 by reduced light.

There are numerous examples of chemical and visual, but not acoustic, communication used by salamanders. Chemical signaling has been described in courtship displays (Arnold & Houck 1982; Houck 1986), sex recognition (Mathis 1990), territoriality (Mathis et al. 1995), assessment of familiar and unfamiliar conspecifics (Gillette et al. 2000; Jaeger & Peterson 2002), as well as in the discrimination between conspecifics and heterospecifics (Houck & Sever 1994; Verrell 2003). Visual cues also can be important, especially in diurnal, sexually dimorphic species during breeding and

aggressive interactions. For example, female smooth newts, *Triturus vulgaris*, accept

more spermatophores from males with greater tail height than those with smaller tail

height (Green 1991; Hosie 1992). The efficacy of sensory cues also can vary based on the

sex of the signaler and receiver. Females of non-breeding 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 & Davis 2003).

The use and relative importance of visual and chemical cues in association

behavior is not known for the threatened San Marcos salamander, *Eurycea nana* (family

Plethodontidae). This neotenic 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 every month of the year suggest that *E. nana* breeds

throughout the year (Bogart 1967; Tupa & 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 poorly defined mental and caudal glands

(Sever 1985) and larger premaxillary teeth than females (Bishop 1941). The presence of

these glandular structures suggests the importance of courtship hormones in the sexual

behavior of these salamanders (Houck 1986).

Aquatic organisms often require multiple cues to communicate because

environmental conditions also affect the mode and efficacy of signals (Endler 1993). In

the white-clawed crayfish, *Austropotamobius pallipes*, high flow conditions in addition to

the low population density and brief mating period may explain why both chemical and

visual stimuli are needed for sex recognition by males (Acquistapace et al. 2002).

Eurycea nana also lives in high flow conditions, but the river within the species' range is

stable in temperature, chemical properties and nutrient concentrations all year (Groeger et

al. 1997). We have observed *E. nana* singly and in small aggregations under rocks and

logs throughout the year, although the sexes of the aggregating individuals are unknown

(J. N. Fries, pers. comm.; pers. obs.). I predict that multimodal sensory cues are important

for *E. nana* for close range social communication in this fast flowing aquatic habitat.

I examined the relative use of different sensory cues in the social communication

of *E. nana*. The first set of experiments were designed to determine if males and females

use (1) visual cues, (2) chemical cues, or (3) both chemical and visual cues to

differentiate between the sexes. I then examined male association preference for gravid

versus non-gravid females as males may prefer females with eggs that can be fertilized or

gravid females may be more receptive. This study provides the first empirical data on the

communication system and social interactions of aquatic salamanders within the genus

Eurycea.

Methods

Collection and Housing

I conducted the 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. Salamanders were sexed using the candling method (Gillette

& Peterson 2001) and only sexually mature individuals > 21 mm in snout-vent length

(SVL), following Tupa & Davis (1976), were used in the experiments. I separated study salamanders 7 mo before the experiments and housed them in a 1.8 x 0.6 x 0.5 m tank divided by an opaque screen that prevented physical contact between the sexes. Among salamanders, the interval between mating and fertilization can range from two days to as long as two years (Halliday 1998). For example, stored sperm can remain viable for eight months in females of the plethodontid salamander *E. quadridigitatus* (Pool & Hoage 1973). During the 7-mo separation, none of the females of *E. nana* laid eggs so the potentially stored sperm from previous matings were likely degraded or flushed out. The large separating tank had re-circulating well water maintained at $22 \pm 1^{\circ}C$ with plastic shelters and aquatic moss native to the San Marcos River. The salamanders were subjected to a reversed 12L:12D light cycle, with 40-W fluorescent lights (General Electric) during the daylight hours of the cycle and were fed commercially raised annelids (*Lumbriculus variegates*) *ad libitum*. Test salamanders were separated in individual tanks (18 x 33 x 18 cm) one week prior to conducting the behavioral observations. All observations were conducted during the "night-hours" with one 25-W red tinted incandescent bulb (General Electric) suspended above the testing chamber to provide enough illumination for the observations to be noted.

Experiment 1: Sensory cues and association preference

To examine preference to associate with individuals of the same or opposite sex, I tested association behavior of male (n = 30) and gravid female (n = 30) salamanders when presented with cues from one individual of each sex simultaneously in three sensory cue treatments: (1) chemical, (2) visual, and (3) both chemical and visual cues.

Tests were conducted in a 38-L aquaria (50 x 20 x 30 cm) with two cylindrical containers (8.5 cm diameter) placed on opposite ends of the tank (Fig. 1). 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 the stimulus salamanders in their containers without physical contact. The three treatments consisted of isolation containers that were either (1) opaque with holes that only allowed the exchange of potential chemical cues, (2) clear without holes that allowed only visual contact, or (3) clear with holes that allowed the exchange of both potential chemical and visual cues between the focal and stimulus salamanders. Pilot studies using dyed water showed that water flow from within the isolating containers into the rest of test tank was constant and symmetrical. The overlap of flow was minimal and would have biased the results towards no preference for chemical cues, as opposed to a preference for stimuli from one end of the container over the other.

In each trial, I first randomly determined the placement of the male or female stimulus individual in its isolation container. I 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, I started a 10-min observation period when the focal individual began to move. Time spent by the focal individual within 44 mm (SVL of the largest individual in the population tested) to each container was recorded and was considered as association. After the first trial, the tank was rotated 180° at the base and the test was repeated. Rotating the sides controlled for potential side bias and prevented chemical cues from being mixed. After each test, the observation tank was drained, scrubbed with 3% hydrogen peroxide solution to remove chemical cues trapped

in the sealant, and rinsed with clean water (McLennan & Ryan 1997). Each focal

individual and the same stimulus male and female were maintained in separate individual

tanks until they were tested in all three sensory cue treatments. The treatments were

conducted for three consecutive days, but the order was randomized. After these three

tests, focal individuals were not retested as focal individuals but were re-used only once

as stimulus individuals in subsequent tests.

Experiment 2: Male preference for gravid versus non-gravid females

To determine whether males (n = 40) differentiate between size-matched (± 2 mm

SVL) gravid and non-gravid females, I examined male preference using the same testing

procedure described in Experiment 1. I only used isolation containers that allowed the

potential exchange of both chemical and visual cues. Again, association preference was

measured as the time spent by males within 44 mm of each container.

Statistical analyses

Within each sensory cue treatment (chemical, visual, chemical and visual) in

Experiment 1, I used a two-tailed Wilcoxon signed ranks test to determine if focal males

and females had a preference for the same or opposite sex. I also compared the strength

of preference (SOP = time spent near opposite sex stimulus – time spent near same sex

stimulus) among males and females between the chemical, and chemical and visual cue

environments using a two-tailed Wilcoxon signed ranks test. I reduced alpha via a

sequential Bonferroni adjustment for the above four analyses (Rice 1989). Male

preference for gravid or non-gravid females (Experiment 2) was determined using a two-

tailed Wilcoxon signed ranks test. Alpha was set at 0.05 for Experiment 2. For statistical

independence, I arbitrarily compared the time each focal salamander associated with the

stimulus individuals on the right side of the tank, comparing within and between

treatments in both trials for Experiment 1 and 2 (see discussion in Gabor 1999).

