

## Palatability of Twelve Species of Anuran Larvae in Eastern Texas

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ABSTRACT.—We tested the palatability of 12 species of anuran larvae that occur in eastern Texas using four common predators. Palatability was determined by offering larvae to predators and recording the behavior of the predator. We also tested for ontogenetic shifts in palatability in twelve species of anuran larvae. *Incilius nebulifer*, *Anaxyrus woodhousii*, *Lithobates catesbeianus*, *L. clamitans* and *L. sphenoccephalus* all showed evidence of unpalatability in laboratory experiments. These noxious larval anurans also showed ontogenetic shifts in palatability to one or more predators by becoming more palatable throughout development. Predator tolerance to noxiousness also played a key role in predation trials. Bluegills were the least tolerant, followed by green sunfish and dragonfly larvae. Crayfish had the highest tolerance to noxiousness.

### INTRODUCTION

North America anurans, depending on species, can remain in the larval stage from 2 wk to 3 y (Skelly, 1997). Anurans with long larval stages are constrained from successfully breeding in ephemeral pools because tadpoles will die when the pools dry. However, hydroperiod cannot explain why many anurans with short larval stages are not readily found in permanent waters. Rather, predation is probably the main cause of mortality for these tadpoles (Alford, 1999), and inadequate anti-predator defenses may explain why many species are excluded from permanent water habitats (Kats *et al.*, 1988). Thus, larval anurans must be able to cope with varying extremes of ponds drying and predation pressures in their respective breeding sites.

In freshwater environments, a gradient of predator species is typically correlated with water permanency (Werner and McPeck, 1994); thus, size and degree of permanence of water bodies have an indirect influence on tadpole community composition through their effects on predator composition (Azevedo-Ramos *et al.*, 1999). In permanent waters, fish are often the dominant predators whereas ephemeral pools are dominated by diving beetles, dragonfly larvae and other invertebrates (Skelly, 1997). Predators in permanent ponds have a greater effect on the survival rate of tadpoles when compared to more ephemeral ponds (Skelly, 1997). Moreover, experiments have shown that predators in ephemeral and permanent systems affect the local distribution of anuran prey species (Wilbur, 1972; Morin, 1981; Smith *et al.*, 1999; Dayton and Fitzgerald, 2001).

Ephemeral sites that dry quickly are better suited to anurans which develop more quickly. However, species that use sites that are of longer duration must cope with predation by using a myriad of anti-predator mechanisms. Two major types of anti-predator mechanisms exist. Primary anti-predator mechanisms are defined as those mechanisms that protect an organism prior to attack (Formanowicz and Brodie, 1982). Some examples of primary anti-predator mechanisms that could be important to larval anurans are cryptic coloration, lack of movement and size. Secondary anti-predator mechanisms are those that protect the organism after the initial attack. Examples of secondary anti-predator mechanisms include

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escape behavior, tail lures, sprint speed and toxins. Toxic skin secretions (unpalatability) have been shown to reduce predation on adult amphibians (Brodie, 1977; Brodie *et al.*, 1979), and unpalatability has also been documented in numerous species of larval anurans (Gunzburger and Travis, 2005).

Tadpoles are vulnerable to a wide range of vertebrate and invertebrate predators (Alford, 1999), with fish playing a key role in determining community composition in permanent habitats since they prey heavily on some amphibian larvae (Macan, 1966; Efford and Mathias, 1969; Heyer *et al.*, 1975; Petranka, 1983; Smith *et al.*, 1999). Vertebrate predators that indiscriminately swallow prey whole and invertebrate predators especially those that suck bodily fluids, are likely less affected by tadpole chemical defenses (Wassersug, 1973; Kiesecker *et al.*, 1996). A recent review of the literature on palatability of amphibian eggs and larvae by Gunzburger and Travis (2005) reports that nine genera of amphibians (five anuran genera) have been found to be unpalatable in at least one trial. The five anuran genera included *Hyla*, *Spea*, *Osteocephalus*, *Rana* and *Bufo*. Some studies have documented ontogenetic shifts in palatability (Brodie *et al.*, 1978; Formanowicz and Brodie, 1982; Brodie and Formanowicz, 1987; Hayes *et al.*, 2009). These studies found greater unpalatability at early stages (*i.e.*, hatchlings) with an increase in palatability thru developmental stages. Other studies have found that late stage tadpoles are the least palatable (Lawler and Hero, 1997; Crossland, 1998). The unpalatability at early developmental stages is usually attributed to remnant toxins from the egg; whereas later stage unpalatability is due to the development of toxins associated with the skin of adult frogs (Brodie *et al.*, 1978; Formanowicz and Brodie, 1982; Brodie and Formanowicz, 1987; Lawler and Hero, 1997).

