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SEX-BIASED PREDATION ON *TARICHA* BY A NOVEL PREDATOR IN
ANNADEL STATE PARK

by

Amber N. Brouillette

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Biology

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2008

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ABSTRACT

Sex-biased Predation on *Taricha* by a Novel Predator in

Annadel State Park

by

Amber N. Brouillette, Master of Science

Utah State University, 2008

Major Professor: Dr. Edmund D. Brodie, Jr.
Department: Biology

Newts of the genus *Taricha* have long been studied due to the powerful neurotoxin found in their skin. Tetrodotoxin (TTX) acts by blocking receptors in sodium channels, ultimately resulting in death via asphyxiation. The only documented predators of species in this genus have been snakes of the genus *Thamnophis*. Recently, predation on *Taricha* in Ledson Marsh in Annadel State Park, Santa Rosa, CA was discovered. Predation was in the form of laceration or evisceration, and tracking of predation from 1998-2008 showed that it was male-biased. Two species of *Taricha* were found living sympatrically at this location, the California newt (*T. torosa*) and the rough-skinned newt (*T. granulosa*). Fluorometric High Phase Liquid Chromatography (HPLC) analysis was used in order to quantify TTX levels in the skin of ten male and ten female newts of each species. Quantification of TTX was done to determine the influence, if any, that TTX levels may have on sex-biased predation in this population. I predicted that levels of TTX would be greater in females than males, and greater in *T. granulosa* than *T. torosa*

since very few *T. granulosa* were preyed upon during the study period. My results indicated that there were significant differences between the sexes, and *T. torosa* were significantly more toxic than *T. granulosa*. An in-depth ecological study of relative abundances of both species and identification of the predator are needed at this site to obtain a clear picture of the predator-prey dynamics at Ledson Marsh.

(46 pages)

ACKNOWLEDGEMENTS

I would like to begin by thanking Kenneth Gobalet and David Germano of California State University, Bakersfield. They encouraged me to continually ask questions, to never stop learning, and brought about my interest in graduate school. Without them, I don't know if I would have found my way. I would also like to thank my committee members, Edmund D. Brodie, Jr., S.K. Morgan Ernest, and Michael E. Pfrender, for all of their support and help throughout this process. They have helped me in ways that I cannot express in such a short amount of space. I would also like to thank all of the students in their labs, especially Kristin Bakkegard, Megan Kanaga, Megan Lahti, Leigh Latta, Meghan Wesolek, and Desaree Williams, for all of their social, moral, and academic support. Kristin Bakkegard has been such a great example of how to produce thorough work. I would also like to thank Megan Lahti for making model newts for me, for helpful comments on the draft of this thesis, and for being an all around great office-mate. Leigh Latta and Susan Durham gave helpful suggestions for some of the statistical analyses for this project. I also thank Daryll Dewald and his students as well as Joseph Li and his students for help in the lab and use of their equipment.

I would also like to acknowledge Edmund D. Brodie, Jr., Judy Brodie, Dave Cook, Charles Hanifin, Cyndy Shafer, and Tyson Stokes for helping me in the field. They made my work much easier, and far more enjoyable. Dave Cook noticed the predation and allowed us to be involved in this research. Additionally, he provided pictures and information that were very helpful in completing this research. Charles Hanifin is owed a great deal of gratitude for helping with my HPLC analysis; without him I would not be finished with this project at this time. I would also like to thank

Becky Williams for her continued help in the lab, and for being such a great teacher. I feel that I have learned so much from her and am a better scientist just by following her example. Funding for this project was provided by the US National Science Foundation (DEB-0315172 to E.D. Brodie Jr. and DEB-0650082 to E.D. Brodie III).

Finally, I would like to thank all of my family for all of their financial, and emotional support. In particular, Jackie and Maurice Brouillette, Lauren Brouillette, Tyson Stokes, Mable and Byron Zeek, and Eugene and Jeannie Brouillette. Without them, I would not have come this far, nor would I be the person who I am today. I would like to thank Tyson Stokes for his continued support through this process. He moved with me to Utah so that I could go to graduate school, and has continued to support me by taking an active interest in my work and helping me to remember to breathe when stress was high. He also has had a big role in keeping a roof over my head and food in my belly.

Amber N. Brouillette

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INTRODUCTION

Newts of the genus *Taricha* have been known to be highly toxic for some time (Mosher et al. 1964; Wakely et al. 1966; Brodie 1968; Brodie et al. 1974; Daly et al. 1987; Hanifin et al. 1999, 2002). Early studies of *Taricha granulosa* (Brodie 1968) showed that many animals were susceptible to the newts' skin toxin, tetrodotoxin (TTX), a potent neurotoxin. TTX acts by blocking sodium channels (Narahashi et al. 1967) and death for exposed animals is usually the result of respiratory failure (Brodie 1968). Until recently, the only known successful predators of *Taricha* (i.e. predators that do not die from ingestion) are garter snakes of the genus *Thamnophis* (Brodie and Brodie 1990, 1991). These predators have been shown to have varying levels of resistance to TTX, and where found sympatric with species of *Taricha* are involved in a coevolutionary arms race (Brodie et al. 2002).

In the past there has been evidence of predation attempts on *Taricha* by various families of birds, but none of those attempts were successful as each resulted in the death of the bird (Storm 1948; Pimentel 1952; McAllister et al. 1997; Mobley and Stidham 2000). Recently, however, successful predation on *Taricha* by great blue herons was documented (Fellers et al. 2008). Additionally, predation attempts on *Taricha* by a skunk have been observed, but the fate of the skunk itself is unknown (M. Edgehouse, personal communication). Successful predation upon *Taricha torosa* and *Taricha granulosa* by an unidentified predator has been extensively documented in Ledson Marsh in Annadel State Park.

Annadel State Park is located on the eastern edge of the city of Santa Rosa, in northern California. In 1835, an 18,833-acre land grant was given to John Wilson, which

included property that is now the park (Krumbein 1993). Through the years, the property changed ownership and was used to raise various types of livestock, fowl, and produce such as grape vineyards and fruit orchards. In 1930, the eastern 1800 acres of the park were sold to a man with the last name of Ledson. He used the property to plant hay and raise cattle, but had problems with water shortages. Sometime in 1930 he built a low dam in an area that may have been a vernal pool to help retain water, which is now known as Ledson Marsh (Cook and Jennings 2007). Today, the entire park is over 5,000 acres and includes a variety of habitat types including oak woodland, fir forest, marshland, chaparral, grassland, and meadow. Ledson Marsh, itself, is approximately 11 ha in size when completely full of water (Cook and Jennings 2007). The marsh is seasonal, and fluctuates in size.

Ledson Marsh has proven to be suitable habitat for amphibians, and serves as the primary breeding ground for many species in the park, including the red legged frog (*Rana aurora draytoni*), the California newt (*T. torosa*) and the rough-skinned newt (*T. granulosa*). During regular surveys of the marsh for *R. aurora* egg masses (winter breeding period in 1998 through the breeding period of winter 2008) *Taricha* mortality has also been recorded. In addition to tracking the numbers of *Taricha* killed, sex was documented as well as classification of the newts to one of three injury types: no injury, laceration, or evisceration. Interestingly, the mortality observed over this ten-year period was male-biased.

Sex-biased predation is not uncommon, however, the causes of such predation as well as the direction of bias (i.e. male-biased vs. female-biased) vary depending on the habits or life history traits of the species of the predator and the prey (i.e. Dickman et al.

