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## PALATABILITY OF THE LARVAE OF THREE SPECIES OF *LITHOBATES*

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**ABSTRACT:** While unpalatability in general is well-known to provide prey protection against predators, the role of unpalatability as an antipredator mechanism in larval anurans and more specifically, *Lithobates catesbeianus* (American Bullfrog) larvae remains contentious. There are three major problems associated with the majority of the studies done to date: (1) failure to incorporate the existence of a range of relative preferences or palatability, (2) failure to effectively control for predator hunger levels, and (3) failure to consider confounding variables such as prey behavior and prey appearance. We sought to alleviate these problems by first training *Lepomis gibbosus* (Pumpkinseed Sunfish) to consume a standardized food ration for 1 wk and then spiking that food ration the following week with only the skin of three different larval anurans that have been hypothesized to range in palatability (namely, *L. catesbeianus*, *L. clamitans* [Green Frog], and *L. sylvaticus* [Wood Frog]). The results of this experiment revealed that a range of palatability does in fact exist, with *L. catesbeianus* being least palatable, *L. clamitans* being somewhat unpalatable, and *L. sylvaticus* being highly palatable. This study was the first to show evidence, devoid of any confounding variables, that *L. catesbeianus* and, to a lesser extent, *L. clamitans* tadpoles are unpalatable to sunfish predators.

**Key words:** Learning; Predator-prey interactions; Tadpoles; Unpalatability

TOXINS and unpalatability are widely used by both plants and animals as a defense against herbivores and predators (e.g., Bowers, 1980; Palo and Robbins, 1991). Toxicity refers to the presence of a known chemical compound that causes some type of physical harm, such as loss of muscle coordination (Liem, 1961), but decreased consumption risk may also be achieved more simply from unpalatability, which refers to a general defense mechanism whereby prey apparently have a taste that a predator perceives as disagreeable (Gunzburger and Travis, 2005). In practice, it is difficult to distinguish between unpalatability and toxicity, because toxicity is nearly always associated with unpalatability (e.g., Bowers,

1992; Brodie and Formanowicz, 1987; Licht, 1968). In vertebrates, amphibians epitomize the use of unpalatability and skin toxins to deter predators (Duellman and Trueb, 1994). In adult amphibians diverse compounds have been identified that are of known toxicity (Daly et al., 2005). Thus, in adults, determination that a group is toxic or unpalatable has been driven by a bottom-up approach—the presence of certain compounds are detected and these compounds are known to be bitter-tasting or toxic to other organisms, and this provides the basis for a presumed avoidance of predation. In larval amphibians (and any number of other animals in which presence of particular toxins is unknown) palatability to potential predators has been inferred only through predator behavior. As a result, knowledge of the compounds that may produce unpalatability is also lacking. Some larval toads in the family Bufonidae, however, are an exception to this, because bufotoxins and other toxic chemical skin secretions have been shown to cause

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unpalatability of these larvae (e.g., Crossland, 2001).

Three general problems are associated with most palatability studies done to date: (1) failure to incorporate the existence of a range of relative preferences or palatability, (2) failure to effectively control for predator hunger levels, and (3) failure to consider confounding variables such as prey behavior (Gunzburger and Travis, 2005). The first problem arises because preference or choice experiments provide little evidence of palatability per se. Blouin (1990) provides a good example of this difficulty. He tested predation rates by fish on *Hyla gratiosa* and *H. cinerea*. When equal numbers of each species were provided to fish, there was no difference in predation rates between the two hylid species. However, when the tadpoles of each species were presented in single-species sets, fish clearly ate more *H. gratiosa* than *H. cinerea*, suggesting that *H. cinerea* was relatively unpalatable. In this example, presence of an unpalatable species appeared to convey some protection to the palatable species present (Blouin, 1990). Equally possible is that a preference observed when two species are in combination simply indicates relative palatability, and not an unwillingness of the predator to eat the apparently less palatable species if it were the only food available.

