THE BEHAVIORAL RESPONSE OF LARVAL COASTAL GIANT SALAMANDERS, *DICAMPTODON TENEBROSUS*, TO CHEMICAL STIMULI

By

Larkin G. Chases

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Approved by the Master's Thesis Committee:

John Reiss, Major Professor	Date
Michael Mesler, Committee Member	Date
Bruce O'Gara, Committee Member	Date
Richard Paselk, Committee Member	Date
Michael Mesler, Graduate Coordinator	Date
Chris Hopper, Interim Dean for Research and Graduate Studies	Date

ABSTRACT

Nearly all animals are able to detect forms of chemical stimuli. In amphibians, chemoreceptive capabilities are used in a wide variety of contexts, including the detection of predators, conspecifics, and food. The focus of this project was the use of chemoreception in the feeding behavior of larval coastal giant salamanders, *Dicamptodon tenebrosus*. I investigated natural chemical cues used by larval salamanders to detect, locate, and assess potential food sources in a laboratory setting. Stimuli used in this study were as follows: bologna, agar blank, guano, lipid component of California black worms (*Lumbriculus variegatus*), cod liver oil, salt, and whole California black worms (*Lumbriculus variegatus*). All stimuli were housed in an agar-based morsel and presented to test subjects at the end of one arm in a Y-shaped test station.

The results of this study provide substantial evidence of coastal giant larvae using chemical cues to detect, assess, and locate a food source. Test subjects actively investigated the test station during trials with all stimuli. All five steps in salamander feeding behavior were observed: orientation, approach, olfactory test, fixation, and snapping. Overall, salamanders showed a general interest in bologna and whole worm morsels. The specific chemical cues used to assess each stimulus were not determined.

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Thesis Committee

John Reiss

Richard Paselk

Michael Mesler

Bruce O’Gara

Marty Reed

Media Services

Anthony Baker

Terry Jones

Rick Brown

Chris House

Rya Rubenthaler

Kjirsten Wayman

My Entire Family

Olivia

Fuzzmerelda

HSU Natural History Museum

Sharyn Marks

Roger Herick

Stephanie Strasser

Beth Banner

Eric Russel

Oliver Miano

Jenny Nyffenegger

Naomi Inoue

Michelle Block

Dawn Goley

Bill Bigg

HSU Biology Small Grant

Colleen Reithmuth Kastak

David Kastak

Margaret Lang

HSU Herpetology Group

DEDICATION

I would like to dedicate this to my mother, Margaret (Gee) Griffin Chases. Without her love, encouragement, and friendship I would have never begun, nor finished, an enormous endeavor such as this. I also have her to thank for my creativity, strong work ethic, love of the wild, determination, and just about everything that makes me who I am. My "Mummy" is, and will always be, my very best friend.

Thank you, Mummy! I love you!

Denali National Park, Alaska 2003

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INTRODUCTION

"Feeding is one of the most essential activities of all animals, so it is no surprise, then, that the lives of most animals are dominated by their never-ending quest for food." –*The Oxford Companion to Animal Behavior (pg. 209).*

Nearly all animals are able to detect chemical stimuli (Zimmer-Faust, 1995). Amphibians, like other tetrapods, have the ability to sample chemicals in their environment using gustatory, olfactory, and vomeronasal senses. In amphibians, chemoreceptive capabilities are used in a wide variety of contexts. Because the specific influence of gustatory, olfactory, and vomeronasal senses in feeding behavior is difficult to distinguish experimentally without invasive procedures, they will be referred to here collectively as "chemical senses."

There is evidence that salamanders use their chemosensory skill to locate predators, conspecifics, and food (Dawley, 1994). Previous work by Amanda Deppe (Senior Research Project, Fort Lewis College, Environmental Biology Department, 2002) showed larval coastal giant salamanders, *Dicamptodon tenebrosus,* to use chemical cues when searching for potential food items. The questions posed in this research are simple questions focusing on the feeding behavior of larval coastal giant salamanders, *Dicamptodon tenebrosus*. More specifically, are larval coastal giant salamanders using chemical cues to detect, locate and assess potential food items, and if so, do they show a different response among stimuli?

Physiology of Salamander Chemoreception

Olfaction & Vomeronasal Chemoreception

In salamanders, chemicals enter the nasal cavity through external nares and pass into the oral cavity through internal nares, or choanae (Heatwole and Dawley, 1998). The nasal cavity is divided into two diverticulae, the main olfactory chamber and vomeronasal organ. The nasal epithelium lining the main olfactory chamber in aquatic salamanders is arranged in folds with sensory epithelium lining the valleys and non-sensory epithelium upon the ridges. The sensory epithelium of aquatic larvae is found in folds similar to that of aquatic salamanders. Functioning Bowman's glands, found in terrestrial adults, secrete an odorant-binding protein. Although Bowman's glands are also found in aquatic larvae, they are not functional (Arzt et al., 1986). Many smells are processed via the main olfactory chamber while the vomeronasal organ is thought to process information regarding sex identification and courtship (Dawley, 1984).

The olfactory epithelium is lined with receptor cells (Heatwole and Dawley, 1998). There are many different kinds of receptor cells, each binding to a specific odorant. There is evidence that receptors on the vomeronasal organ are sensitive to high molecular weight odorant molecules (e.g., pheromones) while the olfactory epithelium in the main olfactory chamber appears to be more sensitive to smaller odorant molecules, for example amino acids. When an

odorant binds to a receptor, transduction occurs, resulting in a cascade of action potentials traveling through nerves to the brain. Axons comprising the olfactory and vomeronasal nerves communicate with the main olfactory and accessory bulbs, respectively. Olfactory nerves travel directly to the main olfactory bulbs while vomeronasal nerves terminate in the accessory olfactory bulbs.

Gustation

The physiological taste system in salamanders is not unlike other vertebrates (Heatwole and Dawley, 1998). It consists of receptor cells arranged in organs called taste buds, located throughout the mouth and pharynx. Taste buds send chemical signals to the central nervous system where information is processed in the hindbrain.

Taste buds are sensitive to numerous chemicals. Aquatic salamanders have been shown to respond to bitter and salty substances. Mudpuppies (*Necturus maculosus*) and axolotls (*Ambystoma mexicanum*) have both been shown to reject usually ingested food when it is treated with a bitter substance. As the most known toxins are bitter tasting, gustation may act as a toxin detector. When the same bitter-treated substances were combined with salt, the rate of rejection declined (Heatwole and Dawley, 1998).

Biological Roles of Chemical Detection in Salamanders

Salamanders use the chemical senses in a number of ways. There is abundant evidence supporting the use of the chemical senses in salamanders to detect predators. Larval salamanders use chemical detection in identifying predators in an aquatic environment (Petranka et al., 1987; Stebbins and Cohen, 1995; Kats, 1988). Aquatic adult red-spotted newts, *Notophthalmus viridescens*, avoid areas containing chemical stimuli from injured conspecifics; thus, warning them of potential predators nearby (Woody and Mathis, 1997). Aquatic adult gray-belly salamanders, *Eurycea multiplicata griseogaster*, moved away and hid in gravel as a response to the presence of chemical stimuli from a predatory fish (Hickman et al., 2004). Individuals did not respond similarly when exposed to chemical stimuli of a control or non-predatory fish. Adult four-toed salamanders, *Hemidactylium scutatum*, have the ability to detect odors of northern red salamanders, *Pseudotriton ruber*, in a terrestrial environment (Cupp, 2001). Northern red salamanders are known to prey upon other salamanders.

Much research has also been done on the use of chemical senses in conspecific detection by salamanders. Chemicals are used to repel mate competitors in terrestrial red-spotted newts, *Notophthalmus viridescens* (Park and Propper, 1999). Male red-spotted newts also have the ability to identify females in an aquatic environment based solely on odor recognition (Dawley, 1984).

Terrestrial California slender salamanders, *Batrachoseps attenuatus*, discriminate, by means of chemical detection, between self and non-self marked substrates, providing evidence for recognition of scent marks (Gillette, 2002). Chemoreception is used by Allegheny mountain dusky salamanders (terrestrial environment), *Demognathus ochrophaeus*, and adult Japanese fire belly newts (aquatic environment), *Cynops pyrrhogaster*, in identifying conspecifics and potential mates (Evans, 1996; Kikuyama et al., 1995). An electrophysiological response to a female-attracting hormone, sodefrin, has been demonstrated in aquatic male Japanese fire belly newts (Toyoda and Kikuyama, 2000). Aquatic San Marcos salamanders, *Eurycea nana*, respond strongly to chemical cues of the opposite sex (Thaker et al., 2006). Chemical cues from conspecifics may influence activity and courtship behavior in axolotls, *Ambystoma mexicanum*, a neotenic aquatic species (Park et al., 2004). This particular study provided evidence of both vomeronasal and olfactory system involvement in discerning "the sex and reproductive condition of conspecifics." Gravid female red-backed salamanders, *Plethodon cinereus*, use olfaction in a terrestrial environment when determining whether or not there is high-quality food in a male's territory by surveying his feces (Jaeger and Wise, 1991).

Although it is considered common knowledge among herpetologists that salamanders have a keen olfactory sense and are able to locate food by this method, surprisingly little research has been done with salamanders and their use of chemical senses in finding food (Artz et al., 1986). Chemoreception is

important for prey detection and initial location of a food source by terrestrial tiger salamanders, *Ambystoma tigrinum* (Lindquist and Bachmann, 1982). There is also evidence of vomeronasal detection of food in terrestrial plethodontid salamanders (Placyk and Graves, 2002). The aquatic blind cave salamander, *Proteus anguinus*, has a strong ability to detect prey based on chemoreception, while its epigean aquatic relative, *Necturus maculosus*, has a very weak ability to detect prey by chemoreception alone (Durand et al., 1982). Artz et al. (1986) found evidence of terrestrial and larval tiger salamanders sensing different items. Interestingly, Sullivan et al. (2000) found no evidence that the aquatic salamander, *Siren intermedia*, responded to chemical cues from prey. During preliminary tests, salamanders in my care detected, located, and attempted to eat immobile prey (bologna and flavorless gelatin chunks).

Chemical Detection of Prey in other Aquatic Vertebrates

Many vertebrates, such as frogs and fish, have been studied in regards to their use of olfaction in prey detection (Dawley, 1994). For example, Shinn and Dole (1978) demonstrated that leopard frogs, *Rana pipiens*, use olfactory cues in feeding. Electrophysiological studies using the channel catfish, *Ictalurus punctatus*, demonstrated olfactory responses to amino acids (Caprio and Byrd, 1984). Catfish have olfactory receptors for acidic, basic, and neutral amino acids. Biochemical studies of odorant recognition in rainbow trout, *Salmo gairdneri*,

provided evidence for sites binding several amino acids (Rhein and Cagan, 1983). Manteifel and Reshetnikov (2002) demonstrated that fish, presumably by chemical detection, would reject noxious tadpoles.

Salamander Feeding Behavior

Active foraging appears to be more common among salamanders than frogs, which are typically sit-and-wait predators (Duellman and Trueb, 1986). For example, the red-backed salamander, *Plethodon cinereus*, has been observed foraging during winter months (Christman and Finkler, 2000). Optimal foraging theory predicts that if salamanders have the ability to rate prey types based on their profitability, they should focus, or specialize, on the most profitable prey (Jaeger and Rubin, 1982). Optimal foraging theory also predicts that individuals will alter their foraging habits depending upon profitability, abundance, and heritable foraging behaviors (Gibbons et al., 2005). Uiblein et al. (1995) demonstrated that larval Pyrenean mountain newts, *Euproctus asper*, chose to forage in areas where prey density was highest. A particularly fascinating discovery resulted from observing the cave salamander, *Eurycea spelaea*, ingesting bat guano (Fenolio et al., 2006). Analysis of the bat droppings found guano to be a “comparable food source” to invertebrate prey.