1991; Norrdahl and Korpimäki 1998; Chirste et al. 2006). Christe et al. (2006) investigated sex-biased predation as a source of extrinsic mortality, which may be a possible cause for differences in the lifespan of males and females. They looked at sex-biased predation by two owl species as well as performed a literature review of sex-biased predation. It was found that sex-biased predation is fairly common, especially by birds, and that in most cases of sex-biased predation by avian predators, the predation was male-biased rather than female-biased. Three potential reasons for male-biased predation were noted. First, sexual dimorphism may play a role. In many cases males are much more visible than females due to showier plumage, coloration, size etc. Second, males often have different behaviors than females, which places them at a higher risk for predation. These behavioral differences are often seen in animals that have parental care, where the female parent stays close to the nest or burrow while the male parent leaves in search of food. Additionally, males often have varying behaviors due to territoriality and mate acquisition. Finally, this study showed that in some mammalian cases, males have higher parasitic loads and are thus unable to escape predation as easily (Moore and Wilson 2002; Christe et al. 2006).

With *Taricha*, the first two hypotheses could have some bearing on sex-biased predation. However, it is unknown if either of them apply to *Taricha* species. In this case, it is likely that differences in toxicity levels play a role in selective predation. Previous research has shown that in some populations, female *T. granulosa* are more toxic than conspecific males (Hanifin et al. 2002). Therefore, it is likely that if toxicity levels in individuals of this population follow the same trend a predator may target males due to decreased risk of encountering TTX.

The pattern of male-biased predation on *Taricha* may be attributed to several possible predators. Studies have shown evisceration by birds of the family Corvidae on toads of the genus *Bufo*, which are also toxic amphibians (Olson 1989; Corn 1993; Brothers 1994). Other studies, however, have noted evisceration and predation on *Bufo* toads by raccoons and skunks, respectively (Hanson and Vial 1956; Wright 1966; Schaaf and Garton 1970). This study looks at the long-term patterns of *Taricha* mortality at Ledson Marsh, and determines whether or not toxicity plays a role through the use of fluorometric HPLC analysis.

MATERIALS AND METHODS

Taricha mortality

Taricha mortality data were collected from Ledson Marsh in Annadel State Park in Santa Rosa, CA. Data were collected during active breeding season (November-March) from winter 1998 to winter 2008 (Figure 1). The exterior edges and pools of the marsh were surveyed haphazardly for *Taricha*. Those that were found dead were sexed and assigned an injury type of either none, punctured, lacerated, dismembered, or eviscerated. Newts that were eviscerated and had another injury type, were classified as eviscerated. Injury types of puncture and laceration were combined, as such a classification was subjective. In addition, the classifications of dismembered or eviscerated were combined, since in both cases the newt was essentially eaten. In some cases newts were collected that had decayed or been torn apart to the point that sex, species, and/or injury were unable to be assigned. These 110 unknown newts only comprised about 18% of the total newts collected, and were excluded from any analyses. Gravidity was also recorded for female newts. During the years of 2007 and 2008, species identifications (*T. torosa* or *T. granulosa*) were also made. All collected newts were preserved.

Since the timing of migration into breeding ponds is affected by rainfall, I compared mortality with precipitation by plotting them together to determine any possible patterns between the level of predation and rainfall over time. Monthly totals for rainfall in Santa Rosa, CA over the 1998-2008 research period were collected from the California Data Exchange Center website (SRO tower) provided by the California

Department of Water Resources (<http://cdec.water.ca.gov/>). Rainfall averages for each year were calculated from the average of the monthly rainfall totals for the months of November through March. Overall average rainfall was calculated as an average of the 1998 – 2008 yearly averages.

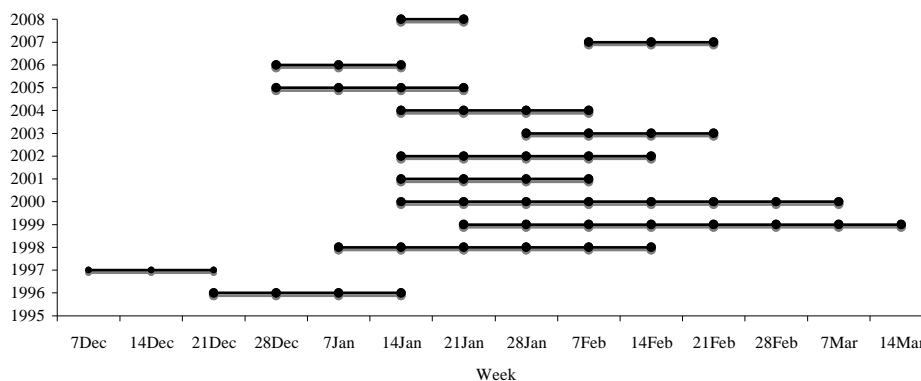


Figure 1. *Taricha torosa* breeding duration in weeks.

Predator identification

Attempts to identify the predator(s) were made using motion activated cameras and clay models of newts. Newt models were made using Premo modeling clay (Sculpey). One male and female “model” newt were made and baked following manufacturers instructions (Figure 2A). These model newts were then pressed into a small block of polymer putty to make a “master” mold of each (Amazing Mold Putty, Aluminate Corp.) (Figure 2B). These master molds were used to make clay newts that were not cooked. The Premo modeling clay used to make the model newts was then pressed into the mold and four male and four female newt “field” models were made

(Figure 2 C and D). These field models were left malleable so that an “attack” by a predator might leave identifying marks (i.e. beak, tooth, claw, etc.) (Brodie 1993).



Figure 2. Materials used to create field models. A) Two model newts. The one on the left is a male, and the one on the right is a female. B) The master mold for male and female newt models. C) Male field model. D) Female field model.

Two male and two female field models were placed on either side of Ledson Marsh in view of a motion-activated digital camera (Cuddeback Excite, Cuddeback Digital). The field models and cameras were within three meters of the edge of the water in two areas of the marsh with newt breeding activity. The cameras were attached to a small bush in each area and secured with zip ties. A 4-inch long hook was made out of 12-gauge wire. The hook was placed through the newt model and into the ground in order to keep the models anchored (Figure 2 C and D). The cameras and models were left in place for 19 consecutive days, and three consecutive days in 2007 and 2008,

respectively. In January 2008, a blind was set up approximately 100 meters from the shore of the marsh. Over a 2-day period for 2 hours at dawn and 2 hours at dusk, the marsh was monitored for predation activity. Evidence of the predator was also searched for on the bodies of collected newts.

Quantification of TTX

Specimens used for quantification of TTX were collected from Ledson Marsh and Bullfrog pond in Santa Rosa, CA in February 2007. Bullfrog pond is located just outside of the north end of Annadel State Park (Figure 3). Both locations were searched haphazardly. A total of 40 newts were collected from the two locations, 10 males and 10 females of each species. All animals were frozen within 3 days at -80°C .

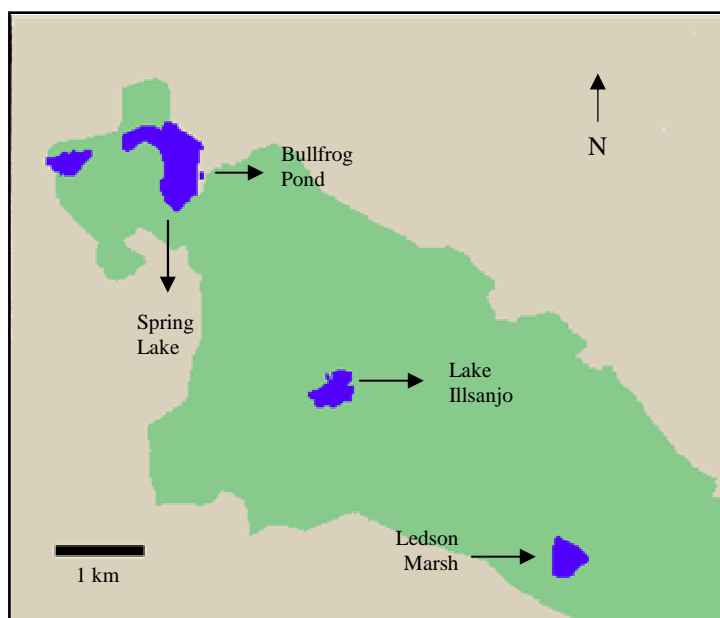


Figure 3. Map of Annadel State Park. Important features of the park are indicated. Green indicates the park's area. The view is from the south.