We assessed the palatability of 12 species of larval anurans by subjecting individuals to predation by four common predators. We evaluated palatability based on predator behavior after a predation event had occurred, under the assumption that the palatability of an individual was based on noxious toxins. We expected the larvae of anurans that coexist with complex predator regimes (*i.e.*, anurans that use permanent habitats for breeding) and the Bufonids to be the most unpalatable.

#### METHODS

Larval anurans used in this study were collected from multiple constructed ponds and/or seasonal wetlands in the Angelina National Forest, Davy Crockett National Forest and Stephen F. Austin Experimental Forest in eastern Texas from Aug. 2002 to Aug. 2006. Larval anurans included in this study were *Lithobates* (formerly *Rana*) *sphenocephalus*, *L. catesbeianus*, *L. clamitans*, *Anaxyrus* (formerly *Bufo*) *woodhousii*, *Incilius* (formerly *Bufo*) *nebulifer*, *Hyla versicolor*/*H. chrysocelis*, *H. cinerea*, *Pseudacris crucifer*, *P. fouquettei*, *Acris crepitans*, *Gastrophryne carolinensis* and *Scaphiopus huerterii*. Tadpoles were captured using dip nets and maintained in 2.5 L plastic tubs with 2 L of tap water aged for at least 24 h. Tadpoles were housed in the laboratory for a maximum of 2 wk and were not fed. Tadpole developmental stages were determined using criteria developed by Gosner (1960) and specimens were grouped into the following developmental classes: class 0 (stages 20–22), class 1 (stages 23–25), class 2 (stages 26–28), class 3 (stages 29–31), class 4 (stages 32–34), class 5 (stages 35–37) and class 6 (stages 38–40).

Four predators were used in the study: *Lepomis cyanellus* (green sunfish), *L. macrochirus* (bluegill), *Procambarus nigrocinctus* (a crayfish) and *Anax junius* (larval dragonfly). Most of the larval anurans used in this study are known to co-occur with these four predators (Table 1). Approximately 100 green sunfish and 100 bluegill were collected throughout the study, using a circular wire mesh funnel trap (118 cm long × 38 cm diameter) with 7 cm

TABLE 1.—Co-occurrence of anuran larvae and predators from 51 sites in eastern Texas

Anuran species	Occurs with			Dragonfly larvae ( <i>Anax</i> )
	Bluegill	Green sunfish	Crayfish	
<i>A. crepitans</i>	X	X	X	X
<i>I. nebulifer</i>	X	X	X	X
<i>A. woodhousii</i>	X	X	X	X
<i>G. carolinensis</i>		X	X	X
<i>H. cinerea</i>	X	X	X	X
<i>H. versicolor</i>		X	X	X
<i>P. crucifer</i>	X	X	X	X
<i>P. fouquettei</i>		X	X	X
<i>L. catesbeianus</i>	X	X	X	X
<i>L. clamitans</i>	X	X	X	X
<i>L. sphenoccephalus</i>	X	X	X	X
<i>S. hurterii</i>				

openings and rod and reel fishing, from multiple constructed ponds in the Davy Crockett National Forest and from two private constructed ponds in Nacogdoches County in eastern Texas. However, we only housed approximately 15 individuals of each sunfish species at any one time. Approximately 100 crayfish were collected throughout the study, using collapsible mesh funnel traps (25 cm × 25 cm × 46 cm), from constructed ponds in the Davy Crockett National Forest. Only 25 individual crayfish were housed in the laboratory at one time. Approximately 200 dragonfly larvae were collected throughout the study, using dip nets, from seasonal wetlands in the Stephen F. Austin Experimental Forest. Only 50 individual dragonfly larvae were housed in the laboratory at one time. Predators were replaced once they had eaten one individual of each larval anuran species, died or would no longer eat. Predators were allowed to acclimate in the laboratory and trained to eat (primarily the two fish species) prior to being used for predation trials. All predators were collected between Aug. 2002 and Aug. 2006.

The two fish species were maintained in 19 L clear square glass aquaria with aged tap water and proper filtration and fed tropical fish flakes (Wardley® Tropical Fish Flakes). Crayfish were maintained in 2 L clear square plastic tubs containing aged tap water and were fed the same tropical fish flakes. Dragonfly larvae were maintained in 0.4 L clear plastic cups containing aged tap water and were fed larval anurans that were known to be palatable. It was necessary to feed dragonfly larvae palatable tadpoles because they are visual predators and primarily require live prey. Fish were fed daily and invertebrates were fed every other day, although predators were not fed for 24 h prior to feeding trials. All predators and larval anurans were kept at room temperature (21 C) on a 12 h light-dark cycle.