Himstedt et al. (1978) studied feeding behavior in terrestrial fire salamanders, *Salamandra salamandra*, and found that if an odiferous stimulus

was associated with a visual stimulus, preference would change. Ultimately the study demonstrated that vision played a greater role in prey detection than olfaction.

Steps in Salamander Feeding Behavior

The following sequence of behaviors has been observed with salamanders and newts when finding and consuming prey items (Roth, 1987)*:

1. Orientation movement – turning of the head towards prey when first noticing a food source
2. Approach – moving towards prey a item
3. Olfactory test – sniffing of prey item to check if palatable; nostrils practically touch food source
4. Fixation – getting positioned to eat
5. Snapping – biting the food item

* Any step has the potential to be duplicated or neglected in the sequence. Feeding can sometimes take place so quickly that no steps can be observed other than the final one, snapping. There is also variation between species and between larval versus adult forms.

Study Animals

Coastal giant salamanders, *Dicamptodon tenebrosus* (Baird and Girard, 1852), were used in this study because they were easily located close to the

Humboldt State University campus. They can be found in both gilled (larval and neotenic) and terrestrial forms (Petranka, 1998), and are the largest salamander in the Pacific Northwest. Terrestrial adults can grow to be 15 cm snout-vent length (Stebbins, 1985). Larvae are generally less than 5.5 cm snout-vent length (Figure 1). Coastal giant salamanders are found in or near semi-permanent to permanent streams in mesic forests (Petranka, 1998; Figure 2). Their distribution ranges from southern Sonoma County, California north to southwestern British Colombia.

Large terrestrial individuals eat a variety of prey including terrestrial invertebrates, small rodents, lizards, and smaller salamanders (Petranka, 1998). Aquatic larvae eat a large variety and size range of prey, the bulk of which is made up of aquatic insects (Parker, 1994). As larvae grow, prey size tends to get larger.

Although there have been many studies involving salamander feeding behavior, few investigate specific chemical cues. This work will contribute to the current body of knowledge since very little is known about chemoreception in larval salamanders (Kats, 1988). To date, no formal study has been done investigating a larval salamander's ability to detect, locate and assess foods using chemical senses alone.

Research Questions

The focus of this project was the use of chemoreception in the feeding behavior of larval coastal giant salamanders, *Dicamptodon tenebrosus*. I investigated potential chemical cues used by seven individuals to detect, locate and assess food in a laboratory setting. Firstly, are larval coastal giant salamanders using chemical cues to detect, locate and assess potential food items? And secondly, if they do use chemical cues to detect potential food items will they show a different response among stimuli if they?

Stimuli were presented in agar "morsels" at the end of one arm within a Y-shaped test station. Stimuli used in this study were as follows: bologna, agar blank (no additional stimulus added), guano, lipid component of California black worms (*Lumbriculus variegatus*), cod liver oil, salt, and whole California black worms (*Lumbriculus variegatus*). Subjects were examined for all steps in salamander feeding behavior outlined on page 21: orientation, approach, olfactory test, fixation and snapping.

Figure 1 Larval coastal giant salamander recently taken into captivity

Figure 2 Larval coastal giant salamander habitat in the Arcata Community Forest.

MATERIALS AND METHODS

Research Animals

Coastal giant salamanders (*Dicamptodon tenebrosus*) were used in this study. Seven coastal giants were collected from within a 1-kilometer radius of Humboldt State University in Arcata, CA (Fish and Game Scientific Collecting Permit SC-007597). All subjects were collected during May, June, and July of 2005. Animals were held in captivity in accordance with and consent from the Institutional Animal Care and Use Committee (IACUC Permit 04/05.B.01-A). Each animal was housed individually in a transparent plastic container (17.5 cm x 12.5 cm x 6.5 cm) filled with 650 mL filtered, dechlorinated water (Figure 3). Aquarium plants (Penn Plax) were added to the enclosures to provide hiding places, theoretically reducing stress. Individuals were fed approximately 10 mL of California black worms (*Lumbriculus variegatus*) every other day. All animals were inspected daily for health, activity level, and variations in behavior.

Acclimation and Conditioning Trials

Acclimation and conditioning trials began in November 2005 and continued through March 2006. Animals were placed in the test station during regular feeding sessions (every other day). Water did not flow into the test station during acclimation or conditioning trials. Each was fed for the first week in an

Figure 3 Larval coastal giant salamander in captivity during the time of data collection.

isolated portion of the test station in order for it to become acclimated to the testing apparatus (Figure 4). Each week individuals were given more access to explore the test station and were fed only in the designated area, the end of either arm. Eventually, salamanders were given access to the entire test station and fed at the end of a randomly chosen arm. Salamanders were given approximately 10 mL of California black worms (*Lumbriculus variegatus*) as a reward once they reached the designated feeding area. Individuals were given 10 minutes to reach the designated area and obtain a reward, thus conditioning them to explore the test station. The conditioning trial was terminated once the reward was obtained and the individual would then be returned to its enclosure. If the salamander did not reach the area within the allowable time frame, it was returned to their enclosure without reward.

Data Collecting Trials

All tests were run between 7:00 and 22:00 using seven individuals (DT501, DT500, DT504, DT505, DT508, and DT509). All individuals were acclimated to laboratory conditions and the presence of researchers prior to experimentation. Data collection trials took place beginning March 11, 2006 and concluded on March 19, 2006. All subjects were healthy and consistent in eating habits (feeding occurred every 48 hours). The average snout-vent length of individuals used was 7.9 cm (SD ± 0.18 cm) at the time data was collected.

Seven stimuli (including agar blanks) were utilized in this study. Each salamander was tested three times on each. In preparation for data collection, the order of the stimuli to be tested was first randomly chosen by blindly choosing stimulus names from a paper bag. Then, the order in which the salamanders would be tested was decided in the same manner. The final randomly chosen variable was the side on which the stimulus morsel would be placed, the left or right arm of the test station. This was done by flipping a coin. Control morsels were assigned to the opposite side.

The test station was created in a Y-shape maze design (Figure 4) using sheets of 0.236 inch black colored and clear cast acrylic from McMaster-Carr (Los Angeles, CA). The test station measured 61.0 cm wide, 71.8 cm long, and 14.6 cm deep. The primary leg of the Y-maze was designated the "neutral chamber" and each blind alley an "arm." The neutral chamber measured 10.2 cm wide and 12.1 cm long. The door of the neutral chamber was fashioned from clear cast acrylic (see above). Arms of the test station were 10.2 cm wide and approximately 41.0 cm long. A strip of orange tape, "initial choice tape," was positioned in both right and left arms 13.0 cm from the neutral chamber gate. The arm or stimulus was deemed chosen by a salamander when its snout crossed this tape.

At the beginning of each test run, the test station was placed on the floor with the draining (neutral chamber) end adjacent to the floor drain (Figure 5).

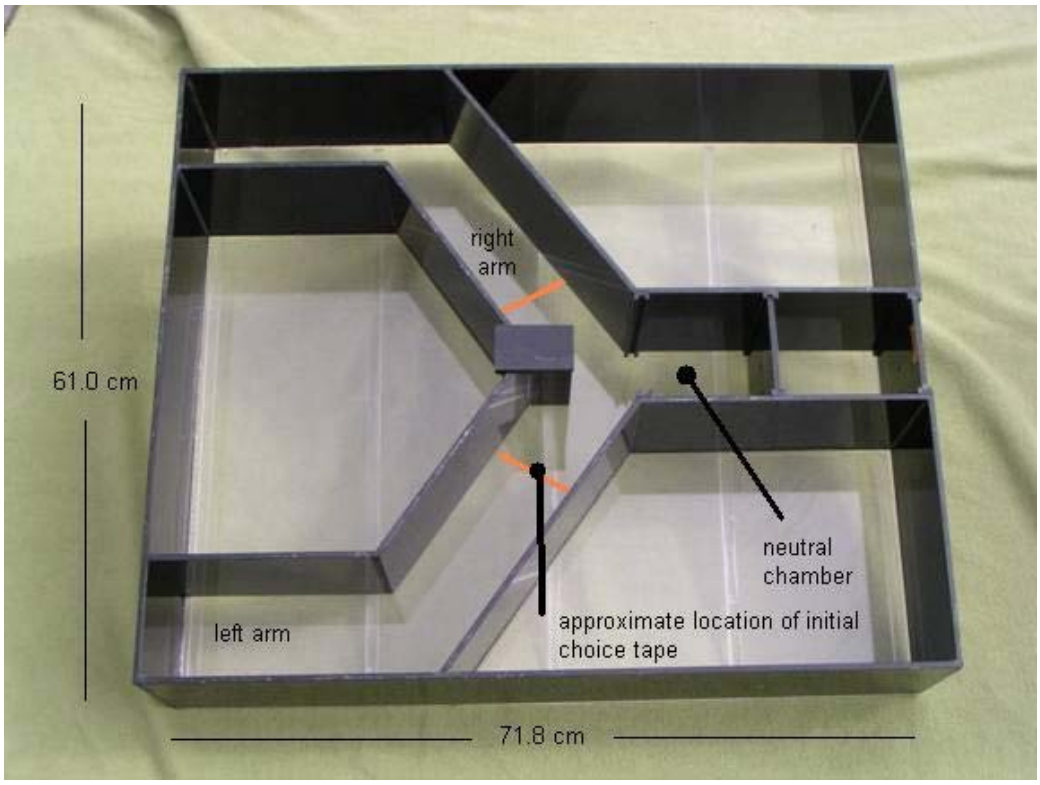

Figure 4 Test station (with door to the neutral chamber removed) used in conditioning and data collecting trials. The chamber to the right of the neutral chamber acted only as a water overflow reservoir. It had no formal function.

Already in place was a 20 gallon tub of filtered, dechlorinated ambient water (17.8^{o}C), located on the opposite side of the test station. The tub was in the same location for each trial. Water flow meters (KOBOLD; Pittsburgh, PA; part KFR-1220V2; Figure 6) were then positioned, the tubing from each extending towards designated arms of the test station. Lastly, waterfall boxes (Figure 6), which help insure a horizontally even flow of water into the test station, were inserted at the end of each arm and connected to the tubing coming from its corresponding water flow meter. Prior to placement, waterfall boxes were draped with netting from an

Figure 5 Experimental setup during data collecting trials.

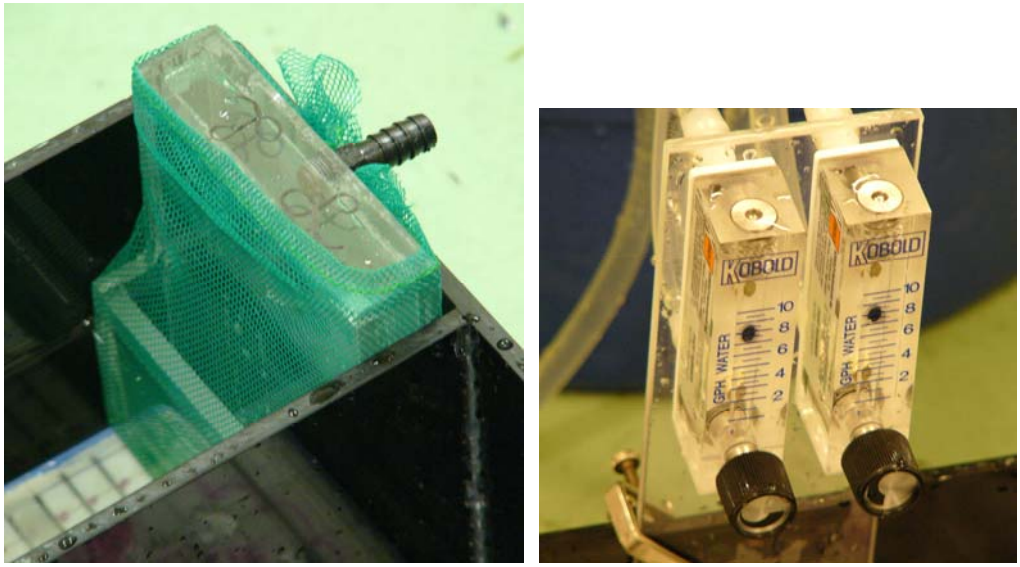

Figure 6 Waterfall box (left) covered in netting to keep salamanders from climbing into it and water flow meters (right).