Procedures for collection of skin tissue as well as extraction and quantification of TTX were performed as in Hanifin et al. (2002) with minor exceptions. Extracts of the tissue were prepared by homogenizing a five millimeter diameter skin punch in 600 μ l of 0.1 M acetic acid using a tissue sonicator (550 Sonic Dismembrator, Fisher Scientific). Standards for flourometric HPLC were prepared from tetrodotoxin with citrate buffer available from Sigma (product number T8024-1MG).

Analyses

All analyses were performed using SAS/STAT version 9.1 (SAS Institute). Differences in the frequencies of injury according to sex were determined using Pearson's chi-squared analysis of a contingency table. This analysis was also done comparing differences in the frequencies of injury according to gravidity in females. All other comparisons between species, sex, or injury type also were done using Pearson's chi-squared analysis. Toxicity data were not normally distributed, so transformations were performed. For the analysis of all 40 newts combined, comparisons of male toxicity levels between the two species, as well as analysis of *T. granulosa* alone, the value of 0.00001 was added to each of the values so as to eliminate zero values. These data were then log transformed. For analysis of *T. torosa* alone, the data were square root transformed. For analyses of female toxicity levels, data were log transformed. Comparisons between species and sex for all 40 newts collected were analyzed using a two-way ANOVA. Within species and within sex comparisons were performed using one-way ANOVA. All ANOVA procedures were conducted using PROC-GLM.

RESULTS

Taricha mortality

Overall, significantly more male than female *Taricha* were found dead ($\chi^2 = 20.86$, $df = 1$, $P < 0.0001$) (Table 1). Figure 4 illustrates some of the common injuries observed. Additionally, there was a significant difference in the number of *T. torosa* vs. *T. granulosa* collected in 2007 and 2008 ($\chi^2 = 88.6567$, $df = 1$, $P < 0.0001$). Specimens from 1998-2006 all appear to be *T. torosa*. While the total number of *Taricha* preyed upon varied across years (Figure 5), overall predation was male-biased. Similarly, the frequency of injury types between the two sexes is significantly different (Table 2, Figure 6). Male *Taricha* were killed significantly more often than females (laceration $\chi^2 = 8.33$, $df = 1$, $P = 0.0039$; evisceration $\chi^2 = 331.74$, $df = 1$, $P < 0.0001$; Figure 7). Conversely, there was a significantly larger number of dead female newts found with no injury than dead male newts ($\chi^2 = 90.04$, $df = 1$, $P < 0.0001$). In 2007 and 2008, a total of 113 newts were collected. Of these 113 newts collected, only 9 were identified as *T. granulosa* (five males, four females).

Most often, dead female *Taricha* that were collected were gravid ($\chi^2 = 166.14$, $df = 1$, $P < 0.0001$). Of the females found, however, there was not a significant difference from what is expected based on proportions of total individuals found in the occurrence of injury types for gravid vs. non-gravid females (Table 3, Figure 8). In addition, comparisons between gravid and non-gravid newts for each injury type were all non-significant (none: $\chi^2 = 0.3191$, $df = 1$, $P = 0.5721$; laceration : $\chi^2 = 0.0004$, $df = 1$, $P = 0.9837$; evisceration: $\chi^2 = 3.6312$, $df = 1$, $P = 0.057$). A plot of newt mortality and

rainfall did not indicate any pattern between the number of *Taricha* killed each year and average rainfall during the breeding season of November – January (Figure 9).

Table 1. Total male and female *Taricha* collected according to injury type and year. Gravid females are the number of females that were found gravid within that year.

Males	Injury Type			Total	Females	Injury Type			Gravid	Total
	N	L	E			N	L	E		
1998	0	1	4	5	1998	24	1	0	25	25
1999	1	3	4	8	1999	1	3	0	4	4
2000	6	2	4	12	2000	46	3	5	52	54
2001	0	0	0	0	2001	0	0	0	0	0
2002	0	1	1	2	2002	16	0	2	18	18
2003	2	1	2	5	2003	11	0	2	12	13
2004	3	4	111	118	2004	40	5	18	44	63
2005	0	2	15	17	2005	11	0	2	10	13
2006	1	4	78	83	2006	5	5	0	9	10
2007	2	0	0	2	2007	18	1	1	16	20
2008	1	11	81	93	2008	12	0	3	12	15
Total	16	29	300	345	Total	184	18	33	209	235

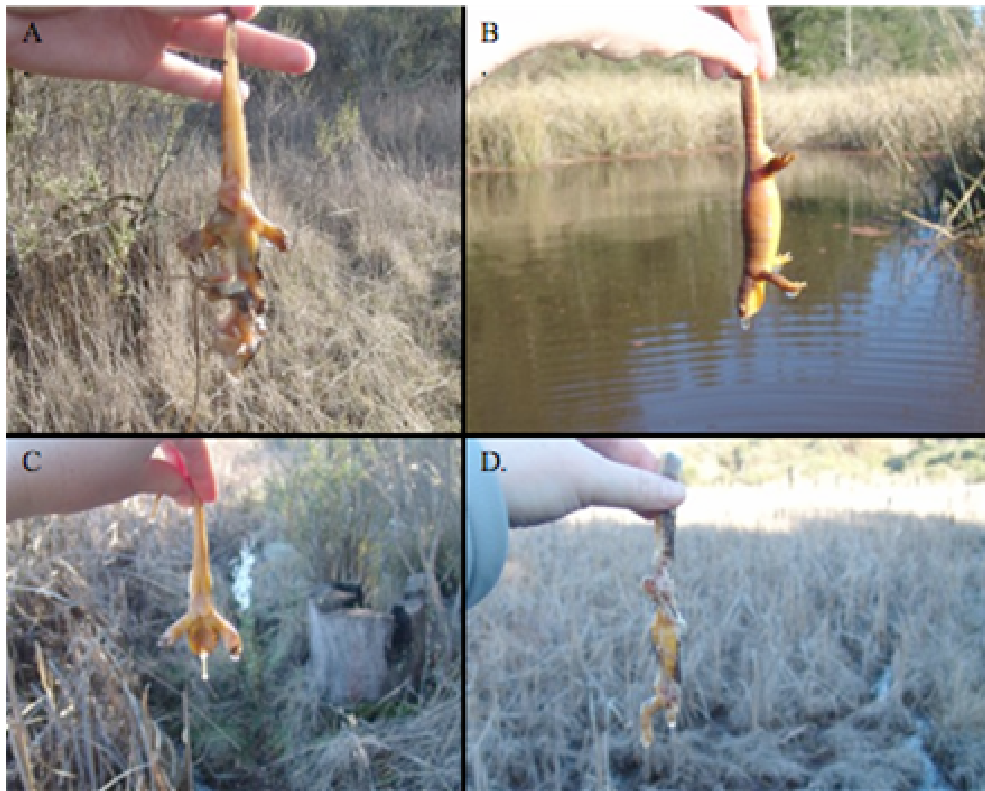


Figure 4. Typical mortality seen in Ledson Marsh. A) Eviscerated male newt. B) Gravid female *T. torosa* with no injury. C) Eviscerated male. D) Male that was both eviscerated and dismembered.

