All Master's Theses

Master's Theses

Winter 2015

Chemical and Behavioral Ecology of the Coastal Giant Salamander (Dicamptodon tenebrosus)

David Reavill
Central Washington University, reavilld@outlook.com

Follow this and additional works at: https://digitalcommons.cwu.edu/etd

Part of the Biology Commons

CHEMICAL AND BEHAVIORAL ECOLOGY OF THE COASTAL GIANT

SALAMANDER (*Dicamptodon tenebrosus)*

_____

A Thesis

Presented to

The Graduate Faculty

Central Washington University

_____

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

Biology

_____

by

David Allen Reavill IV

December 2015

CENTRAL WASHINGTON UNIVERSITY

Graduate Studies

We hereby approve the thesis of

David Allen Reavill IV

Candidate for the degree of Master of Science

APPROVED FOR THE GRADUATE FACULTY

_____   _____
                        Dr. R. Steven Wagner, Committee Chair

_____   _____
                        Dr. Robert Weaver

_____   _____
                        Dr. Wayne Quirk

_____   _____
                        Dean of Graduate Studies

ABSTRACT

ROLES OF CHEMICAL CUES AND INTRASPECIFIC COMPETITION IN THE

BEHAVIOR OF THE COASTAL GIANT SALAMANDER (*Dicamptodon tenebrosus)*

by

David Allen Reavill

December 2015

Intraspecific interactions are an important factor in shaping the population structure of terrestrial salamanders. Both physical interactions and scent-marking are vital components to the establishment of territories and influence dispersion of individuals. However, little is known about the terrestrial interactions of many salamander species, particularly that of the Coastal Giant Salamander (*Dicamptodon tenebrosus*). In this study, I observed the first evidence for the use chemical cues by terrestrial *D. tenerosus* in regards to shelter selection. I also observed the first accounts of agonistic and display behavior of terrestrial *D. tenebrosus*. These results suggest that terrestrial *D. tenebrosus* utilize chemical cues in their selection of refuge sites, and that they also display aggressive, territorial behavior.

ACKNOWLEDGMENTS

.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER II

CHAPTER III

CHAPTER I

INTRODUCTION

Animal behavior has long been a field of interest in the Biological Sciences, after
Nikolas Tinbergen's seminal book, *The Study of Instinct* . Tinbergen's work launched the
study of behavioral ecology, which attempts to determine the evolutionary basis of
animal behavior, with respect to the environmental forces that shape such behaviors. A
significant aspect of behavioral ecology is the study of social behavior in animals. Social
behavior, whether cooperative or antagonistic, can mediate many aspects of an animal's
life history, such as mating, resource acquisition, territorial defense, and predator
defense, among others. Animals can use behavioral interaction to either increase their
own fitness by excluding competitors from a particular resource, or increase the fitness
of the group or close kin by acting altruistically. In either case, most social interactions
are mediated through some form of communication. Communication can be defined as
the release of one or more stimuli by one individual that alters the likelihood of reaction
by another, with the reaction being of benefit to the stimulus emitter, the stimulus
receiver, or both (Wilson 1975). There are a number of modalities utilized by animals in
order to communicate with both conspecifics and heterospecifics, such as; visual,
auditory, olfactory, electro, or tactile. Many functions of communication have been
studied, some of which include communication during contests, mating rituals,
territorial defense, food-related signals, and alarm calls. In this study, I aim to determine
how communication is involved in the behavior of an understudied salamander species,

Commented [OU1]: Provide a reference

1

the Coastal Giant Salamander (*Dicamptodon tenebrosus*), with emphasis on territorial behavior.

Use of Chemical Communication in Urodeles

Communication through chemical cues was perhaps the first mode of communication to evolve between individual organisms. Nearly all vertebrate taxa exhibit some form of chemical communication (Wyatt 2003). Within these taxa, communication via chemical cues is used in various ways and serves as a means of transferring different types of information, which then typically elicit specific behavioral or physiological responses from the receiver. Pheromonel research has led to many useful discoveries and interesting scientific results.. For example, mate-attracting pheromones extracted from the male sea lamprey (*Petromyzon marinus*) have been utilized in controlling this specie's invasion of the Great Lakes without using environmentally harmful techniques (Johnson et al., 2013). The use of pheromones has led to successful control of a common moth pest in cotton fields (Carde & Minks 1995). Studies of pheromones have also yielded important insights into the ecology and evolution of many species, especially within the more well studied 'model' organisms (Houck 1986; Kikuyama et al. 2002; Houck and Arnold 2003; Houck 2009).

However, these broader insights and practical applications are not possible without first describing the behavioral responses of animals to chemical cues in order to determine their higher function. These types of studies are the necessary foundation of future research and have formed a major component of behavioral and ecological

Commented [OU2]: Do you think chemical communication among individuals directly evolved from cell-cell communication?

Commented [RW3]: Add scientific name

Commented [RW4]: I think it reads fine

Commented [D5]: Combine these two sentences?

Commented [D6]: Give examples of said model organisms

studies. Salamanders are a model organism for the study of chemoreception because it is thought that chemoreception is their primary modality for communication. Unlike frogs and toads that extensively utilize acoustic signals to communicate, the vast majority of salamander species do not, or are incapable of vocalizing. Many are also nocturnal or live in aquatic environments, reducing the efficacy of visual information. Such natural histories and ecologies reduce the available modes of communication to salamanders, which likely contributed to the evolution of a highly developed chemosensory system.

There is extensive literature regarding the function of chemical signals in the behavior of salamanders. This suggests that chemical cues are an integral component in nearly all major aspects of salamander life history, including foraging, homing, migration and breeding (Dawley, 1998). A widely conserved function of chemoreception among salamander families is inter and intra-specific identification. Species recognition through chemical signals has been proposed as a pre-mating reproductive isolation mechanism in sympatric plethodontid salamanders, and has possibly contributed to species divergence (Dawley, 1986; Ovaska 1989).

Conspecific recognition is also commonly exhibited in salamanders, and evidence of some level of conspecific recognition has been found in four of the ten extant salamander families (Houck, 2009). Most of the studies on conspecific recognition involved simple avoidance/attraction responses to conspecific or self marked substrates. However, some species of plethodontid salamanders have also been show to

have relatively complex intra-specific social systems (Bruce et. al., 2000). Extensive study of this family has led to the discovery that plethodontid salamanders are able to assess a wide variety of information about conspecifics through chemical signals alone, and that this information appears vital to the functioning of their complex social systems. Female plethodontid salamanders have been shown to be able to obtain information regarding the quality of potential mates, such as information about their diet and parasite load, potentially reflecting the territorial quality as well as the overall health and resource holding ability of the potential mate (Walls et al., 1989, Maksimowich and Mathis 2001). These studies among others indicate chemical signals play an important role in general recognition, location, and quality assessment of potential mates. Thus, understanding of the roles of chemical communication among conspecific salamander species is necessary to understand breeding behavior, which could more accurately inform management decisions regarding conservation.

Chemical cues can also influence the community structure and spatial distribution of salamander populations. This is done primarily through the use of territorial markings. Among Plethodontid salamanders, advertisement of a defended area is one of the "essential characteristics" of territorial behavior (Jaeger 1986). Substrate-borne (non-volatile) chemical cues are an economical means of marking territories and are useful for several reasons. Because non-volatile signals are perceived by the receiver through physical contact, there are fewer limitations to the size of the specific molecules, other than the cost to manufacture by the signaler. Therefore it has

Commented [OU8]: Might state somewhere perhaps below that the majority of studies involve Plethodontids or salamandra, NONE of decamps.

4

been hypothesized that more information may be contained by these non-volatile signals compared to volatile signals (Alberts 1992). This allows territory owners to convey more information to intruders about the defensive ability of the resident and influence their subsequent decisions concerning escalating the territorial contest (Simmons et. al., 1997). For example, Mathis (1990) demonstrated evidence that *P. cinereus* may be able to detect the size of other conspecific males through territorial cues, because males were more aggressive in the presence of chemical signals from similarly sized males and more submissive when exposed to cues from larger individuals. Furthermore, intruders may be able to assess the defensive ability of territory holders based on physical condition. In *P. cinereus,* tail condition (i.e. tailed or autotomized) was apparently conveyed in the scent markings of the territory holder (Wise et al., 2004). The ability to convey such information is beneficial to both the signaler and the receiver because ability to ascertain the defensive ability of the territory holder before escalating conflict would allow both animals to avoid physical damage due to the ensuing battle if there is high asymmetry and the intruder was unlikely to evict the resident.

Though the field of pheromone research has made great advances in determining the social structure of salamanders, the majority of the work in this field has focused on a few families. Even within the most commonly studies families, only a handful of species have published evidence concerning the utilization of chemical communication. A review by Kiemnec (2009) shows that only seven of the ten extant salamander families have any published evidence suggesting the use of any sort

Commented [OU9]: Clarify if this is size?

chemical communication. The families Amphiumidae, Cryptobranchidae and Sirenidae have yet to be studied, and even within the families with published data, only Ambystomatidae, Plethodontidae and Salamandridae have been studied extensively.

Territoriality in Urodeles

Territorial behavior has been observed in most vertebrate groups and has been a subject of extensive study (Brown and Orians 1970). Territories are used as a means to secure some sort of limiting resource, whether that is food, access to mates or nesting sites, shelter, or all-purpose territories. One of the most common definitions of a territory is a 'defended area' (Maher and Lott 1995), and Gergits (1982) proposed that there are four essential characteristics by which territorial behavior can be identified; animals (1) exhibit site tenacity to an area that (2) is advertised by the owner and (3) territory owners defend a site using overt aggression in which (4) there is a high probability of expelling an intruder. All of these criteria have been observed in some species of salamanders, confirming that salamanders can and do exhibit territoriality (Gergits 1982).

The most compelling evidence for territoriality in terrestrial salamanders comes from the study of salamanders in the family Plethodontidae, the lungless salamanders. The social behavior of this group has been studied extensively, with considerable attention to territorial behavior (**reviewed by Jaeger and Forester, 1993).** Territoriality mediates inter- and Intraspecific competition in numerous Plethodontid species, which thereby shapes distributions, regulates salamander communities and even leads to

6

extinction and replacement in some populations (Hairston, 1987, 1996; Jaeger and

Forrester, 1993). Specifically, *Plethodon cinereus*, the Western Red-Backed Salamander,

has served as a model organism for the study of social behavior in salamanders. This

species lives in forested areas throughout much of the eastern United States (Highton,

1972). Typical of the genus *Plethodon, P. cinereus* does not go through an aquatic larval

stage, but instead undergoes direct development and hatches into an immature stage

that looks similar to the adults. Females lay small clutches of five to seven eggs in the

spring (Fraser, 1980), which they guard for about a month. The life history

characteristics of *P. cinereus* are typical of a K-selected species, which suggests that it

likely inhabits a highly competitive environment, and studies have shown this to be the

case.

Both male and female *P. cinereus* establish territories under rocks and logs on

the forest floor (Jaeger et al., 1982). Resident holders exhibit a high degree of site

fidelity indicated by frequent recaptures under the same cover object (Gertis and

Jaeger, 1990; Mathis, 1990). Home ranges of adult *P. cinereus* show very little overlap,

particularly intrasexually (Mathis, 1991), and cohabitation is rare (Mathis, 1990). It

appears that food resources are the primary driving factor in the establishment of

territories (Gabor, 1995; Walls et al., 1989) particularly with females, as food availability

affects fecundity (Fraser, 1980; Majorana, 1976). Males may defend high-quality food

resources in an attempt to attract females (Walls et al. 1989). Competition for space

(Fraser 1976), and cover object quality (Mathis, 1990) have also been shown to lead to

territory establishment. However, in many cases in which territorial behavior is observed, the exact limiting resource that elicits the behavior is often not known (Nishikawa, 1990; reviewed in Wells 2007).

Although other species of salamanders have not been studied as extensively or in as great of detail as *P. cinereus*, there is evidence that aggressive and territorial behavior is widespread throughout the family Plethodontidae (Mathis et. al 1995). Other members of the genus *Plethodon*, which inhabit similar habitats in the Eastern United States as *P. cinereus*, have shown similar territorial behavior, including *P. serratus* (Mathis, Deckard and Duer 1998), *P. websteri* (Camp 1999) and *P. angusticlavis* (Mathis and Britzke 1999). Two *Plethodon* species in the Western United states, *P. dunni* and *P. vehiculum*, which are very similar in appearance and ecology to *P. cinereus*, exhibit contrasting behavioral patterns. *P. dunni* exhibits many of the same territorial behaviors as *P. cinereus* (Ovaska and Davis 1992; Ovaska 1993), while *P. vehiculum* does not show these behaviors (Ovaska, 1988; Ovaska 1993). Two other genera of plethodontine salamanders have been shown to exhibit high levels of aggression and evidence of territoriality. Members of the genus *Aneides* have exceptionally large jaw-closing muscles, large teeth and strengthened skulls, which allow them to deliver powerful and damaging bites. *Aneides* from both the Eastern and Western United States actively defend retreat sites such as rocks, logs, and tree holes (Cupp 1980; Staub 1993; T, Davis 2002). Similar to *Aneides,* desmognathine salamanders have large jaw-closing muscles and can inflict similarly powerful bites. These salamanders have been observed

> **Commented [OU12]:** Dave Darda has a scar on his finger from an Aneides bite!

8

defending retreat sites in both laboratory settings and in the field by using biting attacks against intruding conspecifics (Keen and Sharp 1984; Keen and Reed 1985; Jaeger 1988; Verrell and Donovan 1991).

Accounts of aggression outside of the family Plethodontidae are less frequent, but not absent. Aggression has been reported in several species of ambystomatid salamanders. When housed together in artificial enclosures, *A. maculatum* tended to occupy different retreats, and individuals defended their retreat by using visual displays and biting (Ducey and Ritsema 1988). Subsequent studies on several other ambystoma species (*A. opacum, A. talpoideum, A macrodactylum* and *A. jeffersoniam*) using the same methods showed similar patterns of aggressive behavior, though the frequency of biting differed (Ducey 1989). Dicamptodontidae, the sister group to ambystomatids, has only anecdotal observations (Nussbaum, Brodie, and Storm 1983). Territorial behavior in the family Salamandridae seems to be less frequent and likely is associated with the defense of sites during the breeding season. Wrestling matches between males in *S. salamandra* have been observed, which appeared to be over defense of habitat features such as piles of rocks or roots, but these interactions did not include biting and only occurred during the mating season (Kastle 1986). Similar behavior was observed in *S. lanai*, in which males occupy elevated perches during the night, and two males have been seen clasped together in an apparent fight (Andreone 1992). Members of the families Cryptobranchidae (Hillis and Bellis 1971), Hynoibidae (Tanaka 1986), Sirenidae (Asquith and Altig 1987), and Proteidae (Parzefall, Durand, and Richard 1981; R. Ashton

9

1985) also have evidence of aggressive or territorial behavior, most often associated with guarding spaces under rocks and generally associated with guarding eggs or competing for mates.