8 inch fishing net (Penn Plax, Inc.; Hauppauge, N.Y.). This insured that salamanders would not climb into the waterfall box during trials.

Salamanders were introduced to the neutral chamber of the test station just prior to the beginning of the trial. The flow of water was then initiated (approximately 0.44 L per minute into each arm) and morsels put into place.

Stimulus and control morsels were placed against the waterfall in the assigned arm. Blinders were positioned on either side of the test station so that the animal could not see, and be influenced by, other persons in the room (Figure 5). The gate was removed when the test subject appeared calm; two to ten seconds from the time the animal was placed in the neutral chamber. The trial began when the gate was removed and the animal was allowed to explore the test station. Salamanders were allowed to freely explore the test station throughout the ten minute trial period.

The individual's behavior was recorded using a hand-held digital camcorder (Panasonic, model PV-GS32) throughout a ten-minute test interval, during which water flowed through the waterfalls and into each arm of the test station. The rate at which water was flowing into the test station was monitored during the test interval to insure even flow into each arm. Water drained from the opposite end, the neutral chamber, into the floor drain (Figure 5).

Sterilization

Between each test run, all supplies that may have come in contact with stimuli (test station, door to neutral chamber, both waterfall boxes, all netting, rubber bands, and weights) were sterilized using the following protocol:

1) all surfaces sprayed with 10% bleach solution

2) all surfaces rinsed with hot water
3) all surfaces sprayed with 10% vinegar solution
4) all surfaces rinsed with hot water
5) all surfaces rinsed with cold water

Data Collected

To review each trial easily, taped footage was transferred to DVDs using PowerDVD (version 4.0, copyright 1997-2003, CyberLink Corp., www.cyberlink.com). Measurements were tabulated while reviewing video footage. Data collected were devised with reference to the basic steps of salamander feeding behavior outlined by Roth (1987), as given in Table 1 below. Several measurements provided evidence for both orientation and approach. Because the fourth step, fixation, would be difficult to verify, it was not examined in this study. The following data were collected during the ten minute trials (Appendix A):

Investigation of Test Chamber & Initial Arm Chosen

Individuals were considered "investigating" the test station once they left the neutral chamber and their snout crossed the orange initial choice tape in either left or right arms. The initial arm chosen was noted as left or right.

Time Spent on Stimulus Side

Time from when an individual entered the arm containing the stimulus morsel to the time the salamander exited the same arm. An individual entered the arm when its snout crossed the orange initial choice tape (heading towards the morsel). Exiting occurred when the salamander's snout passed outside the orange initial choice tape in the opposite direction (heading towards the neutral chamber). It had a maximum time of 600 seconds, the length of the trial.

Table 1 Steps in feeding behavior and supporting data

Step	Feeding Behavior	Data Supporting Occurrence
1	Orientation Movement	initial stimulus side chosen, time spent in arm housing stimuli, distance from stimulus, time to reach stimulus, sniffing (nosing)
2	Approach	initial stimulus side chosen, time spent in arm housing stimuli, distance from stimulus, time to reach stimulus, sniffing (nosing)
3	Olfactory Test	snout touching
4	Fixation	assumed occurring if snapping behavior is observed
5	Snapping	bites

Average Distance from Stimulus

Distance (centimeters) salamanders positioned themselves (based upon snout location) from a stimulus morsel. This was noted at one minute intervals. The resulting eleven measurements of distance were averaged.

Time to Reach Stimulus

Time (seconds) it took a salamander (based upon snout location) to come in contact with, or parallel to, a stimulus morsel. If an individual never reached the stimulus, the time was noted as 600 seconds.

Nosing (Sniffing)

Notation of whether or not nosing, or sniffing, occurred. Nosing, or sniffing, was defined as an obvious lowering of the salamander's head and snout touching, or almost touching, the floor of the test station, and moving the head from side to side.

Snout Touches

Notation of whether or not snout touching occurred. Snout touching was noted if a salamander appeared to deliberately touch its snout to a stimulus morsel during a trial. Snout touching was not noted when individuals darted down an arm and bumped into a stimulus morsel; these observations were not considered snout touches.

Bites

Notation of whether or not an individual took a bite of a stimulus morsel. Biting was recorded when a salamander took a bite of the morsel.

Analyses

Contingency tables, multinomial goodness of fit, and repeated measure ANOVA were analyzed using the statistical program Number Cruncher Statistical System, NCSS 2004 (NCSS 2004 and PASS Trial; December 12, 2005; Copyright 2005; Kaysville, UT; www.ncss.com). Contingency test data resulting in expected frequencies of less than five were analyzed using Monte Carlo simulations with StatXact 6 (Cytel Software Corporation; Cambridge, MA, copyright 2004). Differences were considered significant when the p-value was 0.05 or less (Appendix A).

Morsel Preparation

Stimuli tested in this experiment were the following: beef bologna, guano, lipid portion of California black worms (*Lumbriculus variegatus*), cod liver oil, salt, and whole California black worms (*Lumbriculus variegatus*) (Figure 7). Agar blanks were included among my trials because I wanted to test for a difference in preference between sides (left or right) of the test chamber and provide a baseline for expected behavior during trials.

Agar Mixture

The following agar mixture was used in making all morsels. The agar mixture was prepared by combining 3.75 g of technical grade agar (Difco; Lawrence, KS) with 250 mL of filtered, dechlorinated water. The mixture was then heated in a microwave (Daewoo Electronics America Inc.; Doral, FL; model KOR-6115, 600 watts) for approximately 4 minutes or until gently boiling. All 250 mL of the agar mixture was used in each morsel batch.

Control and Agar Blank Morsels

The control and agar blank morsel mixtures were prepared by combining 105 mL of filtered, dechlorinated water and heated agar mixture, detailed above, in a blender (Hamilton Beach/Proctor-Silex, Inc., Canada; model 58130). This final mixture was poured into a standard ice-cube tray and allowed to solidify at room temperature or stored in a refrigerator to be used within 48 hours.

Worm Morsels

Worm morsels were prepared by first draining water from a small amount of live California black worms (*Lumbriculus variegatus*) purchased from Balanced Aquarium in Arcata, CA. Then 89 cc of the worms were blended with 16 mL filtered, dechlorinated water for 3 minutes, or until liquefied. The heated agar mixture was then added to the worm mixture and blended until combined.

This final mixture was poured into a standard ice-cube tray and allowed to solidify at room temperature or stored in a refrigerator to be used within 48 hours.

Bologna Morsels

Bologna morsels were prepared with beef bologna (Oscar Mayer; Kraft Foods, Inc.). Three slices of bologna, 88 cc (84 grams: 264 calories, 0.99 grams sodium, 24.3 grams fat, 9.3 grams protein), were blended with 17 mL of filtered, dechlorinated water. The heated agar mixture was then added to the bologna mixture and blended until combined. This final mixture was poured into a standard ice-cube tray and allowed to solidify at room temperature or stored in a refrigerator to be used within 48 hours.

Guano Morsels

Guano morsels were prepared with guano (Sparetime Organics Nitrogen Bat Guano, Northern California). Ten grams of guano was blended with 95 mL of filtered, dechlorinated water. The heated agar mixture was then added to the guano mixture and blended until combined. This final mixture was poured into a standard ice-cube tray and allowed to solidify at room temperature or stored in a refrigerator to be used within 48 hours.

Cod Liver Oil Morsels

Oil morsels were made with cod liver oil (Twinlab; American Fork, UT). 89 mL of oil was blended with 16 mL filtered, dechlorinated water. The heated

agar mixture was then added to the cod liver oil mixture and blended until combined. This final mixture was poured into a standard ice-cube tray and allowed to solidify at room temperature or stored in a refrigerator to be used within 48 hours.

Salt Morsels

Salt morsels were made from plain non-iodized salt (Safeway, Inc.). 1.25 grams of salt was blended with 105 mL of filtered, dechlorinated water. The heated agar mixture was then added to the salt mixture and blended until combined. This final mixture was poured into a standard ice-cube tray and allowed to solidify at room temperature or stored in a refrigerator to be used within 48 hours.

Lipid Morsels

Forty mL of lipid extract (see below) was blended with 65 mL of filtered, dechlorinated water. The heated agar mixture was then added to the lipid mixture and blended until combined. This final mixture was poured into a standard ice-cube tray and allowed to solidify at room temperature or stored in a refrigerator to be used within 48 hours.

Lipid Extract: The lipid extraction was taken from California black worms (*Lumbriculus variegatus*) purchased from a local pet store (Balanced Aquarium; Arcata, CA). Water was first drained from a small amount of worms.

Figure 7 Stimuli and stimulus morsels in various stages of preparation. Spiraling clockwise and inward from the left: guano morsels in tray, control/agar morsels in tray, lipid extract of worm morsels, fish oil morsels, control/agar morsels, agar in powder form, bologna morsels, worm morsels, guano morsels, guano in powdered form, live worms (*Lumbriculus variegatus*) in water.

Eighty-nine cc of worms was then blended with 15 mL filtered, dechlorinated water, and transferred to a separatory funnel containing 150 mL distilled water. The funnel was then placed in a ring stand. A long-stem glass funnel lined with Whatman #1 filter paper was situated in a round-bottom flask. Three-four grams of sodium sulfate was placed on the filter paper. Fifty mL of methylene chloride was added to the separatory funnel containing the worm mixture and allowed to sit for one minute.

The mixture was gently shaken, periodically releasing pressure, several times. The pressure was released one last time and the funnel returned to the ring stand. The mixture was allowed to sit for approximately 15 minutes. After this time, the methylene chloride portion of the solution (on the bottom) was poured through the long stem funnel holding sodium sulfate and collected in the flask below. Sodium sulfate removed any excess water from the solution as it passed from the separatory funnel and into the round bottom flask. The previous steps, beginning with the shaking, were repeated two more times for a total of three repetitions in all.

A rotovaporator (Büchi Rotovapor-R; Rinco Instrument Company, Inc., Greenville, IL) was used to separate the lipid component of the worm mixture from the methylene chloride solution. After accelerated evaporation, the lipid component of the worm mixture remained in the flask. The flask was then removed from the rotovaporator and allowed to cool. Once at room temperature, the lipid residue was suspended in 6 mL of 100% ethanol, swirled, and an additional 114 mL of filtered, dechlorinated water added. The final lipid extract mixture was then swirled and placed aside for use in preparing the lipid morsels as described above.

Morsel Packaging

Each morsel was prepared using two cubes sliced in half and placed in a bag fashioned from a three inch aquarium net (Tetra) along with two ¾ ounce

tungsten weights (Excalibur; Fort Smith, AZ), and secured with a small rubber band (Figure 8). Tungsten weights were chosen because they would not harm salamanders. The size of each morsel was approximately 224 cm^3. The height of each, including the extra netting at the apex, was approximately 7 cm. Morsels were soft in consistency, and although covered in netting, easy to bite into. All morsels were brought to room temperature before being used in a data collection trial.