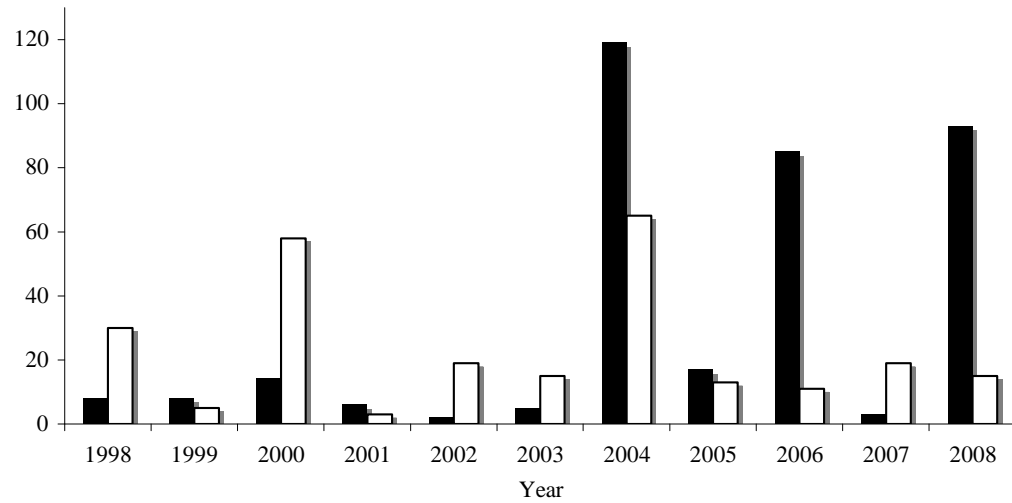


Figure 5. Total numbers of male (black bars) and female (white bars) newts collected from 1998 to 2008.

Table 2. Contingency table comparing injury types for male and female *Taricha*. Italicized numbers are expected values based on probability. N, L, and E represent newts with the following injury types: none, lacerated, or eviscerated.

Sex	Injury Type			Total	χ^2	P
	N	L	E			
Male	16	29	300	345		
	<i>118.97</i>	<i>27.96</i>	<i>198.08</i>			
Female	184	18	33	235		
	<i>81.03</i>	<i>19.04</i>	<i>134.92</i>			
Total	200	47	333	580	349.48	<< 0.0001

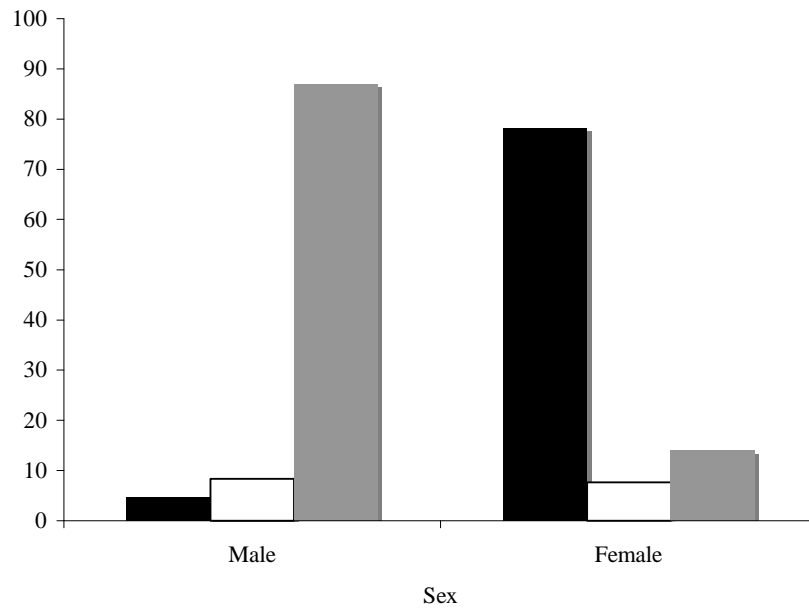


Figure 6. Percentage of male and female *Taricha* collected with no injury (black bars), laceration (white bars), or evisceration (grey bars).

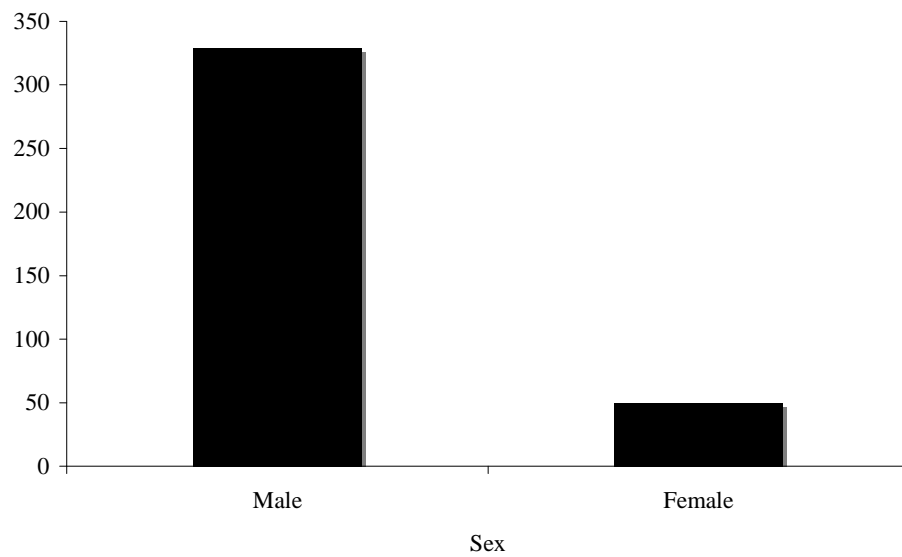


Figure 7. Total number of male and female *Taricha* found that had been lacerated or eviscerated.

Table 3. Contingency table comparing injury types for gravid and non-gravid female *Taricha*. Italicized numbers are expected values based on probability. N, L, and E represent newts with the following injury types: none, lacerated, or eviscerated.

Gravidity	Injury Type			Total	χ^2	P
	N	L	E			
Gravid	171	16	15	202		
	<i>169.12</i>	<i>15.97</i>	<i>16.91</i>			
Non-gravid	9	1	3	13		
	<i>10.88</i>	<i>1.03</i>	<i>1.09</i>			
Total	180	17	18	215	3.9215	0.1408

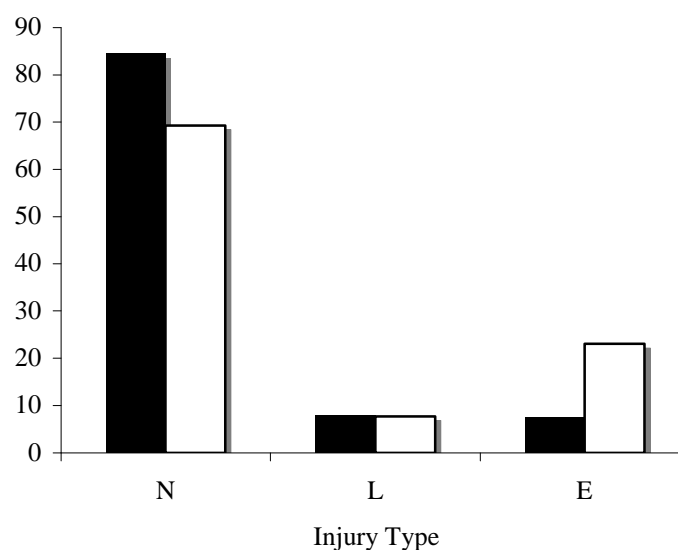


Figure 8. Percentage of total gravid (black bars) and non-gravid (white bars) *Taricha* collected according to injury type. Injury type is denoted as follows: N = none, L = laceration, E = eviscerated.

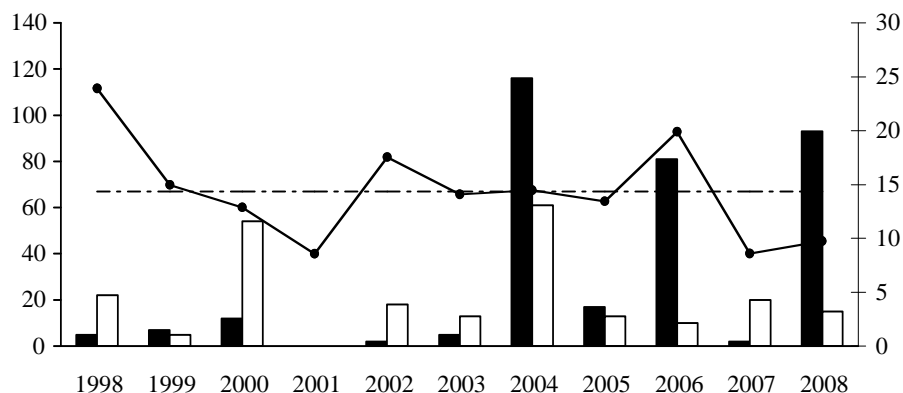


Figure 9. Comparison of yearly rainfall to *Taricha* mortality. Black and white bars represent total male and female *Taricha* collected, respectively. The solid line represents the total rainfall (cm) in November - March, the dashed line is the average rainfall (cm) during the same time period.