For each anuran species, we attempted to present five tadpoles from each developmental class to each predator species (5 tadpoles × 7 classes × 4 predators = max 140 tadpoles/species). However, not all classes were represented for all anuran species. A palatability trial consisted of introducing one tadpole into a container containing one predator. Containers were 19 L, 2 L and 0.4 L, respectively, for trials with fish, crayfish and dragonfly larvae. If the predator did not attempt to eat the tadpole the trial was excluded. If the predator successfully captured the tadpole, palatability was scored based on six levels of predator behavior (ranging from unpalatable to most palatable): 1 – the predator attempted to eat the tadpole, expelled it, and did not try to eat it again, 2 – the predator repeatedly

attempted to eat the tadpole but did not consume it, 3 – the predator repeatedly attempted to eat the tadpole before it was consumed, 4 – the predator attempted to eat the tadpole, expelled it once and consumed it, 5 – the predator consumed tadpole; however, the predator moved mouthparts in a manner suggesting irritation and 6 – the predator consumed the tadpole on the first attempt suggesting no irritation. Trials were terminated when the predator turned away from the tadpole, indicating loss of interest in the tadpole, or the tadpole was consumed. Tadpoles were not eaten in trials scored 1–2 and tadpoles were eaten in trials scored 3–6.

The predators used were generally of sufficient size to easily consume individuals of most tadpole species; however, tadpoles of some species were too large for even the largest predator to consume. For example, the largest dragonfly larvae could not consume late stage *Lithobates clamitans*. Where possible, the same individual predators were used in trials for each anuran species. However, if predators died during the study they were replaced with similarly sized individuals. Individual predators were used in only one trial within each anuran species. For example, one green sunfish could only be fed one individual *L. clamitans* tadpole. Once the green sunfish consumed or attempted to consume the *L. clamitans* tadpole that individual could no longer be used for additional *L. clamitans* trials. However, that individual green sunfish could be used for other anuran species palatability trials. Ideally, we would have only used each individual predator in one palatability trial to help reduce the potential effects on feeding trials but due to space requirements of predators, acclimation time and training time this was not a viable option.

Overall palatability scores were determined for each anuran species by combining all palatability scores, excluding trials with crayfish and taking the mean for each anuran species. Crayfish consumed all species in all classes except for two trials with *Scaphiopus hurterii* and one trial with *Incilius nebulifer*. Therefore crayfish trials were excluded from the overall palatability score. Species with an overall palatability score of 5.5 or less were classified as unpalatable.

We also wanted to determine if there was a difference in predator tolerance to noxiousness. We did this by combining all palatability scores from the 12 species of larval anurans for each predator species and comparing the means.

Because our response variable (palatability) is ordinal, we used Kruskal-Wallis tests (Agresti, 1984) to test the statistical hypotheses of no differences in palatability among anuran species, no differences in overall palatability of each anuran species and no difference in predator tolerance to noxiousness. Each test was followed by Tukey's post-hoc test to separate species ( $P < 0.05$ ). To determine which species showed ontogenetic shifts in palatability we collapsed scores 1–2 (not eaten) and scores 3–6 (eaten) within a class and used a Cochran-Armitage trend test ( $P < 0.05$ ). If a class had fewer than two replications then that class was excluded from the analysis.

## RESULTS

A total of 1324 predation trials were conducted using the 12 species of larval anurans and four predator species. Twenty-four predation trials were scored as 1, 37 trials as 2, 6 trials as 3, 8 trials as 4, 66 trials as 5 and 1183 trials as 6. Scores of 2, 3 and 4 were assigned to predation trials when a predator repeatedly tried to consume a tadpole. This behavior was exhibited primarily by the two fish species but was also seen in trials with invertebrates, primarily dragonfly larvae. This behavior was only documented in 51 of the 1324 predation trials. When predators were exposed to noxious individuals it was clear to us that the

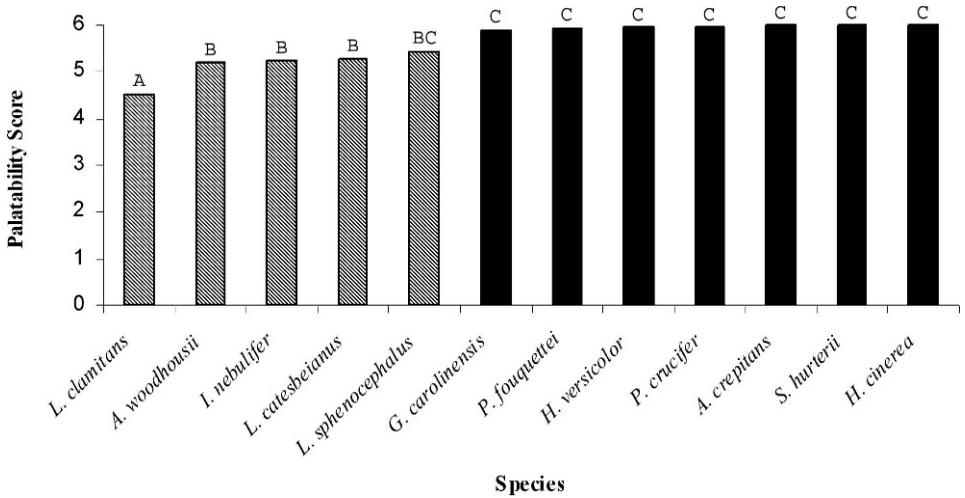


FIG. 1.—Overall mean palatability scores for 12 anuran species larvae based on trials with green sunfish, bluegill and dragonfly larvae as predators. Striped bars indicate a mean palatability score of less than 5.5. Significant differences are indicated by differing letters between species