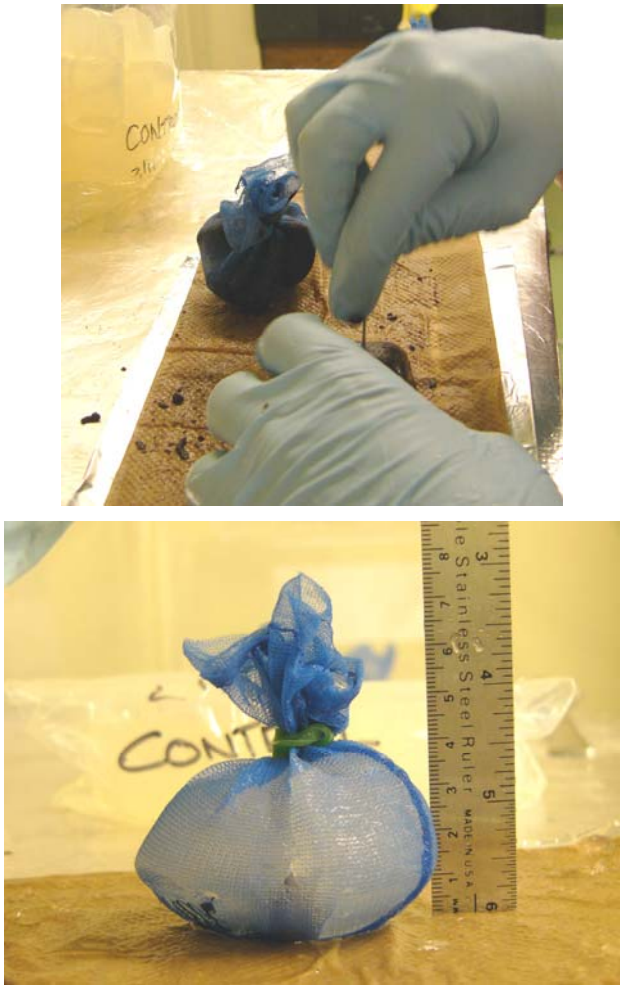

Figure 8 Guano stimulus morsels in preparation (top) and control/agar blank morsel ready for use in a test trial (bottom).

RESULTS

Anecdotal Trial Observations

In general, salamanders seemed to be fairly relaxed throughout all trials, as indicated by lack of darting from one arm to the next. However, salamanders were excellent climbers and escaped the test station on numerous occasions. Animals were replaced in the same end from which they originally escaped and the trial continued. When placed back in the test station, it was not uncommon for individuals to dart back and forth. Another sign of stress observed during trials was a full-body twitching behavior. This behavior was only observed in the presence of fish oil. The twitching would stop soon after being placed back in their enclosures. Subjects were also observed hiding underneath or behind stimulus morsels, which could have also been a sign of stress.

There were several trials during which salamanders were observed calmly remaining in one place, often in the neutral chamber, and would seem to be watching the camera person. There is a great deal of video footage showing this behavior. Although partitions were in place, salamanders could see the camera person and may have been able to sense the presence of another human on the right hand side of the test station. This was where the sink was located and much of the stimulus morsel preparation took place. Human activity behind this blind may have affected the behavioral outcome.

General Observations

Salamanders chose to venture from the neutral chamber and investigate the test station 74% of the time. Individuals investigating the test chamber chose the right arm slightly more often than the left, 62 versus 50 times, but this difference was not significant (multinomial goodness of fit, $p = 0.257$). Figure 9 shows significant variation among individual salamanders in the initial direction chosen: left, neither, or right (Monte Carlo, $p < 0.001$). In general, test subjects were wandering throughout the test station throughout trials. Individuals DT506 and DT509 went neither direction (did not explore the test station) a total of 26 times, approximately 18% of the total number of trials. When individuals DT506 and DT509 were removed and the data reanalyzed, a significant difference did not exist ($p = 0.554$).

Orientation and Approach

The most commonly observed steps in feeding behavior were orientation and approach. Analyses of initial general choice, initial stimulus side chosen, time spent in arm housing stimuli, distance from stimuli, time to reach stimulus, and sniffing (nosing) provided evidence for both orientation and approach.

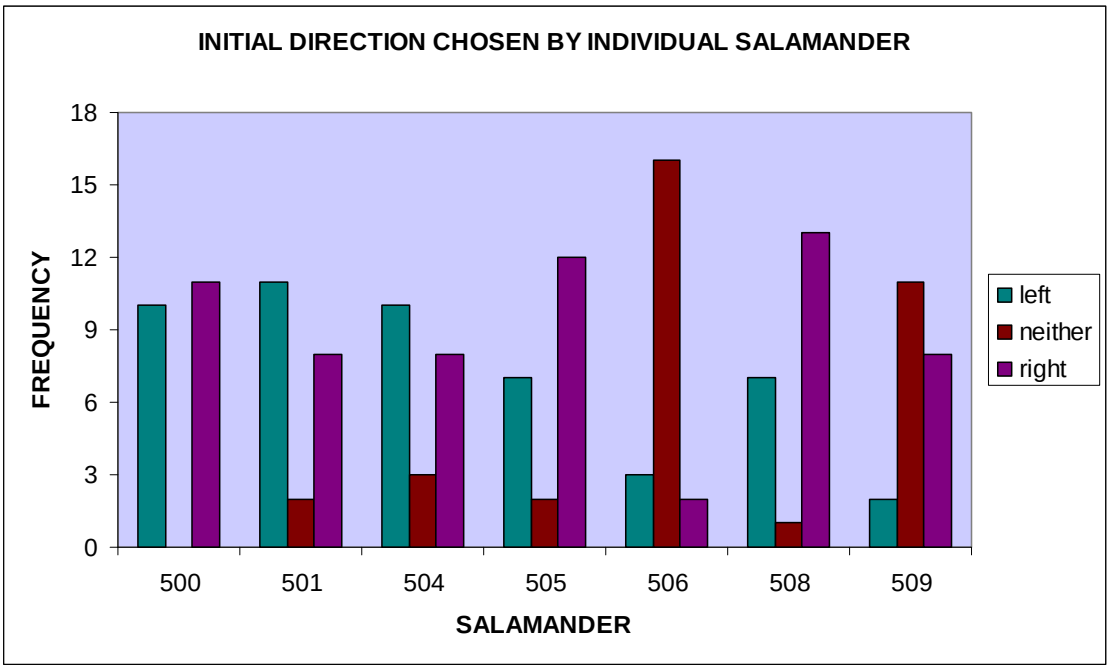

Figure 9 Number of trials (frequency) side chosen (left, neither, or right) by specific salamander (DT500, DT501, DT504, DT505, DT506, DT508, DT509). Note that DT506 and DT 509 chose not to investigate the chamber the majority of the time.

For the initial general choice made by salamanders for the stimulus or control side across all trials where a chemical stimulus was present (i.e. omitting trials with agar morsels on both sides, where no difference should exist), salamanders chose the stimulus arm 57 times and the control arm 39 times. This difference was not significant (multinomial goodness of fit test, $p = 0.066$). Table 2 illustrates variation in the initial choice salamanders made among the different stimuli. Whole worms versus control was the only stimulus showing a significant difference in side chosen, with stimulus preferred over control (multinomial goodness of fit, $p = 0.018$). There was an apparent trend for

salamanders to choose bologna and salt over control morsels, but a similar "choice" was made for randomly designated agar blanks (Table 2).

Nosing, or sniffing, was observed at least once during trials containing every stimulus, including agar blanks, other than oil and lipid (Figure 10). There was a statistically significant difference among stimuli in the frequency of nosing, or sniffing, behavior observed (Monte Carlo, $p = 0.001$).

There was no significant difference among stimuli in the amount of time (seconds) salamanders spent on a stimulus side (Figure 11; repeated measures ANOVA, $p = 0.144$).

Table 2 Multinomial goodness of fit testing significance of stimulus versus control. In the case of the agar blank, the "stimulus" side was randomly designated.

Stimulus	Frequencies			p-value
	stimulus	control	total	
Bologna	11	5	16	*p = 0.134*
Guano	7	9	16	*p = 0.617*
Lipid	8	9	17	*p = 0.808*
Oil	6	7	13	*p = 0.782*
Salt	11	5	16	*p = 0.134*
Worms	14	4	18	*p = 0.018*
			96	