Predator identification

The predator(s) responsible for this sex-biased predation was not identified, as the field models were never “attacked.” However, the digital cameras photographed two possible predators, a raccoon (*Procyon lotor*) and a skunk (*Mephitis mephitis*) (Figure 10 A and B). Other possible predators observed during surveys of the marsh include ravens (*Corvus corax*) and Steller’s jays (*Cyanocitta stelleri*). Bodies of the recovered newts did not provide definitive evidence regarding the identity of the predator(s). A juvenile western fence lizard (*Sceloporus occidentalis*) with a total length of 64.48 mm was also found eviscerated at the edge of the marsh. In some cases dead newts were found on top of stumps around the edges of the marsh. Most often, newts were collected in the shallow pools of the marsh or on the shore not far (within 3 meters) from the water. No predation event was noted during observation from the blind.



Figure 10. Pictures taken of a raccoon (A) and a skunk (B) near the marsh using motion-activated cameras.

Quantification of TTX

HPLC analysis showed that there was a significant difference in the levels of TTX between all 40 male and female newts ($F = 4.71$, $df = 1$, $P = 0.0367$), with females having greater toxicity than males (Figures 11 and 12; Table 4). Additionally, *T. torosa* were significantly more toxic than *T. granulosa* ($F = 8.48$, $df = 1$, $P = 0.0061$) (Figure 13). However, within each species there were no significant differences between the sexes (*T. torosa*: $F = 0.02$, $df = 1$, $P = 0.8796$; *T. granulosa*: $F = 3.90$, $df = 1$, $P = 0.0639$). Although *T. torosa* males had a greater mean toxicity than females, males also had a much larger variance than that of the females (Table 4, Figures 11 and 14). In contrast, the standard error for *T. granulosa* was much larger for females than for males. It was also found that there were no significant differences between *T. torosa* and *T. granulosa* females ($F = 2.79$, $df = 1$, $P = 0.1119$; Figure 14), however, *T. torosa* males were significantly more toxic than those of *T. granulosa* ($F = 5.74$, $df = 1$, $P = 0.0276$) (Figure 14).

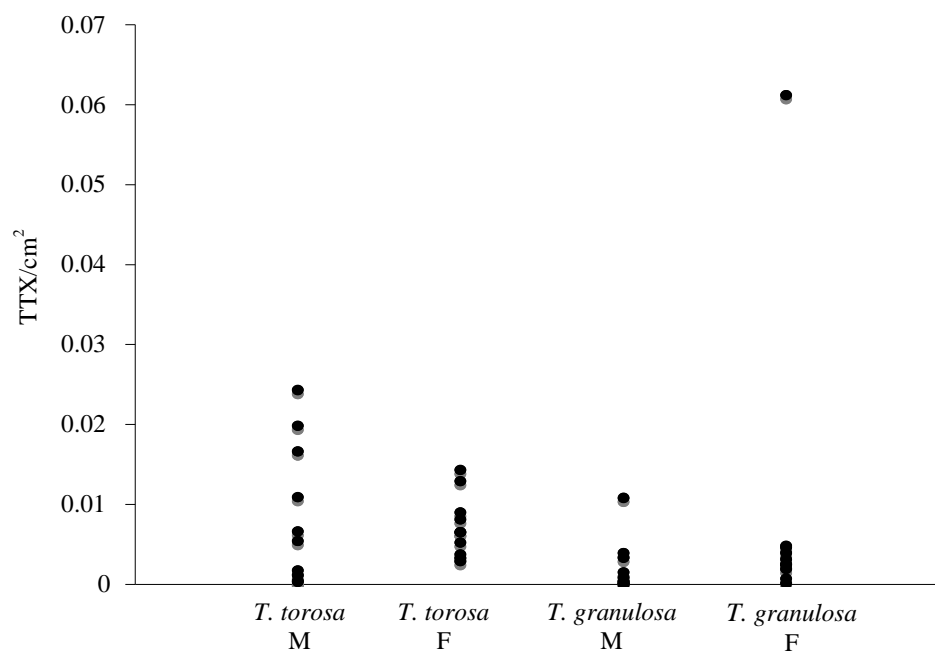


Figure 11. Plot of individual values of TTX/cm² for every individual within each species and sex. The letters M and F indicate males and females, respectively.

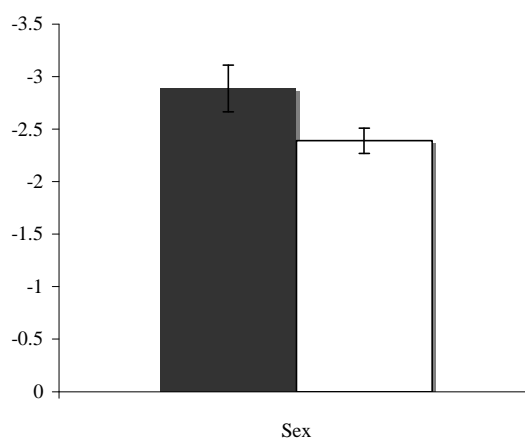


Figure 12. Comparison of mean log values of TTX/cm² for male and female *Taricha*, with standard error bars. This difference is significant at a significance level of 0.05.

Table 4. Comparison of the levels of TTX in *T. torosa* and *T. granulosa* males and females. Sample sizes within each group were equivalent, with N = 10 for each sex.

Species/Sex	TTX/plug (mg, mean \pm SE)	TTX/cm ² (mg, mean \pm SE)	Predicted whole newt toxicity (mg, mean \pm SE)	Range TTX/cm ² (mg)
<i>T. torosa</i>				
Male	0.00171 \pm 0.00055	0.00871 \pm 0.00278	0.3266 \pm 0.10606	0.0003 – 0.0243
Female	0.00142 \pm 0.00024	0.00724 \pm 0.00124	0.2203 \pm 0.04745	0.0029 – 0.0143
<i>T. granulosa</i>				
Male	0.00041 \pm 0.00021	0.00211 \pm 0.00106	0.0583 \pm 0.02911	0.0000 – 0.0108
Female	0.00167 \pm 0.00115	0.00852 \pm 0.00587	0.2285 \pm 0.15813	0.0001 – 0.0612

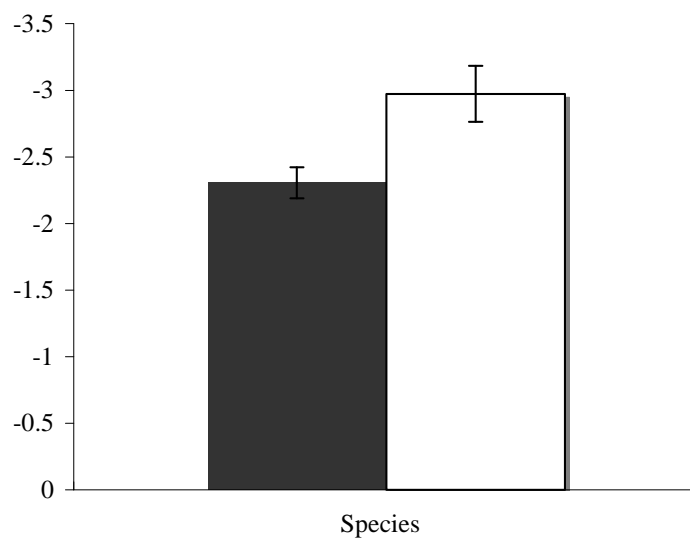


Figure 13. Comparison of mean log values for TTX/cm² for *T. torosa* (grey bars) and *T. granulosa* (white bars), with standard error bars. The difference is significant at $P = 0.05$.