Study Species

Dicamptodontidae is one of the most understudied families of salamanders and very little is known about their ecology and behavior. *Dicamptodon tenebrosus* represents one of the four endemic Pacific Northwest species of the family Dicamptodontidae. Their range extends from extreme southwestern British Columbia, south to Mendocino County, California. *Dicamptodon tenebrosus* are generally associated with mesic forests and headwater streams and have been found in a variety of lentic and lotic habitats (Nussbuam and Clothier 1973). They are obligately aquatic as larvae, but exhibit facultative paedomorphosis as adults, with some populations containing both terrestrial and aquatic adults (pers. obs). The larvae can reach high densities (>2 individuals per m$^{2)}$ in headwater streams, and replace fish species as the primary vertebrate predators (Murphy and Hall 1981; Corn and Bury 1989; Parker 1994). As the primary predators, *D. tenebrosus* larvae can directly affect the composition of benthic invertebrate communities (Parker 1992). Comparatively, little is known about the ecology or behavior of the terrestrial form of *D. tenebrosus* or any other Dicamptodon species. This is likely due to the cryptic nature of the terrestrial adults and the rarity in which they are encountered during standard biological surveys (Corn and

> **Commented [OU13]:** This is pretty well established now.

10

Bury 1991; Wilkins and Peterson 200; Welsh et al. 2007). What is known of terrestrial adults stems mainly from anecdotal accounts, museum specimens and encounters during multi-species surveys (Stebbins 1951; Wilson 1970; Bury 1972; McComb et al. 1993). Two radio-telemetry studies by Johnston and Frid (2002) and Fessler (2010), conducted in southwestern British Columbia and the Central Washington Cascades, respectively, have greatly increased our understanding of the ecology and aspects of the behavior of this species. These studies have shown that *D. tenebrosus* are generally active at night and inhabit burrows in which the exhibit classic sit-and-wait predatory strategies. Fessler (2010) noted that animals made occasional movements away from refuge sites, generally associated with high levels of precipitation, and would return to these sites before dawn. Individuals would also move between sites within an 'activity center' over the course of a season, making frequent returns to previously occupied refuges, with all salamanders returning to previously used locations. The time and distance moved between returns varied, with animals generally returning within 50 days and <50m traveled, though some animals would return to previously occupied locations after >200 days and a maximum distance traveled of 259m. Individuals would commonly move away from an activity center to establish a new activity center, and return to the previously used activity center either within or between seasons. This behavior suggests that *D. tenebrosus* are able to re-locate previously used refuges, suggesting the possibility of olfaction as the homing mechanism, as seen in other salamander species (Barthalmus and Bellis 1972; Madison 1972; Grant et al. 1968).

Commented [D14]: See Kleeberger and Werner 1982

However, very little is still known about the behavior of *D. tenebrosus*, particularly of the terrestrial adults and their utilization of chemical cues, as well as, their social behavior. Only one publication by Rundio et al. (2003) is available concerning the larval response to predatory fish cues. There are no studies concerning the behavior or utilization of chemical communication in terrestrial adults. In order to better understand this group, these types of studies must be carried out. The goals of my thesis are to determine whether or not *D. tenebrosus* are able to detect chemical cues deposited by themselves and conspecifics, and to determine whether they exhibit territorial behavior in direct agonistic interactions. It has been found that in the highly territorial plethodontid species, *Plethodon cinereus*, there are three lines of defense against intruders. First, an individual will mark the substrate of its territory with pheromones, advertising ownership of the area (Jaeger and Gertis 1979; Jaeger et al. 1986). Second, if an intrusion occurs residents and intruders will exchange aggressive and submissive visual displays (Jaeger 1984). If these displays do not deter an intruder, the resident will often attack and expel the intruder by biting the nasolabial grooves (a chemosensory organ) or the tail of the intruder (Jaeger 1981). I designed my study to mimic this progression, first looking at the response of individual *D. tenebrosus* to pheromonal markers laid by both themselves and conspecifics, and then looking at direct behavioral interactions during territorial contests.

REFERENCES

Alberts AC. 1992. Constraints on the design of chemical communication systems in terrestrial vertebrates. American Naturalist S62-S89.

Andreone F. 1992. Observations on the territorial and reproductive behaviour of Salamandra lanzai and consideration about its protection (Amphibia: Salamandridae). British Herpetological Society Bulletin 39:31–33.

Ashton D. 1985. Field and laboratory observations on microhabitat selection, movements, and home range of Necturus lewisi (Brimley). Brimleyana 10:83-106.

Asquith A, Altig R. 1986. Osmoregulation of the lesser siren, Siren intermedia(Caudata: Amphibia). Comparative Biochemistry and Physiology 84A:683–85.

Barthalmus GT, Bellis ED. 1972. Home range, homing and the homing mechanism of the salamander, Desmognathus fuscus. Copeia 1972:632-642.

Brown JL, Orians GL. 1970. Spacing patterns in mobile animals. Annual review of ecology and systematics 1:239-262.

Bruce RC, Jaeger RG, Houck LD. 2000. The Biology of Plethodontid Salamanders. New York, New York: Kluwer Academic/Plenum Publishers. 485 p.

Bury RB. 1972. Small mammals and other prey in the diet of the pacific giant salamander (Dicamptodon ensatus). American Midland Naturalist 87:524–526.

Camp CD. 1999. Intraspecific aggressive behavior in southeastern small species of Plethodon: Inferences for the evolution of aggression in terrestrial salamanders. Herpetologica 55:248–54.

Carde RT, Minks AK. 1995. Control of Moth Pests by Mating Disruption: Successes and Constraints. Annual Review of Entomology 40:559-585.

Corn PS, Bury RB. 1989. Logging in Western Oregon: Responses of headwater habitats and stream amphibians. Forest Ecology and Management 29:39-57.

Corn PS, Bury RB. 1991. Terrestrial amphibian communities in the Oregon Coast Range. In: Ruggiero LF, Aubry KB, Carey AB, Huff MH, editors. Wildlife and Vegetation of Unmanaged

Douglas-fir Forests. Portland, OR: USDA Forest Service. General Technical Report No. PNW-GTR-285. P. 305--317.

Cupp PV. 1980. Territoriality in the green salamander. Copeia 1980:263–468.

Davis TM. 2002. Microhabitat use and movements of the wandering salamander, Aneides vagrans,on Vancouver Island, British Columbia, Canadian Journal Herpetology 36:699–703.

Dawley EM. 1986. Behavioral isolating mechanisms in sympatric terrestrial salamanders. Herpetologica 42:156-164.

Dawley EM. 1998. Olfaction. Amphibian biology 3. Chipping Norton, New South Wales: Surrey Beatty and Sons. p. 711-742

Ducey PK, Ritsema P. 1988. Intraspecific aggression and responses to marked substrates in Ambystoma maculatum (Caudata: Ambystomatidae). Copeia 1988:1008–13.

Ducey PK. 1989. Agonistic behavior and biting during intraspecific encounters in Ambystoma salamanders. Herpetologica 45:155–60.

Fessler B. 2010. Spatial ecology, behavior, and habitat use of terrestrial Coastal Giant Salamanders (Dicamptodon tenebrosus) in the central Washington Cascades. Ellensburg, WA: Central Washington University. 99 p.

Fraser DF 1976. Coexistence of salamanders of the genus Plethodon: A variation on the Santa Rosalia theme. Ecology 57:238–51.

Fraser R. 1980. On the environmental control of oocyte maturation in a plethodontid salamander. Oecologia46:302–307.

Gabor CR. 1995. Correlational test of Mathis' hypothesis that bigger salamanders have better territories. Copeia 1:729–35.

Gergits WF, Jaeger RG. 1990. Site attachment by the red-backed salamander, Plethodon cinereus. Journal of Herpetology 24:91–93.

Gergits WF. 1982. Interference competition and territoriality between the terrestrial salamanders Plethodon cinereus and Plethodon Shenandoah [thesis]. Albany, NY: State University of New York.

Grant D, Anderson O, Twitty VC. 1968. Homing orientation by olfaction in newts (Taricha rivularis). Science 160:1354–55.

Hairston NG. 1987. Community ecology and salamander guilds. Cambridge University Press. 224 p.

Hairston NG. 1996. Long term studies of vertebrate communities. Academic Press. 464 p.

Highton R. 1972. The distributional history of the biota of the southern Appalachians. Part III: vertebrates. Volume 4, Distributional interactions among eastern North American Salamanders of the genus Plethodon. Blacksburg: VA. Virginia Polytechnic Institute and State University.

Hillis RE, Bellis ED. 1971. Some aspects of the ecology of the hellbender, Cryptobranchus alleganiensis alleganiensis in a Pennsylvania stream. Journal of Herpetology 5:121–26.

Houck LD, Arnold SJ, Sever D. 2003. Courtship and mating behavior. In: Reproductive Biology and Phylogeny of Urodela. Sever DM, editor. Enfield, New Hampshire: Science Publishers p. 383-424.

Houck LD. 1986. The evolution of courtship pheromones. In: Duvall D, Müller-Schwarze D, and Silverstein RM, editors. Chemical Signals in Vertebrates IV. New York, New York: Plenum Press p. 173-190.

Houck LD. 2009. Pheromone communication in amphibians and reptiles. Annual Review of Physiology 71:161-176.

Jaeger RG, Forester DC. 1993. Social behavior of plethodontid salamanders. Herpetologica 1:163-175.

Jaeger RG, Goy JM, Tarver M, Marquez CE. 1986. Salamander territoriality: pheromonal markers as advertisement by males. Animal Behaviour 34:860-864.

Jaeger RG, Goy JM, Tarver M, Marquez CE. 1986. Salamander territoriality: Pheromonal markers as advertisement by males. Animal Behavior 34:860–64.

Jaeger RG, Kalvarsky D, Shimizu N. 1982. Territorial behaviour of the red-backed salamander: Expulsion of intruders. Animal Behavior 30:490–96.

Jaeger RG. 1981. Dear enemy recognition and the costs of aggression between salamanders. American Naturalist 117:962–74.

Jaeger RG. 1984. Agonistic behavior of the red-backed salamander. Copeia 1984:309–14.

Jaeger RG. 1988. A comparison of territorial and non-territorial behaviour in two species of salamanders. Animal Behavior 36:307–10.

Jaeger, R.G., and W.F. Gergits. 1979. Intra- and interspecific communication through chemical signals on the substrate. Animal Behavior 27:150-56.

Johnston B, Frid L. 2002. Clearcut logging restricts the movements of terrestrial Pacific giant salamanders (Dicamptodon tenebrosus Good). Canadian Journal of Zoology 80:2170–2177.

Kastle W. 1986. Rival combats in Salamandra salamandra. In Studies in herpetology,ed. Z. Rocˇek, 535–28. Prague: Charles University.

Keen WH, Reed RW. 1985. Territorial defense of space and feeding sites by a plethodontid salamander. Animal Behavior 33:1119–23.

Keen WH, Sharp S. 1984. Responses of a plethodontid salamander to conspecific and congeneric intruders. Animal Behavior 32:58–65.

Kiemnec K. 2009. Chemical cues and the molecular basis of olfactory chemoreception in caudate amphibians [dissertation]. Corvallis, OR: Oregon State University. 156 p.

Kikuyama SK, Yamamoto T, Iwata, Toyoda F. 2002. Peptide and protein pheromones in amphibians. Comparative Biochemistry and Physiology Part B 132:69-74.

Madison DM. 1972. Homing and orientation in salamanders: a mechanism involving chemical cues. In: . Galler SR, Schmidt-Koenig K, Jacobs GJ, Belleville RE, editors. Animal orientation and navigation Washington, DC: NASA. p 485–948.

Maher R, Lott F. 1995. Definitions of territoriality used in the study of variation in vertebrate spacing systems. Animal Behavior 49(6):1581-1597.

Maiorana VC. 1976. Size and environmental predictability for salamanders. Evolution 1:599-613.

Makisimowich DS, Mathis A. 2001. Pheromonal markers as indicators of parasite load: parasite mediated behavior in salamanders (Plethodon angusticlavius). Acta Ethologica 3:83-87.

Mathis A, Britzke E. 1999. The roles of body size and experience in agonistic displays of the Ozark zigzig salamander, Plethodonangusticlavius. Herpetologica 55:344–52.

16

Mathis A, Jaeger RG, Keen WH, Ducey P.K, Walls SC, Buchanan BW. 1995. In Amphibian biology. Volume 2, Social behavior. Aggression and territoriality by salamanders and a comparison with the behaviour of frogs. ed. H. Heatwole and B.K. Sullivan, 633–76. Chipping Norton, New South Wales, Australia: Surrey Beatty and Sons.

Mathis A. 1990a. Territorial salamanders assess sexual and competitive information using chemical signals. Animal Behaviour 40:953-962.

Mathis A. 1990b. Territoriality in a terrestrial salamander: the influence of resource quality and body size. Behaviour 112:162–75

Mathis A. 1991. Territories of male and female terrestrial salamanders: Costs, benefits and intersexual spatial associations. Oecologia 86:433–40.

Mathis, A, Deckard K, Duer C. 1998. Laboratory evidence for territorial behavior by the southern red-backed salamander, Plethodon serratus: influence of residency status and pheromonal advertisement. Southwestern Naturalist 43:1-5.

McComb WC, McGarigal K, Anthony RG. 1993. Small mammal and amphibian abundance in streamside and upslope habitats of mature Douglas-fir stands, western Oregon. Northwest Science 67:7-15.

Murphy ML, Hall JD. 1981. Varied effects of clear-cut logging on predators and their habitat in small streams of the Cascade Mountains, Oregon. Canadian Journal of Fisheries and Aquatic Sciences 38:137-145.

Nishikawa KC. 1990. Intraspecific spatial relationships of two species of terrestrial salamanders. Copeia 1:418–26

Nussbaum RA, Brodie ED, Storm RM. 1983. Amphibians and reptiles of the Pacific Northwest. Moscow, ID: University Press of Idaho 331 p.

Nussbaum RA, Clothier GW. 1973. Population structure, growth, and size of larval Dicamptodonensatus (Eschscholtz). Northwest Science 47:218–27.

Ovaska K, Davis TM. 1992. Faecal pellets as burrow markers: intra- and interspecific odour recognition by western plethodontid salamanders. Animal Behavior 43:931–39.

Ovaska K. 1988. Spacing and movements of the salamander Plethodon vehiculum. Herpetologica 44:377–86.

Ovaska K. 1989. Pheromonal divergence between populations of the salamander Plethodon vehiculum in British Columbia. Copeia 1989:770-775.

Ovaska K. 1993. Aggression and territoriality among sympatric western plethodontid salamanders. Canadian Journal of Zoology 71:901–7.

Parker MS. 1992. Feeding ecology of larvae of the Pacific Giant Salamander (Dicamptodon tenebrosus) and their role as top predator in a headwater stream benthic community. [dissertation]. University of California, Davis.

Parker MS. 1994. Feeding ecology of stream-dwelling Pacific Giant Salamander larvae (Dicamptodon tenebrosus). Copeia 1994:705-718.

Parzefall J, Durand JP, Richard B. 1981. Aggressive behaviour of the European cave salamander Proteus anguinus (Proteidae, Urodela). Proceedings of the 13th International Congress of Speleology 8:415–19.

Rundio DE, Olson DH. 2003. Antipredator defenses of larval Pacific giant salamanders (Dicamptodon tenbrosus) against cutthroat trout (Oncorhynchus clarki). Copeia 2003:402–7.

Simons RR, Jaeger RG, Felgenhauer BE. 1997. Competitor assessment and area defense by territorial salamanders. Copeia 1997:70-76.

Staub NL. 1993. Intraspecific agonistic behavior of the salamander Aneides flavipunctatus(Amphibia: Plethodontidae) with comparisons to other plethodontid species. Herpetologica 49:271–82.

Stebbins RC. 1951. Amphibians of western North America. Berkeley, CA: University of California Press

Tanaka K. 1986. The territorial behavior of Hynobius takedai in the breeding season (Amphibia: Hynobiidae). Japan Journal of Herpetology 11:173–81.