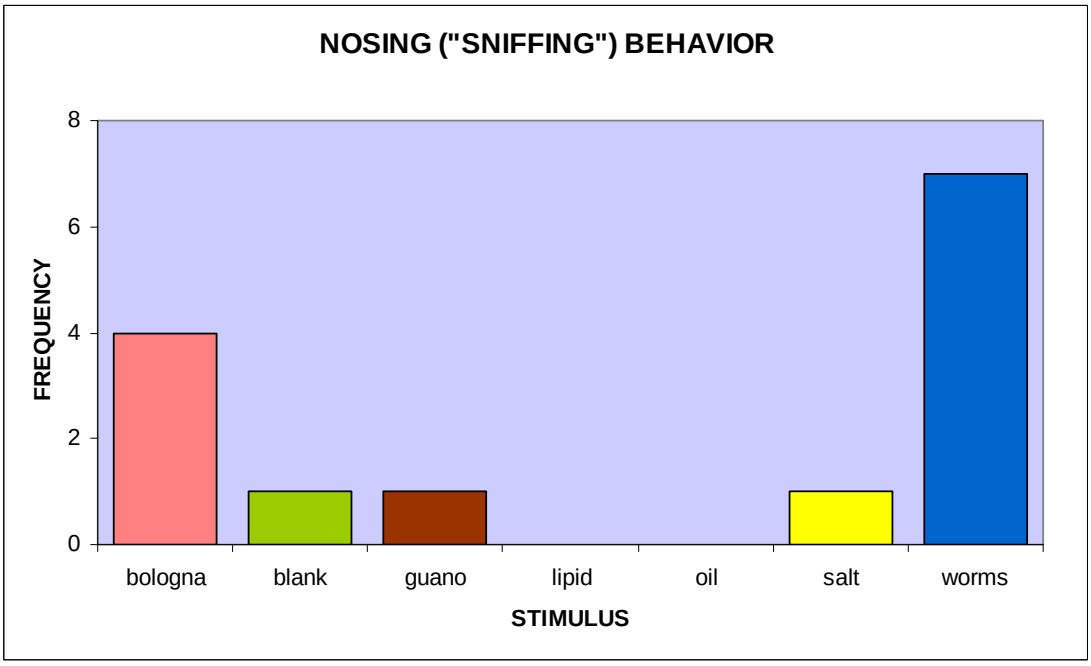

Figure 10 Number of trials (frequency) nosing, or sniffing, behavior observed

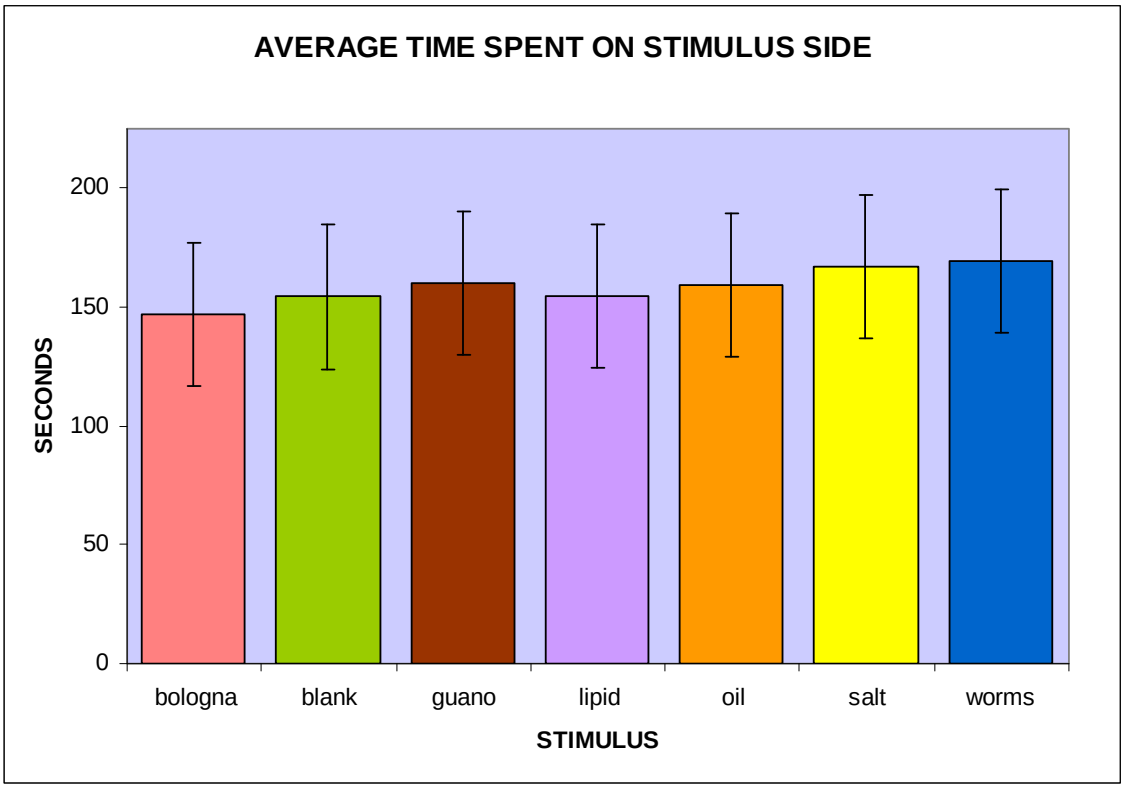

Figure 11 Average time (seconds) spent on stimulus side. Error bars represent the standard error of the mean, estimated across all stimuli (± 30.37 sec). In the case of the agar blank, the "stimulus" side was randomly designated.

There was a trend of salamanders spending their time closer to the agar blank, guano, bologna and whole worm morsels than other stimuli, but this trend was not significant (Figure 12; repeated measures ANOVA, $p = 0.114$).

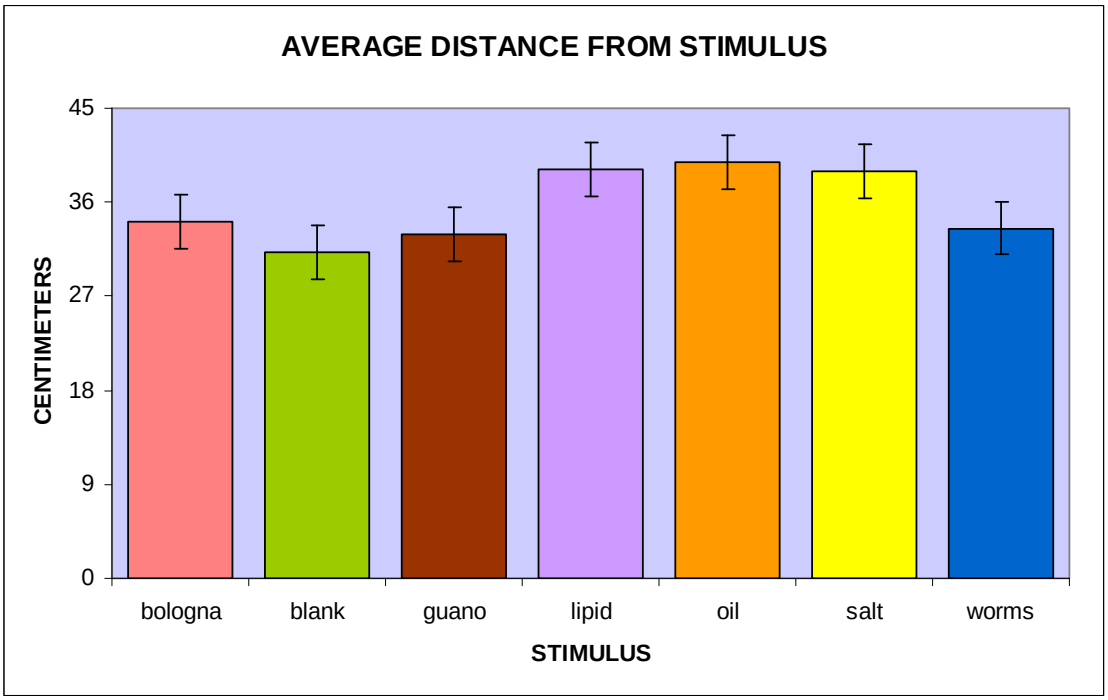

Figure 12 Average distance (centimeters) from a stimulus; error bars reflects the standard error of the mean, estimated across all stimuli (± 2.57 cm). In the case of the agar blank, the "stimulus" side was randomly designated.

Salamanders reached stimuli at different rates (Figure 13; repeated measures ANOVA, $p = 0.032$). It took the least amount of time to reach the agar blank, guano, worm, and bologna morsels and the longest amount of time to reach morsels containing lipid, oil, and salt stimuli.

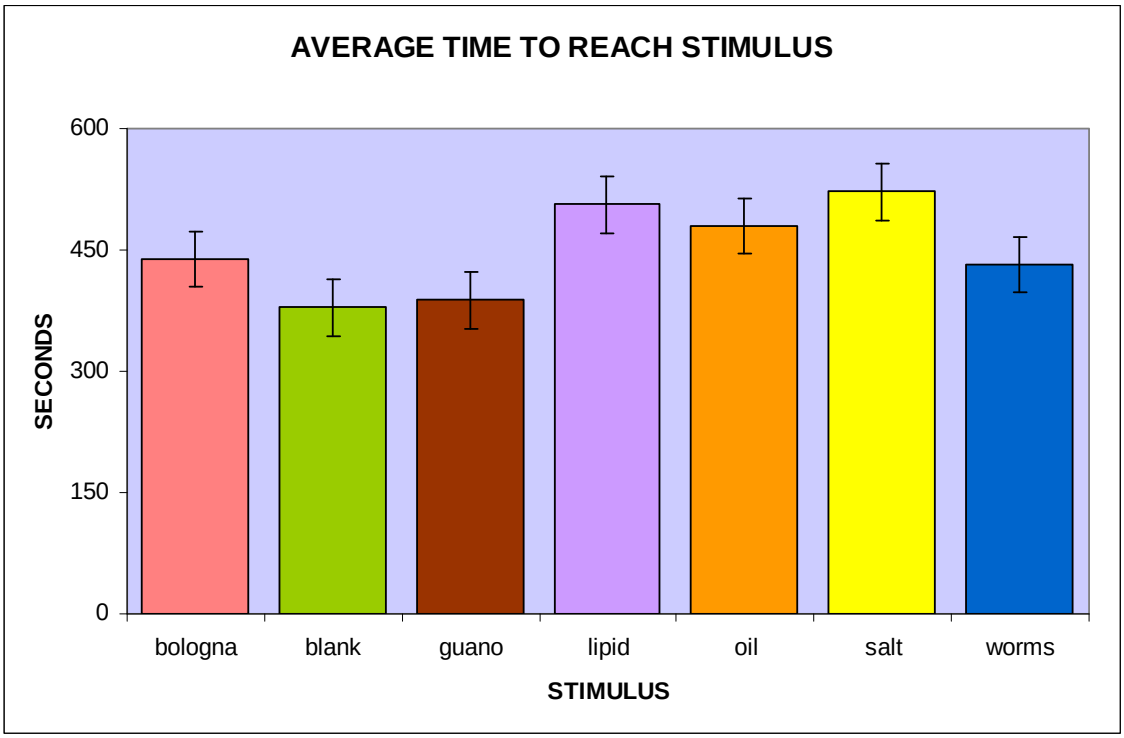

Figure 13 Average time (seconds) for salamanders to reach stimulus. Error bars represent the standard error of the mean, estimated across all stimuli (± 35.04 sec). In the case of the agar blank, the "stimulus" side was randomly designated.

There was a significant difference among individual salamanders in the time it took them to reach stimuli (Figure 14; repeated measures ANOVA, $p = 0.006$). This was likely due to the lack of movement by DT506 and DT509. When these individuals were removed from the analysis, no significant difference existed ($p = 0.066$).

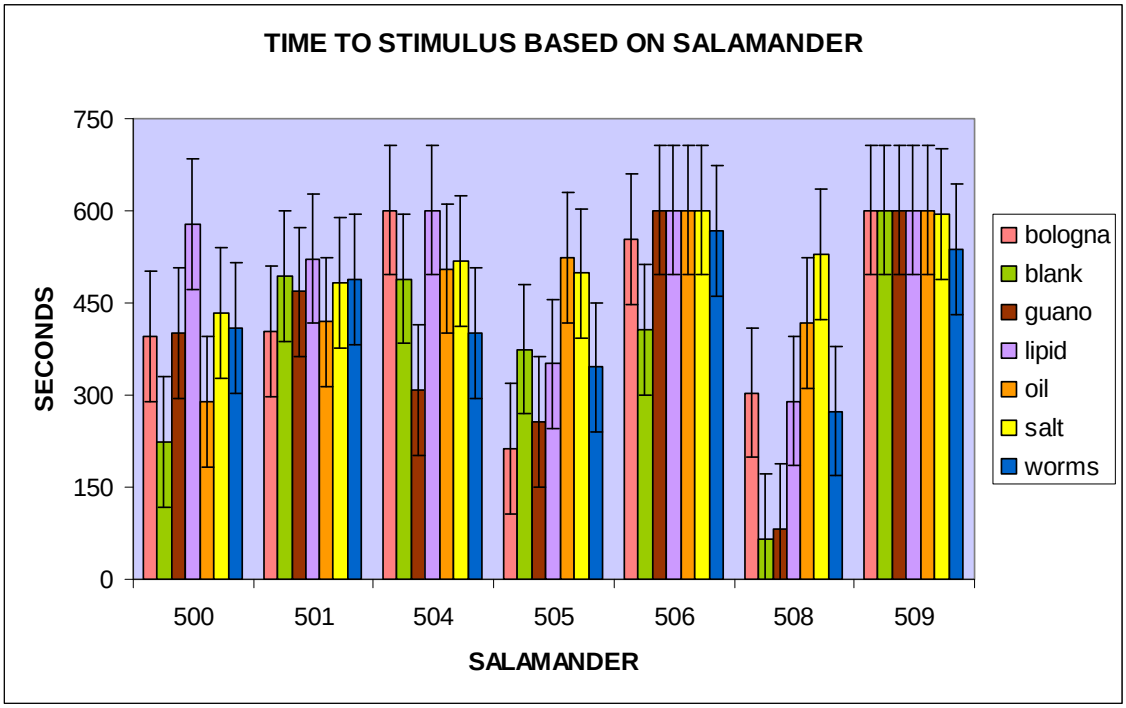

Figure 14 Time (seconds) for individual salamanders to reach a particular stimulus; error bars represent the standard error of the mean, estimated across all individuals and stimuli (± 105.80 sec). In the case of the agar blank, the "stimulus" side was randomly designated.

