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Prey Preference as a Function of Feeding Experience and Prey Type in Neonate Gartersnakes (Colubridae: *Thamnophis*)

By

KATHRYN B. HALE

THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

MASTER OF SCIENCE IN BIOLOGICAL SCIENCES

IN THE GRADUATE SCHOOL, EASTERN ILLINOIS UNIVERSITY
CHARLESTON, ILLINOIS

2010

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ABSTRACT

Several species of potential prey have been introduced into the geographic range of the Two-striped Gartersnake, *Thamnophis hammondii*. Some of these prey species possess defenses that present novel challenges to the snake predator. Despite novel predator defense mechanisms (including secretions from skin glands), adult *T. hammondii* ingest several of these prey species without adverse effects. Although adult *T. hammondii* are dietary generalists, almost nothing is known about prey preferences in newborn individuals that lack feeding experience. My study determined whether or not *T. hammondii* innately exhibits a preference among native and introduced prey types. Furthermore, I compared the degree of prey preference expressed by *T. hammondii* to that seen in another species of *Thamnophis*.

I presented neonate snakes of both species with chemical cues from three prey types, two of which have been introduced within the range of the snake, and one of which is native. I used distilled water and cologne as visual and olfactory controls, respectively. The total number of tongue-flicks and latency to attack was recorded. Following five daily consecutive presentations, all subjects were fed exclusively White Cloud Minnows (*Tanichthys* sp.) for two weeks and then presented again with the chemical cues (assessing responses following a feeding bias). Compared to the introduced prey types, both snake species preferred the native Pacific Treefrog in both naïve and experienced presentations. Both *T. hammondii* and *T. sirtalis* only attacked cotton swabs with Pacific Treefrog odor, and bite frequency was similar for both species; however, females of *T. sirtalis* attacked more frequently than males. Only *T. hammondii* neonates showed a preference for cues from non-native African Clawed Frogs. A stronger response to cues from clawed frogs was also elicited by *T. hammondii* during naïve following experienced presentations. My study indicates that *T. hammondii* is a generalist throughout its life history, and that the species appears to exhibit a preference for toxic prey types not seen in other *Thamnophis* species. I further discuss the development of an innate predatory response in *T. hammondii*, and how this species might be a useful resource in eradication efforts of invasive prey species.

DEDICATION

This thesis is dedicated to my wonderful fiancé Ryan J. Anderson. I appreciate him more than he knows for helping me feed the close to one-hundred neonate snakes, understanding the long hours, bringing my dinner to the lab, but most importantly, always believing in me. His unconditional love, support, and motivation has seen me through my Master's of Science degree.

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INTRODUCTION

Many behaviors of organisms can be categorized as either innate or learned. An innate behavior is defined as any behavior that is instinctive; whereas, a learned behavior is an adaptive change in the individual's behavior that is traceable to a specific experience in the individual's life (Tinbergen 1951). Most predatory behaviors are innate because successful capture and ingestion of prey is necessary for survival. For this reason, I categorize as innate those predatory behaviors that are expressed prior to any exposure to prey.

Innate behaviors can be subquently modified by prey abundance (Jaeger and Rubin 1982) or by experiences that occur after the first expression of the behavior (Arnold 1978a, Burghardt 1992). Arnold (1978a) found that snake feeding responses to particular prey could be changed by feeding experience. Similarly, Burghardt (1992) found that repeatedly exposing neonate gartersnakes to a stimulus representing a specific prey type reduced snake response to stimuli from other prey types; thus, innate behaviors of snakes can be modified towards a single prey type.

Preferences for particular prey types exhibited by neonate snakes typically parallel feeding preferences of other ontogenetic stages of the species. These preferences can vary across populations within a species as well as among species belonging to the same genus (Burghardt 1967, 1968, Dix 1968, Arnold 1977, Drummond and Burghardt 1983). Burghardt (1971) also found that maternal influence on the prey preferences of neonate snakes is minimal, and that neonates will respond with equal vigor to prey types, regardless of the prey type offered to their mothers during gestation. This lack of maternal influence indicates that these predatory responses are innate and largely independent of any stimuli that are detected during embryonic development (Burghardt 1968).

Snakes have been the subjects of research concerning prey preference primarily because of the sensitivity of their vomeronasal organ (VNO) to chemical cues (Halpern 1992, Ford 1995). Snakes conduct chemical information to the VNO by use of their tongue. The nasal cavity is lined with non-sensory (respiratory) and sensory epithelium (Halpern 1992). The VNO opens into the anterior roof of the mouth and is comprised of a pair of sacs with olfactory cells in the epithelial lining (Parsons 1970). As a snake flicks its tongue to assess their environment, the tongue passes over these paired openings and delivers chemical information to the VNO (Parsons 1970). The importance of the tongue and vomeronasal ducts in regulating feeding and responses to prey extracts in snakes has been well documented (Wilde 1938, Burghardt and Pruitt 1975, Kubie and Halpern 1979). The number of tongue flicks elicited by a snake to any given chemical cue provides a quantifiable metric for assessing predatory interest (Burghardt and Pruitt 1975, Ford 1995).

Gartersnakes are a long-favored group for the study of VNO function and prey preference because the Genus is wide-spread throughout North America and exhibits interspecific variation in physiology and behavior (Burghardt 1993). My study is the first to examine the responses of neonate Two-striped Gartersnakes (*Thamonphis hammondii*) to the introduced African Clawed Frog (*Xenopus laevis*), and the first to compare these responses to other native and introduced prey types. The results of this study will add to what is currently known about the natural history of this species and a related species, the Common Gartersnake (*Thamnophis sirtalis*). I will also discuss the connection between my results and conservation efforts for *T. hammondii*, and illustrate a potential means by which the invasive African Clawed Frog could be controlled.

Natural History

The Genus *Thamnophis* is comprised of approximately 30 species which have varying distributions in the United States, Canada, and portions of Central America (Rossman et al. 1996). Although some species within this group are dietary specialists (*e.g.*, the Short-headed Gartersnake [*T. brachystoma*] and Butler's Gartersnake [*T. butleri*] both prefer earthworms), most others are generalists and feed on invertebrates, fish, amphibians, reptiles, birds, and mammals (Rossman et al. 1996). The diet can vary with ontogenetic stage (Arnold 1993), seasonal changes (Garcia and Drummond 1988), or geographic differences among species (Arnold 1977).

The species of interest in my study, *T. hammondii*, ranges from Monterey County, California, southward along the Pacific coast to northwestern Baja California (Ernst and Ernst 2003) and has an elevational range up to around 2450 m above sea level (Jennings and Hayes 1994). Populations have disappeared from approximately 40% of their historic range and this species is currently listed as a species of conservation concern by the California Department of Fish and Game (California Department of Fish and Game 2009).

Two-striped Gartersnakes are among the most aquatic species within the Genus, and are primarily found associated with aquatic habitat. Adults of the species are dietary generalists (Mullin et al. 2004), with prey species that include fish, anurans, and annelid worms (Ernst and Ernst 2003, Ervin et al. 2003). In addition to native prey, such as the Pacific Treefrog (*Pseudacris regilla*; Ervin and Fisher 2001), several introduced species of prey have been found in the gut of *T. hammondii*, including the African Clawed Frog (*Xenopus laevis*; McCoid and Fritts 1993, Ervin and Fisher 2001) and Bullfrog (*Lithobates catesbeianus*; Ervin et al. 2003).

Common Gartersnakes have the greatest geographical distribution of any member of the Genus (Fitch 1965), and are among the most extensively studied. Pertaining to my thesis, research has examined how the species' response to chemicals is affected by geographical variation (Dix 1968), sex, diet, or litter (Lyman-Henley and Burghardt 1995), early feeding experience (Arnold 1978a), and genetic influences on feeding experience (Burghardt et al. 2000). Similar to their distribution, this species' diet is extensive and includes terrestrial and aquatic invertebrates, fish, amphibians, mammals, and birds (Rossman et al. 1996). For these reasons, this snake species was used as a basis for comparison in my examination of *T. hammondii* responses.

Toxin resistance in Thamnophis

Some *Thamnophis* species can tolerate toxins produced by certain prey types – the most extensively studied of these chemicals is tetrodotoxin (TTX). This toxin is secreted from integumentary glands in some amphibians and is a potent neurotoxin that blocks sodium-gated channels in nerve and muscle tissue, resulting in paralysis (Lipkind and Fozzard 1994). Despite the presence of TTX in the Rough-skinned Newt (*Taricha granulosa*), it is consumed by *T. sirtalis*. Moreover, resistance to TTX varies among individual snakes, has a genetic basis (Brodie and Brodie 1990), and is not affected by short or long term exposure to the toxin (Ridenhour et al. 1999). A related species in California, the Sierra Gartersnake (*T. couchii*), has also developed a resistance to TTX from *Taricha torosa* (Brodie et al. 2005). Skin secretions of the Eastern Newt, *Notophthalmus viridescens*, also contain TTX and *T. sirtalis* has voluntarily consumed these newts with no signs of distress (Hurlbert 1970, McCallum 1994). In Michigan, *N. viridescens* has also been found in the guts of Eastern Ribbonsnakes (*T. sauritis*; Carpenter 1952), demonstrating resistance among several *Thamnophis* species occupying geographically distinct areas.

While consumption of toxins typically produces adverse side effects, gartersnakes eating newts can acquire a benefit from the ingested TTX. Williams et al. (2004) showed that individuals of *T. sirtalis* that regularly consume newts harbor active toxin in their tissues for at least one month, and that the amount present could incapacitate or kill their own avian or mammalian predators. Phylogenetic reconstruction of the evolution of TTX has also revealed that species within *Thamnophis*, and possibly natricine snakes in general, are 10 times more resistant to TTX than other colubrid snakes (Motychak et al. 1999). Such a predisposition suggests that snakes of this Genus might possess resistance to other toxins as well. Although initially synonymized, *T. couchii* and *T. hammondii* have more recently been shown to be distinct species that are closely related (Rossman and Stewart 1987, DeQueiroz and Lawson 1994, DeQueiroz et al. 2002), suggesting *T. hammondii* might also display this same resistance to TTX.

Introduced prey species sympatric with T. hammondii

There are approximately 120 native amphibian and reptile species in California and, in recent years, the quality of many aquatic habitats available to them has been compromised due to the introduction of various species. At least seven species of introduced amphibians and reptiles have established breeding populations and an additional 30 exotic or transplanted species have been recorded (Bury and Luckenbach 1976).

Although the Bullfrog is a native species to certain parts of the United States, it is non-native to areas west of the Rocky Mountains (Jennings and Hayes 1985). In California, the frogs were initially introduced in 1896 as a source of food for humans after local native populations of the Red-legged Frog (*Rana aurora*) were over-harvested (Jennings and Hayes 1985). Bullfrogs can survive in a variety of habitats and can grow to large sizes, with a 200-g adult capable of consuming 2.1 times its body mass in a season (Hewitt 1950). This larger size (compared to other frog species) enables Bullfrogs to ingest a variety of large prey types, including snakes (Hewitt 1950). In California, competition between Bullfrogs and native anurans, such as the Pacific Treefrog, has been shown to reduce survivorship of the native species, especially at the larval stage (Kupferberg 1997). Additionally, the presence of Bullfrogs has contributed to population declines of *T. hammondii* both by direct predation on neonate snakes and indirectly, through consumption of native prey species (Jennings and Hayes 1994).

The African Clawed Frog is of particular interest in this study due to its relatively recent introduction (1968; Bury and Luckenbach 1976, McCoid and Fritts 1993) and the toxins which are secreted from its integumentary glands. These toxins are comprised of the indolealkylamines serotonin (5-HT) and bufotenidine (BF), and the peptides cholecystokinin octapeptide (CCK-8), caerulein (CRL), thyrotropin-releasing hormone (TRH), and xenopsin (XN; Barthalmus and Zielinski 1988). Although not as toxic as tetrodotoxin, XN, CCK-8, and CRL all have neuroleptic properties (Bissette et al. 1986, Phillips et al. 1986), while BF and TRH induce a gaping response (Seeman and Brown

1985, Scarpignata et al. 1986) by increasing serum prolactin and oxytocin levels. 5-HT can induce both neuroleptic and gaping responses (Ennis et al. 1981). As such, these toxins render *X. laevis* unpalatable to most predators native to North America that readily consume frogs and, upon consumption, produces a gaping response in natricine snakes. Additionally, writhing tongue movements, gular and chewing movements, and climbing behavior have been observed post-consumption (Barthalmus 1989). These toxins act within 30 sec of ingestion, presumably facilitating the frog's escape (Barthalmus and Zielinski 1988).

Despite these toxins, adult *T. hammondii* readily consume *X. laevis* without impairment of behavior or locomotor function (Ervin and Fisher 2001, Foster and Mullin 2008). The ability to consume this anuran has been observed in only this species of *Thamnophis*; however, the mechanism by which *T. hammondii* is able to tolerate *X. laevis* toxins is not yet known. Co-existence of *T. hammondii* and *X. laevis* in natural habitats has occurred for approximately 40 years. This relatively brief sympatry might indicate that rapid evolution has occurred in response to this toxic prey species. Alternatively, *T. hammondii* might possess an innate resistance to the toxins secreted by *X. laevis*, similar to TTX resistance in some members of *Thamnophis*.

Regardless of their collection locality, the predatory responses elicited by adult *T. hammondii* are similar for both native and introduced species (Mullin et al. 2004). Moreover, *T. hammondii* uses a variety of different foraging behaviors to successfully capture these diverse prey taxa (Dunn 2004), and is able to reduce pupil aperture when submerged underwater to forage in low light conditions (Fontenot 2008). Given these behavioral and physiological traits, *T. hammondii* would appear to be an effective predator on this introduced species of aquatic prey.

Whereas adult *T. hammondii* show predatory interest in both native and introduced chemical cues (Mullin et al. 2004), the development of this behavior has yet to be explored. This is particularly important for this species of gartersnake because the degree to which neonate snakes respond to these introduced prey species will provide valuable information regarding the natural history of this snake of conservation concern. Furthermore, should neonates elicit a predatory response to these introduced species, *T. hammondii* may be of assistance in eradication efforts of these invasive anurans by means of predation.

Research Objectives

The purposes of my research were to determine the degree of preference among neonate Two-striped Gartersnakes for native and introduced prey species, and if a bias in preference develops after exposing the neonate subjects to a single prey type. I performed similar experiments with neonate *T. sirtalis* and compared their predatory behaviors with those expressed in neonate *T. hammondii*. I addressed the following null hypotheses: 1. Neonate *T. hammondii* respond to chemical cues from different prey types with equal vigor; 2. Predatory responses of neonate *T. hammondii* do not change following feeding experience involving the same prey species; and, 3. Neonate *T. sirtalis* respond to chemical cues from different prey types in a fashion similar to that expressed in *T. hammondii*. Neonate *T. hammondii* might respond to prey odors in the same fashion as adult individuals (Mullin et al. 2004), but could be more readily influenced by feeding experience. Based on differences in biogeography and natural history, I expect that predatory responses of both garersnakes species to chemical cues will differ.

METHODS

Study Organisms

Collecting trips to field localities in San Diego County, California, resulted in captive colonies of both predator and prey species (California Department of Fish and Game permit #802017-02). I maintained three female and six male adult *T. hammondii* in the lab and housed them in 30x30x60 cm plastic cages (IACUC protocol #04-005). These enclosures were lined with newspaper substrate and each contained a glass water dish, black plastic shelter, and shedding substrate. Water was provided *ad libitum*, and snakes were fed either fish (mostly *Pimephales* sp. or *Notropis* sp.) or anuran larvae (*Lithobates* sp.) on a bi-weekly basis.