Verrell P, Donovan A. 1991. Male-male aggression in the plethodontid salamander Desmognathus ochrophaeus. Journal of Zoololgy London 223:203–12.

Walls S, Mathis CS, Jaeger RG, Gertis WF. 1989. Male salamanders with high-quality diets have faeces attractive to females. Animal Behaviour 38:546-548.

Wells KD. 2007. The ecology and behavior of amphibians. Chicago, IL: The University of Chicago Press. 1162 p.

Welsh HH, Fellers GM, Lind AJ. 2007. Amphibian populations in the terrestrial environment: Is there evidence of declines of terrestrial forest amphibians in Northwestern California? Journal of Herpetology 41:469-482.

Wilkins R, Peterson NP. 2000. Factors related to amphibian occurrence and abundance in headwater streams draining second-growth Douglas-fir forests in southwestern Washington. Forest Ecology and Management 139:79-91.

Wilson RL. 1970. Dicamptodon ensatus feeding on a microtine. Journal of Herpetology 4:93–93.

Wise SE, Verret FD, Jaeger RG. 2004. Tail autotomy in terrestrial salamanders influences scent marking by residents and behavioral responses of intruders to resident chemical cues. Copeia 2004:165-172.

CHAPTER II

INFLUENCE OF CHEMICAL CUES ON SHELTER SELECTION OF THE COASTAL GIANT

SALAMANDER (*Dicamptodon tenebrosus*)

DAVID ALLEN REAVILL

Submitted to Northwestern Naturalist for Publication

Abstract

Chemical cues are used as ubiquitous markers in salamanders (Urodela), and serve as the primary modality for conveying inter and intra-specific information such as species identity, kinship, and individual information, and are used as territorial markers. Although the role of chemical cues has been widely studied in many Urodele families, particularly plethodontids, studies on the family Dicamptodontidae are very scarce. In order to test if Coastal Giant Salamanders (Dicamptodon tenebrosus) are able to use chemical cues to discriminate among self-marked, conspecific marked and blank substrates, we performed unforced two-choice trials. One of 29 individuals was provided with two shelters (plastic tubes), and tested under three scenarios: (i) self-marked vs control, (ii) conspecific marked vs control, and (iii) self-marked vs conspecific marked. Trials were filmed for 12 hour periods and the video analyzed to determine time spent in each shelter. Our results show that D. tenebrosus (i) did not discriminate between self-marked vs blank shelters, (ii) preferred a shelter marked by a conspecific over a blank shelter and (iii) preferred a self-marked over a conspecific marked shelter. These data suggest that *D. tenebrosus* were able to discriminate between their own scent vs that of a conspecific, but did not show an attraction to their own scent when offered alongside a blank shelter. *D. tenebrosus* were also attracted to the scent of conspecifics when offered alongside a blank, suggesting a possible

Introduction

Use of chemical cues as means of gathering information is ubiquitous across multiple animal taxa (Wyatt 2003). Caudate amphibians have been of particular interest in the study of chemical cues because these animals cannot vocalize and are commonly nocturnal; thus, limiting the efficacy of visual transmission of information (Palmer and Houck 2005). Studies have shown that salamanders can use chemical cues in a wide variety of ways, such as predator avoidance (Sih and Kats 1994; Mathis and Vincent 200), species and sex recognition (Dawley and Dawely,1986; Dantzer and Jaeger 2007; Gautier et al. 2006), general recognition of conspecifics (Ducey and Ritsema 1998; Marvin et al. 2004), mate stimulation and attraction (Park et al. 2004; Houck et al. 2004) and as territorial advertisement (Jaeger et al. 1986). Particularly, the study of intra-specific interactions in the family Plethodontidae demonstrated that chemical cues mediate a variety of complex social behaviors, such as the establishment, advertising, and defense of territories (Jaeger and Gertis 1979; Mathis 1990).

Outside of the families Plethodontidae, Ambystomatidae, and Salamandridae, studies of behavioral responses to chemical cues are scarce. The family Dicamptodontidae has had only one study concerning the response of *D. tenebrosus* larva to predatory cutthroat trout (Rundio and Olson 2003). No other studies have been published on Intra-specific communication through chemical cues in either the larval or terrestrial forms.

In general, very little is known about terrestrial adult *D. tenebrosus* despite the larva being the dominate stream predator throughout much of their range (Corn and Bury 1989; Kroll et al. 2010). This is likely due to the cryptic nature of terrestrial forms and the rarity in which they are encountered during standard biological surveys (Corn and Bury 1991; Welsh et al. 2007). *D. tenebrosus* is one of four

Commented [D16]: See Kinnemec paper

species belonging to the family Dicamptodontidae and is usually associated with mesic forests in the Pacific Northwest. They are mostly nocturnal, living under rocks, logs, small mammal burrows and natural crevices which they emerge from to hunt and move. Fessler (2012) conducted a radio-telemetry study during which 12 terrestrial *D. tenebrosus* were tracked between June 2010 and September 2011 in the Eastern Cascades. The results suggested salamanders maintained activity centers where and individual would move between shelters in a small area. Individuals would return to these activity centers either nightly within seasons or even among seasons. In some cases, an individual would move long distances (< 150 m) before returning to a previously occupied activity center and sometimes to the exact habitat feature. This suggests that *D. tenebrosus* are able to home to previously used refuges and this study proposes that self-recognition of chemical cues may be the mechanism of this homing ability.

> **Commented [OU17]:** This shouldn't be written in first person.

D. tenebrosus is the largest terrestrial salamander, generally transforming between the sizes of 92 and 166 mm total length, reaching a maximum size of 330 mm total length (Nussbaum et al. 1983; Jones et al. 2005). Therefore, there is a large size discrepancy between animals that may interact and compete for resources, such as food, shelter or access to mates. It is also commonly observed that *Dicamptodon spp.* are cannibalistic (Parker 1994; Anderson 1960; pers. obs by DR). This could put pressure, particularly in newly metamorphosed animals, to develop a heightened response to conspecific chemical cues to avoid being eaten by larger individuals. The goal of this study is to determine whether or not *D. tenebrosus* react to chemical cues deposited by itself and conspecifics.

> **Commented [OU18]:** Give initials

23

Methods

Collection and Housing

The Coastal Giant salamander is endemic to the Pacific Northwest region of the United States, ranging from Mendocino County, California, and north into extreme southwestern British Columbia. For this study, terrestrial individuals were collected from the Wenatchee National Forest, on the east slope of the Cascade Mountains along Snoqualmie Pass. A total of 27 individuals (SVL: $\overline{x} \pm 1$ SD = 106.2 $\pm$ 17.69) were collected between the months of June – October, in the years 2012, 2013, and 2014. Salamanders were transported back to Central Washington University and housed individually in large plastic containers (45 x 25cm) lined with moist peat moss. Salamanders were fed 3 - 5 crickets weekly and were kept in a temperature controlled room, maintained at 15˚C on a 12L:12D photoperiod.

Experimental Design

This study was designed to test the role of scent marking on shelter selection in terrestrial *D. tenebrosus*. Three experiments were designed to test the following hypotheses:

1. H_1: Salamanders choose self-marked shelters over non-marked shelters. Previous studies suggest that shelter fidelity of salamanders is often mediated by chemical cues (Jaeger and Forester, 1993). Therefore, we infer that *Dicamptodon tenebrosus* will follow the same pattern.

2. H_2: Chemical cues deposited by a conspecific caused an individual to avoid the conspecific marked shelter and prefer a non-marked shelter. We based this prediction on previous

24

Commented [OU19]: Since this will go to NW Naturalist put species name here.

Commented [OU20]: Need to mention permit and IACUC here somewhere.

Commented [OU21]: Say something more about the experimental design: two choice, Y maze, or random something more descriptive than just experimental design.

Commented [OU22]: Hypotheses should be constructed to test against them one-way the other would be an alternative. You have two choices here....one is to call these prediction and then you collect evidence to support or refute your prediction or make these more testable.

studies that chemical cues laid by conspecifics can act as territorial advertisements and can be used to deter intruders from entering an individual's territory.

3. H_3: Salamanders selected shelters marked with their own chemical cues over those marked by a conspecific. If territorial behavior exists, it is expected that there will be a preference for self-marked shelters (Gosling, 1982). However, in aggregative social systems, any chemical source (self or conspecific) would be selected for. .

Because *D. tenebrosus* is nocturnal*,* all experiments were conducted in the late evening at 20:00, two hours after the lights turned off. Chemical marks were obtained by lining an artificial shelter in the form of a plastic tube (l = 20cm, d = 8cm) with a damp paper towel and housing a donor animal in the tube for six hours immediately prior to the test. The control shelters were prepared in the same manner, with the exception that the shelter did not contain a donor animal. A trial would begin when the focal salamander was placed in a large rectangular arena (60 x 45cm) under an opaque habituation chamber. Two artificial shelters containing chemical marks were then randomly placed at opposite ends of the testing arena. After habituating for ten minutes the habituation chamber was removed and the salamander was allowed to move freely about the arena. Trials were run for 12 hours and filmed via an Infra-red camera suspended directly overhead. I collected data on the total time spent in each shelter, the frequency of entrance in each shelter, the latency to enter a shelter, and the overall choice of the salamander, determined by whichever shelter the salamander spent > 50% of the total time spent in either shelter. All trials were conducted in darkness to minimize the influence of visual stimuli on the animal's shelter choice, and all test arenas were set up in exactly the same manner. Thus, it is assumed that the chemical stimulus was the primary factor affecting shelter choice. After the

Commented [OU23]: This seems more an inference.

Commented [OU24]: The hypotheses in this section need further refine. You want to make sure they are testable and clear and mirror your experiments.

Commented [OU25]: I am not sure what this means. Explain this more.

completion of each trial, the arenas and shelters were thoroughly cleaned with mild dish soap and water to eliminate any residual chemical traces.

Using the methods described, focal salamanders were exposed to three different scent combinations in order to test the three hypotheses. In the first experiment, the focal salamander was presented with a shelter containing its own scent vs a control shelter. In the second experiment, the focal salamander was presented with a shelter containing the scent of a conspecific vs a control shelter, and in the final experiment, the focal salamander was presented with a shelter containing its own scent vs a shelter containing the scent of a conspecific. Each focal salamander was used in each experiment three times, yielding a total of nine trials per individual. In order to prevent the effects of an animal's previous behavior on future trials, each trial was separated by at least three days, and a different scent donor was used for each trial. The order in which an individual participated in each experiment was also randomly determined by assigning each individual a number and using a random number generator to assign the experimental sequence to each individual. In the wild, salamanders are likely to encounter individuals of various sizes, so no attempt was made to size-match donor and focal pairs, and thus a variety of size differences were tested in order to gain a more thorough representation of possible interactions. Terrestrial *D. tenebrosus* do not exhibit sexual dimorphism and thus it was not possible to sex the individuals before the trials, thus all analyses are carried out without regard to sex.

In each trial, the total time the focal salamander spent in each shelter, the number of times a focal salamander entered each shelter, and the overall choice of a focal salamander was recorded. The choice was assigned to whichever shelter the focal salamander spent >50% it's time in. This yielded

26

Commented [OU26]: Hmm this might be a sticking point. I thought you ran some post stats tests with sex? You could mention that here.

three choices for each salamander, which were combined into a single data point based on whichever shelter the salamander chose in two or more of the three trials. This data point was deemed the overall choice of a salamander for a given experiment. Testing the salamanders three times in each trial eliminated the possibility of ties, and any trial in which a salamander never entered a shelter was excluded from the dataset. Chi-Square goodness of fit tests were used to test whether the overall choice differed from the expectation of 0.5 against the assumption that salamanders choose shelters randomly.

Statistical Analysis

Differences in total time spent in shelters within experiments were compared using the Mann-Whitney U test. Entrance frequencies both within and between trials were analyzed using Chi-square tests. All analyses were carried out using program R.

Results

There was no significant difference in the mean time spent in either shelter for the first experiment ($P = 0.098$), the second experiment ($P = 0.478$) or the third experiment ($P = 0.16$).
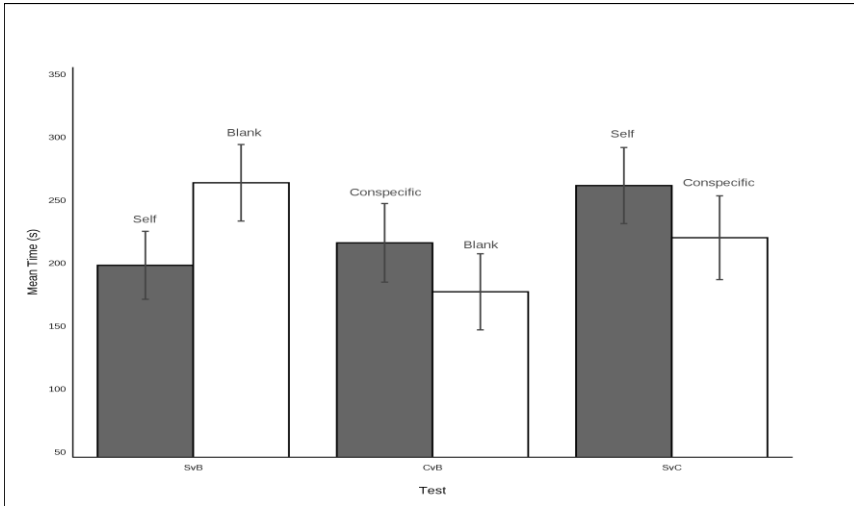
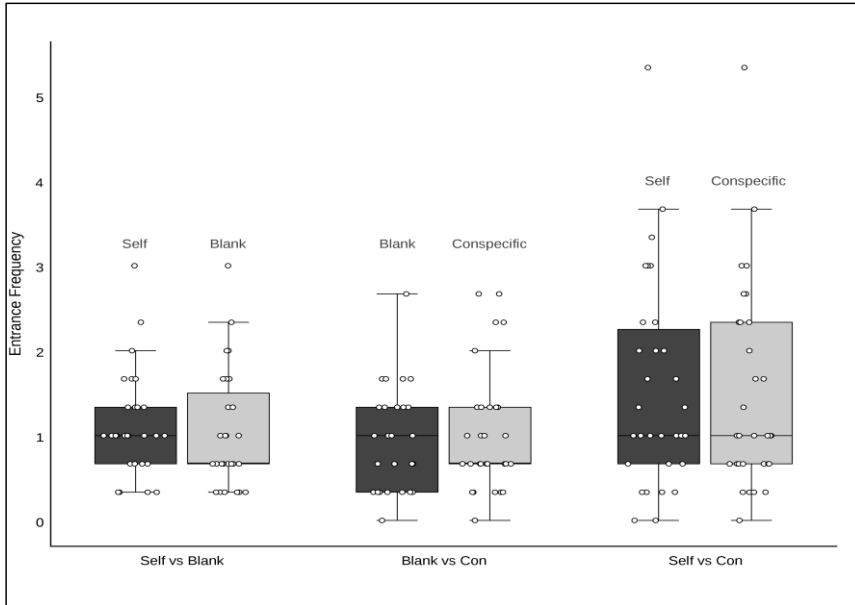FIGURE 1. Mean time spent in shelters for each experiment