predator response was due to an undesirable “taste” and not differential foraging or feeding behavior.

Palatability trials with late stage Ranids were difficult to perform due to the large size of the tadpoles. Bluegill were unable consume developmental classes 4, 5 and 6 *Lithobates catesbeianus* and classes 5 and 6 *L. sphenoccephalus*. Dragonfly larvae were unable to consume classes 4, 5 and 6 *L. catesbeianus* and *L. clamitans* and classes 5 and 6 *L. sphenoccephalus*. Since predators were unable to consume the tadpoles these, trials were not included in any analyses.

Palatability scores were combined for each anuran species for all predators, except crayfish, to get an overall palatability score. Our analysis indicated a significant difference in palatability between the 12 species of larval anurans when palatability scores were combined (Kruskal - Wallis,  $H_{11,983} = 19.35$ ,  $P < 0.001$ ). *Lithobates clamitans* had the lowest mean palatability score and was significantly different from all other species (Fig. 1). *Anaxyrus woodhousii*, *I. nebulifer*, *L. catesbeianus* and *L. sphenoccephalus* had similar palatability scores. *L. sphenoccephalus* did not differ from the remaining species.

Our analysis also revealed a significant difference in predator tolerance to noxiousness (Kruskal - Wallis,  $H_{31,320} = 25.18$ ,  $P < 0.001$ ). Bluegill had the lowest mean tolerance score and were significantly different from all other predator species (Fig. 2). Dragonfly larvae and green sunfish had the next lowest mean tolerance scores, whereas crayfish had the highest tolerance.

Green Sunfish trials indicated a significant difference in palatability among the 12 larval anuran species (Kruskal - Wallis,  $H_{11,346} = 11.13$ ,  $P < 0.001$ ). *Anaxyrus woodhousii* and *Incius nebulifer* had the lowest mean palatability scores of 4.8 and 5.1, respectively, and were the most unpalatable to green sunfish (Table 2). These two species were significantly different from all other species ( $P < 0.05$ ) but not from each other.

Bluegill behavior also indicated significant variation in palatability among the 12 larval anuran species (Kruskal - Wallis,  $H_{11,315} = 41.10$ ,  $P < 0.001$ ). *Lithobates clamitans* had a mean

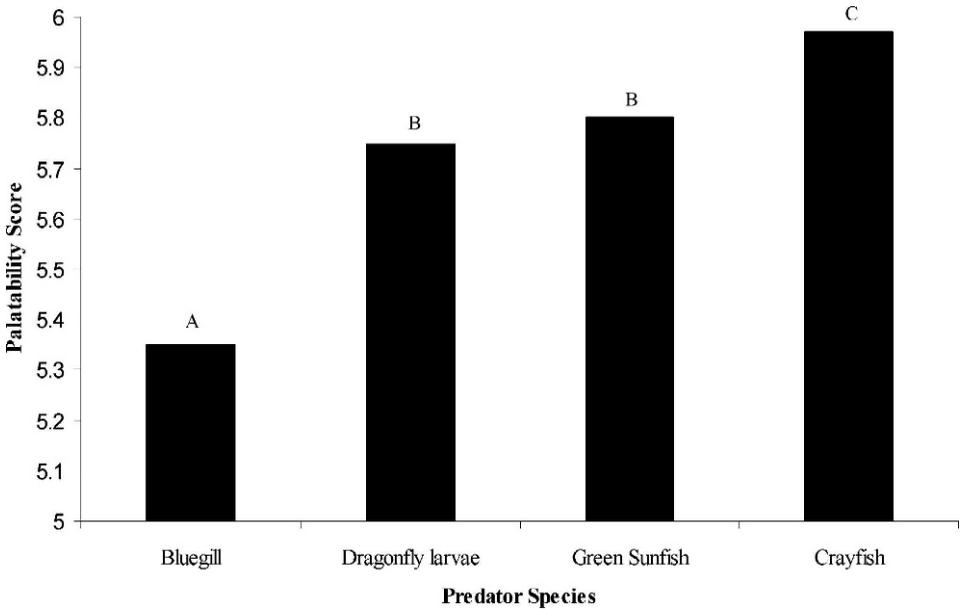


FIG. 2.—Predator tolerances to noxiousness exhibited by anuran species larvae. The lower the palatability score, the lower the tolerance. Significant differences are indicated by differing letters between species

palatability score of 2.3, making it the most unpalatable species (Table 2). The next most unpalatable species was *L. catesbeianus*, with a mean palatability score of 3.7. *L. sphenoccephalus* and *Anaxyrus woodhousii* were similarly unpalatable (4.7 and 5.3, respectively). The latter species was not significantly less palatable than the remaining species.