To obtain neonate *T. hammondii*, I placed all adult snakes in individual containers within an environmental chamber (11 °C and constant darkness) for a brumation period lasting from October 2008 to March 2009. Females can store sperm for up to 53 months (Stewart 1972); therefore, two males were paired with each female both before and after this brumation period. Male and female snakes were paired at least two times to enhance the likelihood of females being inseminated. All three females produced offspring in July and August 2009 with clutch sizes of 8, 8, and 12 neonates that lacked prior exposure to any chemical stimuli. After birth, I housed the neonate subjects by clutch in plastic containers measuring 41x27x15 cm, and provided them with newspaper cage lining, a shelter, and water *ad libitum*.

From May to June 2009, I collected gravid *T. sirtalis* from localities in Coles County, Illinois; following capture, these females (n=3) were housed and fed in the manner described for adult *T. hammondii* above. In June 2009, these female subjects gave birth to clutches totaling 19, 23, and 32 neonates.

At birth, I recorded the gender of each subject and marked each on the parietal scales with a unique combination of small (*circa* 0.5 mm) dots of inert water-soluble paint. Prior to any feeding trials (within 10 days of birth), I recorded the snout-vent length (SVL; ±1 mm) and mass (±0.1 g) of all neonates for both species.

General Testing Procedure

All *T. hammondii* and *T. sirtalis* neonates were tested following their first ecdysis (Porter and Czaplicki 2003) for their responses to chemical cues extracted from the following prey types: 1. Adult *Pseudacris regilla* (Pacific Treefrog; native); 2. adult *Lithobates catesbeiana* (Bullfrog; introduced); and, 3. adult *Xenopus laevis* (African Clawed Frog; introduced). Distilled water and English Leather™ cologne were used as visual and olfactory controls, respectively. The water was a negative control that contained no scent, and the cologne (diluted 1:3 with distilled water to minimize aversive responses) was a positive control for a novel scent that had no meaning in a predatory context.

I prepared each prey extract following procedures described by von Achen and Rakestraw (1984) and Cooper and Vitt (1989). After rinsing a prey item with distilled water to remove any residual odors, I rubbed the tip of a 15 cm cotton swab several times across its dorsal body surface. Excess liquid from each swab was shaken off prior to its presentation to a neonate subject. To minimize responses from other stimuli, I moved each neonate subject from its home cage to a smaller (20x20x6 cm) plastic container (which was sealed between presentations), and allowed a 5 min acclimation period in this testing chamber before any odor presentation. I washed the testing chambers with soap and hot water prior to placing different subjects in them.

Each trial commenced when I moved a swab slowly to within 2 cm of a subject's snout. For a period of 60 sec, I recorded the number of tongue flicks directed the swab. I also recorded the latency (±1 sec) to bite the swab (Burghardt 1993). If no tongue flicks were directed at the swab within the first 30 sec of presentation, I moved the swab closer and touched the labial scales of the subject. Prey extracts were presented in series, with an interval of 5 – 10 min between presentations. All odor types except the positive control were presented sequentially in random order (Ford 1995). The cologne scent was presented to each subject only two times over the course of the study – once at the beginning of the series of trials and once at the end. Limiting the presentation of cologne served to minimize any effects that this pungent scent might have had on the neonates' predatory responses to any subsequent chemical cues.

Data for the number of tongue-flicks and latency to attack cotton swabs was combined into a single metric – the tongue-flick-attack-score (TFAS; Cooper and Burghardt 1990), where:

$$\text{TFAS} = [\text{number of tongue flicks} + (60 - \text{latency to attack})] \qquad \text{[Eq. 1]}$$

Higher TFAS scores denote greater predatory interest towards a particular chemical cue. Values for TFAS have been shown to reliably predict predatory interest by a variety of snake species (Cooper and Burghardt 1990).

Testing Naïve Subjects

To address the first null hypothesis that neonates will respond to chemical cues of different prey types with equal vigor, each neonate subject experienced its first trial on the day following the first ecdysis (a maximum of 8 days following birth). Initially, I replicated odor presentations to each subject daily (with a different presentation order) over a 6-day period. Due to logistic problems that arose following the initial trials with *T. sirtalis* (n=74), I reduced the number of replicate presentations to a 5-day period for all subsequent trials (including all trials involving *T. hammondii*; n=28).

Testing Experienced Subjects

To address the second null hypothesis that predatory responses of neonates will not change following feeding experience, at the end of day 6 (or 5 for *T. hammondii*), each neonate was offered White Cloud Minnows (*Tanichthys* sp.) to test its willingness to feed. Data from individuals refusing to eat after their initial testing period were discarded. To test the second hypothesis concerning the effect of experience, *T. hammondii* (n=21) and *T. sirtalis* (n=26) subjects were fed only White Cloud Minnows for a two-week period. The amount of prey offered per week approximated 50 % of each subject's body mass. I used this food source because none of the anurans providing chemical cues could be obtained in sufficient numbers, and in a size class that could be ingested, to feed the snakes at this rate. After two weeks of this feeding regime, each subject was considered as having obtained feeding experience and again was presented with chemical cues from the same sources as described above. This resulted in replicate trials occurring over five consecutive days and a different order of stimulus presentation on each day.

Statistical Analyses

My data set consisted of TFAS values for five or six (*T. sirtalis* only) replicate sets of trials for naïve subjects, and five replicate sets of trials for experienced subjects. Because the number of replicate trials for *T. sirtalis* differed between odor presentations made to naïve versus experienced subjects, I analyzed data from these two time periods in separate models. Similarly, because data sets representing each species had different sample sizes, I analyzed data contributed from each species in separate models.

I controlled for multiple presentations of chemical stimuli to the same subject by using both analyses of variance (ANOVA) and a repeated measures analyses of variance (ANOVAR) to detect differences in TFAS values (Cooper and Burghardt 1990, Zar 1999). Within SAS software, (SAS v9.1.3, SAS Institute, Inc., Cary, NC), I first used ANOVA with Proc-GLM (general linear model), followed by ANOVAR with Proc-Mixed models to account for any non-independence of the measurements. The combined use of these two models allowed me to test for correlation within subjects, and for temporal effects (of experience), across repeated presentations of the same stimuli. In those analyses where both the GLM and mixed models produced the same outcome, I report the F-statistic and p-value for the GLM model as this indicates within subject correlations and temporal effects were not significant. For those analyses where the two models produced different F-statistics and p-values, I report the mixed model output.

I also analyzed the bite frequency as a separate metric of predatory interest by neonate gartersnakes. For only those snakes which bit a cotton swab, the number of trials in which the individual bit was summed and the mean number of bites was calculated for each species (across all replicates for a particular odor). Individuals who never bit a swab were not included in this analysis. The means of these frequencies were subjected to an ANOVA as described above. The level of statistical significance for these analyses was set at $\propto = 0.05$.

For any differences detected in either of the response variables (TFAS or mean bite frequency), I conducted *post-hoc* comparisons of treatment means, using Bonferroni adjustments to significance values (Zar 1999). Below, I report least-squares means ± 1 standard error (SE).

RESULTS

Reproductive and morphometric traits

During the two-week feeding period, several neonates of both species died, resulting in a smaller sample size for experienced presentations compared to naïve presentations. Deaths occurred at different times and did not seem to be related. The clutch sizes for *T. hammondii* tended to be smaller than *T. sirtalis* clutches. Clutches in *T. hammondii* were male or female experienced, compared to the *T. sirtalis* clutches that were closer to a 50:50 sex ratio (Table 1). The mean (±1 SE) mass of *T. hammondii* neonates (2.53 ± 0.14 g) tended to be greater than that of *T. sirtalis* neonates (1.38 ± 0.01 g). The mean SVL values for both species followed a similar pattern of neonates (171.32 ± 3.50 mm and 151.05 ± 0.58 mm for *T. hammondii* and *T. sirtalis*, respectively; Table 1).

Responses of T. hammondii

Neonate *T. hammondii* exhibited different mean TFAS as a function of odor type ($F_{4,1068} = 207.54$, $p < 0.0001$) and feeding experience ($F_{1,1068} = 10.0$, $p = 0.002$), and the interaction between these variables ($F_{4,1068} = 5.91$, $p < 0.0001$). The mean TFAS was highest when snakes were presented with Pacific Treefrog odor (21.55 ± 0.56), followed by that in response to African Clawed Frog odor (5.77 ± 0.56). TFAS values for these two odor types were higher than when snakes were presented with other odors. The mean TFAS value for snakes presented with Bullfrog odor was similar to that of both control odors (Table 2). The TFAS in subjects without feeding experience (7.71 ± 0.38) was greater than in those subjects after two weeks of experience feeding on minnows

(5.90 ± 0.43; $p = 0.002$). This difference in TFAS was driven by a decreased response to African Clawed Frog odor after two weeks of feeding on minnows ($p < 0.0001$; Table 3).

There was no difference in TFAS as a function of subject sex ($F_{1,1058} = 0.06$, $p = 0.81$), trial ($F_{4,1051} = 1.11$, $p = 0.35$), clutch ($F_{2,25.4} = 1.51$, $p = 0.24$), or the interactions between these variables and most of their interactions with other variables ($F \leq 1.57$, $p \geq 0.09$). The one exception was a significant interaction between subject clutch and odor type ($F_{8,1032} = 12.20$, $p < 0.0001$) where the mean TFAS to Pacific Treefrog odor in subjects from one clutch was higher than in subjects from the other two clutches. There were no differences in TFAS values for the other odor types as a function of subject clutch (Table 4).

Responses of T. sirtalis

The mean TFAS for *T. sirtalis* differed as a function of odor type ($F_{4,2486} = 213.29$, $p < 0.0001$) and the interaction between odor type and feeding experience ($F_{4,2486} = 8.47$, $p < 0.0001$). Pacific Treefrog odor elicited a stronger response (TFAS = 13.69 ± 0.34) than any other odor type (Table 5), and subjects responded to this particular odor with greater vigor after they had acquired feeding experience. TFAS for other odor types and experience levels were similar ($p \geq 0.16$; Table 6).

Mean TFAS differed as a function of subject gender ($F_{1,2481} = 18.57$, $p < 0.0001$), and the interaction between gender and odor type ($F_{4,2481} = 10.31$, $p < 0.0001$). Female subjects (5.33 ± 0.24) exhibited greater responses than male subjects (4.05 ± 0.22) regardless of odor type, and responded with greatest vigor to Pacific Treefrog odor (16.28 ± 0.46; Figure 1).

Although *T. sirtalis* TFAS were similar across clutches ($F_{2,105} = 2.03$; $p = 0.14$), there was a significant interaction between clutch, odor type, and feeding experience that affected TFAS ($F_{8,231} = 3.17$, $p = 0.001$). This was driven by subjects in one clutch responding to Pacific Treefrog odor with greater vigor after acquiring feeding experience, as compared to those same subjects before feeding, or in comparison to subjects from other clutches (Table 7).

Mean TFAS differed as a function of replicate trial ($F_{5,2448} = 4.15$, $p = 0.001$), and the interaction between trial, odor type, and feeding experience ($F_{12,2448} = 4.81$, $p < 0.0001$). When presented with Pacific Treefrog odor, naïve subjects exhibited greater response in the first trial compared to the others. After feeding experience, however, these subjects responded to the same odor with greater vigor in later trials (Table 8).

Interspecific comparisons

Mean TFAS differed between the two snake species ($F_{9,3564} = 56.80$ $p < 0.0001$) and as a function of odor type ($F_{9,3564} = 472.72$, $p < 0.0001$). Regardless of odor type, the TFAS for *T. hammondii* (6.93 ± 0.24) were greater than *T. sirtalis* values (4.71 ± 0.16; $p < 0.0001$). Neonates of both snake species responded to the different odors in similar fashion, with Pacific Treefrog odor eliciting the greatest response (17.17 ± 0.29), followed by African Clawed Frog odor (4.84 ± 0.29) and Bullfrog odor (3.33 ± 0.29). All three prey odors elicited greater TFAS than either of the control odors, and mean TFAS for these controls did not differ from each other (Figure 2). The interaction of snake species and odor type also influenced TFAS ($F = 39.10$, $p < 0.0001$). Neonate *T. hammondii* responded to African Clawed Frog and Pacific Treefrog odors with greater vigor than neonate *T. sirtalis* ($p < 0.0001$; Figure 2). The mean TFAS also varied as a function of an interaction between snake species and feeding experience ($F_{1,3570} = 4.85$, $p = 0.03$), but this effect was driven by the decreased response in *T. hammondii* subjects after having been fed minnows for two weeks ($p < 0.0001$; Figure 3).

Regardless of snake species, subjects bit swabs scented only with Pacific Treefrog odor. The average bite frequency per individual did not differ between the two species ($F_{1,28} = 0.61$ $p = 0.44$). In *T. hammondii,* the average bite frequency did not vary by sex ($F_{1,11} = 0.49$, $p = 0.50$) or clutch ($F_{1,11} = 1.62$, $p = 0.24$), but was greater after gaining feeding experience (2.41 ± 0.36) compared to naïve presentations (1.27 ± 0.37; $F_{1,21} = 5.0$, $p = 0.04$). In *T.sirtalis*, the average bite frequency per individual did not vary as a function of any of these three independent variables ($F \leq 1.31$, $p \geq 0.28$).

DISCUSSION

Neonates born to *T. hammondii* females tended to be larger than *T. sirtalis* in both mass and snout-vent length; the number of neonates per clutch in *T. hammondii*, however, was smaller than that in *T. sirtalis*. These differences could be attributed to stress in captivity (Fitch 1987) or food availability (Ford and Seigel 1989). Offspring having larger initial body size typically experience greater fitness when compared to smaller siblings (Smith and Fretwell 1974) because larger size confers advantages such as ease of escaping predation or competing for resources. The greater variation in offspring size in neonate *T. hammondii* might be a function of a higher quality maternal diet in captive females (Seigel and Ford 1992). Energy resources might have been partitioned towards increasing embryo size (Stewart et al. 1990). Fewer embryos *in utero* might have resulted in more energy being allocated per embryo; thus, small clutch sizes with larger individual mass were produced by the *T, hammondii* females. Lastly, the difference in reproductive experience between captive *T. hammondii* and wild-caught *T. sirtalis* might also have contributed to differences in clutch size and neonate body condition, as has been shown in birds (Pyle et al. 1991, Komdeur 1996).

Prey Preference

The greatest predatory response, as measured either by TFAS or bite frequency, was elicited when both *T. hammondii* and *T. sirtalis* were presented with Pacific Treefrog odors. The response by *T. hammondii* demonstrates the importance that prey sympatry has in regulating predatory interest. Several snake species have shown similar patterns of differential response to prey species depending on the degree of sympatry between them (Gove and Burghardt 1975, Arnold 1977, Cooper et al. 1990, Cooper et al. 2000). While a predatory response was not exclusive to this prey species, snakes bit the swab (attacking behavior) only if Pacific Treefrog odor was present, further indicating a strong preference for this anuran by both *T. hammondii* and *T. sirtalis*.