FIGURE 2. Mean entrance frequency for each shelter for each experiment

There was no significant difference in entrance frequency between the shelters for the first experiment ($X^2 = 0.35$, $P = 0.55$), the second experiment ($X^2 = 0.58$, $P = 0.45$), or the third experiment ($X^2 = 0.17$, $P = 0.68$).
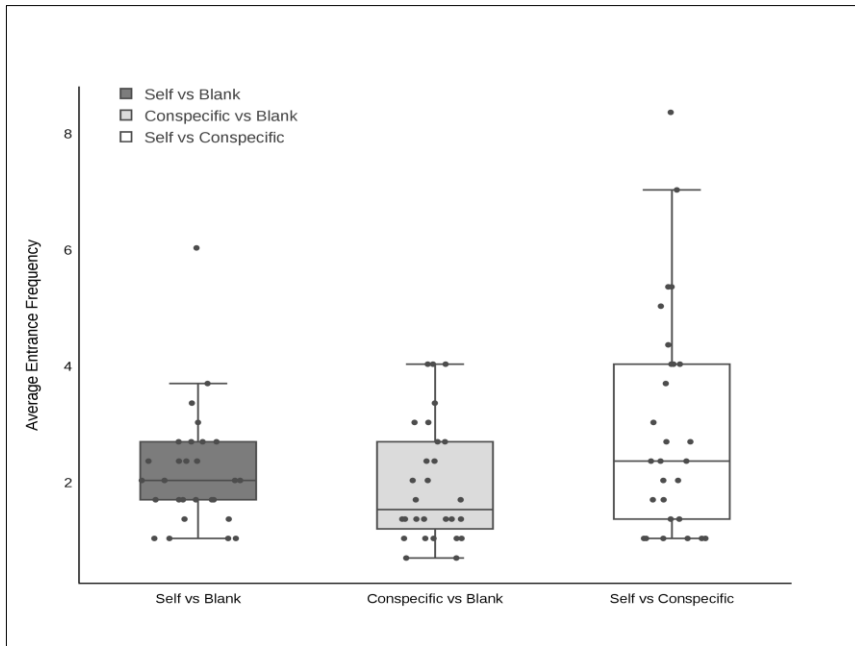
FIGURE 3. Mean entrance frequency for either shelter in each experiment

Total entrances into either of the shelters were summed for each experiment and compared. There was no significant difference between experiment one and experiment two ($X^2= 1.05$, $P = 0.31$), but there were significant differences in entrance frequency between experiments one and three, and experiments two and three ($X^2= 9.85$, $p = 0.002$; $X^2= 17.21$, $p = <0.001$, respectively).
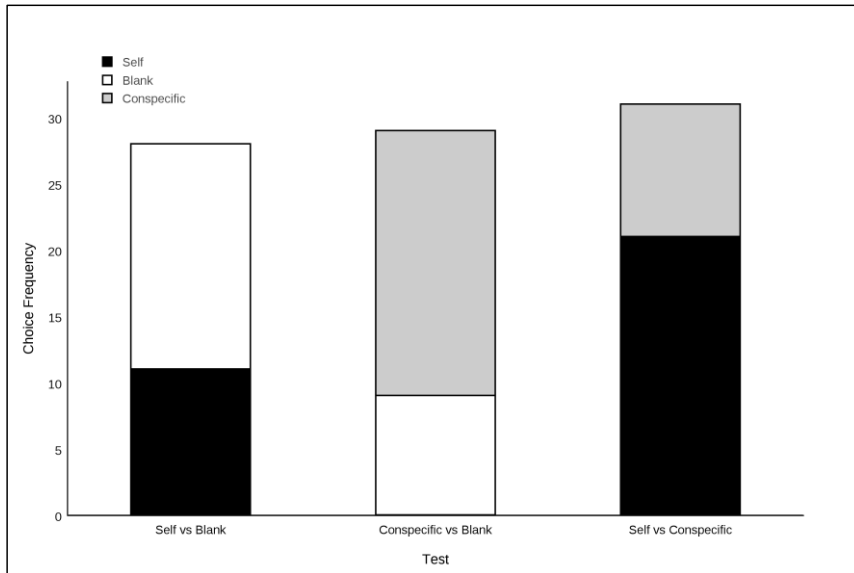
FIGURE 4. Overall choice of salamanders in each of the three experiments

Salamanders showed no difference in the overall choice between the two shelters ($X^2 = 1.286$, $P = 0.257$). When salamanders had the choice between a shelter containing the scent of a conspecific and a shelter containing no scent, they chose the shelter containing the scent of the conspecific significantly more often than the blank shelter ($X^2 = 4.172$, $P = 0.041$). When salamanders had the choice between a shelter containing their own scent and a shelter containing the scent of a conspecific, they chose the shelter containing their own scent significantly more often than the shelter containing the scent of a conspecific ($X^2 = 3.90$, $P = 0.048$).

Discussion

Salamander species that exhibit shelter fidelity are able to relocate previously used shelters after being displaced (Madison 1969, Barthalmus and Bellis 1972, Jaeger et al. 1993, Gautier and Miaud 1999) and many species are able to use their own chemical cues to identify shelters previously inhabited by themselves (reviewed in Mathis et al. 1995, Keen et al. 1987, Gautier and Olgun 2006). In the first experiment of this study, *D. tenebrosus* did not show a preference for shelters previously marked by themselves over blank shelters. This suggests that either these salamanders are not able to distinguish their own cues, or that they simply do not show a particular attraction to these cues in the experimental condition. *D. tenebrosus* have been shown to re-locate previously used burrows, even after moving long distances away or for long periods of time (Fessler, 2012). This behavior suggests that coastal giants may use some other means of relocating previously used areas, though self-recognition should not be discounted (see exp. three).

> **Commented [OU32]:** I think this needs fleshed out more. It seems like their could be priorities 1 use your own marked, 2 use any marked, 3 use any.

Territorial behavior is commonly mediated by chemical cues in terrestrial salamanders, and Gertis (1981) stated that the advertisement (i.e. through chemical cues) of a defended is one of the four essential conditions of territoriality. However, the degree in which salamanders exhibit territorial behavior can vary greatly and only a handful of species respond to the cues left by a territorial resident (Mathis et al 1995). If *D. tenebrosus* exhibits territorial behavior it would be expected that individuals would have generally avoided areas marked by a conspecific. This is contradictory to the behavior observed in experiment two of this study because animals were significantly attracted to the cues left by conspecifics. This suggests *D. tenebrosus* do not use chemical cues to advertise territories, or at least individuals do not seem to be repelled by such advertisements. Contrary to the expectations

> **Commented [OU33]:** Where they significantly attracted or did you see lack of evidence they chose conspecifics as frequently as there own? I think you can still make the argument but you want to make sure: If they are attracted that should be based on statistical evidence that they choose more frequently or spend more time. Perhaps I am interpreting it wrong but I thought the evidence suggested it is just as equal.

under a territorial system, some evidence suggests that certain salamanders exhibit gregarious behavior (Gautier and Olgon, 2006, Romano and Ruggiero 2008). Gregarious behavior may be selected for in harsh environments to form "survival groups" in which animals are drawn to similar high-quality habitat features (Brown and Orians 1970) or in the formation of "mutualistic groups" in which animals benefit from the presence of conspecifics (Graves and Duvall 1995). Gregarious behavior seems unlikely in the case of *D. tenebrosus* due to the rarity of encountering multiple individuals under the same cover object in the field (per. obs. DR and SW) and the aggressive behaviors of animals during direct interactions (Reavill 2015). However, it should be noted that gregarious groups of *D. ensatus* have been observed in California in association with the removal of a deteriorating culvert (Fellers et al 2010). The authors suggest that the culvert may have provided unusually high-quality habitat and that such aggregations may not be uncommon in *Dicamptodon*, but only rarely observed. Without information on the sexual identities of the donor and receiving animals, the motivations behind this pattern of conspecific attraction are confounded.

> **Commented [OU34]:** Since this the second chapter you can cite it as Chapter 3 for the submission to NWN you .

In experiment three, focal salamanders had the option of choosing a shelter marked with their own cues and the cues of a conspecific. Salamanders choose shelters containing their own cues significantly more often than shelters containing the cues of a conspecific. This suggests that *D. tenebrosus* are able to recognize cues laid by themselves, or it would have been expected that animals would not have shown a preference for one shelter over the other. Self-recognition is not uncommon in salamanders (Gautier and Olgun 2006, Gillete 2002, Tristram 1977, Graves 1994) and is a basic element of chemical communication (Jaeger and Gertis 1979, Simon and Madison 1984). The evidence for self-recognition in experiment three suggests that the pattern of animals not being attracted to

their own scent in experiment one is likely not due to the inability to recognize their own scent, but rather to an unforeseen motivation by the animal to not orient towards previously occupied shelters.

Simple attraction-avoidance studies such as this one are advantageous in that they document a response of an individual to chemical cues, and demonstrate that individuals can and do gain information about conspecifics. However, the motivations behind such behaviors are difficult to understand, and what specific information an animal is receiving through conspecific chemical cues is uncertain. This study provides the first evidence of intra-specific communication through chemical cues in any species of the family Dicamptodontidae, and suggests that *D. tenebrosus* may use chemical cues to mediate social behavior. Further studies are needed to better understand exactly what this social behavior is and how it is mediated through chemical cues

REFERENCES

Brown CW. 1968. Additional observations on the function of the nasolabial grooves of plethodontid salamanders. Copie 1968:728-731.

Bury RB, Corn PS. 1991. Sampling methods for amphibians in streams in the Pacific Northwest. Portland, Oregon

Bury RB. 1972. Small mammals and other prey in the diet of the pacific giant salamander (Dicamptodon ensatus). American Midland Naturalist 87:524–526.

Corn PS, Bury RB. 1989. Logging in Western Oregon: Responses of headwater habitats and stream amphibians. Forest Ecology and Management 29:39-57.

Corn PS, Bury RB. 1991. Terrestrial amphibian communities in the Oregon Coast Range. In: Ruggiero LF, Aubry KB, Carey AB, Huff MH, editors. Wildlife and Vegetation of Unmanaged Douglas-fir Forests. Portland, OR: USDA Forest Service. General Technical Report No. PNW-GTR-285. P. 305--317.

Davis TM. 2002. An ethogram of Intraspecific agonistic and display behavior for the wandering salamander, Aneides vagrans. Herpetologica 58(3):371-382

Dawley EM, Bass AH. 1988. Organization of the vomeronasal organ in a plethodontid salamander. Journal of Morphology 198:243–55.

Dawley EM. 1998. Olfaction. Amphibian biology 3. Chipping Norton, New South Wales: Surrey Beatty and Sons. p. 711-742

Ducey PK, Ritsema P. 1988. Intraspecific aggression and responses to marked substrates in Ambystoma maculatum (Caudata: Ambystomatidae). Copeia 1988:1008–13.

Ducey PK. 1989. Agonistic behavior and biting during intraspecific encounters in Ambystoma salamanders. Herpetologica 45:155–60.

Fellers GM, Wood LL, Carlisle S, Pratt D. 2010. Unusual subterranean aggregations of the California Giant Salamander, Dicamptodon ensatus. Herpetological Conservation and Biology 5(1):149-154.

Fessler B. 2010. Spatial ecology, behavior, and habitat use of terrestrial Coastal Giant Salamanders (Dicamptodon tenebrosus) in the central Washington Cascades. Ellensburg, WA: Central Washington University. 99 p.

Gabor CR, Jaeger RG. 1995. Resource quality affects the agonistic behaviour of territorial salamanders. Animal Behavior 49:71–79.

Gergits WF, Jaeger RG. 1990. Site attachment by the red-backed salamander, Plethodon cinereus. Journal of Herpetology 24:91–93.

Gillette JR, Kolb SE, Smith JA, Jaeger RG. 2000. Pheromonal attractions to particular males by female redback salamanders (Plethodon cinereus). In: Bruce RC, Jaeger RG, Houck LD, editors. The biology of plethodontid salamanders. New York, NY: Kluwer Academic/Plenum. P 431–440.

Griffis MR, Jaeger RG. 1998. Competition leads to an extinction-prone species of salamander: interspecific territoriality in a metapopulation. Ecology 79:2494–2502.

Jaeger RG, Kalvarsky D, Shimizu N. 1982. Territorial behaviour of the red-backed salamander: Expulsion of intruders. Animal Behavior 30:490–96.

Jaeger RG, Prosen ED, Adams DC. 2002. Character displacement and aggression in two species of terrestrial salamanders. Copeia 2002:391–401.

Jaeger RG. 1984. Agonistic behavior of the red-backed salamander. Copeia 1984:309–14.

Jaeger RG. 1988. A comparison of territorial and non-territorial behaviour in two species of salamanders. Animal Behavior 36:307–10.

Lang C, Jaeger RG. 2000. Defense of territories by male-female pairs in the red-backed salamander (Plethodon cinereus). Copeia 2000:169–77.

Lawrence LC, Jones WP, Leonard, Olson DH. 2005. Amphibians of the Pacific Northwest. Seattle, WA: Seattle Audubon Society. 227 p.

Mathis A, Jaeger RG, Keen WH, Ducey P.K, Walls SC, Buchanan BW. 1995. In Amphibian biology. Volume 2, Social behavior. Aggression and territoriality by salamanders and a comparison with the behaviour of frogs. ed. H. Heatwole and B.K. Sullivan, 633–76. Chipping Norton, New South Wales, Australia: Surrey Beatty and Sons.

Mathis A. 1990b. Territoriality in a terrestrial salamander: the influence of resource quality and body size. Behaviour 112:162–75

Murphy ML, Hall JD. 1981. Varied effects of clear-cut logging on predators and their habitat in small streams of the Cascade Mountains, Oregon. Canadian Journal of Fisheries and Aquatic Sciences 38:137-145.

Nishikawa KC. 1990. Intraspecific spatial relationships of two species of terrestrial salamanders. Copeia 1:418–26

Nishikawa KC.. 1985. Competition and the evolution of aggressive behavior in two species of terrestrial salamanders. Evolution39:1282–94.

Ovaska K. 1987. Seasonal changes in agonistic behaviour of the western red-backed salamander, Plethodon vehiculum. Animal Behavior 35:67–74.

Ovaska K. 1993. Aggression and territoriality among sympatric western plethodontid salamanders. Canadian Journal of Zoology 71:901–7.

Parker MS. 1994. Feeding ecology of stream-dwelling Pacific Giant Salamander larvae (Dicamptodon tenebrosus). Copeia 1994:705-718.

Simons RR, Felgenhauer BE. 1992. Identifying areas of chemical signal production in the red-backed salamander, Plethodon cinereus. Copeia 1992:776–81.

Staub NL. 1993. Intraspecific agonistic behavior of the salamander Aneides flavipunctatus(Amphibia: Plethodontidae) with comparisons to other plethodontid species. Herpetologica 49:271–82.

Welsh HH, Fellers GM, Lind AJ. 2007. Amphibian populations in the terrestrial environment: Is there evidence of declines of terrestrial forest amphibians in Northwestern California? Journal of Herpetology 41:469-482.

Wilkins R, Peterson NP. 2000. Factors related to amphibian occurrence and abundance in headwater streams draining second-growth Douglas-fir forests in southwestern Washington. Forest Ecology and Management 139:79-91.

Wrobel DJ, Gergits WF, Jaeger RG. 1980. An experimental study of interference competition among terrestrial salamanders. Ecology 61:1034–39.