Additionally, I measured the responsiveness of focal individuals in both Experiments 1

and 2 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 &

Travis 1997).

Results

In those trials with mix-sex presentations, females significantly preferred to

associate with males over females when chemical cues only ($Z = -2.497$, $p = 0.012$, $n = 30$) and both chemical and visual cues ($Z = -3.416$, $p = 0.001$, $n = 30$) were available

(Fig. 2a). Similarly, males significantly preferred to associate with females over males

when chemical cues only ($Z = -3.772$, $p = 0.002$, $n = 30$), and both chemical and visual

cues ($Z = -4.036$, $p < 0.0001$, $n = 30$) were available (Fig. 2b). When only visual cues

were presented, neither females nor males showed a preference to associate with either

sex (females: $Z = -0.711$, $p = 0.477$, $n = 30$, Fig. 2a; males: $Z = -0.663$, $p = 0.507$, $n = 30$,

Fig. 2b). For both sexes, the combination of chemical & visual cues did not significantly

increase the strength of preference for the opposite sex compared to the chemical cue

only treatment (females: $Z = -0.011$, $p = 0.9914$, mean SOP $\pm$ SD in chemical only

treatment $= 64.13 \pm 183.20$, mean SOP $\pm$ SD in chemical and visual treatment $= 75.13 \pm$

117.98; males: Z = -0.250, p = 0.8022, mean SOP ± SD in chemical only treatment =

176.56 ± 193.64, mean SOP ± SD in chemical and visual treatment = 148.70 ± 183.041).

When exposed to the chemical and visual cues of both gravid and non-gravid

females, males do not significantly associate with either one (Z = -0.643, p = 0.5203, n =

40, mean ± SD time with gravid = 18.85 ± 34.17 s, mean ± SD time with non-gravid =

32.18 ± 60.67 s). Males generally were unresponsive to both females in Experiment 2,

spending only a mean of 10% of the total treatment time associating with either female

(range: 0 - 39%). Whereas in Experiment 1, focal males and females were more

responsive, spending a mean of 32% of the total treatment time associating with the

stimulus individuals in the chemical and visual treatments (range: 2 - 95%).

Discussion

Males and females of *E. nana* preferred to associate with individuals of the

opposite sex versus same sex primarily using chemical cues, as they did not differentiate

between the sexes when only visual cues were available but did when both chemical and

visual stimuli were present. The simultaneous inclusion of both visual and chemical cues

did not significantly increase the strength of preference (time spent close by) for the

opposite sex. Furthermore, while males preferred to associate with chemical cues of

gravid females over those of males, my data does not support the hypothesis that males

can discriminate between gravid and non-gravid females when provided with both visual

and chemical cues. These results suggest that although association preferences of *E. nana*

are primarily based on chemical cues, these cues may not be sufficient for males to distinguish between potentially receptive gravid females and non-gravid females.

According to Rowe (1999), multimodal signals provide more reliable information, and are more effective at eliciting responses from receivers. Our results demonstrate that chemical cues only can be sufficient for sex identification, and that visual cues do not provide back-up or additional information. The specific evaluation of reproductive states by males of *E. nana*, however, may require an alternate unimodal signal or another signal mode in addition to the bimodal chemical and visual cues. Alternatively, necessary visual cues, related to female behavior, may not have been expressed in this experimental design.

Chemical signaling via sex attractors is important for numerous other aquatic breeding salamanders and newts (e.g., Arnold 1972; Verrell 1985; Houck & Verrell 1993). Adult male red-spotted newts, *Notophthalmus viridescens*, during the breeding season not only preferred the odors of females over unscented water, but preferred larger over smaller females using either olfactory or visual cues alone (Verrell 1985). Similarly, male smooth newts, *Triturus vulgaris*, during the breeding season preferred the odors of females but not males over unscented water, and differentially preferred larger over smaller females when provided with only visual cues (Verrell 1986). Males and females of both of these diurnal species of newts are sexually dimorphic and there is obvious visible variation (e.g., body size) amongst 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. Alternatively, the predominant use of chemosensory communication in close range is expected in *E. nana* given that these salamanders are nocturnal, completely

aquatic, and have little visible intrasexual morphological variation. Whether male and female responses indicate preference for the opposite sex or avoidance of the same sex in not known.

Male responsiveness to both gravid and non-gravid females was very low and they did not significantly prefer either female. I suggest that the use of tactile information by males may be necessary to evaluate female receptivity in *E. nana*. Adults of the Pyrenean salamander, *Euproctus asper*, when in their aquatic stage do not identify their mates using chemical cues, but mating is initiated when males use mechanical and visual stimuli to capture passing females (Guillaume 1999). Additionally, males of many salamander species exhibit a variety of physical contact behaviors that increase female receptivity during courtship (reviewed in Houck 1986; Houck & Verrell 1993). I have observed males of *E. nana* rapidly rubbing their mental glands across the nares of a female when courtship was disrupted. This behavior is similar to that exhibited by other plethodontid salamanders and increases female receptivity in those species (e.g., Houck & Reagan 1990). In *E. nana*, females respond to this male contact by resuming courtship and tail-straddling walk. Thus, I predict that in *E. nana*, male-motivated tactile interactions, such as snout contacts or moving under and over a female, may be important for evaluating female breeding status or receptivity.

In this study, I found that males and females of *E. nana* use chemosensory communication to distinguish between the sexes at close range. By using unimodal (chemical) cues, both males and females exhibited preference to associate with individuals of the opposite sex. Patterns of chemosensory associations in *E. nana* need to be further explored to determine whether the preference for the opposite sex is related to

mating preference or to avoidance of individuals of the same sex. The lack of support for the hypothesis that males' differentially prefer to associate with gravid or non-gravid females suggests five possible explanations: (1) chemical & visual cues may not be sufficient to evaluate gravidity, (2) association preference is not related to mate choice, (3) gravid females are not emitting distinct reproductive pheromones, (4) gravidity does not indicate sexual receptivity or sexual attractiveness, (5) male mating preferences may only be expressed in the context of male competition, or (6) the experimental design testing association preference was too coarse-grained. I cannot distinguish between these hypotheses with our current data, but I suggest that males may need additional signaling modes, such as tactile cues, to differentiate between females in different reproductive states.

References

Acquistapace, P., Aquiloni, L., Hazlett, B. A., & Gherardi, F. 2002: Multimodal communication in crayfish: sex recognition during mate search by male *Austropotmobius pallipes*. Can. J. Zool. **80**, 2041-2045.

Arnold, S. J. 1972: The evolution of courtship behavior in salamanders. Ph.D. Thesis, University of Michigan, Ann Arbor, Michigan, USA.

Arnold, S. J. & Houck, L. D. 1982: Courtship pheromones: evolution by natural and sexual selection. In: Biochemical Aspects of Evolutionary Biology (Nitecki, M. H., ed). University of Chicago Press, Chicago, Illinois. pp. 173-211.

Atema, J. 1996: Eddy chemotaxis and odor landscapes: exploring of nature with animal

sensors. Biol. Bull. Woods Hole, Massachusetts **191**, 129-138.