The second problem associated with most studies of palatability is that hunger levels of predators are insufficiently controlled (Gunzburger and Travis, 2005). Motivation to forage may vary greatly in predators and we might expect a predator that refuses a slightly unpalatable prey species when it has energy reserves remaining, will rapidly take the same prey when energy reserves are depleted. The difficulty comes in determining what qualifies as being "sufficiently hungry" for the potential predator. For example, Kats et al. (1988) reported both *Lithobates catesbeianus* (American Bullfrog) and *L. clamitans* (Green Frog) tadpoles to be unpalatable, but fail to indicate how much alternative food the fish received.

The third difficulty in studies of palatability is a failure to control for confounding variables, such as prey behavior, which can also alter predation rates (Gunzburger and Travis, 2005). This can be especially problem-

atic in choice experiments, where two species of prey are offered simultaneously to a predator. In a visually oriented predator, such as fish, subtle differences in activity level or body coloration might attract the predator toward one prey item in particular. This, in turn, can skew the results of a palatability study. For example, if the predator is drawn to one species due to its behavior or appearance and does not attempt to consume the other species, the observer will incorrectly conclude that the predator has selected the more palatable prey item (when selection was actually based on prey appearance) and may even deem the other prey species unpalatable. Another confounding variable often neglected in studies using intact anuran larvae is size. For example, some anuran larvae, such as *L. catesbeianus*, become quite large, making them inaccessible to gape-limited predators. Again, this would skew the results of a palatability study, because an observer may erroneously conclude that larvae of a particular anuran species are unpalatable rather than considering the simpler explanation—the larvae are too large for the predator to consume.

Here, we use the methodology suggested by Gunzburger and Travis (2005) to test the palatability of three species of larval anurans to fish predators. We quantified rates of consumption by *Lepomis gibbosus* (Pumpkinseed Sunfish) when fed a common food staple (Chironomidae, midge larvae) spiked with skin from tadpoles of three species of frogs (*L. catesbeianus*, *L. clamitans*, and *L. sylvaticus* [Wood Frog]). This methodology allowed us to isolate change in consumption rates based solely on the taste of skin, while avoiding the three problems outlined above.

#### MATERIALS AND METHODS

*The system.*—*Lithobates catesbeianus* individuals are found in permanent water bodies with fish and have been labeled unpalatable by previous researchers (Kats et al., 1988; Kruse and Francis, 1977; Werner and McPeck, 1994). Not surprisingly, the unpalatability of *L. catesbeianus* has been used by previous researchers to explain low mortality rates despite high activity levels when exposed to predatory fish (Eklov and Werner, 2000;

Werner and McPeck, 1994). Individuals of *L. clamitans* are typically found in permanent water bodies lacking fish predators (Richardson, 2001; Werner and McPeck, 1994). In our study area, we have only found *L. clamitans* tadpoles in lakes with fish where tadpoles were spatially segregated from fish by a shallow, densely vegetated area that fish avoided. In addition, *L. clamitans* larvae have also been considered somewhat unpalatable to fish by previous researchers (Kats et al., 1988; Werner and McPeck, 1994). Unlike *L. catesbeianus* larvae, *L. clamitans* larvae decrease activity when in the presence of fish (Werner and McPeck, 1994) and invertebrate predators (Eklov and Werner, 2000; Werner and McPeck, 1994). This overall decrease in activity may be a general response that enables this species to exist in both permanent and semipermanent pond types (Werner and McPeck, 1994).

Finally, *L. sylvaticus* larvae inhabit temporary ponds (such as vernal ponds, flooded areas, wooded swamps, and quiet stream backwaters; Harding, 2006) that do not contain fish predators and, thus, are expected to lack evolved defense mechanisms against fish (Kats et al., 1988; Walters, 1975). Even though *L. sylvaticus* does not commonly encounter predatory fish in the wild, they do exhibit a sharp decrease in activity when exposed to chemical cues from a fish predator (Chivers and Mirza, 2001; Thiemann and Wassersug, 2000).