This behavioral pattern provides strong evidence for a higher degree of predatory interest existing in both gartersnake species for Pacific Treefrogs as compared to other prey types in this study. Similar attacking behavior directed only at a preferred prey type was reported by Cooper et al. (2000). Although the bite frequencies were similar in both species when presented with Pacific Treefrog odors, females of *T. sirtalis* attacked more frequently than males. The higher number of attacks from females indicates that these neonates were more aggressive in responding to odors from this prey type. Sexual size dimorphism, with larger females, has been documented in neonates of several snake species. Large body size is directly correlated with increased reproductive success in females (Shine 1986). As such, a female should aggressively seek food resources early in life, which might explain my results. Higher rates of food consumption increase body mass, ultimately increasing the overall fitness of a female individual.

When presented with African Clawed Frog odor, predatory responses of neonate *T. hammondii* were also higher than for control odors. The preference for this introduced prey species is of further interest because it indicates that an innate predatory response exists in *T. hammondii*. My results corroborate the description by Mullin et al. (2004) of the generalist nature of this species as a predator. The alternative explanation for this response is one of rapid co-evolution (Phillips and Shine 2006); however, given the relatively recent introduction of this anuran within the geographic range of *T. hammondii*, the former interpretation of my results is more probable. For this reason, I suggest that *T.*

hammondii might also show innate predatory responses to other introduced prey species, assuming that these prey populations are established in sympatry with the snake. Neonate *T. sirtalis* did not exhibit a similar response to this prey type. This finding supports those of previous studies examining interspecific variation in prey preference attributed to differences in biogeography (Burghardt 1967, Arnold 1981). Burghardt (1967) suggested that these interspecific differences correspond to the normal feeding preference within the region occupied by each species.

The generalist nature of *T. sirtalis* was supported by the preference for the non-native Pacific Treefrog. This result was unexpected given that these snakes were collected in Illinois, well outside the range of this prey item. Arnold (1978a) found that neonate snakes readily ingested non-native prey; moreover, neonates consumed larval and adult frogs of multiple species regardless of their level of experience with other prey types. This illustrates the preference for anuran prey species, including those that are not sympatric with local populations, as was the case in my study. A similar response to Pacific Treefrogs was observed in naïve neonate Terrestrial Gartersnakes (*T. elegans*) from populations allopatric to the distribution of the prey species (A. Bronikowski, pers. comm.).

Neither *T. hammondii* nor *T. sirtalis* showed predatory interest to Bullfrog odors. This lack of response was unexpected as adults of both species readily consume this anuran in the lab (pers. obs.). This result might be attributed to a variation in chemical cues existing during different life stages of the Bullfrog, and/or a neonate snake's inability to consume prey larger than its gape size.

Snakes having a large gape size are typically able to capture and ingest large prey items (Shine 1991). As such, the larger size of a Bullfrog, compared to a Pacific Treefrog, would prevent a neonate gartersnake from consuming an adult Bullfrog in the field and, as previously noted, adult Bullfrogs depredate neonate gartersnakes in the field. Although adult stages of the three anurans were used as odors sources in my study, the size of the Pacific Treefrog was smaller than that of the Bullfrog or African Clawed Frog. Furthermore, neonate gartersnakes have been shown to prefer Bullfrog larvae (Rayburn 1990), whereas adult gartersnakes tend to exhibit greater predatory interest towards metamorph stages of this same prey species (Arnold 1978b). This suggests that gartersnake preferences for different life history stages of Bullfrogs can change over the ontogeny of both species. Given that the sole mechanism by which neonates were capable of perceiving prey types was through chemical cues on a cotton swab (*i.e.*, no visual confirmation), it is possible that neonate gartersnakes might be capable of differentiating between life history stages of Bullfrogs based on chemical cues alone and that this bias exists innately in neonate snakes. Biochemical analyses are required to determine the degree to which chemical cues differ between anuran life stages and should be examined in future studies. My study also supports the importance of chemoreception compared to vision in natricine snakes.

There was a differential response to Pacific Treefrog odor among clutches with one clutch within each snake species responding more strongly. This result is supported by other studies that have shown behavioral variation between clutches and individuals within a species, specifically differential response to the same prey item (Burghardt 1993, Lyman-Henley and Burghardt 1995). Furthermore, within a local population of snakes, some individuals might not accept prey that is readily accepted by a majority of others

(Dix 1968).

As compared to subsequent trials, the higher TFAS exhibited by *T. sirtalis* in the first trial could be a conditioning response to the strong odor of cologne, as cologne was only presented to these subjects during trials 1 and 6. Cologne scents (even when diluted) have produced aversion effects, with certain brands increasing tongue-flicking rates in lizards (Cooper et al. 2003). The strong odor of the cologne might also have resulted in neonates eliciting a higher TFAS to other prey odors. Cooper et al. (2003) reported that, as a positive control, cologne can suppress the response to food chemicals, which was why I chose to restrict the presentation of this odor to only the first and last replicates within each treatment.

Responses by naïve and experienced snakes

After feeding on minnows for two weeks, TFAS in *T. hammondii* changed only when presented with African Clawed Frog odor. The decrease in TFAS during the presentations following feeding experience suggests several possible scenarios: 1. neonate *T. hammondii* respond to clawed frog odors innately but, upon consuming a different prey type, the predatory response decreases (*i.e.*, a possible bias induced by feeding on minnows; Fuchs and Burghardt 1971, Burghardt 1992); 2. neonate preference for clawed frog odor decreases because responses during initial presentation of the odor were not reinforced by ingestion of that prey type (*i.e.*, predatory interest was extinguished by not feeding on *Xenopus*; Burghardt 1992); 3. neonate prey preference can change quickly within the early life history of the snake (*i.e.*, differential responses that are independent of feeding experience; *sensu* Arnold 1978, Mushinsky and Lotz 1980); 4. some habituation to clawed frog prey odor occurred (*i.e.* failure of neonates to respond to clawed frog odor presented in the usual manner; Cooper and Hartdegen 2000); or, 5. a synergistic interaction between more than one of the above explanations.

Given the findings of my study, the most probable explanation for this behavior is the development of a bias away from African Clawed Frog odor after gaining feeding experience (*i.e.*, extinguishing the initial preference). Reinforcement of prey preference by feeding does not appear to be a likely scenario because neonate response to Pacific Treefrog odors increased without having consumed this prey type. Furthermore, these increased responses after feeding preclude habituation to African Clawed Frog odors because habituation would have also occurred to Pacific Treefrog odors. Lastly, while a shift in prey preference early in the life history of the snake is plausible, neonates were less than one month old, and a shift within this time frame is unlikely. Such a shift in a generalist predator would be disadvantageous as it would limit food resources for a neonate needing to grow quickly (*i.e.*, a larger body size can be a deterrent against other predators). Further research examining effects of feeding experience should attempt to elucidate the causes for shifts in predatory response as seen in these snake species.

While only statistically discernible in *T. sirtalis*, both species elicited stronger predatory responses to Pacific Treefrog odor after acquiring two weeks of feeding experience. Dunbar (1979) suggested that the innate chemical preference for sympatric prey species supercedes feeding experience, which supports the strong initial predatory response that I observed in *T. hammondii*. Furthermore, Gove and Burghardt (1975) provide evidence that such a predisposition has a genetic basis. The strong response observed in *T. sirtalis* might be explained by a predisposition for anuran prey types, regardless of sympatry (Arnold 1978a). Alternatively, this species might exhibit a preference for anurans belonging to the Genus *Pseudacris*, and future studies examining this relationship are warranted.

The increase in TFAS for *T. sirtalis* in trials that followed feeding experience could be driven by hunger. Increased responsiveness to chemosensory cues in snakes can be mediated by hunger (Chiszar et al. 1981). I considered all snakes that ate any minnows as having acquired feeding experience. I did not, however, assess rates of growth or energy uptake, and assumed that snakes were assimilating energy from the minnows with equal efficiency. If *T. sirtalis* neonates were less satiated by these feedings, their level of predatory interest after this experience might have been higher than in *T. hammondii.* Alternatively, the increase in TFAS might have been a function of the stochastic nature of behavior observed in neonate gartersnakes and a limited sample size. This stochasticity demonstrates the importance of repeating behavioral studies to discern between such effects.

Management Implications

The results of my study provide evidence of the broad range of diet for two species of *Thamnophis*, of which *T. hammondii* is of greater conservation concern. In addition to providing further natural history information for Two-striped Gartersnakes, the nature of this species' responses as a naïve predator reinforces its description as a dietary generalist (Mullin et al. 2004). Of particular interest is the response pattern to African Clawed Frogs, a prey species refused by other North American snakes that regularly eat anurans. Given that adult *T. hammondii* depredate clawed frogs in the field (Ervin and Fisher 2001), and that neonate snakes respond to clawed frog odors, the ability of young snakes to capture larval or subadult stages of clawed frogs should be assessed.

These findings should then be compared to those of Dunn (2004) to determine if prey handling and foraging behavior of neonate *T. hammondii* is similar to those behaviors expressed by adults. By depredating the introduced prey in earlier life history stages, Two-striped Gartersnakes could serve as an appropriate biological control for existing populations of clawed frogs in southern California. Additionally, increased management of *T. hammondii* populations (including repatriation of new populations within their historic range, for example) might also minimize the colonization ability of clawed frogs to adjacent wetlands. Wider distribution and high density of *T. hammondii* in these areas might limit the expansion of clawed frogs if suitable habitat is available (Appendix I).

Thamnophis sirtalis populations in northern California depredate the toxic newt, *Taricha torosa* and have evolved a resistance to TTX (Brodie and Brodie 1990), a more powerful toxin than that found in *Xenopus* skin. Ingesting clawed frogs does not appear to impede physiological function in *T. hammondii* (Foster and Mullin 2008), but tests examining neonate performance are lacking. Given the response to *Xenopus* odor by neonate *T. hammondii*, their innate recognition of this species as potential prey might be coupled with an innate physiological mechanism for reducing its toxicity (*e.g.*, denaturing skin compounds). Future research should include behavioral studies of neonates post-consumption to determine if such a resistance exists at all ontogenetic stages. If neonates do not display adverse effects after exposure to the toxins of African Clawed Frogs, this introduced anuran presents an additional food resource in aquatic habitats where native prey types are lacking (possibly through competitive exclusion or predation by clawed frogs). Such willingness to accept recently introduced prey species might allow for flexibility of establishing populations in new habitats with different prey items or adaptation to long-term environmental changes.

Conclusions and future research

My study provides evidence that variation in predatory responses exists between two species of *Thamnophis* from geographically distinct regions, and that this variation is attributable to prey type. Responses in ingestively-naïve individuals are high for both native and introduced prey species, and can vary by clutch or gender. Furthermore, depending on the prey type, feeding experience might facilitate a subsequent decrease in response to a particular prey type, or fail to change an already maximal response.

Future studies should examine prey preferences of neonate *T. hammondii* from different geographical regions as all neonates in this study were born to females collected from the same locality. Studies should also attempt to detect any preference for the different life history stages of African Clawed Frogs, and thus, if earlier ontogenetic stages elicit a stronger response in neonate *T. hammondii*. Future research with this gartersnake species should also examine if a predatory response is elicited for other toxic prey species, including species of newts (*e.g.*, *Notophthalmus* and *Taricha*). Given the fact other gartersnakes respond with predatory interest to newts, these amphibians might additionally provide *T. hammondii* with an alternative prey type (as yet undocumented).

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Table 1. Total clutch sizes, sex ratios, and mean (±1 SE) and range of the snout-vent lengths (SVL; mm) and masses (g) for neonate Two-striped Gartersnakes (*Thamnophis hammondii*) and Common Gartersnakes (*T. sirtalis*) that were tested for responses to prey odors in 2009. Neonate *T. hammondii* were born to female snakes that had been maintained in captivity, whereas neonate *T. sirtalis* were born to females collected that year in Coles Co., Illinois.

				Mass (g)			SVL (mm)		
Species	clutch	N	Sex Ratio (M:F)	Mean	Minimum	Maximum	Mean	Minimum	Maximum
T. hammondii	H7	12	10:2	1.86 ± 0.16	0.98	2.74	154.33 ± 3.80	130	174
	H18	8	3:5	3.16 ± 0.10	2.78	3.60	185.13 ± 2.70	171	195
	H26	8	1:7	2.89 ± 0.18	1.72	3.45	183.00 ± 3.91	156	189
T. sirtalis	S2	23	12:11	1.33 ± 0.02	1.20	1.61	151.12 ± 1.08	141	161
	S3	32	16:16	1.44 ± 0.02	1.19	1.64	150.22 ± 0.02	140	164
	S4	19	13:6	1.33 ± 0.02	1.09	1.52	152.26 ± 1.04	143	160

Table 2. A matrix of Bonferroni adjusted p-values for all comparisons by prey type of tongue-flick attack scores in neonate Two-striped Gartersnakes (*Thamnophis hammondii*). Subjects, born to female snakes that had been maintained in captivity, were presented with prey odors between June and September 2009.

	Cologne	Water	Bullfrog	African Clawed Frog	Pacific Treefrog
Cologne	-				
Water	1.0	-			
Bullfrog	1.0	0.99	-		
African Clawed Frog	0.0003	0.0001	0.0074	-	
Pacific Treefrog	<0.0001	<0.0001	<0.0001	<0.0001	-

Table 3. Least-squares means (±1 SE) of tongue-flick attack scores and Bonferroni adjusted p-values of naïve and experienced prey presentations in neonate Two-striped Gartersnakes (*Thamnophis hammondii*) born in captivity. Different superscript letters denote differences between prey types regardless of feeding experience, whereas the asterisk denotes a difference between naïve and experienced presentations for a single prey type. Odor presentations occurred between June and September 2009.

	Naïve	Experienced	p-value
Cologne^{a}	1.82 ± 1.16	0.86 ± 1.34	1.0
Water^{a}	2.72 ± 1.74	1.87 ± 0.85	1.0
Bullfrog^{a}	4.25 ± 0.74	1.90 ± 0.85	1.0
African Clawed Frog^{b}	8.85 ± 0.74	2.69 ± 0.85	< 0.0001*
Pacific Treefrog^{c}	20.91 ± 0.74	22.18 ± 0.85	1.0

Table 4. Least-squares means (±1 SE) of tongue-flick attack scores of each clutch of Two-striped Gartersnakes (*Thamnophis hammondii*) to the different prey odors presented between June and September 2009. The asterisk denotes a higher response to that prey type (see text).

	Cologne	Water	Bullfrog	African Clawed Frog	Pacific Treefrog
Clutch 7	2.0 ± 1.56	2.75 ± 1.1	3.74 ± 1.1	6.20 ± 1.1	15.99 ± 0.93
Clutch 18	1.16 ± 1.61	2.31 ± 1.16	2.91 ± 1.16	5.98 ± 1.16	19.48 ± 0.93
Clutch 26	1.03 ± 1.61	1.99 ± 1.16	2.75 ± 1.16	5.30 ± 1.16	29.19 ± 0.93*

Table 5. A matrix of Bonferroni adjusted p-values for all comparisons by prey type of tongue-flick attack scores in neonate Common Gartersnakes (*Thamnophis sirtalis*) born to females maintained in captivity. Odor presentations occurred between June and September 2009.