Olfactory Test

Evidence for the olfactory test was collected by measuring the frequency of trials during which snout touching, or tapping, was observed. Snout touching of the stimulus morsel was observed while individuals were in the presence of only four stimuli: bologna, guano, salt, and whole worm (Figure 15). There was a significant difference among stimuli in the number of snout touches observed (Monte Carlo, $p = 0.001$).

Fixation and Biting

If bites occur to a stimulus morsel, it can be assumed that the "fixation" step in salamander feeding behavior has taken place. Of the stimulus morsels presented, salamanders bit only two: bologna and whole worm, resulting in a significant difference among stimuli (Figure 16; Monte Carlo, $p = 0.001$).

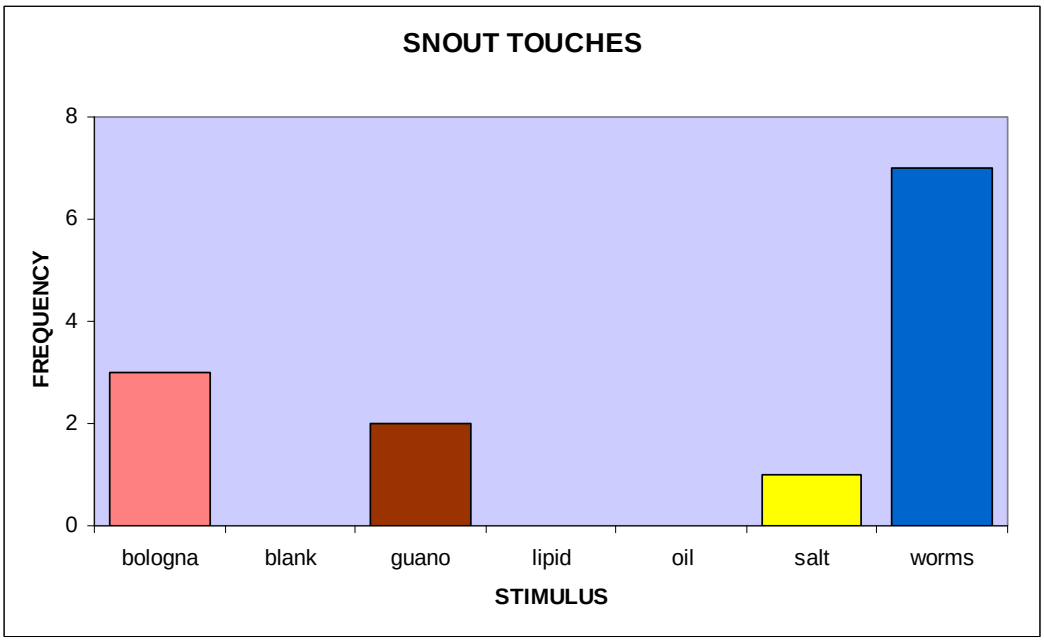

Figure 15 Number of trials (frequency) snout touches were observed

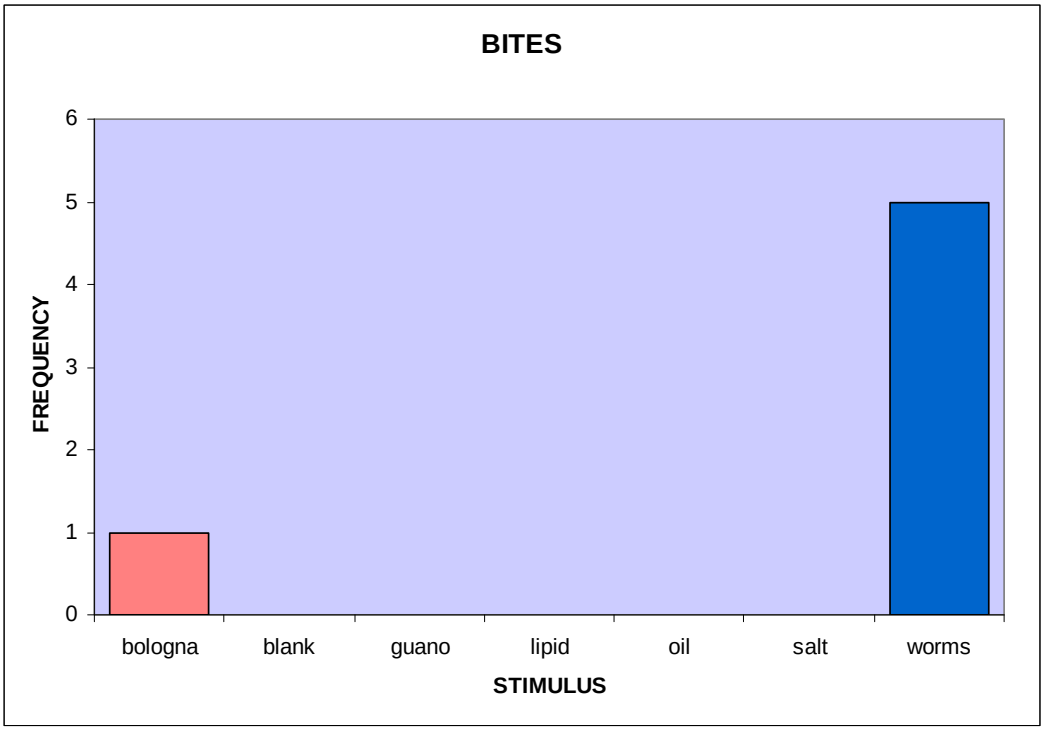

Figure 16 Number of trials (frequency) biting was observed

DISCUSSION

Salamanders appeared to detect chemical cues, use them to locate potential food sources, assess the item, and decide whether or not to ingest it. I was able to see a trend in stimulus choice. There were also several observations during my research which merit discussion as well as recommendations for future research.

Use of Chemical Cues to Detect and Locate Stimuli

There was substantial evidence that test subjects were using chemical cues to locate food sources. Statistically significant results were seen with initial stimulus side chosen (worm vs. control), nosing (sniffing), and the time to reach stimuli (Table 2; Figures 10 and 13). The use of chemical cues among salamanders to find a potential food source is not news. Several researchers have demonstrated this, including Nicholas (1922), Lindquist and Bachman (1982) and Durand et al. (1982).

Although not all species appear to utilize chemical senses in prey detection and location, it appears that aquatic coastal giants do. Durand et al. (1982) suggests that different species depend on chemical cues in prey detection depending on their habitat. For example, visual detection of prey in the cave-dwelling salamander, *Proteus anguinus*, would not be useful in its pitch black habitat. Durand et al. (1982) demonstrated in the same study *Necturus*

maculosus, a non cave-dwelling salamander, may not respond to chemical cues because it is adapted to a lighted environment. Aquatic coastal giants would benefit from well developed chemical senses during certain times of the year, such as the rainy season, when its habitat becomes clouded with sediment. Heavy sediment in the water would make visual detection of a potential food source virtually impossible. It would be advantageous for them to use their chemical senses as a tool in finding food during these times.

General Interest in Stimuli

Figure 17 summarizes the top four choices taken from each of the feeding behaviors observed regardless of overall statistical significance. The result of the agar blank in this graph provides a baseline for expected behavior during trials (denoted by the purple line). The results seen in guano and salt follow this expected behavior. The general interest in bologna and worm falls above the baseline, therefore showing a preference for bologna and whole worm stimuli. Because there was no interest in oil or lipid stimuli, and their results fall far below the expected, there is a possible aversion to these stimuli.

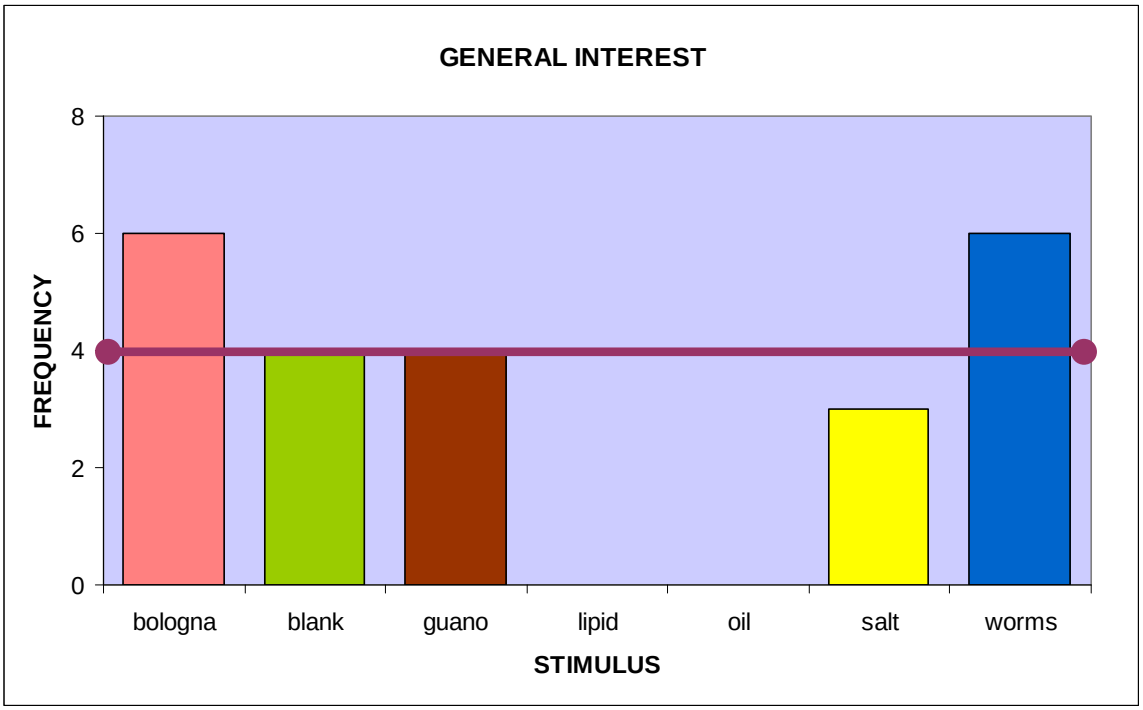

Figure 17 Number of tests (frequency) an interest (= test in which stimulus fell within top four choices) was shown to stimuli. The purple line represents the baseline or expected behavior (based on results of trials using agar blank)

Chemical Cues Used to Assess and Choose Stimuli

Ultimately, I was unable to show which specific chemical cues salamanders were using to assess their food choices, but I was able to provide evidence of basic food preference based upon chemical detection alone. A genuine interest in a potential food source is apparent if an animal decides to chemically sample, or snout touch, the potential food item. Salamanders were genuinely interested in four stimuli: bologna, guano, salt, and whole worms

(Figure 18). The snout touch, or chemical sampling, is the point at which Heatwole and Dawley (1998) suggests a salamander will make the final choice whether or not to ingest, or bite, a food item. Salamanders chose to bite only two stimuli, bologna and whole worms (Figure 16). Aquatic coastal giants seem to prefer bologna and whole worms.

Use of Agar to House Stimuli

Using agar to house stimuli was a novel technique. Not reflected in the results, an agar control morsel was sampled (bitten) in one trial. Therefore salamanders may be attracted to the agar (a carbohydrate) as a food source. Additional trials would be necessary to determine its effectiveness as a blank substrate in which to house a stimulus. A series of tests investigating the interest in agar versus nothing, a true blank, should be run. A true blank that would work well in this case would be the netting used to house the other morsels balled up to the approximate size of the other morsels and wrapped in another piece of netting.

Trials involving agar blank versus agar control do provide information regarding the initial choice between left and right sides (Figure 18). It appears that individuals may be choosing the right side more often than the left but there is not a statistically significant difference (multinomial goodness of fit, $p = 0.134$). Ultimately, trials involving agar blank and agar control morsels provide a behavioral baseline with which to compare other stimuli.