Our analysis revealed a significant difference in palatability between the 12 larval anuran species when fed to dragonfly larvae (Kruskal - Wallis,  $H_{11,298} = 4.08$ ,  $P < 0.001$ ). *Incilius*

TABLE 2.—Mean palatability of 12 species of anuran larvae to predators. Means in columns followed by the same letters are not significantly different ( $P < 0.05$ )

Species	Green sunfish			Bluegill			Dragonfly larvae			Crayfish		
	N	Mean	SE	N	Mean	SE	N	Mean	SE	N	Mean	SE
<i>A. crepitans</i>	25	6.0 A	0.00	25	6.0 A	0.00	25	6.0 A	0.00	25	6.0 A	0.00
<i>I. nebulifer</i>	35	5.1 B	0.20	33	5.6 A	0.09	29	5.1 B	0.31	35	6.0 A	0.03
<i>A. woodhousii</i>	25	4.8 B	0.35	25	5.3 AB	0.18	25	5.4 AB	0.27	25	6.0 A	0.00
<i>G. carolinensis</i>	30	6.0 A	0.00	28	5.7 A	0.14	27	6.0 A	0.00	20	6.0 A	0.00
<i>H. cinerea</i>	25	6.0 A	0.00	26	6.0 A	0.00	25	6.0 A	0.00	24	6.0 A	0.00
<i>H. versicolor</i>	35	6.0 A	0.00	35	5.9 A	0.63	31	6.0 A	0.00	35	6.0 A	0.00
<i>P. crucifer</i>	30	6.0 A	0.00	30	5.9 A	0.06	30	6.0 A	0.00	30	6.0 A	0.00
<i>P. fouquettei</i>	31	5.9 A	0.06	30	6.0 A	0.00	35	5.9 AB	0.14	28	6.0 A	0.00
<i>L. catesbeianus</i>	23	5.6 A	0.22	11	3.7 C	0.68	12	6.0 A	0.00	15	6.0 A	0.00
<i>L. clamitans</i>	35	6.0 A	0.00	28	2.3 D	0.32	20	5.1 B	0.42	32	6.0 A	0.00
<i>L. sphenoccephalus</i>	34	5.9 A	0.00	26	4.7 B	0.38	21	5.4 AB	0.31	30	6.0 A	0.00
<i>S. hurterii</i>	30	6.0 A	0.00	30	6.0 A	0.00	30	6.0 A	0.00	30	5.7 A	0.19

*nebulifer* and *Lithobates clamitans* were significantly less palatable than seven of the anuran species; *L. sphenoccephalus*, *Anaxyrus woodhousii* and *Pseudacris fouquettei*, were intermediate in palatability and did not differ from any species (Table 2). We did not detect a difference in palatability between the 12 larval anuran species when fed to crayfish (Kruskal - Wallis,  $H_{11,317} = 1.79$ ,  $P = 0.055$ ) (Table 2).

Some species showed significant trends in palatability (*i.e.*, ontogenetic shifts) by becoming more palatable through development whereas others showed variation in palatability but showed no trend. In predation trials with green sunfish, *Inciilius nebulifer* and *Anaxyrus woodhousii* became more palatable through development (Cochran-Armitage trend test,  $P = 0.035$  and  $P = 0.017$ , respectively) (Table 3). The remaining species were palatable to green sunfish and no ontogenetic shifts were apparent (Table 3).

In predation trials with bluegill, *Hyla versicolor*, *Pseudacris crucifer*, *Gastrophryne carolinensis*, *Inciilius nebulifer*, *Anaxyrus woodhousii* showed variation in palatability through development whereas *L. catesbeianus* and *L. sphenoccephalus* showed ontogenetic shifts (Table 3). *Lithobates catesbeianus* and *L. sphenoccephalus* became more palatable through development (Cochran-Armitage trend test,  $P = 0.001$  and  $P < 0.001$ , respectively) (Table 3). All developmental classes of *L. clamitans* were very unpalatable, whereas the remaining anuran species were palatable to bluegill (Table 3).