	Cologne	Water	Bullfrog	African Clawed Frog	Pacific Treefrog
Cologne	-				
Water	1.0	-			
Bullfrog	0.45	0.16	-		
African Clawed Frog	0.50	0.17	1.0	-	
Pacific Treefrog	< 0.0001	< 0.0001	< 0.0001	< 0.0001	-

Table 6. Least-squares means (±1 SE) of tongue-flick attack scores and Bonferroni adjusted p-values of naïve and experienced prey presentations in neonate Common Gartersnakes (*Thamnophis sirtalis*) born in captivity. Different superscript letters denote differences between prey types regardless of feeding experience, whereas the asterisk denotes a difference between naïve and experienced presentations for a single prey type. Odor presentations occurred between June and September 2009.

	Naïve	Experienced	p-value
Cologne[a]	2.35 ± 0.56	1.0 ± 0.95	1.0
Water[a]	1.79 ± 0.32	1.79 ± 0.60	1.0
Bullfrog[a]	3.80 ± 0.32	2.09 ± 0.60	0.53
African Clawed Frog[a]	3.92 ± 0.32	1.96 ± 0.60	0.18
Pacific Treefrog[b]	12.23 ± 0.32	15.15 ± 0.60	0.0008*

Table 7. Least-squares means (±1 SE) of tongue-flick attack scores of each clutch of Common Gartersnakes (*Thamnophis sirtalis*) to the different prey odors presented between June and September 2009. Values are reported for subjects during both naïve and experienced presentations. The asterisk denotes a difference in response between clutches for that prey type during presentations that followed feeding experience (see text).

		Cologne	Water	Bullfrog	African Clawed Frog	Pacific Treefrog
Clutch 2	Naïve	2.48 ± 0.96	1.92 ± 0.55	4.41 ± 0.55	4.0 ± 0.55	12.08 ± 0.55
	Experienced	0.50 ± 2.06	1.51 ± 1.30	2.17 ± 1.30	1.89 ± 1.30	15.23 ± 1.31
Clutch 3	Naïve	2.88 ± 0.81	1.67 ± 0.47	4.24 ± 0.47	4.41 ± 0.47	12.09 ± 0.47
	Experienced	1.45 ± 1.73	2.02 ± 1.09	2.30 ± 1.09	2.34 ± 1.10	18.87 ± 1.09*
Clutch 4	Naïve	1.32 ± 1.05	1.82 ± 0.61	2.34 ± 0.61	3.0 ± 0.61	12.64 ± 0.61
	Experienced	0.88 ± 1.82	1.76 ± 1.15	1.80 ± 1.15	1.60 ± 1.15	10.98 ± 1.15

Table 8. Least-squares means (±1 SE) of tongue-flick attack scores directed at Pacific Treefrog odors by neonate Common Gartersnakes (*Thamnophis sirtalis*) as a function of replicate trial. Values are reported for subjects during both naïve and experienced presentations; the asterisks denote greater values for those replicate trials in the same column. Odors were presented between June and September 2009.

	Naïve	Experienced
Trial 1	18.21 ± 0.74*	11.15 ± 1.49
Trial 2	9.89 ± 0.74	10.96 ± 1.49
Trial 3	10.99 ± 0.74	12.08 ± 1.49
Trial 4	12.86 ± 0.74	20.54 ± 1.49*
Trial 5	11.12 ± 0.74	21.04 ± 1.49*
Trial 6	10.31 ± 0.74	N/A

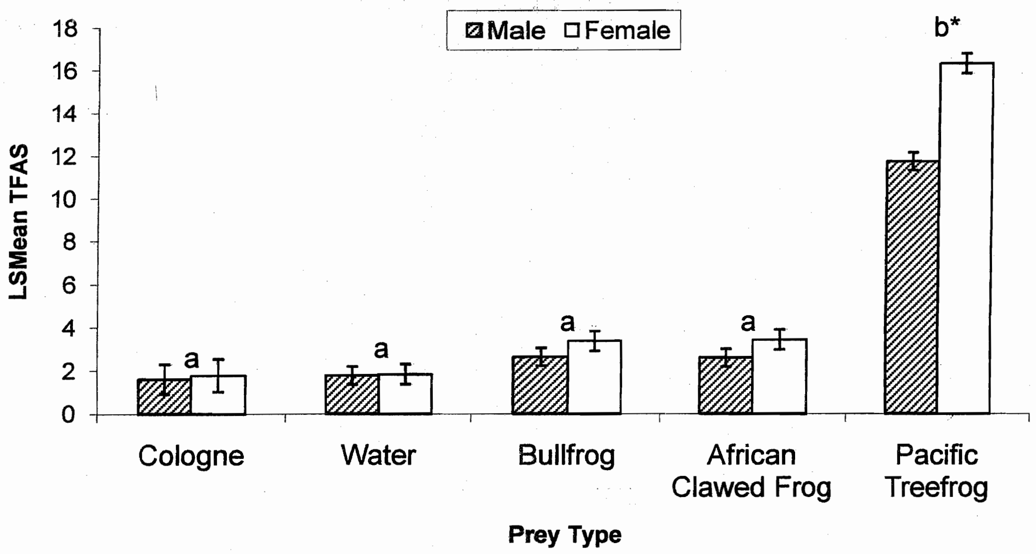

Figure 1. Least-squares means (±1 SE) of tongue-flick attack scores (TFAS) of neonate Common Gartersnakes (*Thamnophis sirtalis*) to odors from introduced and native prey types as a function of subject gender. Odors were presented between June and September 2009. Different letters denote differences in response values regardless of subject gender, whereas the asterisk indicates a greater response in female snakes.

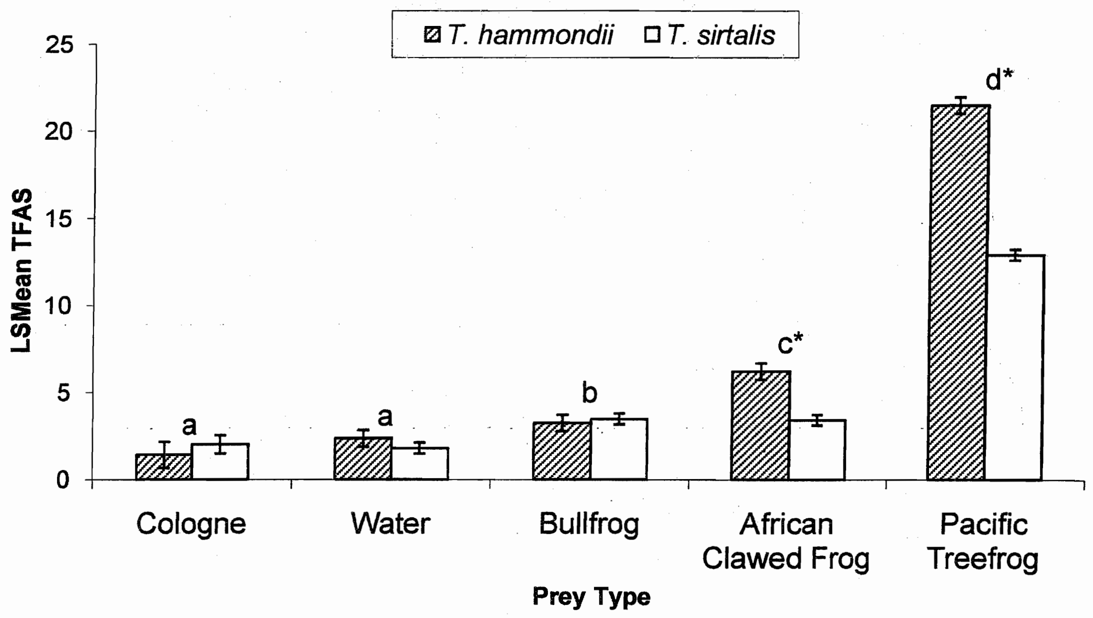

Figure 2. Least-squares means (±1 SE) of tongue-flick attack scores (TFAS) of neonate Two-striped Gartersnakes (*Thamnophis hammondii*) and Common Gartersnakes (*T. sirtalis*) as a function of prey odor. Odors were presented between June and September 2009. Different letters denote differences in response values regardless of snake species, whereas asterisks indicate a greater response in *T. hammondii*.

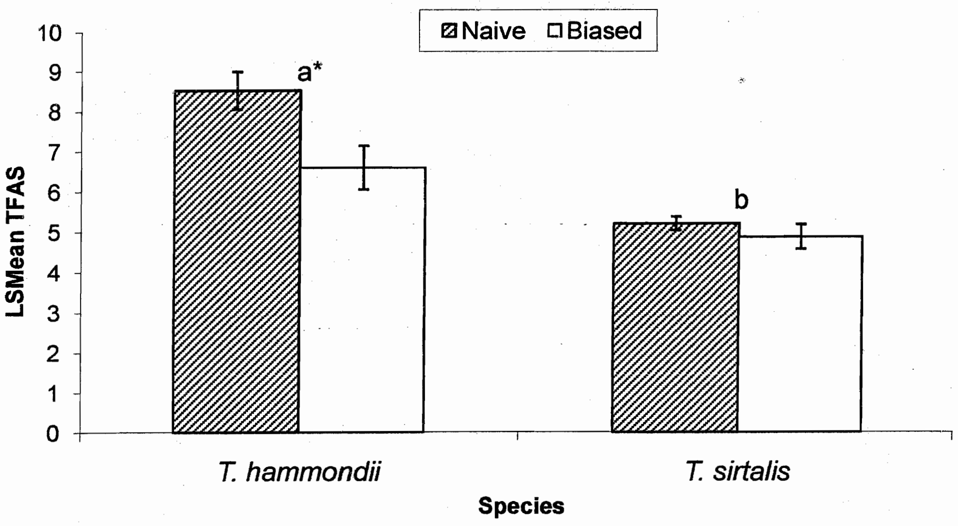

Figure 3. Least-squares means (±1 SE) of tongue-flick attack scores (TFAS) of neonate Two-striped Gartersnakes (*Thamnophis hammondii*) and Common Gartersnakes (*T. sirtalis*) as a function of feeding experience. Odors were presented between June and September 2009. Different letters denote differences in response values regardless of feeding experience, whereas the asterisk indicates a greater response in naïve snakes.

APPENDIX I

The Influence of Wetland Type and Distribution on the Dispersion of the Invasive African Clawed Frog in Southern California

Abstract

The aquatic African Clawed Frog (*Xenopus laevis*) has been introduced to wetland habitats within California and has posed numerous threats to these ecosystems due to their predatory and competitive abilities. Because of this anuran's dependence on aquatic habitat, understanding the potential for *X. laevis* to disperse from its current range to adjacent wetlands will be crucial to the management of this species. Using National Wetland Inventory data within a Geographic Information System, I analyzed wetlands both within and outside the distribution of *X. laevis* in California. The area weighted mean shape index (AWMSI) was calculated for all wetland attributes as was the diversity, dominance and evenness indices for wetlands within and outside the anuran's distribution. Lower diversity, higher dominance and lower evenness were found for those wetlands within the range of *X. laevis*; in contrast, higher diversity, lower dominance and higher evenness was found for areas where the species is unlikely to occur. Riverine intermittent wetlands dominate the area in which *X. laevis* is found, while palustrine scrub-shrub is the dominant habitat where *X. laevis* is not established. Control of these feral populations of *X. laevis* should focus in riverine habitats with relatively low diversity of the biotic community.

INTRODUCTION

Introduced species can have a wide array of negative impacts on native fauna including competitive, predatory, or trophic level effects (Livo et al. 1998). Competition can be observed in the form of apparent competition, exploitative competition, or indirect mutualism (White et al. 2006). Apparent competition occurs when a predator becomes more numerous or efficient at attacking a prey type in the presence of another. Exploitative competition occurs when an introduced species is more efficient in finding or utilizing a resource; thus, out-competing the native species. Lastly, indirect mutualism occurs when other species are affected by the interaction between the native and introduced species (White et al. 2006). Some trophic level effects can also be included as indirect because a species at a lower trophic level can be negatively impacted by the predation of its consumers belonging to higher trophic levels (White et al. 2006).

The adverse side effects of biological invasions of aquatic ecosystems (particularly in riverine habitats) have been documented across several clades, including invertebrates, fish, and amphibians (Kupferberg 1997a). Amphibians are well suited bioindicators in such systems as they are philopatric (especially to wetland habitats for reproduction), relatively long-lived and, if undisturbed, exist in relatively stable populations (Welsh and Ollivier 1998). Species introductions that decrease amphibian populations are of concern because amphibians can serve as better indicators of water quality than fish or macroinvertebrates (Welsh and Ollivier 1998). For example, the larvae of Foothill Yellow-legged Frogs *(Rana boylii*; Kupferberg 1997b) and Web-footed Frogs (*Rana palmipes*; Flecker et al. 1999) have been shown to influence riverine ecosystems by altering algal communities. In turn, primary productivity and organic matter dynamics are altered. Following removal of the larvae primary production, nutrient cycling, leaf litter decomposition, and invertebrate populations were negatively altered within the respective riverine system (Flecker et al. 1999; Kiffney and Richardson 2001; Ranvestal et al 2004), further illustrating the importance of these organisms in aquatic ecosystems.

Amphibian introductions can present novel challenges to many organisms that inhabit an aquatic ecosystem. Their skin contains granular glands that secrete toxins that serve as a defense mechanism (Duellman and Trueb 1994). The African Clawed Frog (*Xenopus laevis*) secretes a toxin comprised of peptide and/or indolealkylamine components (some with neuroleptic properties) from its integumentary glands (Barthalmus and Zielinski 1988). These toxins deter native predators in North America that readily consume frogs from ingesting *X. laevis* (Barthalmus and Zielinski 1988).

Xenopus laevis is a dietary generalist, and consumes crayfish, aquatic insects and larvae, small fish, and other amphibians (including conspecifics), and can survive in brackish waters (Mahrdt and Knefler 1972; Lafferty and Page 1997). In Chile, the frogs inhabit a diverse array of habitats with regard to water temperature, dissolved oxygen, pH, electrical conductivity, and elevation with altitudes reaching up to 620 m above sea level (Lobos and Jaksic 2005).

In addition to being a popular species in the pet trade, the African Clawed Frog was initially shipped globally for use in human pregnancy testing during the 1940's and 1950's, leading to exotic populations establishing in parts of Europe, North America, South America, and new areas in Africa (Tinsley and McCoid 1996). Specifically, populations have been established in Sicily, Italy (Faraone et al. 2008), western France

(Fouquet and Measey 2006), South Wales, UK (Measey 2001) and Chile (Lobos and Measey 2002). Its introduction to the United States is relatively recent (1968) and is of specific concern for the geographical area of the southern west coast of California (Bury and Luckenbach 1976; McCoid and Fritts 1993).

In its native habitat, the African Clawed Frog has been noted for rapidly invading man-made water bodies and spreading throughout anthropogenically-disturbed habitat (Evans et al. 1997). When ponds or rivers dry up during periods of drought, the African Clawed Frog aestivates in underground fissures 30-40 cm deep, allowing it to withstand periods without standing water for many months (Tinsley and McCoid 1996). This species has become established in a relatively diverse range of aquatic habitats, and is able to move overland between water bodies (typically during rain events; Tinsley et al., 1996, Measey and Tinsley 1998). In Chile, a juvenile African Clawed Frog was captured in a dry irrigation canal, 1500 m away from the nearest pond, and 170 m from the nearest standing water (Lobos and Garin 2002). These overland migrations might explain how African Clawed Frogs have dispersed to isolated ponds in California (McCoid and Fritts 1980, 1989; Measey and Tinsley 1998).