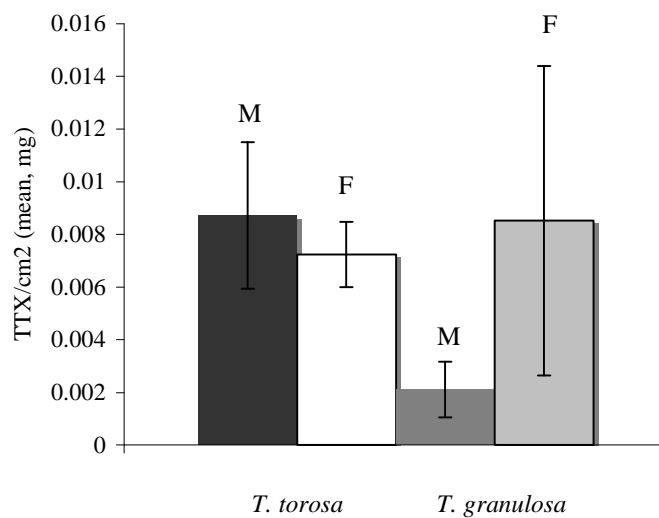


Figure 14. Comparison of mean TTX/cm² for *T. torosa* and *T. granulosa* males and females, with standard error bars. The species is denoted below each bar, with the sex denoted as an M or F above each bar for male or female, respectively.

DISCUSSION

Toxicity may be the driving factor behind male-biased predation at Ledson Marsh. Overall, female *Taricha* are more toxic than males, which is consistent with previous work on populations of *T. granulosa* (Hanifin et al. 2002). Females of the two species do not differ significantly from one another in levels of toxicity, which may be attributed to females investing TTX into their eggs in order to protect them from predation. Hanifin et al. (2003) found that levels of TTX in *T. granulosa* eggs are highly correlated with TTX levels in the dorsal skin of the mother. This suggests that females may invest TTX into their eggs, presumably as a source of protection.

The significant differences in TTX levels between the two species of *Taricha* are opposite from what I expected. *Taricha granulosa* was killed much less frequently than *T. torosa*, and is the less toxic species at this location. Furthermore, *T. torosa* males are significantly more toxic than *T. granulosa* males. As I anticipated that *T. granulosa* would be more toxic than *T. torosa*, since only nine of 113 newts in 2007 and 2008 were identified as *T. granulosa*, these results were also surprising. It is noteworthy, however, that one female *T. granulosa* had very high toxicity, and two males had undetectable levels of TTX (Figures 15, 16, 17). If one out of 10 newts are highly toxic, and two out of 10 have zero toxicity, it is possible that there are many more highly toxic females and non-toxic males in this population.

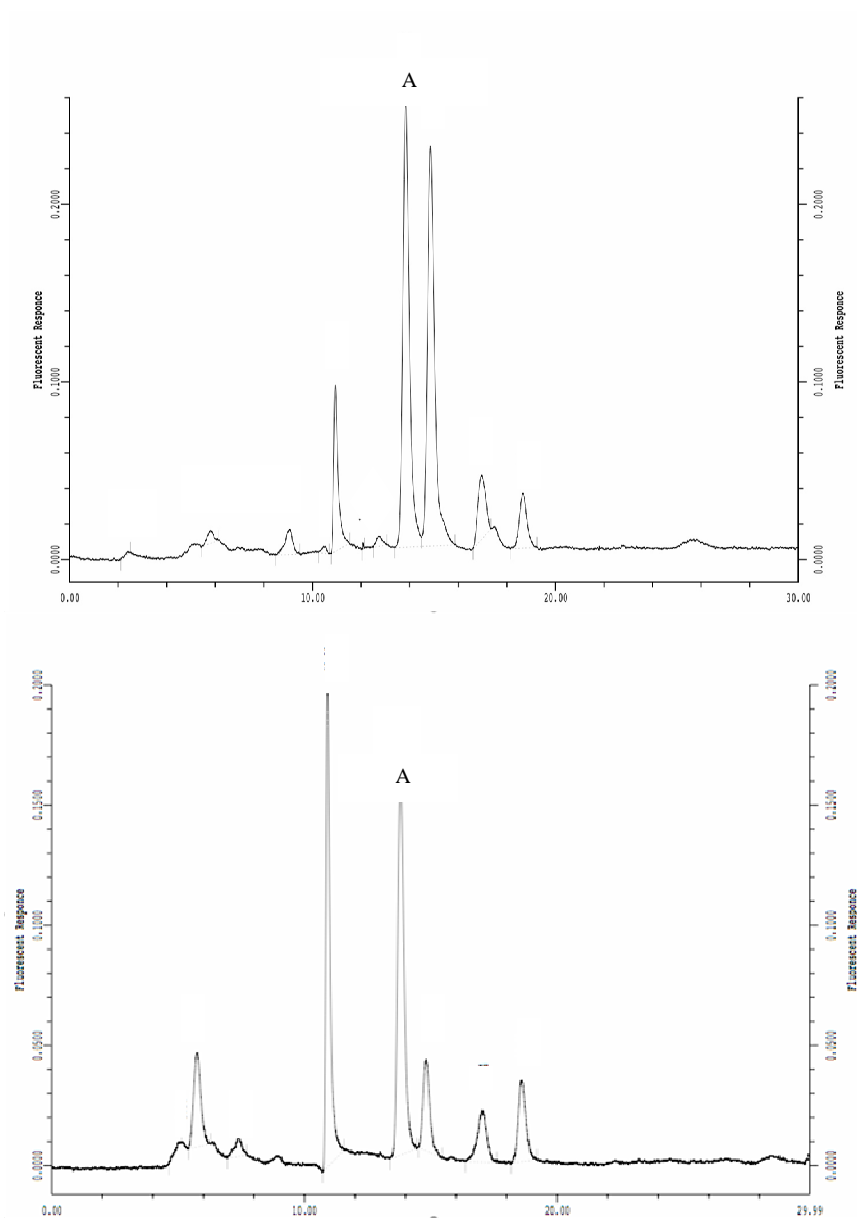


Figure 15. HPLC chromatograms for one male (top) and one female (bottom) *T. torosa*. The letter A denotes the TTX peak.