In predation trials with dragonfly larvae *Pseudacris fouquettei* showed variation in palatability through development, whereas *Inciilius nebulifer*, *Anaxyrus woodhousii*, *Lithobates clamitans* and *L. sphenoccephalus* showed ontogenetic shifts in palatability, becoming more palatable throughout development (Cochran-Armitage trend test,  $P = 0.002$ ,  $P = 0.041$ ,  $P = 0.008$  and  $P = 0.023$  respectively) (Table 3). The remaining anuran species were very palatable to dragonfly larvae. When fed to crayfish, *Scaphiopus hurterii* and *I. nebulifer* were the only species that showed variation in palatability (Table 3).

Only two of the five replicates of class 6 *Scaphiopus hurterii* and one of the five replicates of class 6 *Inciilius nebulifer* indicated any unpalatability to crayfish. These trials were the only trials that showed potential noxiousness across all species when fed to crayfish and can likely be explained as simple variation in predator-prey interactions rather than toxicity.

## DISCUSSION

Chemical defenses have been documented in many larval amphibians (Liem, 1961; Voris and Bacon, 1966; Wassersug, 1971; Kruse and Francis, 1977; Brodie *et al.*, 1978; Formanowicz and Brodie, 1982; Kruse and Stone, 1984; Brodie and Formanowicz, 1987; Kats *et al.*, 1988; Peterson and Blaustein, 1991; Peterson and Blaustein, 1992; Lawler and Hero, 1997; Gunzburger and Travis, 2005). We clearly demonstrated the occurrence of significant noxiousness in several species in this study. However, not all species showed noxiousness to all predators, and there was much variation among individuals within anuran species. Also, it was obvious that there was a difference in response to noxiousness among predators. Bluegill were the least tolerant predators followed by dragonfly larvae and green sunfish. Crayfish were the most tolerant and consumed tadpoles in all but three trials. These findings follow the general trend that fish predators are more sensitive to unpalatable individuals than invertebrate predators (Voris and Bacon, 1966; Brodie *et al.*, 1978; Formanowicz and Brodie, 1982; Kruse and Stone, 1984; Brodie and Formanowicz, 1987; Kats *et al.*, 1988; Gunzburger and Travis, 2005).

We did not demonstrate noxiousness in any species of Hylid, Pelobatid or Microhylid used in this study. To our knowledge, no studies have found larval anurans within these families to be noxious. We documented noxiousness, at the tadpole stage, in two species of

TABLE 3.—Mean palatability scores for each developmental class<sup>a</sup> of 12 species of larval anurans when fed to predators. Blanks indicate no tadpoles in that developmental class were available for use in trials. Numbers in parentheses denote sample size

Anuran species	Predator						
	Class 0	Class 1	Class 2	Class 3	Class 4	Class 5	Class 6
<b>Green Sunfish</b>							
<i>A. crepitans</i>			6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)
<i>I. nebulifer</i> *	3.8 (5)	5.2 (5)	6.0 (5)	4.4 (5)	5.8 (5)	5.2 (5)	5.0 (5)
<i>A. woodhousii</i> *			4.4 (5)	3.6 (5)	4.6 (5)	6.0 (5)	5.6 (5)
<i>G. carolinensis</i>		6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)
<i>H. cinerea</i>			6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)
<i>H. versicolor</i>	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)
<i>P. crucifer</i>		6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)
<i>P. fouquettei</i>	6.0 (1) <sup>b</sup>	6.0 (5)	5.6 (5)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)
<i>L. catesbeianus</i>		4.4 (5)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (3)	
<i>L. clamitans</i>	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)
<i>L. sphenoccephalus</i>	6.0 (4)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)
<i>S. hurterii</i>		6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)
<b>Bluegill</b>							
<i>A. crepitans</i>			6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)
<i>I. nebulifer</i>	5.2 (5)	5.6 (5)	6.0 (5)	5.6 (5)	5.7 (3)	5.6 (5)	5.4 (5)
<i>A. woodhousii</i>			5.0 (5)	5.0 (5)	5.6 (5)	4.8 (5)	6.0 (5)
<i>G. carolinensis</i>		6.0 (5)	6.0 (5)	6.0 (5)	5.7 (3)	5.0 (5)	5.4 (5)
<i>H. cinerea</i>		6.0 (1) <sup>b</sup>	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)
<i>H. versicolor</i>	5.8 (5)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	5.6 (5)
<i>P. crucifer</i>		6.0 (5)	6.0 (5)	5.4 (5)	6.0 (5)	6.0 (5)	6.0 (5)
<i>P. fouquettei</i>		6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)
<i>L. catesbeianus</i> *		1.6 (5)	5.4 (5)	6.0 (1) <sup>b</sup>			
<i>L. clamitans</i>	1.2 (5)	3.8 (5)	1.6 (5)	2.4 (5)	1.3 (3)	2.0 (2)	3.3 (3)
<i>L. sphenoccephalus</i> *	2.2 (5)	3.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (1) <sup>b</sup>	
<i>S. hurterii</i>		6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)
<b>Dragonfly larvae</b>							
<i>A. crepitans</i>			6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)
<i>I. nebulifer</i> *		2.2 (5)	6.0 (5)	4.6 (5)	6.0 (5)	5.8 (4)	6.0 (5)
<i>A. woodhousii</i> *			5.2 (5)	4.4 (5)	5.6 (5)	6.0 (5)	6.0 (5)
<i>G. carolinensis</i>		6.0 (5)	6.0 (5)	6.0 (2)	6.0 (5)	6.0 (5)	6.0 (5)
<i>H. cinerea</i>			6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)
<i>H. versicolor</i>	6.0 (1) <sup>b</sup>	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)
<i>P. crucifer</i>		6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)
<i>P. fouquettei</i>	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	5.0 (5)
<i>L. catesbeianus</i>		6.0 (2)	6.0 (5)	6.0 (5)			
<i>L. clamitans</i> *	4.4 (5)	4.0 (5)	6.0 (5)	6.0 (5)			
<i>L. sphenoccephalus</i> *	3.6 (5)	6.0 (5)	6.0 (5)	6.0 (4)	6.0 (2)		
<i>S. hurterii</i>		6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)
<b>Crayfish</b>							
<i>A. crepitans</i>			6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)
<i>I. nebulifer</i>	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	5.8 (5)
<i>A. woodhousii</i>			6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)
<i>G. carolinensis</i>		6.0 (5)	6.0 (5)			6.0 (5)	6.0 (5)