Xenopus embryos can tolerate temperatures ranging from 10 - 35 °C and, after hatching, attain reproductive maturity in as little as eight months (Tinsley et al. 1996). In California, breeding occurs during most of the year (McCoid 1985) and is triggered by warm water temperatures (typically greater than 20.8 °C; McCoid and Fritts 1989). This breeding pattern results in the African Clawed Frog breeding at a young age when they are still growing and allows for population expansion at a rate of up to 5.4 km/yr, as has been documented in Chile (Lobos and Jaksic 2005). Thus, the African Clawed Frog is a robust organism with tremendous potential to establish populations in aquatic habitats. Given their feeding habits and ability to inhabit different types of aquatic habitat, the relationship between wetland availability and the frog's ability to disperse as a function of wetland type is of interest.

The purpose of my study was to determine the influence that wetland distribution and type as a function of complexity and National Wetland Inventory categorization have on the potential for dispersal by *X. laevis* within the state of California. The objective of my study was to compare wetland habitat type as well as wetland landscape composition in areas inhabited by *X. laevis,* versus adjacent watersheds that do not have records of the species occurrence within the state of California using a geographic information system (GIS; ArcGIS ver 9.3 ESRI 2010). Therefore, this analysis is at a watershed scale.

METHODS

I obtained GIS-distribution data for *Xenopus laevis* in California from the International Union for Conservation of Nature (IUCN) Red List of Threatened Species website from the Worldwide Amphibian-Anuran range dataset (available from <http://www.iucnredlist.org/technical-documents/spatial-data#amphibians>). I also obtained wetland type and distribution data from the National Wetlands Inventory (NWI) of 7.5 min coverages for the State of California (available from <http://www.fws.gov/wetlands/Data/DataDownload.html>). The distribution of *X. laevis* within California and wetlands found within that range were clipped from the source GIS data sets (hereafter referred to as "shapefiles"). From this, I created two new shapefiles: one shape file for current *X. laevis* distribution; and, one shape file for wetlands within and outside that distribution (Figure 1).

NWI coverages are at a scale of at least 1:24,000 where some quadrants are enhanced and verified through satellite imagery and digital photography (available from <http://www.fws.gov/wetlands/Data/Mapper.html>). Only quadrants with digital data enhancement were used, which restricted analysis to certain regions of California (Figures 2 and 3). From the available areas in the dataset, I selected wetlands based upon areas that contained the most enhancement for each watershed as this provided the most data (Figure 3), and by areas of varying elevation using a topographic map (Figure 4). By sampling areas of varying elevations found both within and outside the range of *X. laevis*, I assumed that elevational barriers did not produce the current pattern of *X. laevis* distribution. To place these sampled points on the map, a shapefile was created and the points were digitized on the screen in mapsets that had the necessary wetland detail. A 5-

km buffer was created around each of these points to quantify and qualify the wetland structure (Figure 3). This buffer size was geographically relevant because it was the largest radius of habitat with digital enhancement in common with all random points created. The new wetland shapefile was then clipped to the 5-km buffers. The clipped wetlands were converted into two new shapefiles: within distribution and outside distribution. The area, perimeter and area weighted mean shape index (AWMSI) were calculated within the GIS using Patch Analyst (Patch Analyst 4, GIS; ArcGIS ver 9.3 ESRI 2010; *sensu* Munger et al. 1998, Knutson et al. 1999) based on wetland attribute as decoded to their most specific descriptor based on Cowardin et al. (1979).

AWMSI values were used to quantify shape complexity. The metric is equal to 1 when wetland type most closely matches a circle in shape and these values increase with increasing patch shape irregularity or complexity. AWMSI equals the sum of each wetland's perimeter, divided by the square root of wetland area (in ha) for each wetland type, and is divided by the total number of wetlands of that type; thus, larger wetlands will carry more weight than smaller ones.

Due to the extensive categorization of the classification system for wetlands used in generating the NWI maps (Cowardin et al. 1979), wetland habitat type was reduced from five descriptors (system, subsystem, class, water regime, and special modifiers) to the first two descriptors (system and subsystem; *sensu* Gaines et al. 1998, 2000). Within SAS software, (SAS v9.1.3, SAS Institute, Inc., Cary, NC), I used a PROC TTEST to examine differences between average AWMSI's for wetlands of interest. The level of statistical significance for these analyses was set at $\propto = 0.05$.

RESULTS

From the 93 original wetland types (five descriptors), 11 wetland types (two descriptors) were identified with 10 types being found both within and outside the current range of *X. laevis* and riverine lower perennial only being found within the range of *X. laevis* (Figure 5). Palustrine emergent, palustrine forested, palustrine scrub-shrub, and riverine intermittent comprised the highest percentage of wetlands found both within and outside the range of *X. laevis* (Figure 5). Wetlands within the range of *X. laevis* were comprised of 4.62% palustrine emergent and 21.02% palustrine forested (Figure 5). Wetlands outside the range were comprised of 8.40% and 17.69%, respectively (Figure 5). 50 different

Within the range of *X. laevis*, riverine intermittent comprised 43.06%, while outside the range, only 24.4% consisted of this wetland type (Figure 5). Within the range of *X. laevis*, the specific wetland type of streambed temporarily flooded comprised 83.4% of these riverine intermittent wetlands (Figure 6). Streambed temporarily flooded wetlands were not observed outside the range of *X. laevis* (Figure 6). Within the range of *X. laevis*, 25.78% of wetlands consisted of palustrine scrub-shrub wetland type, while outside the range this type comprised 43.96% of all wetlands (Figure 5). Within the range of *X. laevis*, palustrine scrub-shrub temporarily flooded wetlands comprised 79.0%, while outside the range 82.11% of these wetlands were temporarily flooded (Figure 7).

The flooding regime of each wetland type was approximately the same for wetlands found within and outside the range of *X. laevis* (Figure 8). The range of flooding regimes found within the distribution of *X. laevis* was between 0.04% to 80.0%, and 0.24% to 82.4% outside the known range, with artificially flooded excavated consisting of the lowest percentages for both ranges (Figure 8). Temporarily flooded wetlands comprised the highest percentage of flooding regimes both within the range (80.0%) and outside the range (82.47%) of *X. laevis* (Figure 8).

The average AWMSI's for wetlands found within the range of *X. laevis* varied from 1.39 to 12.94, compared to variation from 1.26 to 12.16 outside the range (Figure 9). There was no difference between the average AWMSI (±1 standard error [SE]) for all wetlands within the range (4.17 ± 0.43) and those wetlands outside the range (3.91 ± 0.65; $t_{96.6} = -0.34$, $p = 0.74$). The average AWMSI's for wetlands found within and outside the range of *X. laevis* were largest in riverine systems, with riverine lower perennial wetlands (12.94 ± 2.78) being greatest within the range of *X. laevis*. This wetland type was also unique to the range within *X. laevis* populations (Table 1). The largest average AWMSI outside the range of *X. laevis* was that of riverine intermittent wetlands (12.16 ± 3.59; Figure 9). These values did not differ ($t_{1.27} = 0.07$, $p = 0.95$). Riverine upper perennial wetlands outside the range of *X. laevis* also were comprised of a large AWMSI (10.60 ± 6.25; Figure 8).

There was a variety of unique wetlands found only within or outside the range of *X. laevis* in each of the broader wetland categories. A total of 2128 unique wetlands were found within the range of *X. laevis* and 36 unique outside the range (Table 1). There was no difference between the average AWMSI for all unique wetlands within the range (4.07 ± 0.67) and those wetlands outside the range (4.79 ± 2.36; $t_{9.34} = 0.29$, $p = 0.78$). Riverine upper perennial wetlands (14.65 ± 2.15) outside the range of *X. laevis* comprised the highest AWMSI of unique wetlands (Table 1).

The diversity, dominance, and evenness indices of all wetland types sampled were 2.52, 0.44, and 0.56, respectively (Table 2). The diversity, dominance, and evenness indices of all wetland types sampled within the range of *X. laevis* were 1.08, 0.71, and 0.29, respectively, and these values tended to differ from those for wetlands sampled outside the range of *X. laevis* (Table 3).

DISCUSSION

My study addressed the suitability of available habitats for dispersing *Xenopus laevis* in southern California, given the context of what is known about their habitat requirements. Although the diversity, dominance, and evenness indices of all wetland types indicated a large amount of diversity, the wetland types within and outside the range of *X. laevis* differ primarily by broader wetland categorization with palustrine and riverine wetland types (Cowardin et al. 1979) being most dominant.

While palustrine emergent, palustrine forested, palustrine scrub-shrub, and riverine intermittent were the dominant general wetland types, the relationship observed within the range of *X. laevis* for palustrine scrub-shrub and riverine intermittent wetlands was of interest because it was opposite to that occurring outside the range, and these two wetlands comprised the highest percentage within their respective ranges. Outside the range of *X. laevis*, there were more palustrine scrub-shrub wetlands, while more riverine intermittent wetlands were found within the range of *X. laevis*. This relationship may indicate *X. laevis* exhibits a preference for riverine intermittent wetland habitats compared to available wetland habitats in the area. Of these riverine intermittent wetlands, those that experience temporary flooding were the most dominant. This appears to be the pattern for most wetlands found in southern California, however, as flooding regime did not differ by distribution of *X. laevis*.

Higher dominance and lower evenness values for those wetlands found within the range supports the finding that riverine intermittent wetlands are more prevalent in the areas where *X. laevis* occurs. The diversity of wetlands was higher for those found outside the range of *X. laevis*, with a lower dominance value and higher evenness value.

These findings indicate that *X. laevis* exhibits a preference for specific wetland habitats, given that heterogeneity is lower where they are currently found compared to where they could potentially disperse. The abundance of riverine intermittent wetlands found within the range of *X. laevis* compared to the lower number of this type of wetland outside their range suggests not only that *X. laevis* exhibits a preference for this wetland type, but also that it is capable of inhabiting a wide array of riverine intermittent habitats. Management within these areas should consider these patterns.

The average AWMSI's indicate that *X. laevis* does not exhibit a preference for aquatic habitats with more complex shapes compared to simpler shapes. The wetlands that were most complex in shape consisted of riverine systems while palustrine systems were simplest. Given that the highest percentage of wetland types within the range of *X. laevis* consisted of palustrine and riverine systems, the average AWMSI vaules indicate that the complexity of a wetland system does not play a role in habitat selection.

Management Implications

These findings support the focused control of the African Clawed Frog populations in riverine wetland habitats. Such control is important as this anuran is negatively affecting aquatic ecosystems at a variety of levels. The rate at which the African Clawed Frogs affect riverine fauna is contingent upon their density, and they are found in areas up to 0.25 frogs m^{-2} (Lobos and Measey 2002). At this density, the African Clawed Frog could directly impact native prey species negatively and indirectly affect species at other trophic levels (Lobos and Measey 2002). Additionally, the activity of the frog in the water column (*i.e.*, disturbing sediment) could alter water turbidity and nutrient levels (Lobos and Measey 2002). Ultimately, this could negatively affect the community dynamics of the riverine systems.

Being dietary generalists, African Clawed Frogs have adversely affected riverine fauna by direct predation. Lafferty and Page (1997) found native Tidewater Gobies (*Eucyclogobius newberryi*) in the stomachs of the introduced African Clawed Frog found in the brackish Santa Clara River in California. This native species has suffered a population decline, and while noted the decline may also be due to other environmental factors, the African Clawed Frog might be contributing to this reduction in population size (Lafferty and Page 1997). Similarly, Tinsley and McCoid (1996) suggested that predation by the African Clawed Frog might threaten survival of the endangered Unarmored Three-spine Stickleback (*Gasterosteus aculeatus williamsoni*) in Placerita Canyon, California.

In addition to predation, the African Clawed Frog has been shown to be a vector of disease. In Chile, African Clawed Frogs have invaded both lotic and lentic systems and in 24% in three of ten sites sampled, these anurans harbored the fungus, *Batrachochytrium dendrobatidis,* that has been plaguing native frog populations and resulting in death in many countries (Rigoberto et al. 2009). Prior to this study, there has been no evidence of this fungus persisting in native fauna, which implicates the African Clawed Frog and possible other introduced species as the vector of disease transmission (Rigoberto et al. 2009). Ultimately, the transfer of this fungus across the country via water systems could result in the spread and infection of the fungus to native fauna and decrease biodiversity in the population as population numbers decline.

In an effort to maintain low densities of this invasive anuran, protect the population dynamics of these aquatic systems, and prevent disease transmission across the state of California, these frog populations should be managed with a focus on the riverine systems. Since 1990, the United States government has increased funding for the management of invasive species and required that states develop nuisance species management plans (Kats and Ferrer 2003). Developing models to predict the sensitivity of habitats to invasive species is critical in developing a management plan (Kats and Ferrer 2003) and the findings of this study could be used as a baseline by which to build a predictive model for the occurrence and potential dispersion of *X. laevis*.

An eradication of the African Clawed Frog was attempted in 1974 by the California Department of Fish and Game by chemically treating ponds (St. Amant 1975). Repeated applications with various chemicals at high concentrations were required for effective removal, and such a method would be less than ideal for larger wetland systems or systems containing sensitive species (St. Amant 1975). The ability of this species to tolerate exposure to toxic chemicals and extended periods on land also suggests that this method is less than ideal.

Conclusions and future research

My study provides evidence that African Clawed Frogs currently inhabit areas dominated by an array of riverine intermittent wetland habitats, while palustrine scrub-shrub dominates the area currently not occupied by this anuran. Furthermore, flooding regime and complexity of wetland shape appear not to be factors in clawed frog habitat selection. While the findings of my study support the focused control of California African Clawed Frog populations in riverine intermittent systems, it is important to consider the datasets that were used in my study. The NWI data available was not digitally enhanced in all areas, and as such, future studies should apply the same methods with a more complete dataset to determine if this pattern is consistent across all areas that present current and potential occupancy by clawed frogs. Furthermore, the densities of these anuran populations within the sampled areas should be assessed in order to examine spatially explicit wetland habitats inhabited by the largest feral populations.

Developing new management plans that consider these variables is important because attempts at eradication have been unsuccessful to date (Tinsley and McCoid 1996). To properly protect native aquatic fauna, management practices should be modified in order to conserve sensitive species found throughout these aquatic ecosystems.

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Table 1. Total number of unique wetland types and average area weighted mean shape indices (AWMSI; ±1 SE) for wetlands within the state of California known to have *Xenopus laevis* (African Clawed Frog) populations or nearby areas with no record of the species occurrence (data obtained from National Wetlands Inventory and International Union for Conservation of Nature).

Wetland Type	Within or Outside range	Number of wetland types	Total number of unique wetlands	Average AWMSI
Lacustrine Limnetic	within	1	1	7.15 ± 0.0
Lacustrine Littoral	within	5	38	2.49 ± 2.21
Palustrine Aquatic Bed	within	1	9	1.19 ± 0
Palustrine Emergent	within	5	16	3.25 ± 0.53
Palustrine Forested	within	5	40	5.04 ± 0.77
Palustrine Forested	outside	1	1	5.41 ± 0.0
Palustrine Scrub Shrub	within	8	102	2.95 ± 0.46
Palustrine Scrub Shrub	outside	3	17	1.63 ± 0.33
Palustrine Unconsolidated Bottom	within	2	8	1.28 ± 0.004
Palustrine Unconsolidated Bottom	outside	2	3	1.16 ± 0.12
Palustrine Unconsolidated Shore	within	5	23	1.84 ± 0.14
Palustrine Unconsolidated Shore	outside	1	1	1.12 ± 0.0
Riverine Lower Perennial	within	2	26	12.94 ± 2.78
Riverine Upper Perennial	outside	2	12	14.65 ± 2.15
Riverine Intermittent	within	6	1865	6.36 ± 0.79

Table 2. Shannon Wiener diversity, dominance, and evenness indices of all wetland types within the state of California known to have *Xenopus laevis* (African Clawed Frog) populations including nearby wetlands with no record of the species occurrence (data obtained from National Wetlands Inventory and International Union for Conservation of Nature).