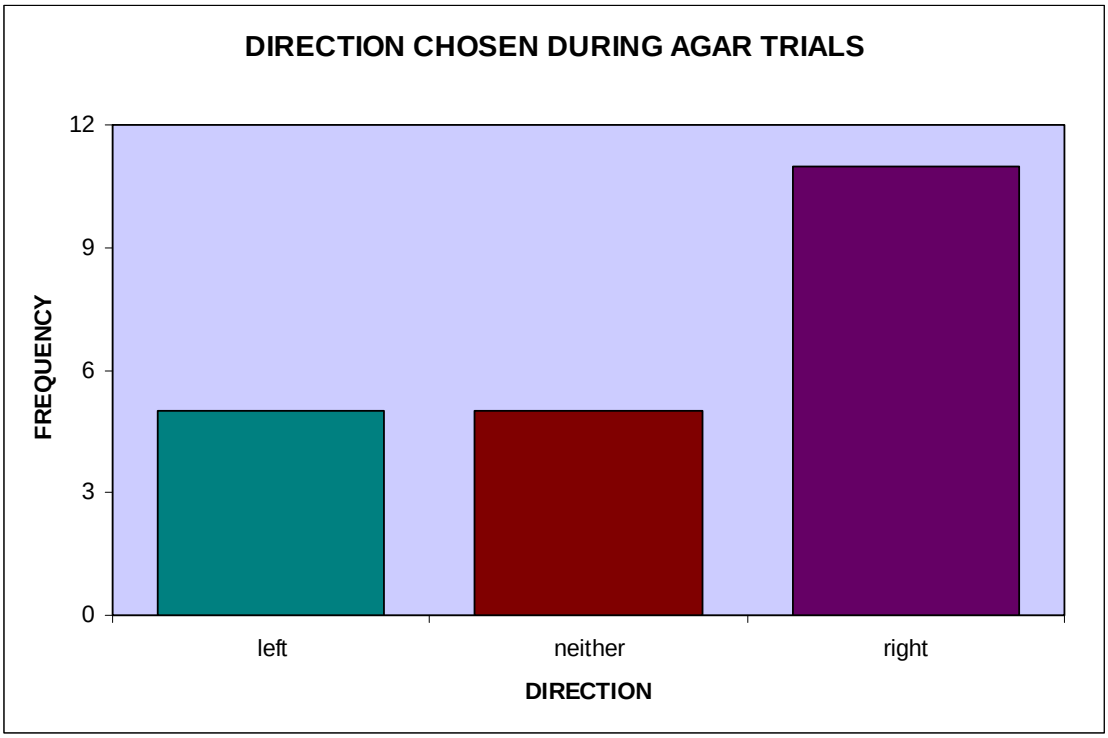

Figure 18 Number of trials (frequency) of the initial direction chosen during agar blank versus agar control trials

Avoidance Behavior

Webster's Student Dictionary defines avoidance as keeping "away or at a distance from" (Landau, 2002). With this in mind, individuals avoiding a particular stimulus would choose to "distance" themselves by either remaining in the neutral chamber or choosing the arm containing the control morsel.

Individuals may have avoided the lipid component of CA black worms and cod liver oil. Because the general interest for these two stimuli fell far below the expect behavior seen in the agar blanks, there may be a possible aversion to these stimuli (Figure 17). Individuals favored least of all, or avoided, these

stimuli throughout all variables analyzed. Individuals were never seen to nose/sniff (Figure 10), spent little time near (Figure 11), chose to stay farther away (Figure 12), took quite a bit of time to reach (Figure 13), and chose to neither snout touch nor bite morsels containing either stimulus (Figures 15 and 16).

Salamanders may have been avoiding the lipid component of CA black worm morsels because of potential residual chemicals, ethanol or methylene chloride, from the extraction process. Salamanders may have avoided cod liver oil morsels for a couple of reasons. It could have been because of a potential predator-prey reaction. Because many fish prey upon salamanders, individuals may have interpreted the presence of fish oil as a risk, hence avoiding it (Cupp, 2001; Hickman, 2004; Kats, 1988; Petranka, 1987). On the other hand, several individuals were observed twitching during cod liver oil trails. The twitching behavior stopped soon after being placed back in their enclosures. Individuals may have avoided the cod liver oil because it was irritating to them. I found the oil morsels to be incredibly pungent. Although I am unable to say for certain that they would be as pungent to an aquatic salamander, I suspect that the concentration used may have been too strong.

Thoughts to Consider

In future food preference studies I recommend that other researchers evaluate several behavioral observations. Feeding behavior is not a simple one-step process. There are a number of steps involved in feeding behavior. Lindquist and Bachmann (1982) included several steps of feeding behavior in their research. The series of steps I chose to evaluate in my research was a simplified sequence noted by Roth (1987). Measuring only one step in feeding behavior, for example bites, provides limited information. An evaluation of several steps in feeding results in a more accurate picture of interest (Appendix B). For example, if I had only looked at initial choice, it would seem that animals were avoiding guano. But, because I measured several steps in feeding behavior I was able to see that individuals may have had an interest in the guano morsels overall and I should therefore consider guano in further investigations.

The concentration of stimuli used must also be considered. Inappropriate concentrations may have been used. This could have been remedied by offering a series of lesser to greater concentrations during test runs and measuring the resulting behavior associated with each. The current concentrations may not have been odoriferous enough for the salamanders to locate them. Although they may have smelled strong to the experimenter, they may not have been desirable to the salamanders. On the other hand, some may have been too strong (e.g. cod liver

oil), thus repelling hungry test subjects. I suspect the concentration was much too strong because several individuals exhibited a twitching behavior, suggesting distress, or agitation, in its presence.

As with many behavioral studies, a painfully small sample size was used. The small sample size used could have been offset by a larger number of replicates, but time did not permit. For example, the distance spent from a particular stimulus may have been found statistically significant if more repetitions had been performed (Figure 12). Both a larger sample size and additional replicates would be ideal.

It would have been interesting to examine the feeding behavior in both dark and light conditions. Previous research using terrestrial fire salamanders, *Salamandra salamandra*, demonstrated that individuals reacted differently to immobile prey differently in light and darkness (Luthardt and Roth, 1983; Roth, 1987). In lighted conditions salamanders did not respond to immobile prey, but in darkness they would respond to the same immobile prey. Uiblein (1992) demonstrated a similar behavior in the aquatic larvae of the Pyrenean salamander, *Euproctus asper*. Although my study provided evidence of aquatic coastal giants using chemical cues to detect, locate and assess potential food items, they may have responded more strongly to stimuli under dark conditions, and better able to illustrate stimulus preference.

The affinity for worms may have been as a result of previous prey experience. Luthardt-Laimer and Roth (1983) suggest that an "imprinting" of a specific prey type may occur within the first few weeks of metamorphosis. After capture, individuals were fed solely upon *Lumbriculus variegatus*. If "imprinting" of prey type does occur, and this may occur before metamorphosis as well, then it is not surprising that they showed a preference for California whole worm morsels. I may have inadvertently conditioned them to look for worms. It would have been interesting, and beneficial to my study, to provide research subjects with a variety of food sources prior to data collection so that if there is some type of prey "imprinting," it would not influence their choice. Testing individuals with only novel food choices may have been interesting.

It may have also been beneficial to allow research subjects the opportunity to sense, assess, and sample all stimuli prior to the study in order for them to learn which would be the most desirable. Jaeger and Rubin (1982) found that red-backed salamanders, *Plethodon cinereus*, learned to forage more efficiently through experience and this learning process was important when measuring the caloric value of items consumed. Additional researchers found that learning, which takes place at various ages, is very important in successful foraging behavior and how well individuals forage may also be hereditary (Gibbons et al., 2005).

All trials took place over a period of only nine days. This may have been too many trials in too short a period of time for salamanders and could have

resulted in stressed individuals, which could ultimately affect their behavior. Although I am uncertain that stress would influence choice across stimuli, the possibility merits mention. For both scientific and ethical reasons, every effort should be taken to limit the amount of stress experienced by test subjects. A certain amount of stress is expected when being transferred from enclosure to test station (and back again). Although there were few signs of stress (darting and/or hiding), animals could have been stressed after so much use in such a short period of time. Ideally, individuals should have been tested only once each day, but this was not possible in my research.

Animals did not undergo a period of fasting prior to each trial. Lindquist and Bachman (1982) starved the animals in their trials 48 hours prior to data collection. Although individuals appeared to always be interested in feeding, regardless of the day, I may have gotten more convincing results if they were just that much hungrier.

All individuals used were well conditioned to human presence during feeding sessions. Study subjects were seen sitting in the neutral chamber directly below the camera person and appeared to be looking up at the individual filming during 13 trials. In the future I would not hand-feed individuals. Conditioning of the animal to eat on their own should begin as soon as they hatch from the egg or come into a laboratory setting. Individuals should have had no association between humans and food.

Another design will need to be created for the waterfall boxes. Although they worked well ensuring an even flow of water into the test stations, they were also the perfect size for a salamander to climb into. To remedy this situation netting was wrapped around the opening. Unfortunately, this remedy caused additional problems because they assisted the salamanders in escaping during trials. There were over 16 escape attempts during the course of data collection. Stress from the escape attempt and being placed back into the test station for the remaining trial period could have affected an individual's behavior. If time permitted, I would rerun each trial during which an individual escaped the test station. Better yet, I would make sure individuals were unable to escape in the first place.

The separation of the lipid component of the worms was only attempted once. In the future several isolations should be done and each tested in order to cover the possibility of a mistake done in the isolation process.

Lastly, the caloric value and nutritional content of all stimuli should have been assessed. This would have aided in determining exactly to which specific cue(s) individuals may have been attracted.

Conclusion

There was substantial evidence of aquatic coastal giant salamanders, *Dicamptodon Tenebrosus*, using chemical cues to detect, locate, and assess a stationary food source. Preliminary observations seen in the lab were supported by these data collected in this experiment. Individuals appeared to actively investigate the test chamber in search for the source of a chemical stimulus. Once they oriented themselves in the direction of the source, they would approach, perform an olfactory test to assess, fixate upon the item if it passed the test, and bite. It appeared that individuals preferred worm morsels over agar blank, bologna, guano, lipid component of worm, cod liver oil, and salt morsels.

REFERENCES

Arzt, A, W Silver, J Mason, and L Clark. 1986. Olfactory responses of aquatic and terrestrial tiger salamanders to airborne and waterborne stimuli. Journal of Comparative Physiology 158: 479-487.

Caprio, J and R Byrd, Jr. 1984. Electrophysiological evidence for acidic, basic, and neutral amino acid olfactory receptor sites in catfish. Journal of General Physiology 84: 403-422.

Christman, T and M Finkler. 2000. Overwintering energetics of the red-backed salamander, *Plethodon cinereus*. American Zoologist 40: 973-974.

Cupp, P, Jr. 2001. Chemical detection of predatory salamanders by the four-toed salamander, *Hemidactylium scutatum*. American Zoologist 41: 1420.

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

Depp, A. 2002. Evaluation of the ability of the aquatic salamander, *Dicamptodon tenebrosus*, to detect olfactory clues that indicate potential food items. Senior Research Project, Fort Lewis College, Environmental Biology Department.

Duellman, W and L Trueb. 1986. Biology of amphibians. McGraw-Hill Book Company. New York.

Durand, J; J Parzefall; and B Richard. 1982. Comparative study of chemical prey detection in the cave living *Proteus anguinus* and its epigean relative *Necturus maculosus* (Proteidae, Urodela). Behavioural Processess 7: 123-134.