TABLE 3.—Continued

Anuran species	Predator						
	Class 0	Class 1	Class 2	Class 3	Class 4	Class 5	Class 6
<i>H. cinerea</i>			6.0 (5)	6.0 (5)	6.0 (4)	6.0 (5)	6.0 (5)
<i>H. versicolor</i>	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)
<i>P. crucifer</i>		6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)
<i>P. fouquettei</i>		6.0 (5)	6.0 (5)	6.0 (4)	6.0 (4)	6.0 (5)	6.0 (5)
<i>L. catesbeianus</i>		6.0 (5)	6.0 (5)	6.0 (5)			
<i>L. clamitans</i>	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (2)
<i>L. sphenoccephalus</i>	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (4)	6.0 (2)	6.0 (4)	6.0 (5)
<i>S. hurterii</i>		6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	6.0 (5)	4.4 (5)

<sup>a</sup> Gosner Stages for class: Class 0 = 20–22, Class 1 = 23–25, Class 2 = 26–28, Class 3 = 29–31, Class 4 = 32–34, Class 5 = 35–37, Class 6 = 38–40

<sup>b</sup> Sample size less than two; not used in analysis

\* Significant positive trend in consumption (Cochran-Armitage trend test,  $P < 0.05$ )

Bufonids and three species of Ranids. Previous studies have documented noxiousness, at the tadpole stage, in 2 of the 12 species in our study; *Lithobates clamitans* and *L. catesbeianus* (Walters, 1975; Kruse and Francis, 1977; Woodward, 1983; Kats *et al.*, 1988; Werner and McPeck, 1994). These studies suggest that *L. catesbeianus* and *L. clamitans* tadpoles exhibit noxiousness, which agrees with our findings. We found that *L. clamitans* larvae are extremely unpalatable to bluegill and hatchlings are somewhat unpalatable to dragonfly larvae. The relationship between *L. clamitans* and bluegill was definitely the most obvious; all stages of *L. clamitans* were unpalatable to bluegill.

In eastern Texas, *Lithobates clamitans* inhabits many different permanent and semi-permanent breeding sites, with and without fish. This species persists particularly well in ponds with fish, when the fish community is made up of *Micropterus* sp. (black basses) and bluegill (C.K. Adams, pers. comm.). *Lithobates catesbeianus* tadpoles were also noxious to bluegill at early stages and also showed some unpalatability to green sunfish. In eastern Texas, *L. catesbeianus* inhabit permanent breeding sites that almost always have predatory fish.

We also documented noxiousness in *Lithobates sphenoccephalus*, *Incilius nebulifer* and *Anaxyrus woodhousii*. The palatability of these species has not previously been explored. *Lithobates sphenoccephalus* uses a wide range of breeding habitats ranging from small ephemeral pools to permanent ponds. This species does particularly well in ephemeral pools dominated by invertebrate predators because the tadpoles of this species grow to a large size and have the ability to escape predation (Saenz, 2004). The unpalatability of this species at early Gosner stages, particularly to dragonfly larvae, likely increases its survival which allows individuals to reach larger sizes. *Anaxyrus woodhousii* typically uses semi-permanent to permanent breeding sites with and without fish, and *I. nebulifer* can be found in permanent and ephemeral pools. Toxicity has been well documented in Bufonid eggs, larvae and metamorphs (Licht, 1968; Wassersug, 1971; Brodie *et al.*, 1978; Kruse and Stone, 1984; Brodie and Formanowicz, 1987; Kats *et al.*, 1988; Henrikson, 1990; Lawler and Hero, 1997; Hayes *et al.*, 2009). As a result, it was not surprising to find that the two species of Bufonids in this study were noxious in some trials.