CHAPTER III

INTRASPECIFIC AGONISTIC AND DISPLAY BEHAVIOR OF THE COASTAL GIANT

SALAMANDER (*Dicamptodon tenebrosus*)

DAVID ALLEN REAVILL

Abstract

Intraspecific interactions are an important factor in shaping the population structure of
terrestrial salamanders. Both physical interactions and scent-marking are vital components to the
establishment of territories and influence dispersion of individuals. However, little is known about the
terrestrial interactions of many salamander species. In this study, I observed the first accounts of
agonistic and display behavior of terrestrial individuals of the Coastal Giant Salamander (Dicamptodon
tenebrosus) in 27 staged laboratory encounters. Territory holders exhibited aggressive behaviors such
as biting and lunging more frequently than intruders. Biting also happened fairly frequently, with bites
being observed in nearly all interactions. Behavioral patterns showed similarities to those seen in
plethodontid salamanders, and certain behaviors seemed to serve the same functions, such as
aggression, agonistic displays, and passive displays. No evidence for marking behavior was observed,
such as vent-rubbing, which is commonly seen in plethodontid salamanders. Similarly, behaviors
facilitating enhanced chemoreception were either not present or not apparent, as there was no
evidence for nose-tapping which is commonly interpreted as a chemoreceptive behavior. The
aggressive nature of these salamanders likely contributes to the dispersion of adults seen in the field.

Intra and inter-specific interactions influence many aspects of the ecology of terrestrial salamanders, such as community structure, terrestrial spacing patterns, and competition for mates and resources (Mathis et al., 1995; Nishikawa, 1985; Wrobel et. al., 1980). Limited or patchy distribution of resources (i.e. prey, mates, shelter) may lead to the development of interference competition in the form of territoriality (Citation). Through staged laboratory interactions and field studies, territorial behavior has been observed in many species of terrestrial salamander particularly within the family plethodontidae (Staub 1993; Jaeger 1988; Wiltenmuth 1996; Ovaska 1993). These salamanders have been shown to utilize scent marks, visual displays, and agonistic behavior such as biting to defend territories (Jaeger et. al., 1982; Mathis, 1990, Nishikawa, 1990).

Despite the large body of research on plethodontids, there is a noted paucity of information regarding territorial behavior in other families with the exception of a handful of studies on salamandridae and ambystomatidae (Ducey and Ritsema 1988, Ducey 1989). Understanding the roles of intraspecific interactions in terrestrial salamanders is essential to better our understanding of the behavioral ecology of these animals particularly in cases when information regarding ecology is limited. Terrestrial adults of the family dicamptodontidae are notoriously understudied which is likely due to the cryptic nature of post-metamorphic adults and the rarity of encounters during biological surveys (Corn and Bury 1991; Wilkins and Peterson 200; Welsh et al. 2007). Until now no studies focusing on agonistic behavior of post-metamorphic adults has been published.

Dicamptodon tenebrosus represents one of four species of the family Dicamptontidae, and are generally associated with mesic forests and headwater streams of the Pacific Northwest. While the larvae can reach densities of >2 individuals per m^2 in headwater streams, the terrestrial forms are

much less abundant (Murphy and Hall 1981; Corn and Bury 1989; Parker 1994; Bury and Corn 1991; Fellers et al. 2010). Terrestrial adults are nocturnal and use habitat features such as rocks, logs, small mammal burrows and natural crevices as refuge (pers. obs.DR). Fessler (2010) radio-tracked individuals on the East slope of the Cascades in Washington State, and found that individual *D. tenebrosus* maintained "activity centers" in which an animal would move between refuges within a small area. This study showed each individual making returns to previously occupied refuges either nightly within seasons or between seasons. However, individuals were never found in close proximity to one another (Fessler 2010), and this pattern of refuge attendance and return combined with the spatial separation observed suggests the possibility of *D. tenebrosus* defending patches of resources and repelling intruding conspecifics. The goals of this study are; to develop an ethogram of behaviors used by *D. tenebrosus* during interactions with conspecifics, experimentally test whether *D. tenebrosus* exhibit agonistic and display behavior towards conspecifics, and test whether residency status has an effect on these behaviors.

Methods

Animal Collection and Care

For this study, salamanders were collected from the Wenatchee National forest on the East slope of the Cascade Mountains along Snoqualmie Pass. A total of 27 Individuals were collected during the active season, May – October in the years 2012, 2013, and 2014. All salamanders were transported back to the lab at Central Washington University where they were weighed to the nearest 0.1 g and their snout-vent length (SVL) was measured to the nearest 0.1 mm. Salamanders were housed individually in large plastic containers (45 x 25cm) lined with moist peat moss. All salamanders were

---

Commented [D38]: How to cite unpublished data?

Commented [OU39]: Just cite it as you would a paper but in the reference section say it is a MS Thesis.

Commented [OU40]: That is a not from Brandon's study? You want to limit the times you use a per obs in a paper.

Commented [D41]: Included development of an ethogram and clarified study goals

41

kept in a climate-controlled room maintained at 15˚C on a 12L:12D photoperiod and fed 3 – 5 crickets

weekly.

Experimental Design

Staged laboratory encounters were conducted between November 2014 and April 2015, using

methods similar to Jaeger (1984). Each salamander participated in three separate encounters serving a

different role in each: as a *resident* and an *intruder* in paired encounters, and once as a *resident* paired

with a surrogate salamander in a control treatment. To control for potential size effects, each resident-

intruder pair was matched to the nearest possible individual based on snout-vent length (SVL). Coastal

Giant Salamanders can grow to considerable size, and thus my sample exhibited a wide range of sizes

($\bar{x}$ = 107.1 mm, SD = 18.9, Range = 81 – 145 mm). Paired salamanders differed by ≤ 5 cm SVL ($\bar{x}$ = 0.89,

SD= 2.4 cm). In the control encounters, a resident was matched with a 'surrogate salamander', which

was made by rolling up a moist dark-colored paper towel into the approximate size and shape of the

resident. This is a commonly used control in behavioral studies of terrestrial salamanders, and is

included to test the behavioral reaction to any novel object in a resident's territory (Gabor and Jaegar

1995; Griffis and Jaeger 1998; Jaeger et al. 2002; Kohn et al. 2005). Treatment order was randomized

for each salamander with each individual used only once in each treatment. Every intruder/resident

pair was unique, and no salamander was paired with the same individual in a subsequent encounter. A

minimum of five days elapsed between each trial and the order in which a salamander participated in

each treatment was randomized. Terrestrial *D. tenebrosus* do not exhibit sexual dimorphism and

individuals were sexed after the completion of the experiment by inspecting the gonads. Therefore, pairings were a combination of male-male, male-female and female-female pairs.

Agonistic Trials

Staged encounters occurred in experimental units that consisted of a glass aquarium (60cm x 30cm) lined with a layer of moist paper towels, a main burrow, and an alternate refuge. The main burrow and alternative refuge consisted of small PVC tubes (l = 15 cm, d = 5cm) lined with moistened paper towels and placed randomly on opposite ends of the aquarium. The purpose of the alternative refuge was to provide a refuge for an intruder to retreat during the trial.

Five days prior to the beginning of a trial, the resident was placed into one experimental unit to allow it to presumably mark the area with pheromones. Previous observations have shown that salamanders were active during this establishment period, moving about the area often, and thus had sufficient opportunity to mark the entire area. During the establishment period, the main shelter of the resident's arena was sealed at one end, while the alternate refuge was sealed at both ends. The purpose of barring access to the alternate refuge was to allow the intruder an unmarked retreat. Every night at ~20:00 the resident was fed three crickets during the five day establishment period. Five days prior to the test, an intruder was placed into an experimental unit identical to the resident's, and treated it with the same protocol with the exception that the main shelter was sealed and the alternate refuge was open. The rationale behind this design is that the intruder would become accustomed to entering and exiting the alternate refuge, which would be open during the trial allowing the animal a retreat.

43

Because of the nocturnal nature of *D. tenebrosus* all trials were conducted at night between 2000-2300 under a dim red light. At the beginning of the trial, the intruder was picked up and placed into the resident's chamber under an opaque habituation dish. At the same time, the resident was similarly picked up and placed back into it's own chamber under an opaque habituation dish. The handling of the resident was included to control for any effects that handling an individual may have on its behavior. Both the resident and intruder were allowed 15 minutes to acclimate, during which time the alternate refuge was replaced with an identical, clean shelter in order to insure that it did not contain any residual chemical cues from the resident. When the alternate refuge was returned to chamber, it was left open allowing access to the intruder. After the acclimation period, both habituation dishes were removed and the salamander's interactions were directly observed from behind a blind, and recorded via two cameras, one placed in front of the chamber and one suspended overhead. Encounters were run for 30 minutes which allowed sufficient time for the two salamanders to interact. Control salamanders were treated with the same protocol, with the exception of introducing a 'surrogate salamander' instead of a live intruder. After each trial, the paper towel substrate was removed and the chambers were thoroughly cleaned with soap and water. Because previous studies have not provided operational definitions for behaviors exhibited by *D. tenebrosus*, all visually obvious behaviors were scored and an ethogram of behaviors was created. .

Commented [OU43]: You want to mention somewhere in the intro about the development of an ethogram. This is an important part of the study.

Commented [OU44]: Need to have a section about statistical analysis.

Results

Description of Behaviors

During 27 staged encounters, 19 distinct behavioral patterns were observed. These behaviors were separated into three general groups; postures, non-contact behaviors and contact behaviors **(Table 2)**.

Postures

Postures were defined as body positions that were maintained by an individual for at least five seconds. The most common was the head raised (HR) posture, in which the entire trunk and tail were pressed against the substrate, with the neck held at a 45° angle and the head held horizontal. In some instances, the head would be lifted to a 45° angle in-line with the neck into snout raised (SR). After several minutes salamanders would often sink into the chin raised (CR) posture, in which the neck and head were lowered to nearly parallel with the trunk, but with the chin resting just above substrate. The head raised and chin raised postures imperceptibly graded with each other, and it was often difficult to distinguish between them. Salamanders would often sink further into the flat (FLAT) posture, where the entire head, trunk and tail were pressed against the substrate. On several occasions, salamanders displayed an arching posture (ARCH), in which the trunk was lifted into a conspicuous arch, with the head and tail angled downward. In this study, *D. tenebrosus* exhibited another likely defensive posture (DF), which generally was elicited by an attack from a conspecific. The bitten animal would lift its entire trunk off the ground, look toward its attacker and curve its body such that the concave flank was exposed to the attacker.

45

Commented [OU45]: What percentage of time was it observed?

Commented [D46]: Total percentage of trials each behavior is used is summed in Table 1, last column. No. of trials (out of the 27) and percent of trials. This data includes both residents and intruders in the experimental condition. Does this need more clarification in the text?

Commented [OU47]: Did you calculate the percentage of time this occurred? Can you? Since it seems to be sequence of events.

Commented [DR48]: Did not calculate this. Indivs would switch between postures very frequently. I measured posture use in time (s) spent in each. I can also include frequency per trial, but It seemed redundant.

Commented [OU49]: Can you include photos of these positions or have someone draw them?

Commented [DR50]: I only have low-quality still shots from my videos at this point. Attempting to make drawings, but are not turning out to the quality I would like.

Commented [OU51]: Again this should be in the discussion.

Non-Contact Behavior

Non-contact behaviors were defined as discrete actions which did not result in physical contact. The most common of these were look toward (LT), in which a salamander turns its head directly toward a conspecific, and turn toward (TT), in which a salamander would pivot its body so that it directly faced a conspecific. Both of these behaviors were often elicited by movement of the opponent. When look toward and turn toward were performed simultaneously, the action was scored as turn toward only. Moving toward (MT) another salamander was often used in conjunction with LT and TT. Looking away (LA) and turning away (TA) involved a salamander turning its head or pivoting its body, respectively, such that visual contact with the conspecific is terminated. Similar to LT and TT, when looking away and turning away were performed simultaneously, the action was scored as turning away only. Moving away (MA) occurred when a salamander moved such that it increased the distance between itself and a conspecific. Both residents and intruders performed lunges (LUNGE) frequently during encounters. A lunge was used as an apparent attempt to bite an opponent, in which an individual would use its rear legs to propel itself with open jaws directly towards a conspecific.

Physical Contact Behaviors

Physical contact behaviors were discrete behaviors that resulted in contact between two salamanders, and the majority of these behaviors seemed to be aggressive in nature. Most common of the physical contact behaviors was biting (BITE). A bite occurred when a salamander grasped an opponent with its jaws, but released its hold immediately. Similarly, salamanders would also grasp

(GRASP) an opponent, which was the same as a bite, except the attacker would maintain its hold for several seconds. Both bites and grasps were directed toward the head, body and tail of the opponent. In a few cases, salamanders would form a circle pattern while biting, in which one salamander would grasp the rear flank or tail of its opponent, and the bitten animal would swing around and grasp the tail or flank of its attacker. Salamanders would wrestle in this manner for several seconds until one would release its hold and retreat from the confrontation. Physical contact behaviors not involving biting were seen less commonly, and included pushing, general contact and chin rubbing. In pushing, one salamander would use its snout to push against the opponent. Pushing was typically directed towards the head or trunk of the opponent, and sometimes animals would push so vigorously that the opponent was flipped over or even completely lifted off the ground. Instances in which physical contact was made, but couldn't be defined as either pushing or chin rubbing, were scored as contact (CONTACT). These included when a salamander contacted any part of a conspecific with its own body, and included walking over, touching etc. None of these other contact behaviors seemed to serve as aggressive actions, and often resulted in an entwinement of two salamanders after a bite, or during an escape attempt.

In the 27 staged trials conducted, there was no evidence for scent-marking or enhanced chemoreception behaviors. Almost universally seen amongst plethodontid salamanders is nose tapping behavior, in which the salamander will tap its snout so that the nasolabial grooves come in contact with the substrate, facilitating the transfer of chemicals into the nares (Brown, 1968). *Dicamptodontidae* does not possess nasolabial grooves, and thus the absence of this behavior is not

surprising. Scent marking behaviors observed in other salamander taxa, such as chin rubbing or vent

rubbing, were also not seen in any of the trials, in either the control or experimental conditions.