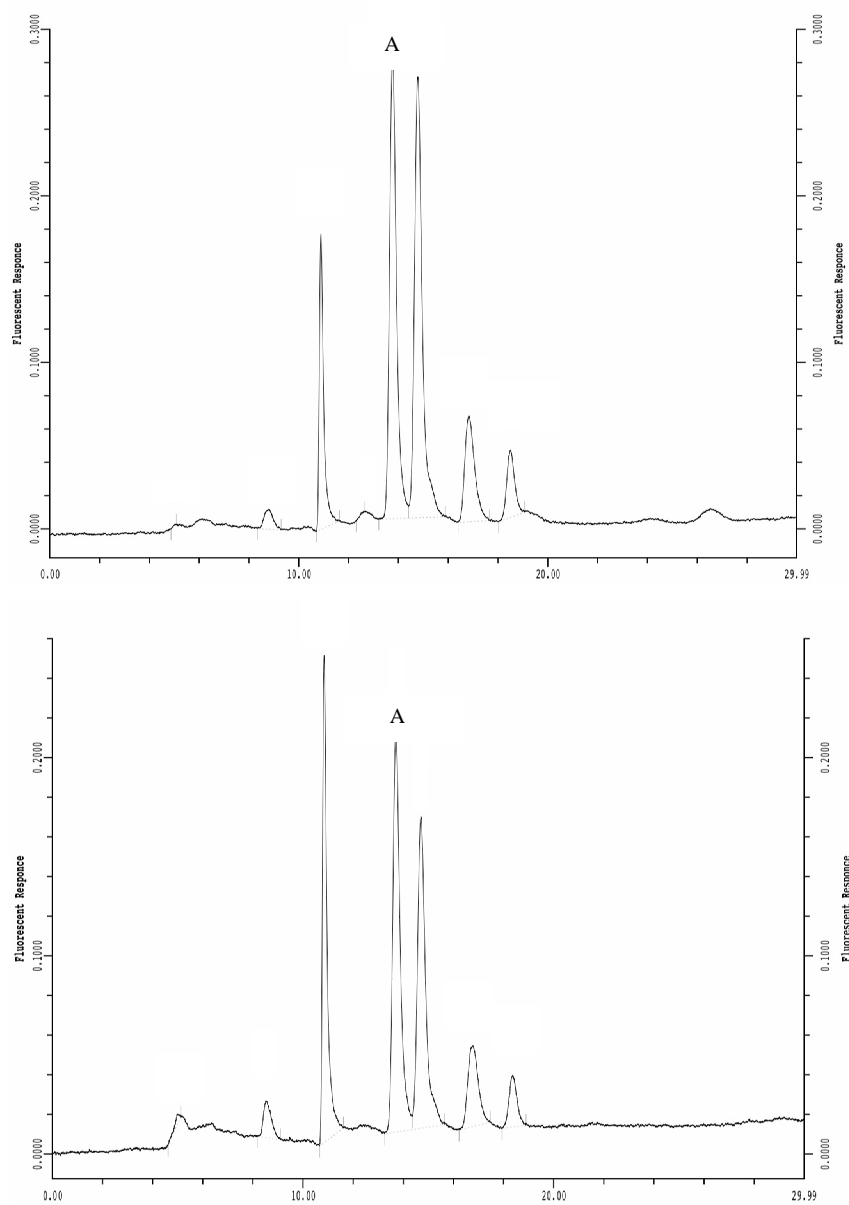


Figure 16. HPLC chromatograms for one male (top) and one female (bottom) *T. granulosa*. The letter A denotes the peak for TTX.

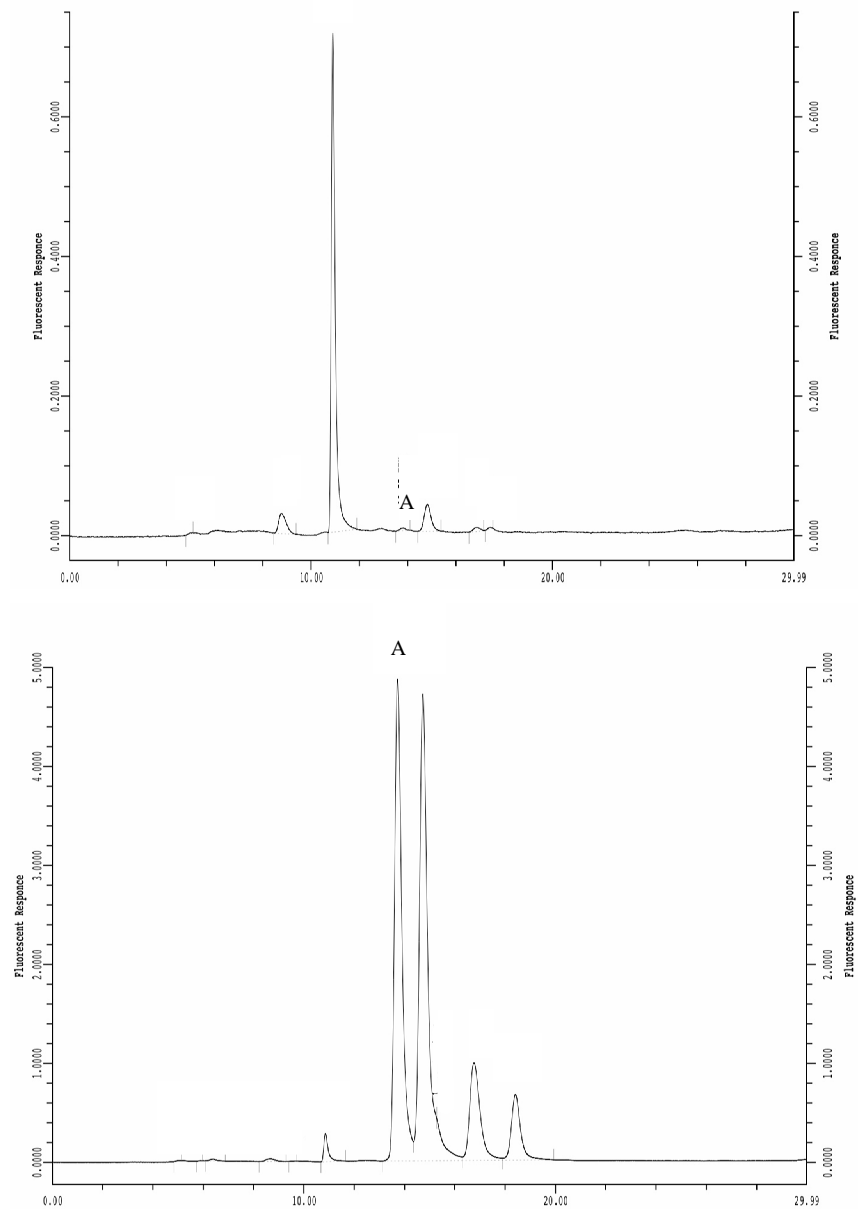


Figure 17. Outlier HPLC chromatograms for *T. granulosa*. Top is a male with non-detectable levels of TTX. Bottom is a female with high levels of TTX.

There is the potential that the differences in the levels of predation within and between species may be due to differences in relative abundances of *Taricha*. These data were not collected during this study due to logistical reasons. There are multiple methods of determining relative abundances for amphibian populations that would be effective for this species including visual encounter surveys, straight-line drift fences and pitfall traps, surveys at breeding sites, drift fences at breeding sites, and quantitative sampling of amphibian larvae (Scott et al. 1994). However, the time and resources needed to effectively monitor this population combined with the sometimes short breeding season made collecting these data not possible at this time. Having noted this potential bias in the data, it is important to point out that collection of the newts for HPLC analysis was not difficult. *Taricha* of both sexes and species were found easily.

Differences in the numbers of male and female newts found dead, without injury may be due to the courting process. In both species it has been noted that several males will simultaneously attempt to mate with one female (Smith 1941; Janzen and Brodie 1988). This behavior was also observed during this study. It is possible that males competing to mate with a female accidentally drown the female in the process of breeding (Briggs and Storm 1970; Kargarise Sherman 1980). The few males found with no injury may also have been drowned in this struggle (Kargarise Sherman 1980).

It is interesting that predation is male-biased, and yet few females are found killed (i.e. laceration), but not consumed (i.e. eviscerated). In handling many live newts in Ledson Marsh, I noted that some of the females had scars (Figure 18). It may be that a predator handles a female and then realizes that she is too toxic, and releases her. Past research has found that snakes of the species *Thamnophis sirtalis* will limit exposure time

to *Taricha granulosa* based on the toxicity of the newt as well as the snake's own resistance (Williams et al. 2003). In other words, a snake that could withstand less toxin than found in its prey item would reject the newt more quickly than a more resistant snake. However, few females were scarred, and it would be difficult to show that scarring was a result of predator handling without actually observing predation attempts.



Figure 18. Female *T. torosa* found with scarring on head, back, and tail.