Bishop, S. C. 1941: Notes on the salamanders with descriptions of several new forms. Occa. Papers Mus. Zool., University of Michigan **451**, 1-21.

Bogart, J. P. 1967: Life history and chromosomes of the neotenic salamanders of the Edwards Plateau. M.S. Thesis, University of Texas, Austin, Texas, USA.

Brönmark, C. & Hannson L. -A. 2000: Chemical communication in aquatic systems: an introduction. Oikos **88**, 103-109.

Endler, J. A. 1993: Some general comments on the evolution and design of animal communication systems. Phil. Trans. R. Soc. Lond. Ser. B-Biol. Sci. **340**, 215-225.

Gabor, C. R. 1999: Association patterns of sailfin mollies (*Poecilia latipinna*): alternative hypotheses. Behav. Ecol. Sociobiol. **46**, 333-340.

Gillette, J. R. & Peterson, M. G. 2001: The benefits of transparency: candling as a simple method for determining sex in red-backed salamanders (*Plethodon cinereus*). Herpetol. Rev. **32**, 233-235.

Gillette, J. R., Jaeger, R. G. & Peterson, M. G. 2000: Social monogamy in a territorial salamander. Anim. Behav. **59**, 1241-1250.

Green, A. J. 1991: Large male crests, an honest indicator of condition, are preferred by female smooth newts, *Triturus vulgaris* (Salamandridae) at the spermatophore transfer stage Anim. Behav. **41**, 367-369.

Groeger, A. W., Brown, P. F., Tietjen, T. E. & Kelsey, T. C. 1997: Water quality of the San Marcos River. Texas J. Sci. **49**, 279-294.

Guillaume, O. 1999: Does the Pyrenean salamander *Euproctus asper* use chemical cues

for sex identification and mating behavior? Behav. Proc. **46**, 57-62.

Halliday, T. 1998: Sperm competition in amphibians. In: Sperm Competition and Sexual

Selection (Birkhead, T. R. & Møller, A. P., eds). Academic Press, San Diego,

California. pp. 465-502.

Hosie, C. 1992: Sexual motivation and mate choice in the female smooth newt, *Triturus*

vulgaris Ph.D. Thesis, The Open University, Milton Keynes, United Kingdom

Houck, L. D. 1986: The evolution of salamander courtship pheromones. In: Chemical

Signals in Vertebrates, Vol. IV (Duvall, D., Müller-Schwarze, D. & Silverstein,

R. M., eds). Plenum Press, New York. pp. 173-190.

Houck, L.D. & Reagan, N.L. 1990: Male courtship pheromones increase female

receptivity in a plethodontid salamander. Anim. Behav. **39**, 729-734.

Houck, L. D. & Sever, D. M. 1994: Role of skin in reproduction and behavior. In:

Amphibian Biology, Vol. 1, The Integument (Heatwole, H. & Barthalmus, G. T.,

eds). Surrey Beatty, Chipping Norton. pp. 351-380.

Houck, L. D. & Verrell, P. A. 1993: Studies of courtship behavior in plethodontid

salamanders: A review. Herpetologica **49**, 175-184.

Jaeger, R. G. & Peterson, M. G. 2002: Familiarity affects agonistic interactions between

female red-backed salamanders. Copeia, 865-869.

Johnstone, R. A. 1996: Multiple displays in animal communication: 'backup signals' and

'multiple messages'. Phil. Trans. R. Soc. Lond. B-Biol. Sci. **351**, 329-338.

Mathis, A. 1990: Territorial salamanders assess sexual and competitive information using

chemical signals. Anim. Behav. **40**, 953-962.

Mathis, A., Jaeger, R. G., Keen, W. H., Ducey, P. K., Walls, S. C. & Buchanan, B. C.

1995: Aggression and territoriality by salamanders and a comparison with the territorial behavior of frogs. In: Social Behavior, Vol. 2 (Heatwole, H. & Sullivan, B. K., eds). Surrey Beatty, Chipping Norton. pp. 633-676.

McLennan, D. A. & Ryan, M. J. 1997: Responses to conspecific and heterospecific olfactory cues in the swordtail *Xiphophorus cortezi*. Anim. Behav. **54**, 1077-1088.

Pool, T. B. & Hoage, T. R. 1973: The ultrastructure of secretion in the spermatheca of the salamander *Manculus quadridigitatus* (Holbrook). Tiss. Cell. **5**, 303-313.

Ptacek, M. B. & Travis, J. 1997: Mate choice in the sailfin molly, *Poecilia latipinna*. Evolution **51**, 1217-1231.

Rice, W. R. 1989: Analyzing tables of statistical tests. Evolution **43**, 223-225.

Rowe, C. 1999: Receiver psychology and the evolution of multicomponent signals. Anim. Behav. **58**, 921-931.

Sever, D. M. 1985: Sexual dimorphic glands of the *Eurycea nana*, *Eurycea neotenes*, and *Typhlomolge rathbuni* (Amphibia: Plethodontidae). Herpetologica **41**, 71-84.

Tupa, D. D. & Davis, W. K. 1976: Population dynamics of the San Marcos salamander, *Eurycea nana* Bishop. Texas J. Sci. **27**, 179-195.

USDI (United States Department of Interior) 1980: Endangered and threatened wildlife and plants: listing of San Marcos salamander as threatened, the San Marcos gambusia as endangered, and the listing of critical habitat for Texas wild rice, San Marcos salamander, San Marcos gambusia, and fountain darter. Federal Register **45**, 47355-47364.

Verrell, P. A. 1985: Male mate choice for larger, more fecund females in the red-spotted newt, *Notophthalmus viridescens*: How is size assessed? Herpetologica **41**, 382-

386.

Verrell, P. A. 1986: Male discrimination of larger, more fecund females in the smooth

newt, *Triturus vulgaris*. J. Herpetol. **20**, 416-422.

Verrell, P. 2003: Population and species divergence of chemical cues that influence male

recognition of females in Desmognathine salamanders. Ethology **109**, 577-586.

Verrell, P. & Davis, K. 2003: Do non-breeding, adult long-toed salamanders respond to