TABLE 1. Ethogram of behaviors observed by residents and intruders during experimental trials

Behavior	Description	No. of Encounters (%)
Postures		
Head Raised	Trunk flat against substrate and neck raised at ~45° angle with head horizontal	27 (100%)
Chin Raised	Trunk flat against ground, head and neck held parallel with chin just slightly above substrate	27 (100%)
Snout Raised	Trunk flat against substrate, both head and neck held at a ~45° angle	5 (18.5%)
Flat	Head, trunk, and tail pressed flat against substrate	16 (59.3%)
Front Trunk Raised	Forelimbs lifting anterior portion of trunk off ground, neck held at ~45° angle and head horizontal	2 (7.4%)
All Trunk Raised	Entire trunk held above substrate, with neck at ~45° angle and head horizontal	4 (14.8%)
Arch	Entire trunk held off substrate in a conspicuous arch, head and tail angled downward	10 (37%)
Defense	After an aggressive interaction, defending salamander will curve its body so concave flank is facing attacker. Head and trunk held off ground with head held parallel to body	1 (7.4%)
Non-Contact Behaviors		
Look toward	Salamander will turn its head in the direction of a conspecific	20 (74.1%)
Turn Toward	Salamander will pivot its body so that it is facing a conspecific	22 (81.5%)
Move Toward	Salamander will move directly toward a conspecific	22 (81.5%)
Look Away	Salamander will turn its head so that it terminates visual contact with a conspecific	11 (40.7%)
Turn Away	Salamander will pivot its body away from a conspecific	13 (48.1%)
Move Away	Salamander will move directly away from a conspecific	26 (96.3%)
Lunge	Salamander will lunge towards a conspecific with open jaws in an apparent attempt to bite	22 (81.5%)
Physical Contact Behaviors		
Bite	Salamander will grasp a conspecific with its jaws, but release immediately	21 (77.8%)
Grasp	Salamander will grasp a conspecific with its jaws and maintain its hold for several seconds	8 (29.6%)
Contact	Salamander will contact a conspecific with any part of its body other than the jaws	4 (14.8%)
Circle Biting	One salamander will grasp the base of the tail of its opponent, and the bitten salamander will turn and grasp the base of the tail of its attacker. The pair will maintain this position until one salamander releases its grip	3 (11.1%)

Control vs Experimental Conditions

In order to determine whether behaviors acted as visual displays the control and experimental conditions were compared. The four most common postures seen in the study were Head Raised (100%), Chin Raised (100%), Flat (59.3%) and Snout Raised (18.5%). Wilcoxon Rank-Sum tests were used to determine whether the amount of time differed between the control and experimental conditions for both males and females. Males showed no significant difference for head raised (Z = -1.08, P = 0.28), chin raised (Z = -0.94, P = 0.35), or flat (W = 6 > crit val 3), and there was not a large enough sample size to test males for snout raised, or females for any of the four postures. The data was then pooled for males and females and the difference between the experimental and control conditions was tested. There was no difference in head raised (Z = -0.067, P = 0.94), chin raised (Z = -0.87, P = 0.384), or snout raised (W = 2 > crit val 0). The only significant difference observed was for the FLAT posture (P = 0.039), with individuals using FLAT more towards a live intruder than the surrogate ($\bar{x}$ (s) exp. = 287, $\bar{x}$ (s) cont. = 52.5). This suggests that FLAT is likely used as a visual display towards conspecific

Commented [OU53]: Give percentages.

Commented [DR54]: Percentages in text, or is summarized in table ok?

Commented [OU55]: Stats need to be described in methods

Commented [DR56]: N in this case was not large enough to test using the Z score to produce a P-value. Instead, the W-score was compared to the critical value. Is there a standard way of reporting this?

Commented [OU57]: How much more often? Compare percentages or give rates. P
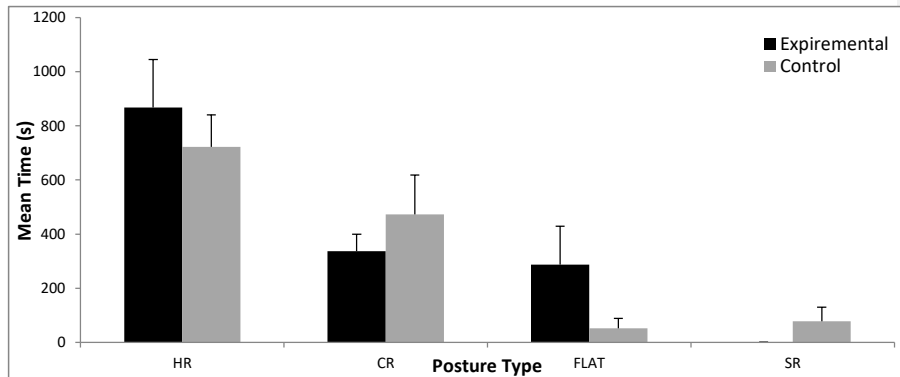
intruders.

FIGURE 1. Pooled data of males and females for time spent in each posture

All discrete behaviors were separated into three categories based on their apparent function (Table 2). Overt aggressive behaviors (Lunge, Snap, Bite, and Grasp) were behaviors which were obvious acts of aggression towards a conspecific. Passive aggressive (look toward, turn toward, move toward, and contact) were actions that have been shown to serve as aggressive or display behaviors in some plethodontid species (**citation**), and seemed to have a similar function in *D. tenebrosus*. When turn toward happened in simultaneously with look toward, the action was scored as turn toward only, similarly, when move toward happened simultaneously with either of the other two behaviors, it was scored as move toward only. Avoidance behaviors (look away, turn away, and move away) have been shown to act as submissive displays (**citation**) in plethodontid species and appeared to have a similar function in *D. tenebrosus*. Similar to passive aggressive, each behavior was scored as only the most escalated behavior when occurring simultaneously with less escalated behaviors.

Commented [OU58]: Move to discussion.

Commented [DR59]: The rationale for separating into these categories is based on their function in other species. I feel this explanation needs to be here in order to explain why I split them up like this.

51

TABLE 2. Salamander behavioral categories

Overt Aggressive	
Lunge	With mouth open, a salamander will lunge at its opponent
Snap	A salamander will make a quick strike at its opponent, but not make contact
Bite	A salamander will quickly bite its opponent, but release immediately
Grasp	A salamander will bite its opponent and hold for several seconds
Passive Aggressive	
Look Toward	A salamander turns its head toward the opponent
Turn Toward	A salamander pivots its body toward the opponent
Move Toward	A salamander moves directly towards the opponent
Avoidance	
Look Away	A salamander turns its head so as to terminate visual contact with its opponent
Turn Away	A salamander pivots its body to face away from an opponent
Move Away	A salamander will move directly away from an opponent

Three behavioral categories were compared for males and females between the control and experimental conditions (**Figures 2 and 3).** Males differed significantly in all three categories, but females did not have a large enough sample size to run the Wilcoxon Rank-Sum test. All data was pooled for males and females (**Figure 4),** and **t**here was a significant difference in all of the behavioral categories between the control and experimental conditions.

FIGURE 2. Frequency of behaviors of males in control vs experimental conditions

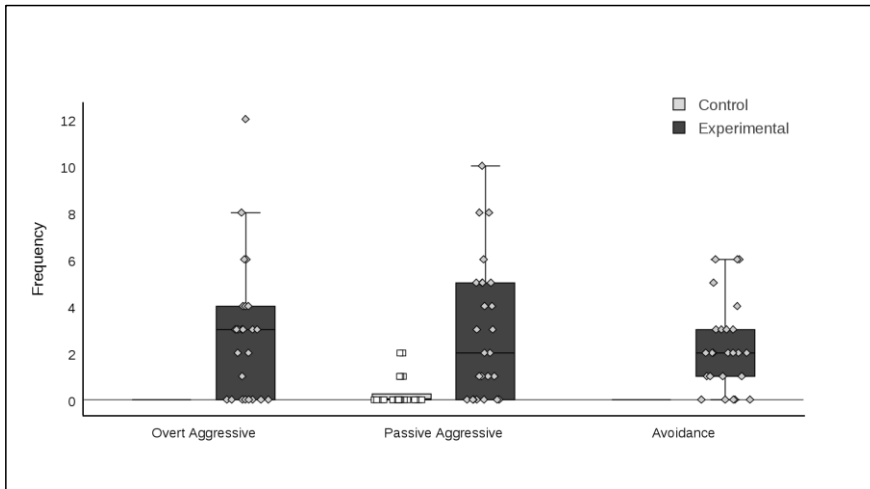FIGURE 3. Frequency of behaviors of females in control vs experimental conditions

FIGURE 4. Pooled data for frequency of behaviors in control vs experimental conditions

Effects of Absolute Body Size on Agonistic Behavior

Using the same three categories of behavior, It was tested whether absolute body size had an effect on the frequency of agonistic and avoidance behaviors. Due to the paired nature of the data, the effects of body size for all animals in both the resident and intruder status were tested. No significant correlation was found between body size and behavior category for either the residents or intruders. For residents, no significant correlation was found between body size and overt aggressive, passive aggressive or avoidance behaviors (Z = -0.02, P = 0.92; Z = -0.17, P = 0.41; Z = -0.23, P = 0.27, respectively). Similarly for intruders, no significant correlation was found between overt aggressive, passive aggressive or avoidance behaviors (Z = -0.05, P = 0.33; Z = 0.21, P = 0.33; Z = -0.09, P = 0.67, respectively).
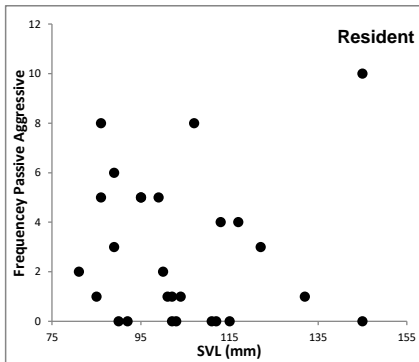
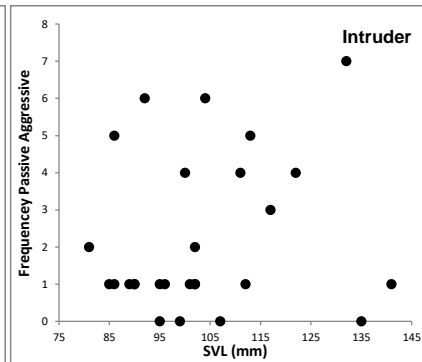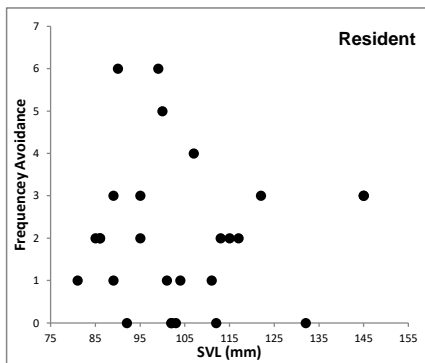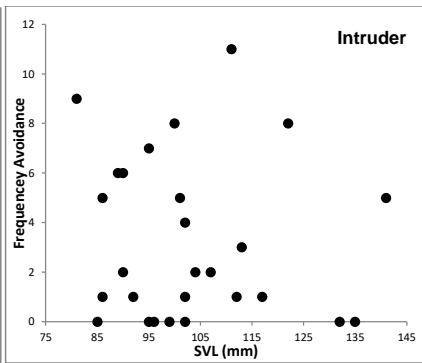**Commented [OU61]:** What are the stats?

A

B

C

D

E

F

55
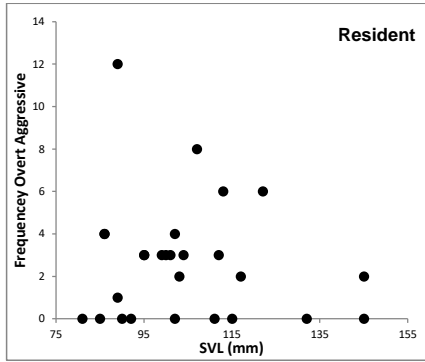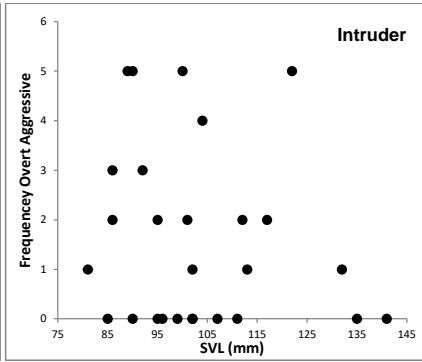
FIGURE 5 A – F. Correlations between size and behavioral category for both residents and intruders

Do Males and Females Differ in Behavior During Agonistic Interactions?

The data was partitioned to test whether resident males and females differed in their behavioral patterns during territorial contests against conspecific intruders. There were no significant differences between male and female residents for time spent in head-raised ($Z = 0.63$, $P = 0.53$), chin-raised ($Z = 0.07$, $P = 0.94$) flat ($Z = 0.55$, $P = 0.58$) or snout-raised ($Z = 0.18$, $P = 0.8$).(**Figure 5).** There was also no significant difference in the frequency of overt aggressive ($Z=-0.52$, $P = 0.6$), passive aggressive ($Z = -0.85$, $P = 0.39$) or avoidance behaviors ($Z = -0.7$, $P = 0.48$) between male and female residents **(Figure 6).** Male and female intruders where then tested to determine whether they differed in their time spent in the postures (**Figure 7)** and the behavioral categories **(Figure 8).** . There were no significant differences between male and female intruders for time spent in head-raised ($Z = 0.61$, $P = 0.54$), chin-raised ($Z = -0.96$, $P = 0.34$) flat ($Z = -0.17$, $P = 0.86$) or snout-raised ($Z = 0.7$, $P = 0.45$). There was also no significant difference in the frequency of overt aggressive ($Z=0.66$, $P = 0.51$), passive aggressive ($Z = 0.04$, $P = 0.97$) or avoidance behaviors ($Z = -0.31$, $P = 0.76$) between male and female intruders.
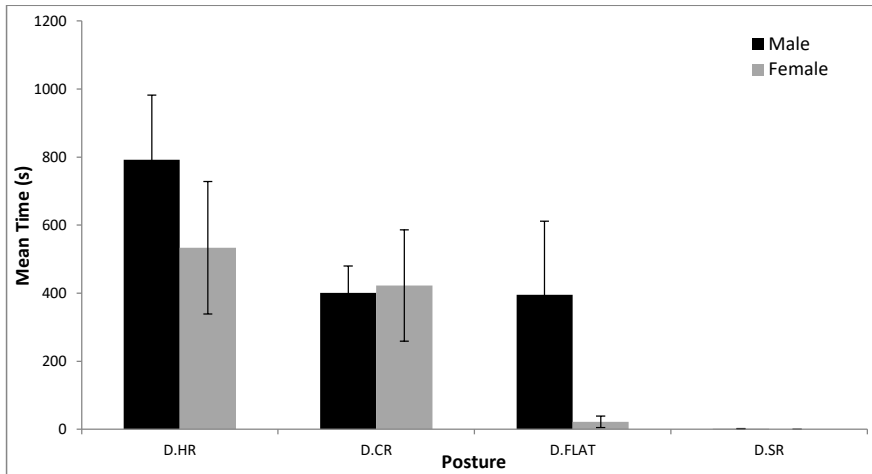
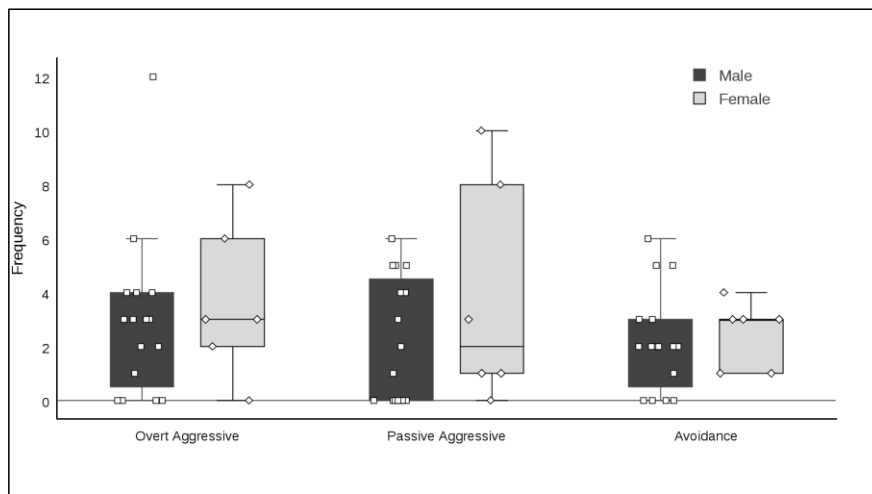FIGURE 6. Time spent in postures by resident males and females