**Animal collection and husbandry.**—Nine *L. sylvaticus* egg masses were collected in May 2008 from Bat Lake at the Wildlife Research Station, Algonquin Park, Ontario, Canada (45°35'N, 78°31'W; datum = WGS84). The egg masses were placed in glass bowls and housed in growth chambers at 5°C prior to hatching (ambient temperature of natural habitat). *Lithobates clamitans* hatchlings (Gosner stage 20; Gosner, 1960) were collected in July 2008 from a pond near Rock Lake in Algonquin Park, Ontario, Canada (45°31'N, 78°24'W). Hatchlings of all species were housed in 38-L (61 × 40.6 × 22.2-cm) Rubbermaid® tubs or 11.4-L (30 × 25 × 15-cm) Sterilite® tubs containing a mixture of filtered pond water and conditioned tap water (carbon-filtered tap water was adjusted to

pH 7.0 and aerated for 24 h). To minimize animal usage, larger (Gosner stages 30–34; Gosner, 1960) *L. catesbeianus* tadpoles were collected using dip nets in September 2008 from the Glenridge Naturalization Site in Niagara region, Ontario, Canada (43°7'N, 79°14'W). *Lithobates catesbeianus* tadpoles were housed in 11.4-L (30 × 25 × 15-cm) Sterilite® tubs filled approximately 3 L of animal-ready water with up to 15 tadpoles per tub for a 1-mo period prior to any *L. catesbeianus* skin feeding experiments. Because both *L. sylvaticus* and *L. clamitans* tadpoles spent approximately 1 mo in common housing conditions prior to experimentation, this 1-mo period helped to maintain a consistent level of pre-experimental conditions across all species.

All tadpoles were maintained on ground Spirulina Algae Discs (Wardley®, Secaucus, New Jersey) and approximately 0.5 L of suspended algae (from a lab culture of mixed unicellular spp.) placed into tubs once per week. Tubs were cleaned of feces every second day and complete water changes were performed weekly. All animals were maintained on a 14:10 light:dark cycle.

Six *Lepomis gibbosus* (approximately 8–10 cm in length) were collected in May 2008 from a pond located within St. John's Conservation Area, Pelham, Ontario (43°3'N, 79°17'W). The fish were housed in transparent 10-L (30.5 × 22.9 × 17.8-cm) aquaria (Tom Pla-House Clear Vue) filled with approximately 8.5 L of animal-ready water and fitted with a sponge filter (Dirt Magnet® Aquarium Filter, Junior Model). All tanks received five droplets of Stress Coat® and five droplets of Kent Freshwater Essential™ (mineral supplement for aquaria), as well as one piece of rounded polyvinyl chloride tubing as cover for fish. The fish were maintained on approximately 2 mL of wet bloodworms three times per week.

**Experimental feeding regime.**—Throughout the experiment, fish were fed using pellets constructed of bloodworms only (control treatment) or bloodworms with tadpole skin (experimental treatment). Pellets were constructed as follows: Wet bloodworms (Chironomidae, San Francisco Bay Brand) were divided into 2-g samples and dried for

approximately 2 h until the consistency of the bloodworms samples was leather-hard. The dried bloodworms were packed into a "pellet mold" (a piece of Plexiglas approximately 6.4 mm in thickness with a 6.4-mm-diameter hole drilled into it). Dried bloodworms were packed tightly together to form uniform pellets that held together once removed from the mold. Experimental pellets with tadpole skin were constructed by packing dried bloodworms into the bottom of the "pellet mold," then placing tadpole skin on top of the dried bloodworms within the pellet mold, and adding more dried bloodworms to cap off the pellet. Tadpole skin was obtained by first euthanizing tadpoles by immersion into liquid nitrogen for approximately 30–45 s. Liquid nitrogen was chosen over chemical agents such as MS-222 because we were concerned that the chemical would either change or mask the true "taste" of the skin. Once euthanized tadpoles had thawed, a small incision in the epidermis was made all around the base of the tail. With a pair of forceps the skin covering the body was gently pulled forward until completely removed. Skin added to pellets was weighed to insure that skin pieces placed into pellets were each  $0.060 \pm 0.002$  g. *Lithobates sylvaticus* and *L. clamitans* tadpoles used were between Gosner stages 27–30 (Gosner, 1960), and *L. catesbeianus* tadpoles used were Gosner stages 30–34 (Gosner, 1960). As with the control pellets, the dried bloodworms/skin samples were packed tightly together to form uniform pellets that stayed together after removal from the mold. Each pellet was weighed to insure all pellets were each  $0.121 \pm 0.01$  g.