conspecifics as friends or as foes? Herpetologica **59**, 1-7.
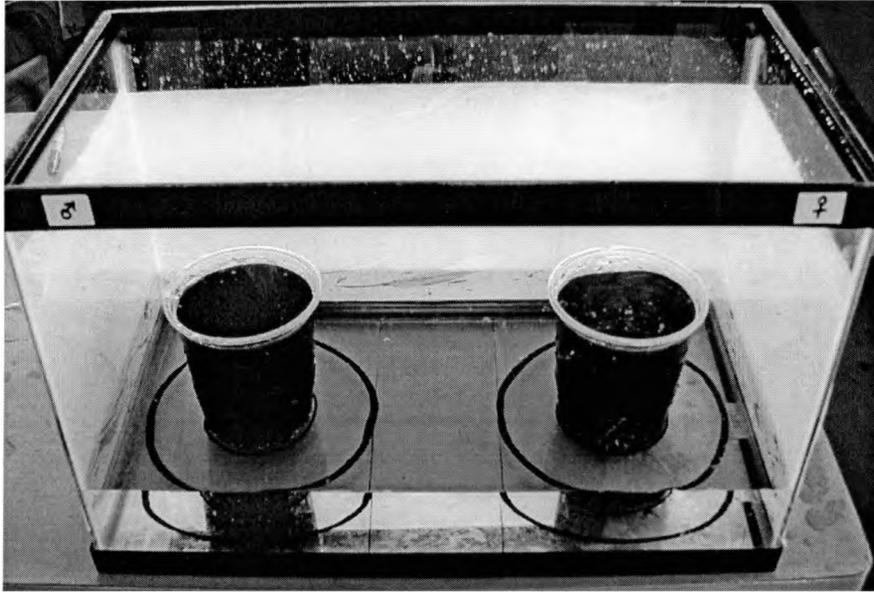
Figure 1. Experimental tank for testing the association preference of females ($N =$ 30) and males ($N =$ 30) for individuals of the same and opposite sex. Each cylindrical container isolated a size-matched stimulus male or stimulus female. The focal male or female could freely move in the aquarium during the 10 min trial and association was recorded when the salamander was within 44 mm to each container, denoted by the ring drawn on the tank. The three treatments consisted of isolation containers that were either (1) opaque with holes that only allowed the exchange of potential chemical cues (shown), (2) clear without holes that allowed only visual contact, or (3) clear with holes that allowed the exchange of both potential chemical and visual cues between the focal and stimulus salamanders.
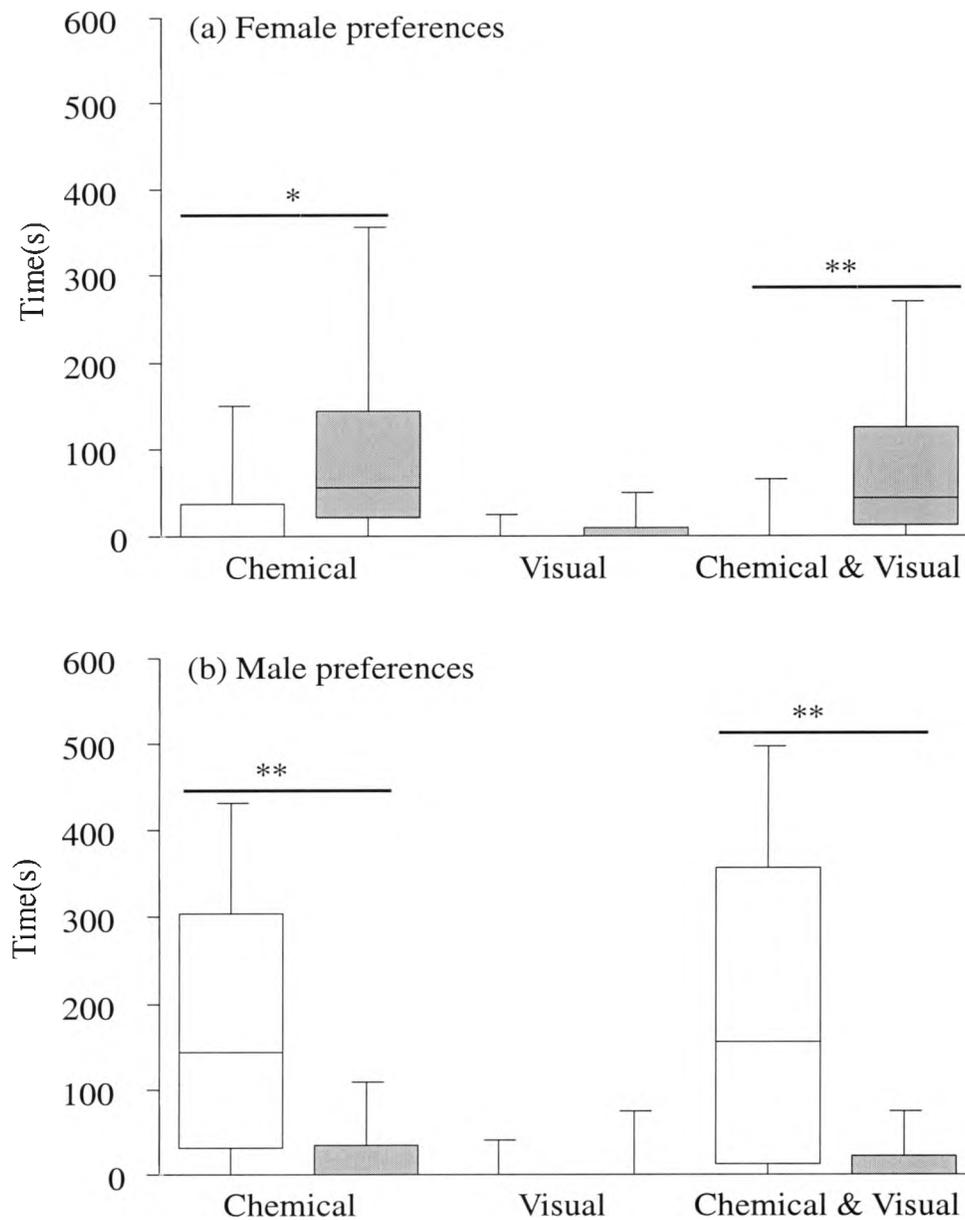
**Figure 2.** Association preference of (a) females (n = 30) and (b) males (n = 30) for individuals of the same and opposite sex in the chemical only, visual only, and chemical & visual cue treatments. White boxes denote preference for females and gray boxes denote preference for males. Box plots represent median, 1st and 3rd quartile, and range; * $P \leq 0.01$, ** $P < 0.001$.

CHAPTER III

AGGREGATION AND NON-TERRITORIALITY

Individuals of caudate amphibians often associate non-randomly with conspecifics in their habitats, where spatial distributions of adults range from territorial spacing to aggregations (see Blaustein and Walls, 1995). A territory is defined as a space where individuals defend a patchy or limited resource, typically food or mates (Noble, 1939). Conspecifics that defend an area with limited resources often experience higher net fitness (Brown, 1964). Aggregations of adults, in contrast, generally can be explained by three main scenarios: attraction to a common and possibly limited resource, anti-predatory tactics via dilution or predator detection, and social interactions with conspecifics (Wilson, 1975).

Territoriality is demonstrated in salamanders when individuals: (1) exhibit site tenacity, (2) advertise these sites, (3) defend these sites, and (4) resist intrusion into these sites by competitors (Gergits, 1982). 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). Less is known about territoriality in aquatic salamanders. Some species of completely aquatic salamanders exhibit two of the fours tenets of territoriality: site tenacity and agonistic behavior towards intruders (Mathis et al., 1995). Anecdotal observations of *Siren intermedia*, for example, report shelter tenacity and expulsion of intruders that are equal or smaller in size (Asquith and Altig, 1987). Similar observations of *Cryptobranchus* spp. (Nickerson and Mays, 1973; Hillis and Bellis, 1971) and *Necturus* spp. (Ashton, 1985) suggest that these neotenic salamanders are territorial in that they exhibit aggression in occupying, advertising, and defending exclusive areas.