Index	Value
Shannon Wiener Diversity	2.52
Dominance	0.44
Evenness	0.56

Table 3. A comparison of Shannon Wiener diversity, dominance, and evenness indices of wetland types within the state of California known to have *Xenopus laevis* (African Clawed Frog) populations and nearby areas with no record of the species occurrence (data obtained from National Wetlands Inventory and International Union for Conservation of Nature).

Index	Value	
	Within range of *X. laevis*	Outside range of *X. laevis*
Shannon Wiener Diversity	1.08	1.47
Dominance	0.71	0.33
Evenness	0.29	0.67

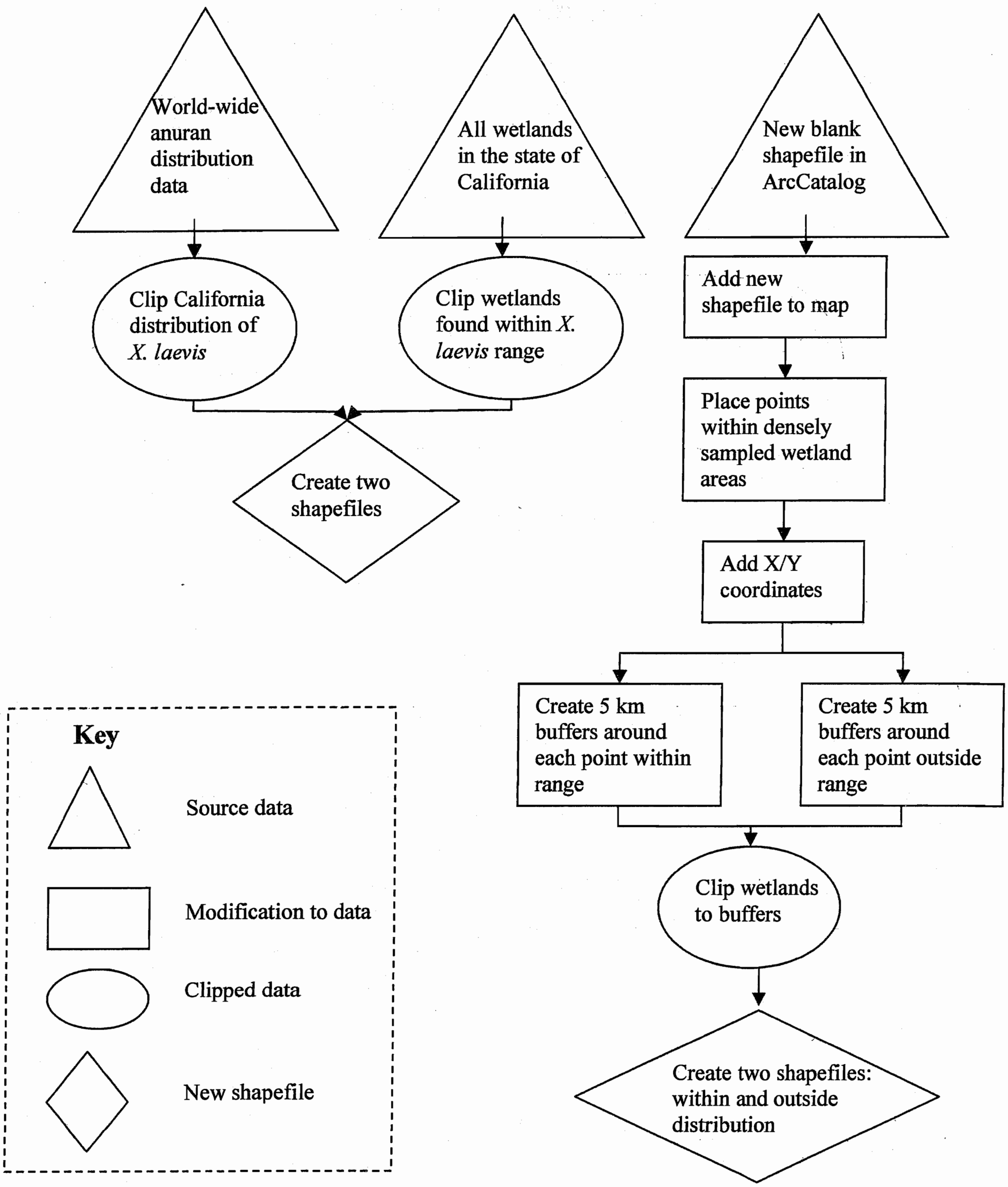

Figure 1. Flowchart showing the procedures used within ArcGIS to construct GIS data layers for the final database used to analyze wetland habitat and structure within the state of California for areas known to have *Xenopus laevis* (African Clawed Frog) populations compared to nearby areas with no record of the species occurrence.

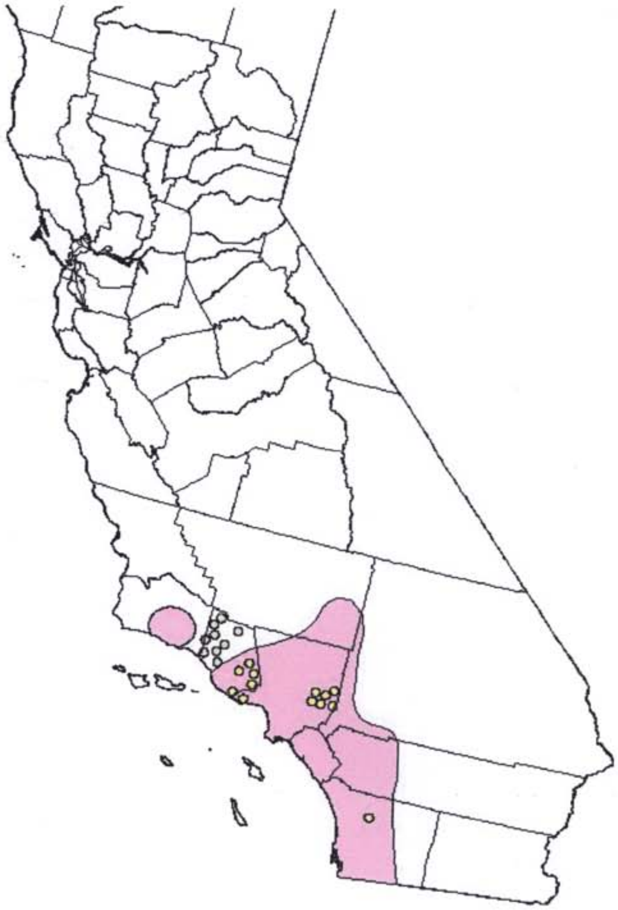

Figure 2. Current distribution of *Xenopus laevis* (African Clawed Frog) within the state of California, denoted by shaded areas. Circles denote sampled National Wetland Inventory (NWI) areas within ArcGIS from digitally enhanced areas. Areas that were not digitally enhanced were not sampled (data obtained from the NWI and International Union for Conservation of Nature).

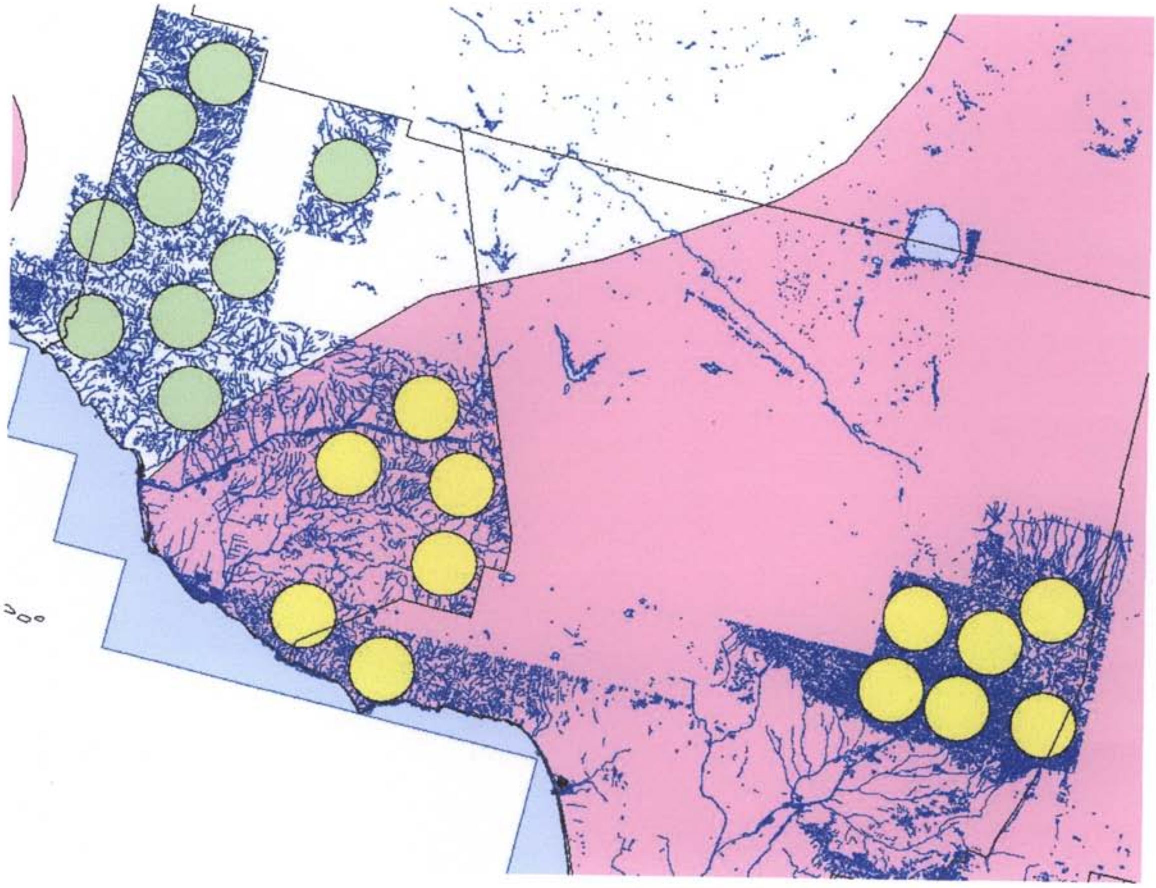

Figure 3. Representation of National Wetland Inventory (NWI) data and wetlands sampled both outside and within the range of *Xenopus laevis* (African Clawed Frog) in the state of California. Yellow circles represent NWI data within the range of *X. laevis* and green circles represent data outside the range. The pink area represents current distribution of *X. laevis* (data obtained from the NWI and International Union for Conservation of Nature).

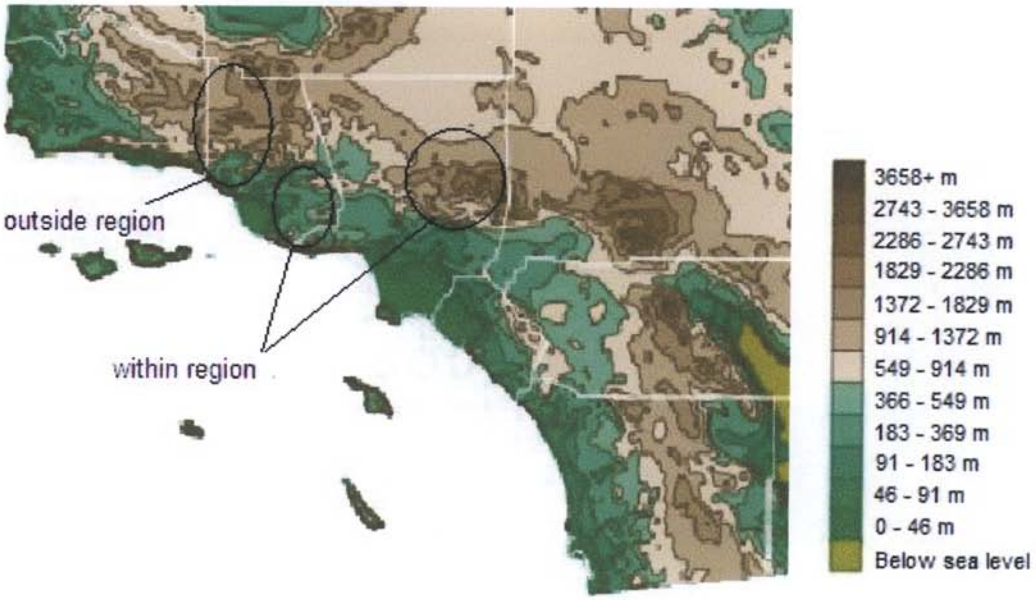

Figure 4. Topographic map of the elevation of the landscape within and outside range of *Xenopus laevis* (African Clawed Frog) in the state of California and sampled sections of land in which wetlands were analyzed in ArcGIS.

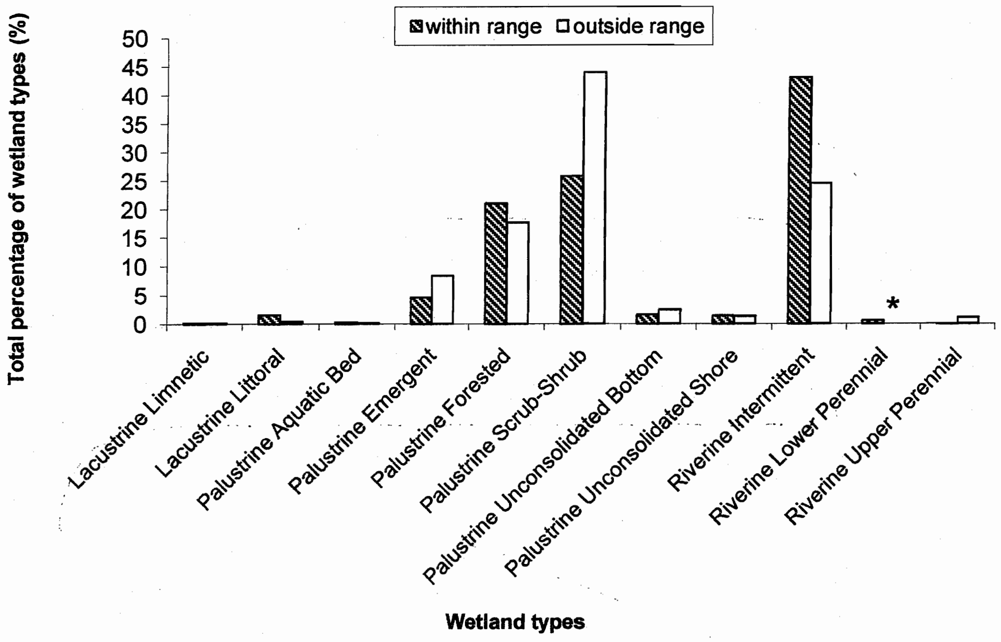

Figure 5. Relative percentages of wetland types found within and outside the current distribution of *Xenopus laevis* (African Clawed Frog) in the state of California. (Asterisk denotes absence of wetland type for either category; data obtained from National Wetlands Inventory and International Union for Conservation of Nature).