We observed ontogenetic shifts in palatability in all five unpalatable larval anuran species; *Incilius nebulifer*, *Anaxyrus woodhousii*, *Lithobates clamitans*, *L. catesbeianus* and *L. sphenoccephalus*. *Incilius nebulifer* and *A. woodhousii* became more palatable throughout development when

fed to green sunfish and dragonfly larvae. We also documented some noxiousness in the intermediate stages of *I. nebulifer* and *A. woodhousii* when fed to bluegill, green sunfish and dragonfly larvae. These results parallel the findings of Brodie *et al.* (1978), Brodie and Formanowicz (1987) and Hayes *et al.* (2009) who saw a general trend of unpalatability declining from early to later stages. Hayes *et al.* (2009) found that newly laid *Bufo marinus* (Marine Toad) eggs contained high levels of bufadienolide (toxin), whereas midstage tadpoles had lower levels and that all stages beyond the egg decreased in toxin diversity. These findings reaffirm the results of our feeding trials.

Three species of Ranids (*Lithobates clamitans*, *L. catesbeianus* and *L. sphenoccephalus*) showed ontogenetic shifts in palatability. *Lithobates clamitans* and *L. sphenoccephalus* larvae became more palatable through development to dragonfly larvae. *Lithobates sphenoccephalus* and *L. catesbeianus* became more palatable through development to bluegill. All stages of *L. clamitans* were unpalatable to bluegill. These three species come from a clade of permanent pond breeders and have the ability to grow to large sizes when compared to other sympatric species (Saenz, 2004). Larger-sized tadpoles have a greater ability to escape predation reducing the need to produce noxious secretions. Several studies have suggested that susceptibility to predation on tadpoles decreases with increased size (Caldwell *et al.*, 1980; Smith, 1983; Travis *et al.*, 1985; Cronin and Travis, 1986; Werner and McPeck, 1994). In our study, predators were unable to consume some late stage *L. sphenoccephalus*, *L. clamitans* and *L. catesbeianus* because the predators were gape limited. Occasionally a dragonfly larvae was able to successfully attack a large *Lithobates* tadpole, but on most occasions the tadpole would "shake" off the dragonfly larvae. Size may serve as a very important anti-predator mechanism for late stage larval anurans of the genus *Lithobates*. Since these species are less susceptible to predation when they reach large size it would be beneficial for them to have protection at smaller sizes. More research on the unpalatability of these species is needed in order to understand the ecological contributions of this anti-predator mechanism.

The present study documents unpalatability in five anuran species in two families and demonstrates ontogenetic shifts in all five species. Noxiousness was previously undocumented in three of the five unpalatable species in this study. This information continues to add to our growing breadth of knowledge of chemical defenses in larval anurans. It has been suggested that larval anurans that live in permanent water should be less palatable (Skelly, 1997). Of the five species found to be unpalatable in this study four are strongly associated with permanent water and fishes; however, three additional species from the current study breed in permanent water and were found to be palatable. These three species were all Hylids. Phylogenetic history likely plays a key role in determining larval anuran palatability. Kats *et al.* (1988) noted that some anuran larvae that coexist with fish are palatable and likely use other anti-predator mechanisms such as behavior in order to escape predation. Other anti-predator mechanisms have been documented in some of the species used in this study (Lawler, 1989; Skelly, 1992; Lefcort, 1996; Bridges and Gutzke, 1997; Anholt *et al.*, 2000; Saenz, 2004; Watkins and McPeck, 2006). It is probable that unpalatability is not the most important mechanism which allows larval anurans to coexist with predators; however, it likely plays a key role in the survival of some species at vulnerable developmental stages.

There were some factors in this study that we did not control for that could influence palatability. Hagman *et al.* (2009) found that alarm cues experienced by *Bufo marinus* larvae can affect post-metamorphic toxin levels. This plasticity may be present in anuran larvae as well, and future research should control for this by collecting larvae from areas with known predator composition or by collecting eggs and rearing larvae in a controlled setting. Our experimental design was set up so that individual predators were not used for more than

one predation trial within an anuran species; however, they could be used across anuran species. As a result, there is potential for predators to learn to eat palatable larval anurans and simply consume unpalatable larvae as if they were palatable or vice versa. This indicates that our palatability results could be skewed towards species being less or more palatable than we observed. If any palatability trials were incorrectly categorized they are likely individuals that are unpalatable that appeared to be palatable since only 10.7% of the trials exhibited unpalatability.

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