Aggregations of adults can be facilitated either by microhabitat selection or social factors, such as breeding and communal egg-laying (Blaustein and Walls, 1995). Regardless of the reasons, the sex of the adult salamander seems to be an important determinant of aggregation patterns across species and seasons. During the non-breeding season, red-backed salamanders (*Plethodon cinereus*) tend to aggregate more in intersexual pairs than intrasexual pairs under artificial cover objects on the forest floor (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 salamanders (*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 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 et al., 1969; Pough and Wilson, 1970; Nussbaum et al., 1983) and *Plethodon* spp. (Heatwole, 1960; Wells and Wells, 1976) form aggregations of conspecifics when habitat conditions are dry, perhaps as a way to reduce desiccation and conserve energy (Alvarado, 1967).

Throughout the year, the aquatic San Marcos salamander (*Eurycea nana*) can be found either singly or in small aggregations of two to four individuals under rocks and logs in their natural habitat (J. N. Fries, pers. comm.; pers. obs.). *Eurycea nana* interacts non-aggressively in captivity, where it usually is seen aggregating in clumps of aquatic moss and under structures provided for shelter. In previous laboratory experiments, I

have shown that *E. nana* prefers to associate with the opposite sex using chemical cues alone or chemical & visual cues together of both sexes (Chapter II). In this study, I tested the first tenet of territoriality in *E. nana* by examining shelter affinity in the laboratory. I also examined aspects of social interactions by testing whether *E. nana* cohabits in intersexual or intrasexual pairs under shelters rather than reside alone. I predicted that *E. nana* will not exhibit shelter affinity. I also predicted that *E. nana* will cohabit with the opposite sex.

MATERIALS AND METHODS

Subjects.— *Eurycea nana* (Caudata: Plethodontidae) is a completely aquatic, neotenic salamander species, that is endemic to the thermostable headwaters of the San Marcos River (Texas) where it breeds year-round (Bishop, 1941; Tupa and Davis, 1976). All individuals of this threatened species (USDI, 1980) used in the experiment were laboratory-reared offspring of wild-caught *E. nana*. I measured snout-vent length (SVL) from the tip of the snout to the posterior end of the vent using digital calipers. I sexed *E. nana* using the candling method (Gillette and Peterson, 2001) and used only males and females > 25 mm SVL, which I presume are sexually mature (Tupa and Davis, 1976).

I uniquely marked every test individual with Visible Implant Fluorescent 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. Fluorescent VIE are faintly visible through the dark skin pigmentation but fluoresce under ultra-violet light.

Experiments were conducted three weeks after the marking procedure, between August 2003 and February 2004 at the National Fish Hatchery and Technology Center (San Marcos, Texas).

Procedure.— To examine cohabitation patterns, pairs of salamanders were assigned to one of three treatments (n = 10 pairs per treatment): (1) male-male pair, (2) female-female pair, or (3) male-female pair. I size-matched individuals in pairs within ± 3 mm SVL (mean difference: 0.37 mm). Salamanders were maintained in sex-specific group tanks, and individuals were separated from each other for at least one week before testing. Salamanders were not tested again once assigned to a treatment. Each pair was simultaneously placed in individual testing tanks and allowed 24 h to habituate. Following habituation, I examined the position of each salamander once daily sometime between 11:00 to 12:00 hours for 20 consecutive days. I recorded the position of each individual, without disturbing them or the tank environment, by identifying the salamanders based on their fluorescent VIE markers using a portable ultra-violet light.

I used testing tanks that were flow-through aquaria (24 x 12.5 x 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. At two ends of each tank, I placed one artificial shelter made from a 10-cm long PVC pipe (2.5 cm diameter) cut in half along its longitudinal axis. Shelter A was placed in the corner closer to the stand-pipe, while shelter B was placed in the diagonally opposite corner of the tank. Each

salamander could cohabit under a shelter, reside alone under a shelter, or not be under either shelter. Shelter location had no effect on shelter use as there was no significant difference between the numbers of times individuals were found under shelter A or shelter B ($Z = -0.464$, $P = 0.643$). Salamanders were fed every five days by placing a small amount of commercially-raised annelids (*Lumbriculus variegatus*) in the center of each tank, equidistant from both shelters. Due to feeding problems, the substrate of the glass tanks was changed from gravel (first six out of 10 pairs) to bare glass (remaining four out of 10 pairs) in all three treatments. There was no significant difference in shelter use ($Z = -0.546$, $P = 0.585$) or cohabitation pattern ($Z = -1.726$, $P = 0.084$) between the trials with gravel and those without, so the data were pooled. On day 20, after the last position was recorded, all individuals were removed and the tanks were scrubbed with 3% hydrogen peroxide solution to remove chemical cues trapped in the sealant (McLennan and Ryan, 1997). I then rinsed the tanks thoroughly with clean water prior to their use in subsequent trials.

Analyses.— I tested for shelter affiliation by comparing the number of times each individual was found under one of the two shelters (shelter A was arbitrarily chosen) with the null expectation of random residency (null = total number of times found under both shelters / 2). I also compared the number of times members of a pair were found cohabiting when using a shelter with the null expectation of random cohabitation (null = total number of times found together and apart under either shelter / 2). As there were 20 repeated observations of the same pair, the non-independent data were analyzed using matched-pairs signed-rank Wilcoxon test at $\alpha = 0.05$. Additionally, I evaluated

movement by comparing the number of times males and females in intrasexual pairs changed positions between shelters during the 20-day observation period using a Mann-Whitney U-test at $\alpha = 0.05$.

RESULTS

Examining shelter use revealed that individuals were under one of the two shelters in at least 75% of the observations ($\leq$ 5 times unsheltered out of 20 days of observation). There was, however, no significant affinity for shelter A by either sex in any of the three treatments (Table 1). Examining patterns of cohabitation under a shelter revealed that female-female pairs and female-male pairs were recorded as cohabiting more often than expected from random during the 20 days of observation (Table 2). In contrast, male-male pairs were found cohabiting in a pattern not significantly different from random expectation (Table 2). Males in the intrasexual pair treatment changed positions (mean $\pm$ SD = 3.5 $\pm$ 2.54 times out of 20 observation days) significantly more often than females (2.1 $\pm$ 1.86) in the intrasexual pair treatments ($Z = -2.160$, $N = 20$, $P = 0.031$).

DISCUSSION

I 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 across a 20-day period. Female pairs and intersexual pairs were found cohabiting more often than expected from random,

whereas pairs of males showed no significant cohabitation pattern. All salamanders exhibited no significant shelter affinity by being equally likely to be found under one shelter or the other, and males in intrasexual pairs affiliated with both shelters equally often during the 20 consecutive days of observation.

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 et al., 1997). It has been suggested that females of long-toed salamanders prefer to associate with males because cohabiting with the opposite sex during the non-breeding season reduces resource competition (Verrell and Davis, 2003). In *E. nana*, the patterns of cohabitation of intrasexual female pairs and intersexual pairs are yet to be explained.