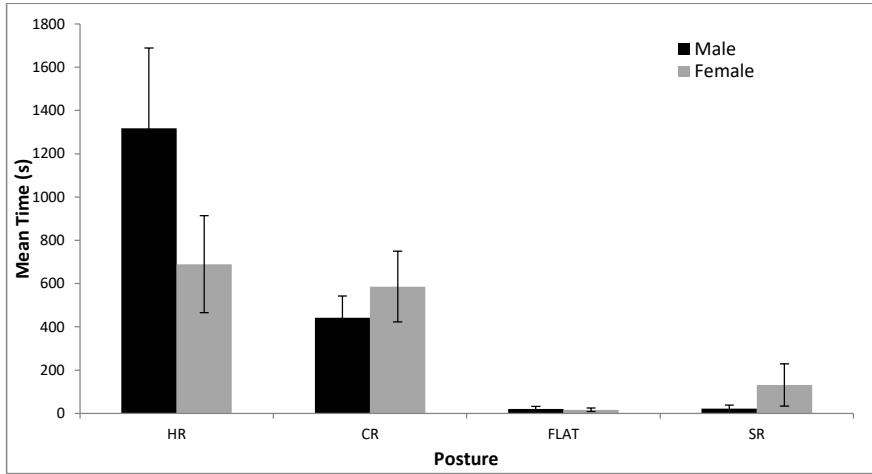FIGURE 7. Frequency of behavioral categories by resident males and females

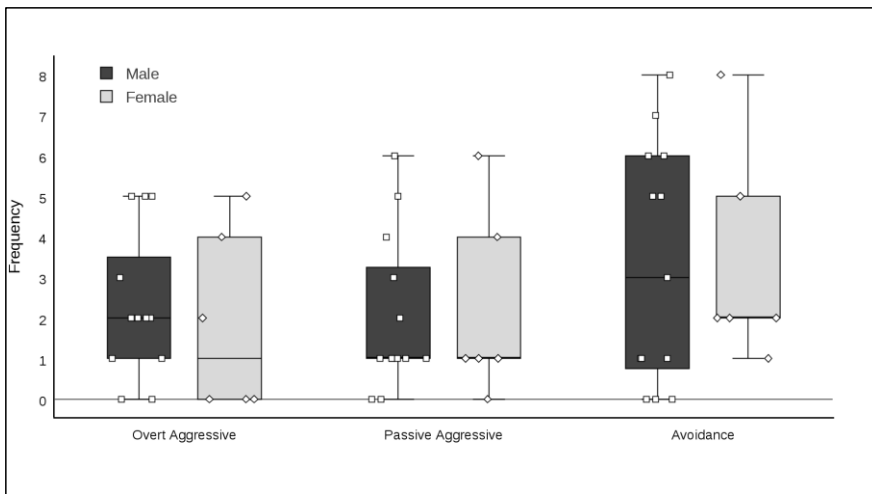FIGURE 8. Mean time spent in postures by intruder males and females

FIGURE 9. Frequency of behavioral categories by intruder males and females

Does residency status have an effect on agonistic behavior?

In order to test whether or not residency status had an effect on behavior, the data for males and females was pooled. There were no significant differences between residents and intruders for time spent in head-raised (Z = -1.11, P = 0.27), chin-raised (Z = -0.086, P = 0.93) flat (Z = -1.85, P = 0.06) or snout-raised (Z = -1.38, P = 0.17).There was also no significant difference in the frequency of overt aggressive (Z= -1.74, P = 0.08), passive aggressive (Z = -0.35, P = 0.73) or avoidance behaviors (Z = -1.43, P = 0.15) between residents or intruders.
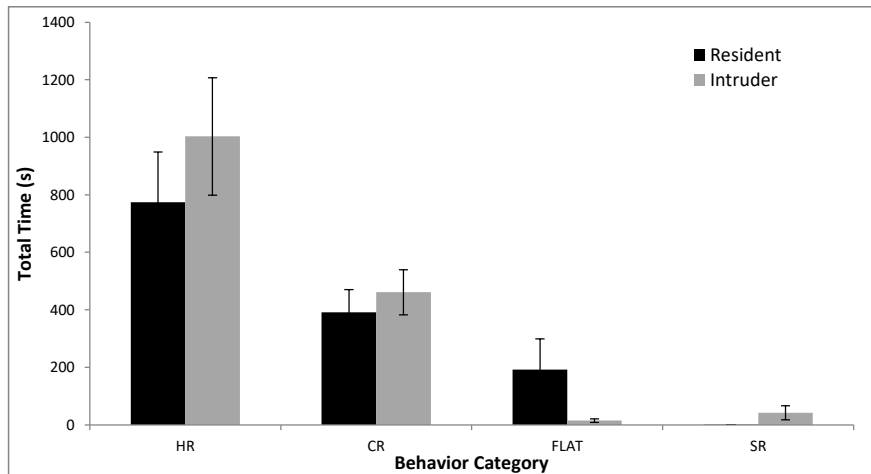

FIGURE 10. Mean time spent in postures in pooled data
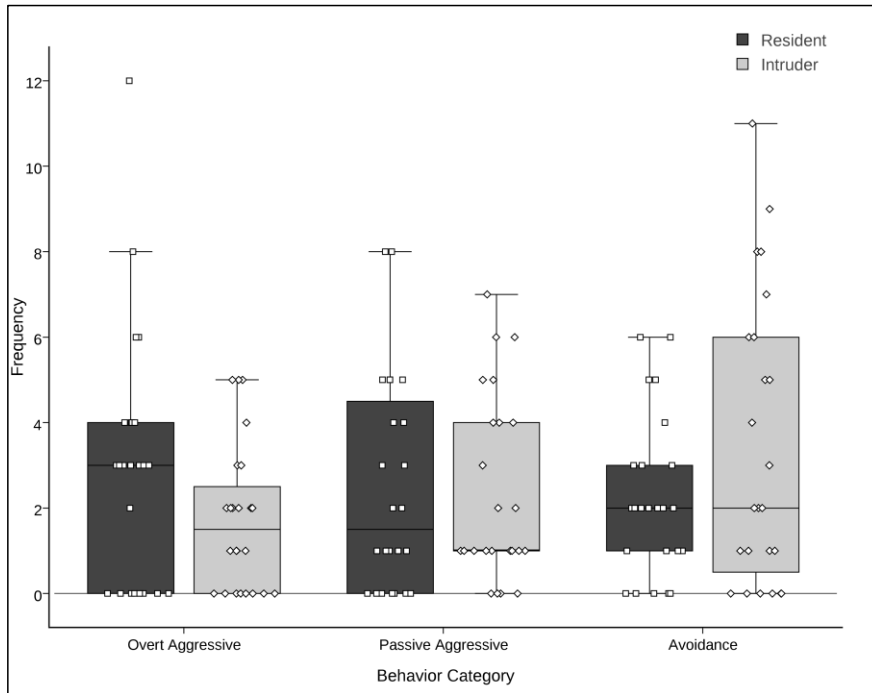
FIGURE 11. Frequency of behavior categories for pooled data

Do male's behavioral patterns differ when paired other males and females?

Sexing *D. tenebrosus* by external features was uncertain, so after the trials all animals were euthanized and sexed by inspecting the gonads, and it was found that there were 16 males and 7 females. Due to the uneven sex distribution and deaths during the course of the study, there ended up being eight male-male, five male-female, one male-female and one female-female resident-intruder pairs. Because there were not enough female-male and female-female pairings, these analyses were limited to

male-male and male-female pairings. The difference in time spent in postures by male residents when paired with male intruders vs female intruders was tested There were no differences between male-male vs male-female pairings in head-raised ($Z = 0.95$, $P = 0.34$), chin-raised ($Z = 1.83$, $P = 0.068$), flat ($Z = 0$, $P = 1$) or snout-raised ($Z = -051$, $P = 0.61$). However, there was a general trend of males spending more time in each of the postures when paired with other males than when paired with females (**Figure 11**). Males did not differ significantly in overt aggressive ($Z = 0.73$, $P = 0.46$), passive aggressive ($Z = -0.58$, $P = 0.55$), or avoidance behaviors ($Z = 0.58$, $P = 0.55$).(**Figure 12**).
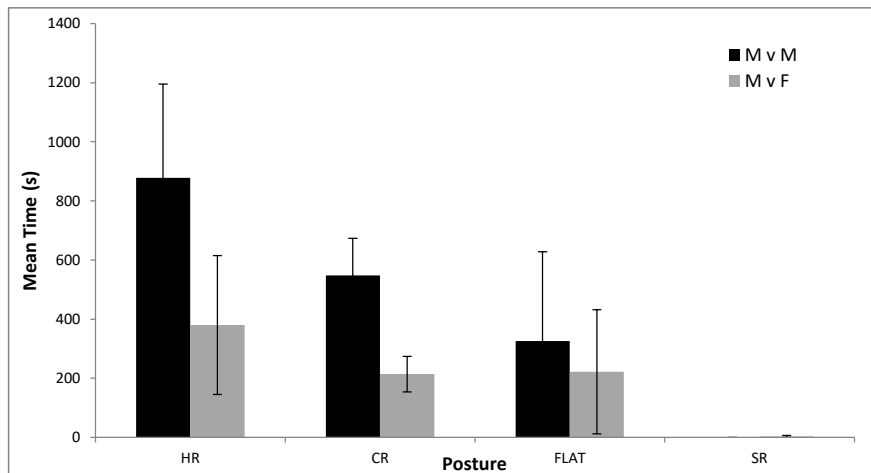
> **Commented [DR64]:** Move to discussion?

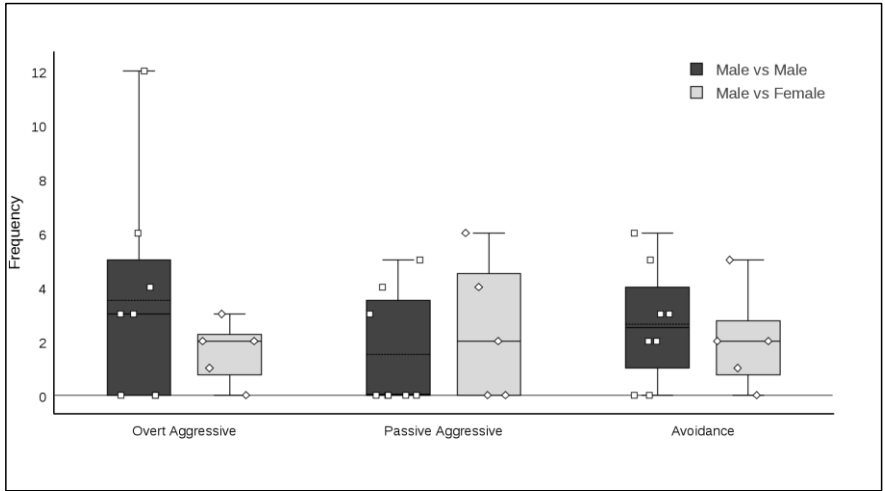FIGURE 12. Mean time spent in postures by male residents against male and female intruders

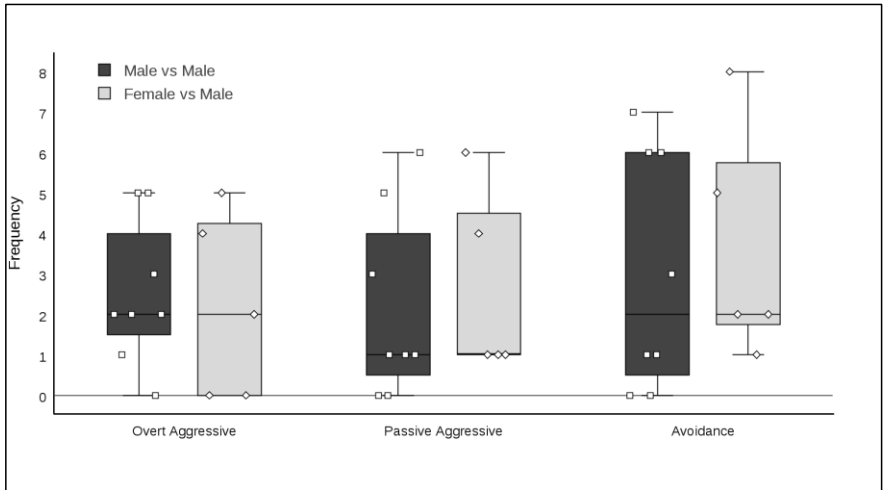FIGURE 13. Frequency of behaviors used by male residents against male and female intruder

FIGURE 14. Frequency of behaviors used by male and female intruders when paired with male residents

Do certain postures act as aggressive or submissive displays?

In order to determine whether certain postures acted as agonistic displays, I partitioned the data by counting the number of times a BITE occurred, and recording the postures of the participants pre and post-bite. If certain postures act as threat displays, then bites should occur most often when both participants are in those postures. After a bite occurs, the attacker's posture should be more threatening, and the bitten animal's posture should be more submissive. This should distinguish the difference between threat and submissive displays. I scored the postures head-raised, chin-raised, flat, defense, all-trunk-raised, front-trunk-raised and retreat. I defined retreat as any time an animal was climbing on the wall, on top of, or into a shelter.

TABLE 3. Frequency of postures used immediately before and after a bite attack

Posture	Pre-Bite		Post-Bite	
	Attacker	Defender	Attacker	Defender
HR	65.4	32.7	42.3	19.2
CR	28.8	40.4	48.1	51.9
FLAT	0	7.7	5.8	9.6
Retreat	1.9	13.5	1.9	7.7
DF	1.9	0	0	3.8
ATR	1.9	3.8	1.9	5.8
FTR	0	0	0	1.9
ARCH	0	1.9	0	0

There were 52 interactions that included bites (**Table 2**). For each posture to be included in the dataset, the individual must have maintained the posture for ≥ 30

seconds before or after the bite. Instances in which multiple bites occurred by the same animal without separation of at least 30 seconds were scored only as one bite. Attackers used HR significantly more often (χ^2 = 5.67, P = 0.017) pre-bite than did defenders (65% and 33%, respectively). This pattern held post-bite, with the attacking individual using HR significantly more frequently (χ^2 = 4.5, P = 0.034) than defenders (42% and 19%, respectively). The use of CR did not differ between attackers and defenders pre-bite (χ^2 = 1, P = 0.32) or post-bite (χ^2 = 0.08, P = 0.78). Post-bite, however, both attackers and defenders increased their use of CR to 48%. This suggests that CR may be a more submissive or defensive posture than HR, since after a bite, and occasionally a brief wrestling match, both attackers and defenders would likely be on alert and adopt a more defensive posture. Many of the other postures had too low of occurrences to draw meaningful conclusions. However, it should be noted that a bite was never administered while the attacker was in FLAT, and post-bite occurrences of FLAT increased for both attackers and defenders. Similar to CR, this could suggest that FLAT is used as a submissive or defensive display (**Figures 13 & 14**).
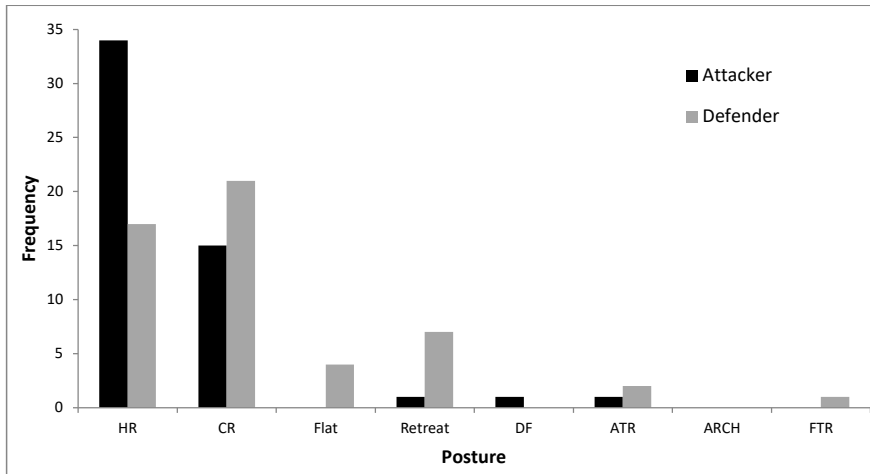
Commented [DR65]: Move to discussion