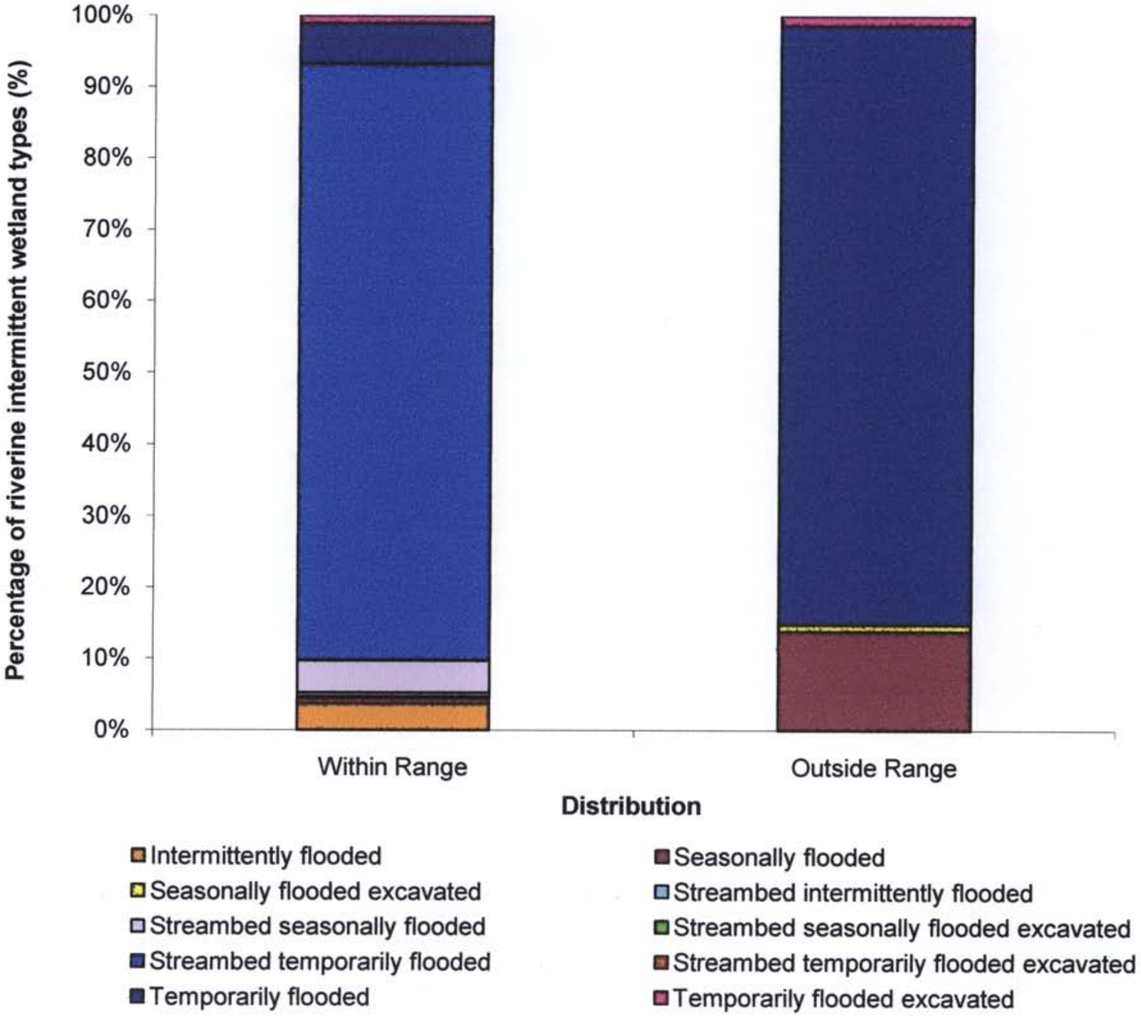

Figure 6. Relative percentages of specific wetland types comprising the broad category of riverine intermittent wetland within and outside the range of *Xenopus laevis* (African Clawed Frog) in the state of California (data obtained from National Wetlands Inventory and International Union for Conservation of Nature).

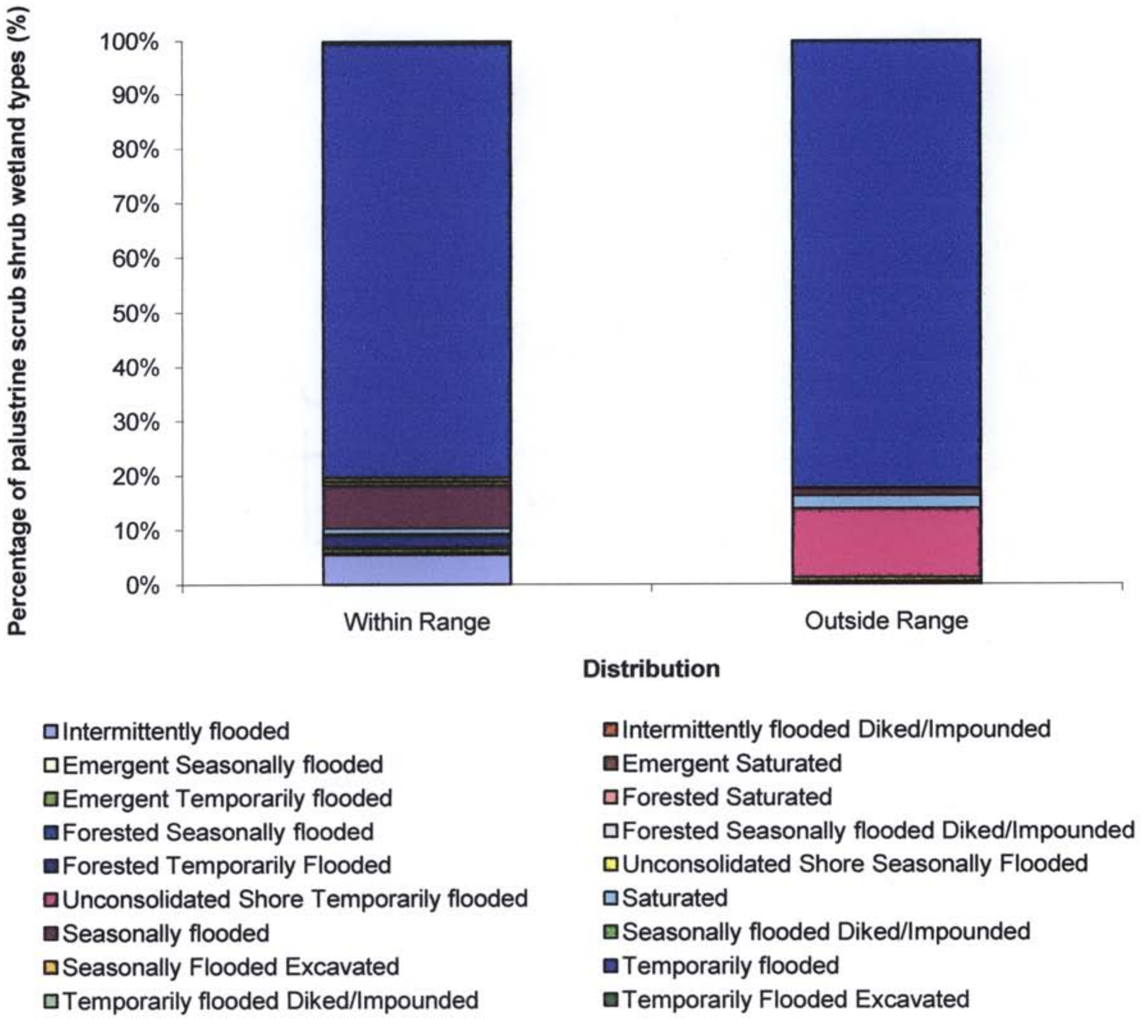

Figure 7. Relative percentages of specific wetland types comprising the broad category of palustrine scrub-shrub wetland within and outside the range of *Xenopus laevis* (African Clawed Frog) in the state of California (data obtained from National Wetlands Inventory and International Union for Conservation of Nature).

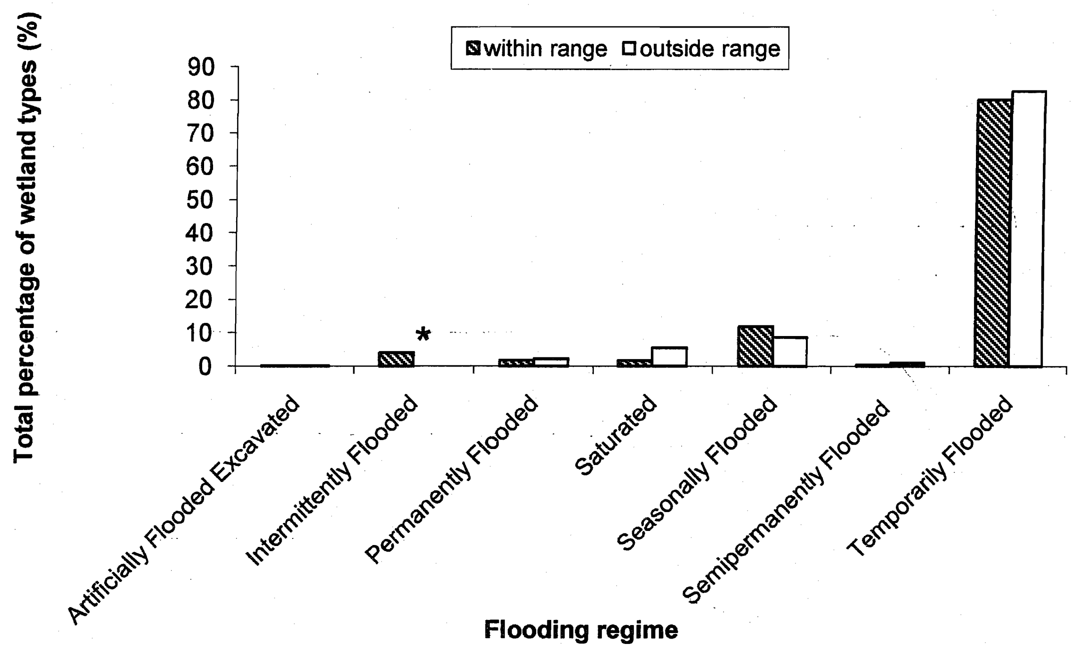

Figure 8. Relative percentages of flooding regimes found within and outside the range of *Xenopus laevis* (African Clawed Frog) in the state of California. (Asterisk denotes absence of wetland type for either category; data obtained from National Wetlands Inventory and International Union for Conservation of Nature).

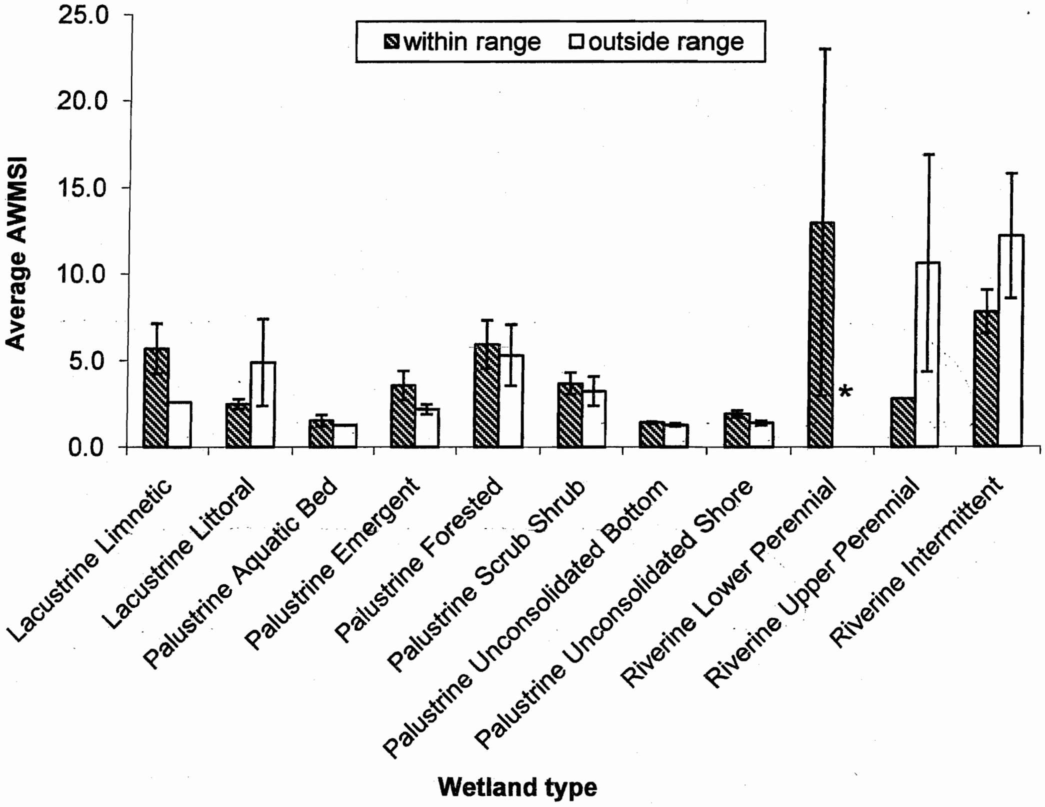

Figure 9. Average area weighted mean shape indices (±1 SE) of all wetlands found within or outside the range of *Xenopus laevis* (African Clawed Frog) in the state of California (Asterisk denotes absence of wetland type for either category; data obtained from National Wetlands Inventory and International Union for Conservation of Nature).

Appendix I-a. All specific wetland types sampled within ArcGIS outside the range of *Xenopus laevis* (African Clawed Frog) in the state of California (data obtained from National Wetlands Inventory and International Union for Conservation of Nature).