The aggregation patterns I observed in the laboratory are not likely attributed to breeding or non-breeding preferences as breeding occurs throughout the year in this species (Tupa and Davis, 1976) and I cannot determine when males or females are sexually receptive. Females may prefer to cohabit with other females and thus avoid males when sexually non-receptive. Intersexual cohabitation preferences may reflect breeding preferences, or that females prefer to cohabit even though males do not. The lack of cohabitation preference exhibited by males in intrasexual pairs is interesting as it may be indicative of a non-aggressive tactic that reduces competition by avoiding other males. Alternatively, it may indicate a difference in movement activity between the sexes, as males in intrasexual pairs moved between shelters more often than females in

intrasexual pairs.

One model to explain cohabitation patterns, especially intersexual association preferences, is that males compete with males for access to feeding territories, whereupon females then associate and mate with males that obtain these territories (see Hom et al., 1997 for *P. cinereus*). This is unlikely to apply to *E. nana* because although males and females cohabited at times, they did not exhibit shelter affinity. Territoriality evolves when there is competition for a resource that can be defended. Among aquatic salamanders, territoriality is unlikely to be seen in species that inhabit fluctuating or ephemeral habitats. For example, larval *Ambystoma* spp. inhabit ephemeral ponds during their short developmental stage, which is a time of high mortality, so the establishment and defense of exclusive sites is not probable (Mathis et al., 1995). Although *E. 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), the shelters (rocks and logs) used by *E. nana* in their habitat are abundant, and thus probably not limiting (pers. obs.). Therefore, the lack of shelter affinity in the laboratory as a test of the first tenet of territoriality was expected for this species because food and shelter resources probably are not limited in their natural habitat. Whether reproductively active females are a limiting resource, however, is unknown.

Future experiments using more than two individuals in different sex ratios with several shelters options would be informative. Such a design would elucidate whether these salamanders are truly aggregating and if cohabitations are indicative of mate choice or avoidance of other individuals. More frequent or even continuous monitoring of individuals would provide greater resolution of patterns for space use. 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.

LITERATURE CITED

ALVARADO, R. H. 1967. The significance of grouping on water conservation in *Ambystoma*. Copeia 1967:667-668.

ANDERSON, J. D. 1961. The courtship behavior of *Ambystoma macrodactylum croceum*. Copeia 1961:132-139.

ASHTON, R. E. 1985. A study of movement, home range, and winter behavior of *Desmognathus fuscus* (Rafinesque). J. Herpetol. 9:85-91.

ASQUITH, A., AND R. ALTIG 1987. Phototaxis and activity patterns of *Siren intermedia*. Southwest. Nat. 32:146-148.

BAILEY, L. L. 2004. Evaluating elastomer marking and photo identification methods for terrestrial salamanders: marking effects and observer bias. Herpetol. Rev. 35:38-41.

BISHOP, S. C. 1941. Notes on the salamanders with descriptions of several new forms. Occa. Papers Mus. Zool., Univ. of Michigan. 451:1-21.

BLAUSTEIN, A. R., AND S. C. WALLS. 1995. Aggregation and kin recognition. p. 568-602. *In*: Social behaviour. H. Heatwole and B.K. Sullivan (eds.). Surrey Beatty and Sons, Chipping Norton, New South Wales.

BROWN, J. L. 1964. The evolution of diversity in avian territorial systems. Wilson Bull. 76:160-169.

GEHLBACH, F. R., J. R. KIMMEL, AND W. A. WEEMS. 1969. Aggregations and body water

relations in tiger salamanders (*Ambystoma tigrinum*) from the Grand Canyon

rims, Arizona. Physiol. Zool. 42:173-182.

GERGITS, W. F. 1982. Interference competition and territoriality between the terrestrial

salamanders *Plethodon cinereus* and *Plethodon shenandoah*. Unpubl. M.S.

Thesis, State Univ. of New York, Albany, NY.

GILLETTE, J. R., AND M. G. PETERSON. 2001. The benefits of transparency: candling as a

simple method for determining sex in red-backed salamanders (*Plethodon*

cinereus). Herp. Rev. 32:233-235.

GROEGER, A. W., P. F. BROWN, T. E. TIETJEN, AND T. C. KELSEY. 1997. Water quality of

the San Marcos River. Texas J. Sci. 49:279-294.

HEATWOLE, H. 1960. Burrowing ability and behavioral responses to desiccation of the

salamander, *Plethodon cinereus*. Ecology 41:661-668.

HILLIS, R. E., AND E. D. BELLIS. 1971. Some aspects of the ecology of the hellbender,

Cryptobranchus alleganiensis alleganiensis, in a Pennsylvania stream. J.

Herpetol. 5:121-126.

HOM, C. L., R. G. JAEGER, AND N. H. WILLITS. 1997. Courtship behaviour in male red-

backed salamanders: the ESS dating game. Anim. Behav. 54:715-724.

JAEGER, R. G. 1980. Microhabitats of a terrestrial forest salamander. Copeia 1980:265-

268.

_____, M. G. PETERSON, G. GOLLMANN, B. GOLLMANN, AND V. R. TOWNSEND. 2001.

Salamander social strategies: living together in female-male pairs. J. Herpetol.

35:335-338.

MATHIS, A. 1991. Territories of male and female terrestrial salamanders: costs, benefits and intersexual spatial associations. Oecologia 86:443-440.

_____, R. G. JAEGER, W. H. KEEN, P. K. DUCEY, S. C. WALLS, AND B. C. BUCHANAN. 1995. Aggression and territoriality by salamanders and a comparison with the territorial behaviour of frogs. p. 633-676. *In*: Social behaviour. H. Heatwole and B. K. Sullivan (eds.). Surrey Beatty and Sons, Chipping Norton, New South Wales.

MCLENNAN, D. A., AND M. J. RYAN. 1997. Responses to conspecific and heterospecific olfactory cues in the swordtail *Xiphophorus cortezi*. Anim. Behav. 54:1077-1088.

NICKERSON, M. A., AND C. E. MAYS. 1973. A study of the Ozark hellbender *Cryptobranchus alleganiensis bishopi*. Ecology 54:1164-1165.

NOBLE, G. K. 1939. The role of dominance in the social life of birds. Auk 56:263-273.

NUSSBAUM, R. A., E. D. BRODIE, JR., AND R. M. STORM. 1983. Amphibian and Reptiles of the Pacific Northwest. Univ. Idaho Press, Moscow, ID.

PETERSON, M. G., J. R. GILLETTE, R. FRANKS, AND R. G. JAEGER. 2000. Alternative life styles in a terrestrial salamander: do females preferentially associate with each other? p. 417-429. *In*: The Biology of plethodontid salamanders. R. C. Bruce, R. G. Jaeger, and L. D. Houck (eds.). Kluwer Academic/Plenum Publishers, New York, NY.

POUGH, F. H., AND R. E. WILSON. 1970. Natural daily temperature stress, dehydration, and acclimation in juvenile *Ambystoma maculatum* (Shaw) (Amphibia: Caudata). Phys. Zool. 43:194-205.

TUPA, D. D., AND W. K. DAVIS. 1976. Population dynamics of the San Marcos

salamander, *Eurycea nana* Bishop. Texas J. Sci. 27:179-195.

(USDI) UNITED STATED DEPARTMENT OF THE INTERIOR. 1980. Endangered and

threatened wildlife and plants: listing of San Marcos salamander as threatened, the

San Marcos gambusia as endangered, and the listing of critical habitat for Texas

wild rice, San Marcos salamander, San Marcos gambusia, and fountain darter. *In*:

Federal register 47355-47364.