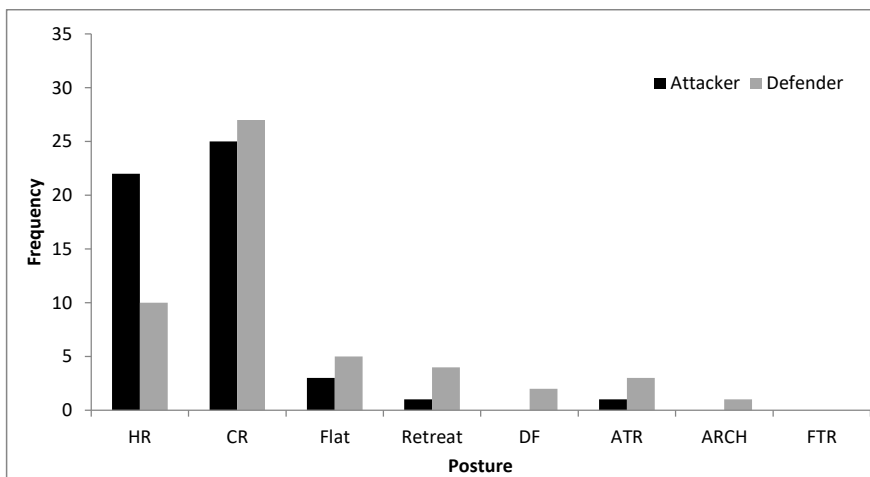FIGURE 15. Postures of attackers and defenders pre-bite

FIGURE 16. Postures of attackers and defenders post-bite

Discussion

Posture utilization

During staged agonistic encounters, *D. tenebrosus* exhibited 19 discrete behavioral categories, which were classified into three groups, postures, non-contact behaviors, and contact behaviors. In order to determine whether postures acted as visual displays, the control and experimental conditions were compared. For the four most common postures; head raised, chin raised, flat and snout raised, the mean times spent were compared, and only flat was significantly different. This suggests that flat is a visual display used by *D. tenebrosus*. This behavior has been observed in other salamander species, and is often interpreted as a submissive display (Jeager 1984). Flat likely serves a similar function in *D. tenebrosus*, as most individuals used flat after and aggressive interaction.

The insignificant difference of use of other postures, however, does not necessarily imply that these postures are not used as visual displays, and it is likely that they serve multiple functions depending on the context of an interaction. Head raised appeared to have the function of an investigatory behavior in both the control and experimental conditions, and also as a threat display during aggressive interactions, as attacking individuals used head-raised more both before and after a bite attack. Chin-raised seemed to serve as a resting posture both during the control and experimental conditions, as salamanders would often slowly sink from the head-raised posture into

Commented [DR66]: Find others

chin-raised, and remain in that position for a long period of time. During aggressive interactions, chin-raised seemed to serve as a defensive posture, as after an attack both the attacking and defending individuals increased their use of chin-raised. Other postures occurred infrequently, and it is difficult to determine their probable functions, however, snout-raised appeared to be an exploratory behavior, as salamanders often entered this posture in response to the observer lifting the habituation chamber. All-trunk-raised and front-trunk-raised were likely threat or defense displays, as they were only used during aggressive interactions, and arch was likely a defense display, as it was only used by defending individuals in response to an attack, and often as an apparent response to handling while setting up an encounter.

Males vs Females

When the sexes were compared, there was no difference in either posture utilization or behavioral category in regards to residency status, or in the case of males, sexual identity of an intruder. However, the power of these conclusions are limited due to the small sample size. It is worth noting that male intruders showed a general trend of increased usage of postures when paired with other males than when paired with females. Males also showed more overt aggressive behaviors when paired with males than with females. This may suggest that males show higher rates of interaction with intruding males than with females, and that this interaction may be more aggressive in

nature. In some wild populations, males of *Plethodon cinereus* tend to form intersexual pairs with females (Gillette et al. 2000; Lang and Jaeger 2000), but in staged laboratory encounters, male *P. cinereus* did not show different levels of aggression towards other males than to non-gravid females (Jaeger 1982). It would not be surprising if male *D. tenebrosus* showed different aggression levels towards other males vs females, however, little is known about the breeding behavior of *D. tenebrosus*, and eggs have been found from spring into autumn, suggesting little synchrony in breeding activity (Lawrence et al. 2005). This study was conducted outside of the likely courtship season, and this combined with the effects of the experimental condition may cloud any meaningful conclusions that can be made.

Effects of Residency Status

There was no significant difference in overt aggressive, passive aggressive or avoidance behavior between residents and intruders, though residents exhibited overt aggressive behaviors slightly more than intruders, and intruders exhibited avoidance behaviors slightly more than residents. This suggests that residency status does not give individuals a higher success rate of excluding intruders. Both residents and intruders bit opponents, and bites were often administered by both in the same trial. It is difficult to determine whether these bites were administered as a defense, or simply as a feeding response. Bites were usually administered in apparent response to the movement of the

Commented [D67]: See Jaeger and Oeterson 2002

opponent salamander, and after a bite the pair would typically separate for a period of time with no apparent behavioral interactions, until the movement of one individual elicited the interest of its opponent. *D. tenebrosus* are voracious predators, Bury (1972) found evidence of mammals, lizards, salamanders and large-sized land invertebrates while examining the gut contents of *D. tenebrosus* (*D. ensatus* at the time). Bury noted that when housed in the same terrarium, individuals would administer damaging bites to conspecifics, bitten individuals losing large portions of their tail. This voracity was mirrored in the present study, as many individuals would lunge at any movement, even the movement of an observer through the walls of the housing containers. This leads one to believe that this high level of aggression is likely due to feeding responses, rather than territorial defense. However, this aggressive behavior likely contributes to the spacing of individuals in the field, and the rarity in which individuals in this population are encountered inhabiting the same refuge (Fessler 2012, pers. obs.).

There was no evidence of enhanced chemoreceptive behaviors such as "nose-tapping" (Dawley and Bass 1989; Gertis and Jaeger 1990) or scent-marking behaviors such as "vent rubbing" (Simons and Felgenhauer 1992; Davis 2002) in any of the trials. Unlike plethodontid salamanders, Dicamptodon do not posses nasolabial grooves, which are involved in the transfer of chemical cues from the substrate into the vomeronasal organ (Dawley 1998). Ducey Ritsema (1988) observed the Ambystomatid species, *Ambystoma maculatum*, touching their snout to the substrate and to conspecifics, which they interpreted as an enhanced chemosensory behavior. This was not seen in

69

the present study, and if *D. tenebrosus* do use enhanced chemoreceptive or scent-marking behaviors, it was not apparent.

These data show that *D. tenebrosus* do exhibit behaviors that may lead to spacing of individuals in the field, though whether these behaviors are indicative of true territoriality is unclear. Obvious use and function of visual displays were also unclear, and may indicate that *D. tenebrosus* do not rely on these behaviors to mediate social interaction. These animals occur at relatively low densities in the field (Corn and Bury 1991; Welsh et al. 2007) and may have little chance of interacting with conspecifics, which may not have led to the development of complex display behaviors, as has been shown in Plethodontid species which can occur in very high densities. While this study presented the first account of Intraspecific aggression of *D. tenebrosus*, more studies are needed to understand the patterns seen here, particularly studies of behavior in natural settings.

REFERENCES

Anderson JD. 1960. Cannibalism in Dicamptodon ensatus. Herpetologica 16:260.

Barthalmus GT, Bellis ED. 1972. Home range, homing and the homing mechanism of the salamander, Desmognathus fuscus. Copeia 1972:632-642.

Brown JL, Orians GL. 1970. Spacing patterns in mobile animals. Annual review of ecology and systematics 1:239-262.

Corn PS, Bury RB. 1989. Logging in Western Oregon: Responses of headwater habitats and stream amphibians. Forest Ecology and Management 29:39-57.

Corn PS, Bury RB. 1991. Terrestrial amphibian communities in the Oregon Coast Range. In: Ruggiero LF, Aubry KB, Carey AB, Huff MH, editors. Wildlife and Vegetation of Unmanaged Douglas-fir Forests. Portland, OR: USDA Forest Service. General Technical Report No. PNW-GTR-285. P. 305--317.

Dantzer BJ, Jaeger RG. 2007. Detection of the sexual identity of conspecifics through volatile chemical signals in a territorial salamander. Ethology 113: 214- 222.

Dawley EM, Dawley RM. 1986. Species discrimination by chemical cues in a unisexual-bisexual complex of salamanders. Journal of Herpetology 20: 114- 116.

Ducey, PK, Ritsema P. 1988. Intraspecific aggression and responses to marked substrates in Ambystoma maculatum (Caudata: Ambystomatidae). Copeia 1988: 1008-1013.

Fellers GM, Wood LL, Carlisle S, Pratt D. 2010. Unusual subterranean aggregations of the California Giant Salamander, Dicamptodon ensatus. Herpetological Conservation and Biology 5(1):149-154.

Fessler B. 2010. Spatial ecology, behavior, and habitat use of terrestrial Coastal Giant Salamanders (Dicamptodon tenebrosus) in the central Washington Cascades. Ellensburg, WA: Central Washington University. 99 p.

Gautier P, Miaud C. 2003. Faecal pellets used as an economic territorial marker in two terrestrial alpine salamanders. Ecoscience 134-139.

Gautier P, Olgun K, Uzum N, Miaud C. 2006. Gregarious behaviour in a salamander: attraction to conspecific chemical cues in burrow choice. Behavioral Ecology and Sociobiology. 59(6):836-841.

Gergits WF. 1982. Interference competition and territoriality between the terrestrial salamanders Plethodon cinereus and Plethodon Shenandoah [thesis]. Albany, NY: State University of New York.

Gillette JR. 2002. Odor discrimination in the Californai slender salamander, Batrachoseps attenuates: evidence for self-recognition. Herpetologica 58(2):165-170.

Gosling LM. 1982. A reassessment of the function of scent marking in territories. Zeitschrift für Tierpsychologie 60:89–118.

Graves BM, Duvall D. 1995. Aggregation of squamate reptiles associated with gestation, oviposition, and parturition. Herpetological Monographs 9: 102–119

Graves BM. 1994. The role of nasolabial grooves and the vomeronasal system in recognition of home area by redbacked salamanders. Animal Behavior 47:1216–19.

Houck LD, Arnold SJ. 2003. Courtship and mating behavior. In: Sever DM, editor. Phylogeny and Reproductive Biology of Urodela (Amphibia). Enfield, NH, Science Publishers: p 383-424.

Jaeger RG, Forester DC. 1993. Social behavior of plethodontid salamanders. Herpetologica 1:163-175.

Jaeger RG, Fortune D, Hill G, Palen A, Risher G. 1993. Salamander homing behavior and territorial pheromones: Alternative hypotheses. Journal of Herpetology 27:236–39.

Jaeger RG, Gergits WF. 1979. Intra- and interspecifc communication in salamanders through chemical signals on the substrate. Animal Behaviour 27: 150-156.

Jaeger RJ, Goy JM, Tarver M, Marquez CE. 1986. Salamander territoriality: pheromonal markers as advertisement by males. Animal Behaviour 34:860-864.

Jones LLC, Bury RB, Corn PS. 1990. Field observation of the development of a clutch of pacific giant salamander (Dicamptodon tenebrosus) eggs. Northwestern Naturalist 71:93–94.

Keen WH, McManus MG, Wohltman M. 1987. Cover site recognition and sex differences in cover site use by the salamander, Desmognathus fuscus. Journal of Herpetology 21: 363-365.

Kroll AJ, Maccracken JG, Mcbride TC, Bakke J, Light J, Peterson P, Bach J. 2010. Basin- scale surveys of stream-associated amphibians in intensively managed forests. Journal of Wildlife Management 74:1580-1587.

Madison DM. 1969. Homing behavior of the red-cheeked salamander, Plethodonjordani. Animal Behavior 17:25–39.

Marvin GA, Whitekiller R, Hutchison VH. 2004. Avoidance of alarm chemicals by plethodontid salamanders (genus Eurycea): importance of phylogeny, ecology, and methodology. Herpetologica 60: 24-33.

Mathis A, Jaeger RG, Keen WH, Ducey P.K, Walls SC, Buchanan BW. 1995. In Amphibian biology. Volume 2, Social behavior. Aggression and territoriality by salamanders and a comparison with the behaviour of frogs. ed. H. Heatwole and B.K. Sullivan, 633–76. Chipping Norton, New South Wales, Australia: Surrey Beatty and Sons.

Mathis A, Vincent F. 2000. Differential use of visual and chemical cues in predator recognition and threat-sensitive predator-avoidance responses by larval newts (Notophthalmus viridescens). Canadian Journal of Zoology 78: 1646- 1652.

Mathis A. 1990. Territorial salamanders assess sexual and competitive information using chemical signals. Animal Behaviour 40: 953-962.

Nussbaum RA, Brodie ED, Storm RM. 1983. Amphibians and reptiles of the Pacific Northwest. Moscow, ID: University Press of Idaho 331 p.

Palmer CA, Houck D. 2005. Responses to sex-and species-specific chemical signals in allopatric and sympatric salamander species. In: Mason RT, Lemaster MP, Müller-Schwarze D, editors. Chemical Signals in Vertebrates 10. New York, NY: Springer: p 32-41.

Park DJ, McGuire M, Majchrzak AL, Ziobro JM, Eisthen HL. 2004. Discrimination of conspecific sex and reproductive condition using chemical cues in axolotls (Ambystoma mexicanum). Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology 190: 415-427.

Parker MS. 1994. Feeding ecology of stream-dwelling Pacific Giant Salamander larvae (Dicamptodon tenebrosus). Copeia 1994:705-718.

Reavill DA, Weaver RO, Wagner S. 2015. Intraspecific agonistic and display behavior of the Coastal Giant Salamander (Dicamptodon tenebrosus) [unpublished thesis]. Ellensburg, WA: Central Washington University

Romano A, Ruggiero A. 2008. Olfactory recognition of terrestrial shelters in female Northern Spectacled Salamanders Salamandra perspicillata (Caudata, Salamandridae). Phyllomedusa: Journal of Herpetology 7(1) 3-10.

Rundio DE, Olson DH. 2003. Antipredator defenses of larval Pacific giant salamanders (Dicamptodon tenbrosus) against cutthroat trout (Oncorhynchus clarki). Copeia 2003:402–7.

Sih A, Kats LB. 1994. Age, experience, and the response of streamside salamander hatchlings to chemical cues from predatory sunfish. Ethology 96: 253-259.

Simon GS, Madison DM. 1984. Individual recognition in salamanders: cloacal odors. Animal Behaviour 32: 1017-1020

Tristram DA. 1977. Intraspecific olfactory communication in the terrestrial salamander Plethodon cinereus. Copeia 1977:597–600.

Welsh HH, Fellers GM, Lind AJ. 2007. Amphibian populations in the terrestrial environment: Is there evidence of declines of terrestrial forest amphibians in Northwestern California? Journal of Herpetology 41:469-482.

Wyatt TD. Pheremones and animal behavior. Cambridge, UK: Cambridge University Press. 391 p.