Specific wetland types outside range of *X. laevis*	Count
LACUSTRINE LIMNETIC UNCONSOLIDATED BOTTOM Permanently Flooded Diked/Impounded	2
LACUSTRINE LITTORAL EMERGENT Seasonally Flooded Diked/Impounded	2
LACUSTRINE LITTORAL UNCONSOLIDATED BOTTOM Permanently Flooded Diked/Impounded	5
PALUSTRINE AQUATIC BED Permanently Flooded Diked/Impounded	2
PALUSTRINE EMERGENT Saturated	36
PALUSTRINE EMERGENT Seasonally Flooded	14
PALUSTRINE EMERGENT Seasonally Flooded Diked/Impounded	17
PALUSTRINE EMERGENT Seasonally Flooded Excavated	4
PALUSTRINE EMERGENT PALUSTRINE SCRUB-SHRUB	4
PALUSTRINE EMERGENT PALUSTRINE UNCONSOLIDATED SHORE	3
PALUSTRINE EMERGENT Temporary Flooded	55
PALUSTRINE EMERGENT Temporary Flooded Diked/Impounded	6
PALUSTRINE EMERGENT Temporary Flooded Diked/Impounded Excavated	3
PALUSTRINE FORESTED PALUSTRINE SCRUB SHRUB Temporary Flooded	13
PALUSTRINE FORESTED PALUSTRINE UNCONSOLIDATED SHORE Temporary Flooded	1
PALUSTRINE FORESTED Saturated	38
PALUSTRINE FORESTED Seasonally Flooded	11
PALUSTRINE FORESTED Temporary Flooded	226
PALUSTRINE FORESTED Temporary Flooded Diked/Impounded	10
PALUSTRINE SCRUB SHRUB Saturated	17
PALUSTRINE SCRUB SHRUB Seasonally Flooded	9
PALUSTRINE SCRUB SHRUB Seasonally Flooded Diked/Impounded	2
PALUSTRINE SCRUB SHRUB Temporary Flooded	606
PALUSTRINE SCRUB SHRUB Temporary Flooded Excavated	1
PALUSTRINE SCRUB-SHRUB PALUSTRINE EMERGENT Seasonally Flooded	1
PALUSTRINE SCRUB-SHRUB PALUSTRINE EMERGENT Temporary Flooded	1
PALUSTRINE SCRUB-SHRUB PALUSTRINE FORESTED Saturated	1
PALUSTRINE SCRUB-SHRUB PALUSTRINE FORESTED Temporary Flooded	6
PALUSTRINE SCRUB-SHRUB PALUSTRINE UNCONSOLIDATED SHORE Seasonally Flooded	5
PALUSTRINE SCRUB-SHRUB PALUSTRINE UNCONSOLIDATED SHORE Temporary Flooded	94

Appendix 1-a, continued.

PALUSTRINE UNCONSOLIDATED BOTTOM Artificially Flooded	1
PALUSTRINE UNCONSOLIDATED BOTTOM Artificially Flooded Diked/Impounded	2
PALUSTRINE UNCONSOLIDATED BOTTOM Permanently Flooded Diked/Impounded	9
PALUSTRINE UNCONSOLIDATED BOTTOM Permanently Flooded Excavated	13
PALUSTRINE UNCONSOLIDATED BOTTOM Semipermanently Flooded	2
PALUSTRINE UNCONSOLIDATED BOTTOM Semipermanently Flooded Diked/Impounded	8
PALUSTRINE UNCONSOLIDATED BOTTOM Semipermanently Flooded Excavated	7
PALUSTRINE UNCONSOLIDATED SHORE Artificially Flooded Diked/Impounded	1
PALUSTRINE UNCONSOLIDATED SHORE PALUSTRINE SCRUB SHRUB Temporary Flooded	11
PALUSTRINE UNCONSOLIDATED SHORE Seasonally Flooded	2
PALUSTRINE UNCONSOLIDATED SHORE Seasonally Flooded Excavated	6
PALUSTRINE UNCONSOLIDATED SHORE Temporary Flooded	1
PALUSTRINE UNCONSOLIDATED SHORE Temporary Flooded Diked/Impounded	1
RIVERINE UPPER PERENNIAL UNCONSOLIDATED BOTTOM Permanently Flooded	6
RIVERINE UPPER PERENNIAL UNCONSOLIDATED SHORE Seasonally Flooded	11
RIVERINE UPPER PERENNIAL UNCONSOLIDATED SHORE Temporary Flooded	1
RIVERINE INTERMITTENT Seasonally Flooded	57
RIVERINE INTERMITTENT Seasonally Flooded Excavated	4
RIVERINE INTERMITTENT Temporary Flooded	346
RIVERINE INTERMITTENT Temporary Flooded Excavated	6

Appendix I-b. All specific wetland types sampled within ArcGIS within the range of *Xenopus laevis* (African Clawed Frog) in the state of California (data obtained from National Wetlands Inventory and International Union for Conservation of Nature).

Specific wetland types within range of *X. laevis*	Count
LACUSTRINE LIMNETIC UNCONSOLIDATED BOTTOM Permanently Flooded Diked/Impounded	6
LACUSTRINE LIMNETIC UNCONSOLIDATED BOTTOM Semipermanently Flooded Diked/Impounded	1
LACUSTRINE LITTORAL EMERGENT LACUSTRINE LITTORAL UNCONSOLIDATED BOTTOM	1
LACUSTRINE LITTORAL EMERGENT Permanently Flooded Diked/Impounded	1
LACUSTRINE LITTORAL EMERGENT Seasonally Flooded Diked/Impounded	9
LACUSTRINE LITTORAL UNCONSOLIDATED BOTTOM Permanently Flooded Diked/Impounded	4
LACUSTRINE LITTORAL UNCONSOLIDATED BOTTOM Semipermanently Flooded Diked/Impounded	6
LACUSTRINE LITTORAL UNCONSOLIDATED SHORE Intermittently Flooded Diked/Impounded	4
LACUSTRINE LITTORAL UNCONSOLIDATED SHORE Seasonally Flooded Diked/Impounded	21
LACUSTRINE LITTORAL UNCONSOLIDATED SHORE Temporary Flooded Diked/Impounded	26
PALUSTRINE AQUATIC BED Permanently Flooded Diked/Impounded	3
PALUSTRINE AQUATIC BED Permanently Flooded Excavated	9
PALUSTRINE EMERGENT Intermittently Flooded	10
PALUSTRINE EMERGENT PALUSTRINE FORESTED Saturated	1
PALUSTRINE EMERGENT PALUSTRINE SCRUB-SHRUB Saturated	1
PALUSTRINE EMERGENT PALUSTRINE SCRUB-SHRUB Seasonally Flooded	2
PALUSTRINE EMERGENT PALUSTRINE SCRUB-SHRUB Temporary Flooded	2
PALUSTRINE EMERGENT PALUSTRINE UNCONSOLIDATED SHORE Seasonally Flooded	1
PALUSTRINE EMERGENT PALUSTRINE UNCONSOLIDATED SHORE Temporary Flooded	1
PALUSTRINE EMERGENT Saturated	15
PALUSTRINE EMERGENT Seasonally Flooded	18
PALUSTRINE EMERGENT Seasonally Flooded Diked/Impounded	15

PALUSTRINE EMERGENT Seasonally Flooded Excavated	20
PALUSTRINE EMERGENT Semipermanently Flooded Diked/Impounded	2
PALUSTRINE EMERGENT Temporary Flooded	96
PALUSTRINE EMERGENT Temporary Flooded Excavated	12
PALUSTRINE EMERGENT Temporary Flooded Diked/Impounded	21
PALUSTRINE FORESTED Intermittently Flooded	20
PALUSTRINE FORESTED PALUSTRINE EMERGENT Seasonally Flooded	2
PALUSTRINE FORESTED PALUSTRINE SCRUB-SHRUB Intermittently Flooded	4
PALUSTRINE FORESTED PALUSTRINE SCRUB-SHRUB Seasonally Flooded	4
PALUSTRINE FORESTED PALUSTRINE SCRUB-SHRUB Temporary Flooded	51
PALUSTRINE FORESTED Saturated	47
PALUSTRINE FORESTED Seasonally Flooded	168
PALUSTRINE FORESTED Seasonally Flooded Diked/Impounded	5
PALUSTRINE FORESTED Temporary Flooded	681
PALUSTRINE FORESTED Temporary Flooded Diked/Impounded	6
PALUSTRINE SCRUB-SHRUB Intermittently Flooded	66
PALUSTRINE SCRUB-SHRUB Intermittently Flooded Diked/Impounded	2
PALUSTRINE SCRUB-SHRUB PALUSTRINE EMERGENT Saturated	2
PALUSTRINE SCRUB-SHRUB PALUSTRINE EMERGENT Temporary Flooded	10
PALUSTRINE SCRUB-SHRUB PALUSTRINE FORESTED Seasonally Flooded	2
PALUSTRINE SCRUB-SHRUB PALUSTRINE FORESTED Seasonally Flooded Diked/Impounded	1
PALUSTRINE SCRUB-SHRUB PALUSTRINE FORESTED Temporary Flooded	26
PALUSTRINE SCRUB-SHRUB PALUSTRINE UNCONSOLIDATED SHORE Seasonally Flooded	1
PALUSTRINE SCRUB-SHRUB PALUSTRINE UNCONSOLIDATED SHORE Temporary Flooded	2
PALUSTRINE SCRUB-SHRUB Saturated	14
PALUSTRINE SCRUB-SHRUB Seasonally Flooded	94
PALUSTRINE SCRUB-SHRUB Seasonally Flooded Diked/Impounded	10
PALUSTRINE SCRUB-SHRUB Seasonally Flooded Excavated	8
PALUSTRINE SCRUB-SHRUB Temporary Flooded	966
PALUSTRINE SCRUB-SHRUB Temporary Flooded Diked/Impounded	7
PALUSTRINE SCRUB-SHRUB Temporary Flooded Excavated	1
PALUSTRINE UNCONSOLIDATED BOTTOM Artificially Flooded Excavated	2
PALUSTRINE UNCONSOLIDATED BOTTOM Permanently Flooded	6

Appendix 1-b, continued.

PALUSTRINE UNCONSOLIDATED BOTTOM Permanently Flooded Diked/Impounded	10
PALUSTRINE UNCONSOLIDATED BOTTOM Permanently Flooded Excavated	42
PALUSTRINE UNCONSOLIDATED BOTTOM Semipermanently Flooded	1
PALUSTRINE UNCONSOLIDATED BOTTOM Semipermanently Flooded Diked/Impounded	5
PALUSTRINE UNCONSOLIDATED BOTTOM Semipermanently Flooded Excavated	8
PALUSTRINE UNCONSOLIDATED SHORE PALUSTRINE EMERGENT Seasonally Flooded Diked/Impounded	1
PALUSTRINE UNCONSOLIDATED SHORE PALUSTRINE EMERGENT Temporary Flooded	2
PALUSTRINE UNCONSOLIDATED SHORE PALUSTRINE SCRUB-SHRUB Seasonally Flooded	4
PALUSTRINE UNCONSOLIDATED SHORE PALUSTRINE SCRUB-SHRUB Seasonally Flooded Diked/Impounded	1
PALUSTRINE UNCONSOLIDATED SHORE PALUSTRINE SCRUB-SHRUB Temporary Flooded	18
PALUSTRINE UNCONSOLIDATED SHORE Seasonally Flooded	10
PALUSTRINE UNCONSOLIDATED SHORE Seasonally Flooded Diked/Impounded	15
PALUSTRINE UNCONSOLIDATED SHORE Seasonally Flooded Excavated	10
PALUSTRINE UNCONSOLIDATED SHORE Temporary Flooded	3
PALUSTRINE UNCONSOLIDATED SHORE Temporary Flooded Diked/Impounded	2
RIVERINE INTERMITTENT Intermittently Flooded	74
RIVERINE INTERMITTENT Seasonally Flooded	16
RIVERINE INTERMITTENT Seasonally Flooded Excavated	7
RIVERINE INTERMITTENT STREAMBED Intermittently Flooded	9
RIVERINE INTERMITTENT STREAMBED Seasonally Flooded	91
RIVERINE INTERMITTENT STREAMBED Seasonally Flooded Excavated	1
RIVERINE INTERMITTENT STREAMBED Temporary Flooded	1688
RIVERINE INTERMITTENT STREAMBED Temporary Flooded Excavated	2
RIVERINE INTERMITTENT Temporary Flooded	112
RIVERINE INTERMITTENT Temporary Flooded Excavated	24
RIVERINE LOWER PERENNIAL UNCONSOLIDATED BOTTOM Permanently Flooded	2
RIVERINE LOWER PERENNIAL UNCONSOLIDATED BOTTOM Seasonally Flooded	24
RIVERINE UPPER PERENNIAL UNCONSOLIDATED BOTTOM Permanently Flooded	2

Appendix I-c. Area weighted mean shape indices (AWMSI) for all attributes of National Wetland Inventory Data sampled in ArcGIS within and outside the range of *Xenopus laevis* (African Clawed Frog) in the state of California (data obtained from National Wetlands Inventory and International Union for Conservation of Nature).

Within Range Attribute	AWMSI	Outside Range Attribute	AWMSI
L1UBFh	7.15	L1UBHh	2.58
L1UBHh	4.23	L2EMCh	2.19
L2EM/UBHh	2.45	L2UBHh	2.58
L2EMCh	1.55	L2USCh	9.92
L2EMHh	1.65	PABHh	1.26
L2UBFh	2.66	PEM/SSA	1.69
L2UBHh	2.10	PEM/SSB	2.29
L2USAh	3.60	PEM/USA	1.67
L2USCh	3.74	PEMA	1.95
L2USJh	2.08	PEMAh	1.37
PABHh	1.84	PEMAx	1.28
PABHx	1.19	PEMB	1.92
PEM/FOB	1.03	PEMb	1.59
PEM/SSA	2.38	PEMC	4.64
PEM/SSB	1.14	PEMCh	2.62
PEM/SSC	6.02	PEMCx	2.91
PEM/USA	1.26	PFO/SSA	5.41
PEM/USC	2.12	PFO/USA	2.00
PEMA	3.24	PFOA	10.44
PEMAh	1.38	PFOAh	1.95
PEMAx	2.81	PFOB	1.32
PEMB	1.74	PFOC	10.70
PEMC	9.51	PSS/EMA	1.30
PEMCh	1.55	PSS/EMC	3.86
PEMCx	12.04	PSS/FOA	2.47
PEMFh	2.14	PSS/FOB	1.27
PEMJ	4.94	PSS/USA	2.09
PFO/EMC	1.94	PSS/USC	2.33
PFO/SSA	12.85	PSSA	8.05
PFO/SSC	13.15	PSSAx	1.13
PFO/SSJ	4.72	PSSB	1.77
PFOA	10.21	PSSC	9.36
PFOAh	1.11	PSSCh	1.84
PFOAx	3.02	PUBF	1.69
PFOB	1.39	PUBFh	1.28

Appendix 1-c, continued.

PFOC	9.47
PFOCh	2.00
PFOJ	5.40
PSS/EMA	3.47
PSS/EMB	2.05
PSS/FOA	7.75
PSS/FOC	6.78
PSS/FOCh	1.17
PSS/USA	2.53
PSS/USC	1.22
PSSA	8.79
PSSAh	1.75
PSSAx	5.29
PSSB	1.25
PSSC	4.01
PSSCh	2.13
PSSCx	2.98
PSSJ	6.10
PSSJh	1.53
PUBF	1.57
PUBFh	1.33
PUBFx	1.30
PUBH	1.28
PUBHh	1.70
PUBHx	1.26
PUBKx	1.27
PUS/EMA	2.32
PUS/EMCh	1.33
PUS/SSA	2.05
PUS/SSC	2.04
PUS/SSCh	2.10
PUSA	1.24
PUSAh	3.31
PUSC	1.93
PUSCh	1.43
PUSCx	1.29
R2UBH	22.93
R2USC	2.96
R3UBH	2.79
R4SBA	14.77
R4SBAx	1.89
R4SBC	8.42
R4SBCx	1.85
PUBFx	1.21
PUBHh	1.19
PUBHx	1.29
PUBK	1.04
PUBKh	1.13
PUS/SSA	1.95
PUSA	1.42
PUSAh	1.32
PUSC	1.33
PUSCx	1.12
PUSKh	1.10
R3UBH	22.89
R3USA	6.42
R3USC	2.48
R4USA	22.86
R4USAx	8.17
R4USC	9.60
R4USCx	8.00

Appendix 1-c, continued.

R4SBJ	4.52
R4USA	9.21
R4USArx	5.56
R4USAx	9.54
R4USC	11.29
R4USCx	12.08
R4USJ	6.71