VERRELL, P., AND K. DAVIS. 2003. Do non-breeding, adult long-toed salamanders

respond to conspecifics as friends or as foes? Herpetologica 59:1-7.

WELLS, K. D., AND R. A. WELLS. 1976. Patterns of movement in a population of the

slimy salamander, *Plethodon glutinosus*, with observations on aggregations.

Herpetologica 32:156-162.

WILSON, E. O. 1975. Sociobiology: The new synthesis. Belknap Press, Cambridge, MA.

TABLE 1: PATTERNS OF SHELTER AFFINITY FOR MALES AND FEMALES IN INTRASEXUAL AND INTERSEXUAL PAIRS. Shelter affinity was the number of times an individual was found under shelter A during the 20 observation days. Observed shelter affinity was compared to the null expectation of random shelter affinity.

Treatment	Sex	Shelter affinity (days) Mean $\pm$ SD	Z	P-value
Female-female	Female ($n = 20$)	7.5 $\pm$ 7.39	-1.531	0.126
Male-male	Male ($n = 20$)	10.0 $\pm$ 6.54	-0.093	0.926
Female-male	Female ($n = 10$)	13.3 $\pm$ 7.69	-1.529	0.126
	Male ($n = 10$)	13.6 $\pm$ 6.92	-1.478	0.139

TABLE 2: COHABITATION PATTERNS OF INTRASEXUAL AND INTERSEXUAL PAIRS. Cohabitation was the number of times a pair was found together under either shelter during the 20 observation days. Observed cohabitation was compared to the null expectation of random cohabitation (n =10 pairs per treatment).

Treatment	Cohabitation pattern (days) Mean $\pm$ SD	Z	P-value
Female-female	17.2 $\pm$ 3.55	-2.701	0.006
Male-male	8.1 $\pm$ 5.59	-0.980	0.326
Female-male	15.9 $\pm$ 3.67	-2.701	0.006

APPENDIX I

ADDITIONAL RESULTS FROM CHAPTER II

The following are additional exploratory analyses from Experiment I that were not included in Chapter II because the resulting trends were not statistically significant and therefore did not contribute sufficient information for publication. They are, however, important to examine because association preferences can be affected by other variables in addition to the sex of the stimulus individuals (see results in Chapter II).

Many studies of plethodontid salamanders indicate that sex influences the social interactions among individuals during breeding and non-breeding seasons (reviewed in Mathis et al. 1995; Dawley 1998). In long-toed salamander species, for example, breeding males are better able to recognize and locate potential mates than breeding females (Verrell et al. 2001). In the non-breeding season, however, females but not males prefer to cohabit with the opposite sex (Verrell & Davis 2003). Therefore, sex of the focal individual may be an important factor for association preferences of *E. nana*, so I compared male and female responses in Experiment I.

Visible phenotypic traits such as body size, tail height, and crest depth in many species of salamanders, also affect female and male mate preferences. For example, female smooth newts, *Triturus vulgaris*, accept more spermatophores from males with greater tail height than those with smaller tail height (Green 1991; Hosie 1992), and significantly prefer to re-mate with males that have deeper crests than their first mates (Gabor & Halliday 1997). Female smooth newts also preferentially associate based on size, where smaller females prefer smaller males and larger females prefer larger males (Verrell 1991). In red-spotted newts, *Notophthalmus viridescens*, males preferentially choose large fecund females (Verrell, 1985). Similarly, males of mountain dusky salamanders, *Desmognathus ochrophaeus*, prefer large females who produce more eggs

than small females (Verrell, 1989). Therefore, body size also may be an important factor in the association behavior of the San Marcos salamander. I analyzed the effects of body size of focal and stimulus salamanders on the association preferences of males and females in Experiment I.

In this experiment, I examined association preferences of males ($N = 30$) and females ($N = 30$) based on (1) chemical cues, (2) visual cues, and (3) chemical & visual cues when simultaneously exposed to a gravid female and a male (refer to Chapter II for methods). The salamanders only exhibited significant association preferences for the opposite sex in the chemical only and chemical & visual treatments, with no preference for either sex in the visual only treatment (see results in Chapter II). Response levels of focal males and females to the stimulus salamanders were very low in visual only treatment, therefore I only report the effects of sex and body size on the strength of preference for the opposite sex in the chemical only and chemical & visual treatments here (Spearman's rank Correlation). First, I will present data on body size for the population that was studied to illustrate variation between and within the sexes.

Results

Body size variation

The snout-vent lengths (SVL) of females of *E. nana* (Mean ± SD = 39.26 ± 1.79 mm, n = 70, Fig. 3a) were significantly longer than in males (Mean ± SD = 37.17 ± 4.06 mm, n = 70, Fig, 3b; Z = -3.432, p = 0.0006). Note that the variation in SVL within the

sexes in this study population was low (SD < 0.5 mm for SVL in both males and females).

Strength of preference and sex, regardless of body size

There was no significant difference in the strength of preference for the opposite sex (SOP = time spent near opposite sex stimulus - time spent near same sex stimulus) between focal males and focal females in the chemical cue treatment ($Z = -1.427$, $p = 0.1535$, $n = 30$, Mean SOP ± SD for females = 64.13 ± 183.20, Mean ± SD for males = 148.70 ± 183.04). I found that males had a slightly greater, but still non-significantly different, SOP for the opposite sex than females in the chemical & visual treatment ($Z = -1.863$, $p = 0.0625$, $n = 30$, Mean SOP ± SD for females = 75.13 ± 117.98, Mean SOP ± SD for males = 176.56 ± 193.64).

Strength of preference and body size

There was no significant correlation between the SOP and the SVL of the focal individual for both females and males in chemical (Female: $R_s = -0.184$, $Z = -0.991$, $p = 0.322$; Male: $R_s = 0.315$, $Z = 1.695$, $p = 0.090$) and chemical & visual treatments (Female: $R_s = -0.017$, $Z = -0.090$, $p = 0.928$; Male: $R_s = -0.255$, $Z = -1.374$, $p = 0.1696$). Similarly, there was no significant correlation between the SOP and the SVL of the stimulus individual for either sex in the chemical (Female: $R_s = 0.067$, $Z = 0.359$, $p = 0.7197$; Male: $R_s = 0.041$, $Z = 0.221$, $p = 0.8251$) and chemical & visual treatments (Female: $R_s = -0.159$, $Z = -0.856$, $p = 0.392$; Male: $R_s = 0.026$, $Z = 0.138$, $p = 0.889$).

Latency to choose and body size

When comparing the latency to choose (time of first preference - start of experiment) and the SVL of the focal individual, there was no significant correlation for focal females or males in both chemical (Female: R_s = -0.206, Z = -1.111, p = 0.2665; Male: R_s = 0.042, Z = 0.225, p = 0.8218) and chemical & visual treatments (Female: R_s = -0.072, Z = -0.388, p = 0.6983; Male: R_s = -0.042, Z = -0.225, p = 0.821). Additionally, there was no significant correlation between the latency to choose and the SVL of the opposite sex stimulus individual for either sex in the chemical (Female: R_s = 0.012, Z = 0.065, p = 0.947; Male: R_s = -0.091, Z = -0.489, p = 0.625) and chemical & visual treatments (Female: R_s = 0.108, Z = 0.583, p = 0.559; Male: R_s = -0.176, Z = -0.950, p = 0.342).