It is possible though, that male-biased predation on this population is also controlled by sexual dimorphism or behavioral differences as mentioned in the introduction. As Christie et al. (2006) note, in some cases of sex-biased predation sexual dimorphism plays a role. Sex-biased predation seems to be most often influenced by sexual dimorphism in cases where males are much larger and/or much more colorful than females. In the case of *Taricha*, there is some sexual dimorphism, however, it is slight. It has been shown that in the case of *T. granulosa*, males are only 1-9% larger than females

(Neish 1971; Taylor 1984). Measurements taken on the 40 newts collected for HPLC analysis indicate that the average total length (TL) of *T. granulosa* males and females in this population was 153.45 mm and 138.47 mm, respectively. The average TL for male and female *T. torosa* in this population was 167.65 mm TL and 143.02 mm TL, respectively. There is, however, a much larger difference between the masses of the sexes and species in this population. The average mass for *T. granulosa* males and females was 12.82 g and 11.31 g, respectively. The average mass for *T. torosa* males and females was 18.74 g and 13.09 g, respectively. These masses indicate that despite small differences in the lengths of the sexes and species, there are differences in the total body size that may be significant when it comes to predation as bigger prey items may be easier to find or may be preferred by the predator.

During breeding, male *Taricha* develop lighter, smoother skin, their tails flatten out to aid in swimming, and they get nuptial pads on their feet, which aid in breeding (Petranka 1998). Physical differences between the two species are also minor, and determining species identity when they are found sympatrically can be difficult. *T. torosa* has light skin that comes in contact with their eye, whereas the skin surrounding the eye of *T. granulosa* is completely dark (Stebbins 1951; Riemer 1958). Furthermore, the eyes of both species are different sizes, with the eyes of *T. torosa* meeting the margin of the head while the eyes of *T. granulosa* do not (Petranka 1998). The most telling method of distinguishing between the two species are differences in the posture of the tail during an unken reflex, in which a newt responds to agitation by arching its back and raising its tail to show aposematic coloration on the ventral side. *Taricha granulosa* will curl their tail at the end, while *T. torosa* maintain a straight tail. Again, these physical

differences are slight, and it is unknown whether or not a predator could avoid either sex or species by sight alone.

The second point of Christie et al. (2006) that may be applicable to this population is behavioral differences between males and females. Depending on the location of the population, there is a lot of variability in the patterns of migration into breeding ponds for both *Taricha torosa* and *Taricha granulosa* (Petranka 1998). It is important to note that these patterns and other life history traits vary according to geographical location, the topography of the area, the climate, and other ecological, and possibly even genetic, factors (Riemer 1958). Nonetheless, there have been general patterns for migration noted for both species (Twitty 1942; Stebbins 1951; Pimentel 1960; Neish 1971; Petranka 1998). In the case of *T. granulosa*, this migration pattern ranges from males and females both moving into the pond in mid-April and then leaving in late October in Canada (Neish 1971), to males migrating into the breeding pond earlier than females in late January to early March (Pimentel 1960). Some populations of *T. granulosa* may be found in breeding ponds year round (Petranka 1998). For *T. torosa*, breeding typically begins in January, although, populations in the Berkley area have been noted to breed as early as the end of September (Stebbins 1951). When migrating into the pond, males arrive at the pond before females and often stay longer following breeding (Twitty 1942; Stebbins 1951). If the males of this population move earlier than females and also stay longer, then male-biased predation may be due to increased exposure time to predators by males. Additionally, given this scenario, there would be a higher ratio of males to females in the marsh, which would also increase the chances of predation occurring on male than female *Taricha*.

It is unclear if there are differences in the timing of migration into the marsh for the two species of *Taricha*. Given the typical breeding times above, it is likely that the breeding period of the two species overlaps and/or occurs concurrently at Ledson Marsh. As the breeding periods were denoted according to the presence of *T. torosa* egg masses in Ledson Marsh (Figure 1) data regarding *T. granulosa* breeding periods were not collected during this study. *Taricha granulosa* lay individual eggs, which are more difficult to observe than the *T. torosa* egg masses. Personal observation over the past two years confirms the presence of *T. granulosa* breeding males and gravid females concurrently with those of *T. torosa*. However, it is not known exactly when each species entered and left the marsh. A difference in the timing of migration into the breeding pond for these two species, may explain the difference in the levels of predation on the two species. Either a later or earlier migration period may not coincide with activity of the predator(s), producing lower numbers of *T. granulosa* attacked due to a lack of contact between predator and prey.

It is unknown what produced the increase in levels of predation between 2003 and 2004 (Figure 5). It was originally thought that the amount of precipitation may have influenced the numbers of newts migrating to the marsh as precipitation does effect when newts migrate (Petranka 1998). However, looking at Figure 9, there does not seem to be any correlation between yearly rainfall and newt mortality. Rainfall in 2004 was above average levels, however, there have been years with far more rainfall and lesser predation. I attempted to correlate this predation with the loss of another food source for the predator(s), such as the shutdown of a nearby landfill; however, I was unable to find such an occurrence.

Although the field models did not experience any predation attempts, field observations over the research period provide some clues as to the identity of the predator(s). Most of the dead newts that were collected were found in the shallow pools at the edge of the marsh or along the shore. Newts were very rarely found in the deeper interior waters of the marsh. In some cases dead newts were found on top of stumps (Figure 19) or on top of the aquatic fern in the water. These observations suggest that these attacks are due to an avian predator. This predator would have to have enough dexterity to cleanly eviscerate a newt or something as small as the sceloperan lizard mentioned in the results. This predator is probably not similar to a great blue heron as seen in Fellers et al. (2008), as the herons were observed eating the entire newt. Herons, to my knowledge, are not known to eviscerate prey prior to consumption. As mentioned above, both Brothers (1994) and Olson (1989) have witnessed predation on *Bufo boreas*, another toxic amphibian, by members of the family Corvidae. In both instances, predation occurred near a breeding pond, and consisted of evisceration of the toad. Brothers reports that in some cases, toads were pulled out of the water onto the shore by the bird. Other Corvids, such as gray jays, Stellar's jays, and Clark's nutcrackers, have been observed eating other amphibian species and/or their larvae (Tordoff 1980; Beiswenger 1981; Turner 1960; Pilliod 2002; Murray et al. 2005).

Despite this evidence, it is not possible to fully rule out other potential predators. There have been multiple descriptions of evisceration of amphibian prey by skunks and raccoons (Hanson and Vial 1956; Wright 1966; Schaaf and Garton 1970; Groves 1980). In all of these cases the prey species was a toxic toad of the genus *Bufo*. As seen in Figure 10, these two possible predators were found very close to the marsh.

Additionally, the dam for the marsh had to be repaired during winter 2008. During that time a group of raccoons would often investigate the construction and were seen harassing western pond turtles (*Clemmys marmorata*) and other animals living in the marsh (Cyndy Shafer, personal communication). Tracks of both species were also found on the trails around the marsh. The substrate in and around the marsh itself, however, was not suitable for identifying tracks of the predator near recovered newts.



Figure 19. Juvenile newt found on top of a stump on the edge of the marsh. A) zoomed out view. B) close up.

As toxicity may be a driving factor for male-biased predation on the Ledson Marsh population, a broad ecological study of the site is needed. Relative abundance data for males and females of both species would allow one to evaluate a skewed sex ratio as a potential explanation for these data. It would also be important to have accurate data regarding the migration patterns of both species of *Taricha* into the marsh for breeding. Additionally, it would be helpful to identify the predator(s) in the area. The predators' identification would provide knowledge about its activity and habits during the newt-breeding season. In turn identification may provide further evidence as to why

there is selective predation at this location. Furthermore, identification may provide some insight into the drastic change in the numbers of newts preyed on between 2003 and 2004. Answers to all these questions may help us distinguish between two hypotheses regarding the predator(s). We would understand whether the predator(s) can detect or capture one sex/species better than the other (as with sexual dimorphism or behavioral differences) or whether the predator is discriminating between highly toxic and non-toxic newts during handling or due to learning. Additionally, we would have a clearer picture of the interactions between the two species of newts and their predator(s).

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