Baird, S and C Girard. 1852. Characteristics of some new reptiles in the museum of the Smithonian Institution. Proceedings of the Academy of Natural Sciences of Philadelphia 6: 125-129.

Evans, L and D Forester. 1996. Conspecific recognition by *Desmognathus ochrophaeus* using substrate-borne odor cues. Journal of Herpetology 30: 447-451.

Fenolio, D and G Graening. B Collier and J Stout. 2006. Coprophagy in a cave-adapted salamander: the importance of bat guano examined through

nutritional and stable isotope analyses. Proceedings of the Royal Society B: Biological Sciences 273: 439-443.

Gibbons, M; A Ferguson and D Lee. 2005. Both learning and heritability affect foraging behaviour of red-backed salamanders, *Plethodon cinereus*. Animal Behaviour 69: 721-732.

Gillette, J. 2002. Odor discrimination in the California slender salamander, *Batrachoseps attenuatus*: evidence for self-recognition. Herpetologica 58: 165-170.

Griffiths, R. 1996. Newts and salamanders of Europe. Academic Press. London.

Heatwole, H and E Dawley. 1998. Amphibian biology: volume three. Surrey Beatty and Sons. Chipping Norton.

Hickman, C; M Stone and A Mathis. 2004. Priority use of chemical over visual cues for detection of predators by grey-belly salamanders, *Eurycea multiplicata griseogaster*. Herpetologica 60: 203-210.

Himstedt, W, P Tempel; and J Weiler. 1978. Responses of salamanders to stationary visual patterns. Journal of Comparative Physiology: A Sensory, Neural, and Behavioral Physiology 124: 49-52.

Jaeger, R and A Rubin. 1982. Foraging tactics of a terrestrial salamanders judging prey profitability. Journal of Animal Ecology 51: 167-176.

Jaeger, R, R Joseph and D Barnard. 1981. Foraging tactics of a terrestrial salamander *Plethodon cinereus*: sustained yield in territories. Animal Behaviour 29: 1100-1105.

Jaeger, R and S Wise. 1991. Reexamination of the male salamander "sexy faeces hypothesis". Journal of Herpetology 25: 370-373.

Kats, L. 1988. Detection of certain predators via olfaction by small-mouthed salamander larvae (*Ambystoma texanum*). Behavioral and Neural Biology 50: 126-131.

Kikuyama, S, F Toyoda, Y Ohmiya, S Tanaka, K Matsuda, and H Hayashi. 1995. Sex attractant in the cloacal gland of the newt, *Cynops Pyrrhogaster*. Netherlands Journal of Zoology 45: 160-162.

Landau, Sidney I. 2002. The new international Webster's student dictionary of the English language. International Encyclopedic Edition. Trident Press International. USA.

Lindquist, S and M Bachmann. 1982. The role of visual and olfactory cues in the prey catching behavior of the tiger salamander, *Ambystoma tigrinum*. Copeia: 81-90.

Luthardt, G and G Roth. 1983. Reduction of visual inhibition to stationary prey by early experience in *Salamandra salamandra*. Zeitschrift für Tierpsychologie: 294-302.

Manteifel, Y and A Reshetnikov. 2002. Avoidance of noxious tadpole prey by fish and invertebrate predators: adaptivity of a chemical defense may depend on predator feeding habits. Hydrobiologie 153: 657-668.

Nicholas, JS. 1922. The reactions of *Amblystoma tigrinum* to olfactory stimuli. Journal of Experimental Zoology: 257-281.

Park, D; J McGuire, A Majchrazak, J Ziobro, and H Eisthen. 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.

Park, D and C Propper. 2001. Male-male repelling function of male pheromone in the red-spotted newt. Journal of Experimental Zoology 289: 404-408.

Parker, M. 1994. Feeding ecology of stream-dwelling Pacific giant salamander larvae (*Dicamptodon tenebrosus*). Copeia: 705-718.

Petranka, J, L Kats, and A Sih. 1987. Predator-prey interactions among fish and larval amphibians: use of chemical cues to detect predatory fish. Animal Behavior 35: 420-425.

Petranka, J. 1998. Salamanders of the United States and Canada. Smithsonian Institution Press, Washington and London.

Placyk, J, Jr and B Graves. 2002. Prey detection by vomeronasal chemoreception in a plethodontid salamander. Journal of Chemical Ecology 28: 1017-1036.

Rhein, L and R Cagan. 1983. Biochemical studies of olfaction: Binding specificity of odorants to a cilia preparation from rainbow trout olfactory rosettes. Journal of Neurochemistry 41: 569-577.

Rossi, J. 1983. The use of olfactory cues by *Bufo marinus*. Journal of Herpetology 17: 72-73.

Roth, G. 1987. Visual behaviour in salamanders: studies of brain function 14. Springer Verlag, Heidelberg Berlin.

Shinn, E and J Dole. 1978. Evidence for a role for olfactory cues in the feeding response of leopard frogs, *Rana Pipiens*. Herpetologica 34: 167-172.

Stebbins, Robert C. 1985. Peterson field guides: western reptiles and amphibians. Houghton Mifflin Company. New York, New York.

Stebbins, R and N Cohen. 1995. A natural history of amphibians. Princeton University Press. Princeton, New Jersey.

Sullivan, A, P Frese and A Mathis. 2000. Does the aquatic salamander, *Siren intermedia*, respond to chemical cues from prey? Journal of Herpetology 34: 607-611.

Takeuchi, H, T Masuda, and T Nagai. 1994. Electrophysiological and behavioral studies of taste discrimination in the axolotl (*Ambystoma mexicanum*). Physiological Behavior 56: 121-127.

Thaker, M, C Gabor and J Fries. 2006. Sensory cues for conspecific associations in aquatic San Marcos salamanders. Herpetologica 62: 151-155.

Toyoda, F and S Kikuyama. 2000. Hormonal influence on the olfactory response to a female-attracting phermone, sodefrin, in the newt, *Cynops pyrrhogaster*. Comparative Biochemistry and Physiology Part B 126: 239-245.

Uiblein, F. 1992. Prey choice behavior in light and darkness in the facultative cave dweller, the Pyrenean salamander, *Euproctus asper*. Alytes 10: 131-136.

Uiblein, F, E Silke and J Parzefall. 1995. Trade-off between visual detectability and nutrient content in the patch choice of the Pyrenean salamander *Euproctus asper*. Ethology 101: 39-45.

Woody, D and A Mathis. 1997. Avoidance of areas labeled with chemical stimuli from damaged conspecifics by adult newts, *Notophthalmus viridescens*, in a natural habitat. Journal of Herpetology 31: 316-318.

Zimmer-Faust, R, C Finelli, N Pentcheff, and D Wethey. 1995. Odor plumes and animal navigation in turbulent water flow: a field study. Biological Bulletin 188: 111-116.

APPENDIX A

Statistical analyses and associated information.

Test	Dependent Variables	Independent Variables	Sample Size (N)	Analysis	Null Hypotheses
Investigation	Behavior (Investigating, Not Investigating)	Trial	147	Descriptive	Not Applicable
Initial Direction Chosen	Side (Left, Right, Neither)	Individual Salamander	147	Monte Carlo Simulation	No difference between individuals
Stimulus vs. Control	Side (Stimulus, Control)	Trial (with bologna)	16	Multinomial Goodness of Fit	No tendency to seek a specific side (two-tailed)
Stimulus vs. Control	Side (Stimulus, Control)	Trial (with guano)	16	Multinomial Goodness of Fit	No tendency to seek a specific side (two-tailed)
Stimulus vs. Control	Side (Stimulus, Control)	Trial (with lipid)	16	Multinomial Goodness of Fit	No tendency to seek a specific side (two-tailed)
Stimulus vs. Control	Side (Stimulus, Control)	Trial (with oil)	17	Multinomial Goodness of Fit	No tendency to seek a specific side (two-tailed)
Stimulus vs. Control	Side (Stimulus, Control)	Trial (with salt)	16	Multinomial Goodness of Fit	No tendency to seek a specific side (two-tailed)
Stimulus vs. Control	Side (Stimulus, Control)	Trial (with worms)	18	Multinomial Goodness of Fit	No tendency to seek a specific side (two-tailed)
Nosing (Sniffing)	Sniffing (Yes, No)	Stimulus (Bologna, Blank, Guano, Lipid, Oil, Salt, Worms)	147	Monte Carlo Simulation	No difference among stimuli in tendency to elicit sniffing behavior
Initial Morsel Chosen by Individuals	Distribution of Choice of Stimulus Side among Different Morsels	Individual Salamander	147	Monte Carlo Simulation	No difference among salamanders in tendency to initially choose different stimuli
Time Spent on Stimulus Morsel Side	Time (sec.)	Stimulus (Bologna, Blank, Guano, Lipid, Oil, Salt, Worms)	7 (with 21 trials / stimulus)	Repeated Measures ANOVA	No differences among stimuli in tendency for salamanders to spend time on the stimulus side
Average Distance from Stimulus Morsel	Distance (cm.)	Stimulus (Bologna, Blank, Guano, Lipid, Oil, Salt, Worms)	7 (with 21 trials / stimulus)	Repeated Measures ANOVA	No differences among stimuli in distance between salamanders and stimulus
Time to Reach Stimulus Morsel	Time (sec.)	Stimulus (Bologna, Blank, Guano, Lipid, Oil, Salt, Worms)	7 (with 21 trials / stimulus)	Repeated Measures ANOVA	No differences among stimuli in time for salamanders to reach stimulus
Time to Reach Stimulus Morsel	Time (sec.)	Individual Salamander	7 (with 21 trials / stimulus)	Repeated Measures ANOVA	No difference between individuals in distribution of times to reach stimuli
Snout Touches to Stimulus Morsel	Snout touch (Yes, No)	Stimulus (Bologna, Blank, Guano, Lipid, Oil, Salt, Worms)	7 (with 21 trials / stimulus)	Monte Carlo Simulation	No differences among stimuli in tendency to elicit snout touches
Bites from Stimulus Morsel	Bite (Yes, No)	Stimulus (Bologna, Blank, Guano, Lipid, Oil, Salt, Worms)	7 (with 21 trials / stimulus)	Monte Carlo Simulation	No difference among stimuli in tendency to elicit bites

APPENDIX B

Test, feeding behavior, and corresponding statistical significance.

Test	Feeding Behavior	Statistical Significance
Investigation	None	n/a
Initial Direction Chosen (by individual salamanders)	Orientation Movement & Approach	-
Initial General Choice (excluding trials with agar blanks)	Orientation Movement & Approach	-
Initial Stimulus Side Chosen		
Stimulus vs. Control (bologna)	Orientation Movement & Approach	-
"Stimulus" vs. Control (agar blank)	Orientation Movement & Approach	-
Stimulus vs. Control (guano)	Orientation Movement & Approach	-
Stimulus vs. Control (lipid)	Orientation Movement & Approach	-
Stimulus vs. Control (oil)	Orientation Movement & Approach	-
Stimulus vs. Control (salt)	Orientation Movement & Approach	-
Stimulus vs. Control (worms)	Orientation Movement & Approach	+
Nosing (Sniffing) (between stimuli)	Orientation Movement & Approach	+
Time Spent on Stimulus Morsel Side (between stimuli)	Orientation Movement & Approach	-
Average Distance from Stimulus Morsel (between stimuli)	Orientation Movement & Approach	-
Time to Reach Stimulus Morsel (between stimuli)	Approach	+
Time to Reach Stimulus Morsel (between individuals)	Approach	+
Snout Touches to Stimulus Morsel (between stimuli)	Olfactory Test	+
Bites from Stimulus Morsel (between stimuli)	Fixation & Snapping	+