Discussion

Males and females of *E. nana* preferentially associated with the opposite sex to similar degrees, although males tended to have a slightly greater strength of preference for the opposite sex (SOP) than females in the chemical & visual cue treatment. In this study the focal *E. nana* were sexually mature but their breeding status was unknown. The slight variation between males and female responses may not necessarily reflect mating preferences, but may be an outcome of other aspects of social interactions, such as male avoidance of males.

Males and females are sexually dimorphic for size, with females being significantly larger in SVL than males. The SOP for the opposite sex and the latency to make the first choice by focal males and females, however, are not based on the size (SVL) of the focal individual or the size (SVL) of the opposite sex stimulus individual. The lack of size-based preferences is expected given that there was little evidence for the use of visual cues to differentiate between the sexes (see results in Chapter II). Therefore, it is unlikely that visual differences in body size of stimulus individuals would affect the response of focal individuals. These results suggest that larger stimulus individuals do not produce more pheromones, or that focal individuals do not choose based on size. It is possible, however, that sample sizes were not large enough to detect a preference. Alternatively, the minimal intrasexual variation in body size for both males and females might not be detectable or relevant in this species. Size-based preferences usually are seen in sexually dimorphic species with greater intrasexual variation. In the sexually dimorphic red-spotted newt for example, Gabor et al. (2000) found that males with deeper tails and larger body size (SVL) were more successful at mating than shallow tailed smaller males. This, however, was not a result of female mate choice but may have been due to intrasexual competitive advantage. It is possible that body size may affect intrasexual competition in *E. nana* but my experimental design and data preclude the ability to test this hypothesis.

I conclude that males and females similarly prefer to associate with the opposite sex in the chemical and chemical & visual cue treatments. The association preferences of males and females using chemical cues only and chemical & visual cues of both sexes simultaneously are unaffected by the size of the focal or stimulus salamanders.

References

Dawley, E. M. 1998: Olfaction. In: Sensory Perception, Vol. 3 (Heatwole, H. & Dawley, E. M., eds). Surrey Beatty, Chipping Norton. pp. 711-742.

Gabor, C. R. & Halliday, T.R. 1997: Sequential mate choice by smooth newts: females become more choosy. Behav. Ecol. **8**, 162-166.

Gabor, C. R., Krenz, J. D. & Jaeger, R. G. 2000: Female choice, male interference, and sperm precedence in the red-spotted newt. Behav. Ecol.**11**, 115-124.

Green, A. J. 1991: Large male crests, an honest indicator of condition, are preferred by female smooth newts, *Triturus vulgaris* (Salamandridae) at the spermatophore transfer stage. Anim. Behav. **41**, 67-369.

Hosie, C. 1992: Sexual motivation and mate choice in the female smooth newt, *Triturus vulgaris*. PhD, The Open University, Milton Keynes, United Kingdom.

Mathis, A., Jaeger, R. G., Keen, W. H., Ducey, P. K., Walls, S. C. & Buchanan, B. C. 1995: Aggression and territoriality by salamanders and a comparison with the territorial behavior of frogs. In: Social Behavior, Vol. 2 (Heatwole, H. & Sullivan, B. K., eds). Surrey Beatty, Chipping Norton. pp. 633-676.

Verrell, P. A. 1985: Male mate choice for larger, more fecund females in the red-spotted newt, *Notophthalmus viridescens*: how is size assessed? Herpetologica **41**, 382-386.

Verrell, P. A. 1989: The sexual strategies of natural populations of newts and salamanders. Herpetologica **45**, 265-282.

Verrell, P. A. 1991: Proximity preferences in female smooth newts, *Triturus vulgaris vulgaris*: do they reveal complex mating decisions? Copeia **1991**, 835-836.

Verrell, P. & Davis, K. 2003: Do non-breeding, adult long-toed salamanders respond to

    conspecifics as friends or as foes? Herpetologica **59**, 1-7.

Verrell, P., Strand, N. & Hanson, E. 2001: Sexual dimorphism of mate location in the

   long-toed salamander *Ambystoma macrodactylum columbianum*. Ethology **107**,

    677-683.
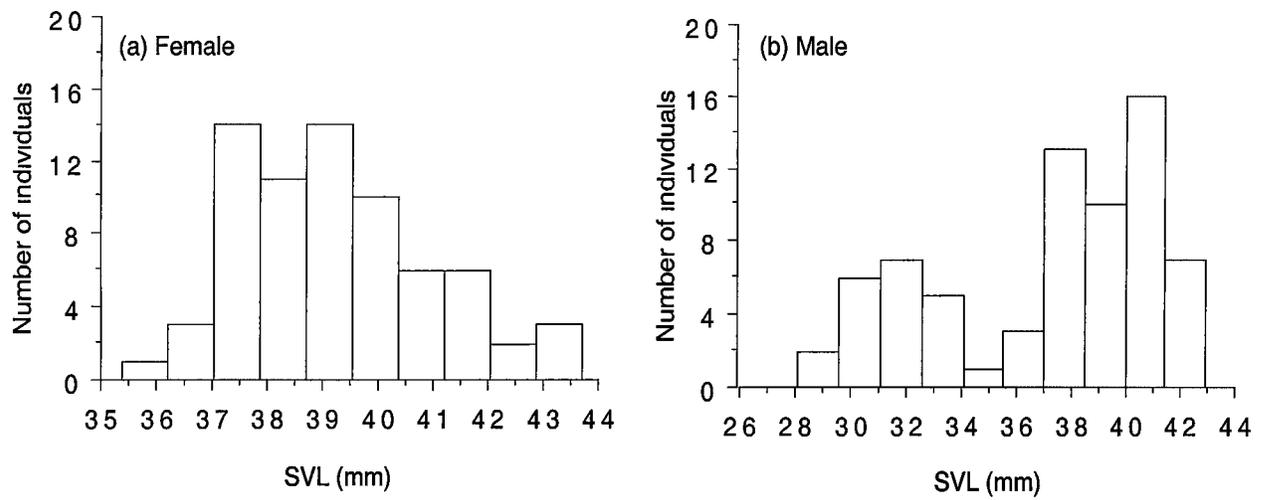
Figure 3. Snout-vent lengths (SVL) of (a) females (n = 70) and (b) males (n =70) of *E. nana* in the study population.

VITA

Maria Thaker was born in Singapore on April 30th, 1979. She moved to New Delhi, India, and graduated from the American Embassy High School there in 1997. She attended William Smith College in Geneva, New York, and received a Bachelor of Science degree in Biology in May 2001. She entered the Graduate College at Texas State University – San Marcos in August 2002 to pursue a Master of Science degree in Biology. During her tenure at Texas State, Maria was employed as a Research Assistant and an Instructional Assistant for the Organismal Biology labs.

Permanent address: 159 Lorong Sarina

Singapore 416717

This thesis was typed by Maria Thaker.