Six fish were first fed five bloodworm-only pellets (control) three times in the first week. The following week, the same fish were fed five *L. sylvaticus* skin-containing pellets three times. The above procedure was replicated for both *L. clamitans* and *L. catesbeianus*, such that all fish received bloodworm-only control pellets for a week prior to the pellets containing anuran skin (Fig. 1). A 1-mo period elapsed between the last *L. sylvaticus* skin feeding and the next bloodworm-only feeding to allow *L. clamitans* tadpoles to grow to a large enough size for use in the experiments. Fish were maintained on bloodworms during

this interval. The control bloodworm-only week of feeding for *L. catesbeianus* followed immediately after the week testing *L. clamitans*. Finally, to determine whether fish would consume bloodworm-only pellets after all the anuran skin feeding experiments were completed, the six fish were fed bloodworm-only pellets for an additional, final week.

Ideally, *L. sylvaticus* tadpoles should have also been tested within the same period of time as *L. clamitans* and *L. catesbeianus* and the order of feedings for the three species should have been randomized; however, due to differences in development and life-history characteristics, this was not possible. One alternative might have been to freeze *L. sylvaticus* tadpoles, but we rejected this because we had no idea how long-term freezing would alter the skin composition and, inevitably, the palatability of the specimens.

Approximately 3 h prior to feedings (to allow fish sufficient recovery time after disturbance in the tank), all waste/debris and aquaria accessories were removed from each of the fishes' home aquaria. On feeding days (3 per wk), each fish was presented with five pellets (the first week they received control pellets and the second week they received experimental pellets; Fig. 1). During feedings, pellets were dispensed into fish tanks one at a time, with a 5-min delay between each pellet to allow fish to fully consume the pellet. After the last pellet was introduced and the 5-min period had elapsed, all uneaten food was reclaimed using a fine mesh fish net and a pipette. All unconsumed food was dried for 24 h in a drying oven at 50°C, after which it was combusted in a muffle furnace. Six pellets were made for each of the six fish for each feeding day. Five of the pellets for each fish were given to the fish during feeding trials as described above, and the sixth pellet was placed into the drying oven for 24 h to obtain an estimate of pellet final dry weight and then into the muffle furnace to obtain an estimate of ash-free dry weight per pellet. For each fish and feeding, the ash-free dry weight of this sixth pellet was multiplied by five to give the total amount of food presented to each fish. To calculate consumption rates, the ash-free food remains for each fish was subtracted from the total amount of ash-free food offered



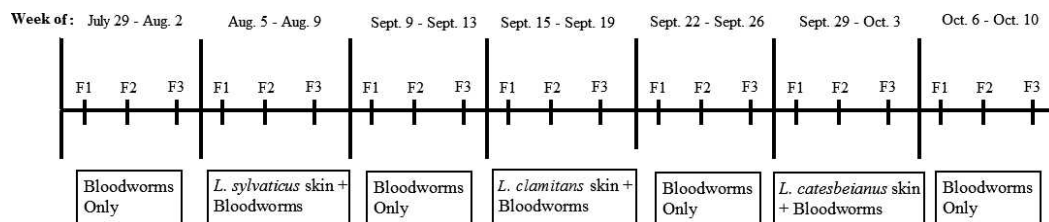


FIG. 1.—Schematic of experimental feeding regime for the larvae of three species of *Lithobates*. Six fish were first fed five bloodworm-only pellets for 3 d (labeled F1–F3) starting in week 1 (29 July–2 August). The same fish were then fed five *L. sylvaticus*–skin-containing pellets three times during the following week (5–9 August). One month passed between the *L. sylvaticus* skin feedings and the next bloodworm-only feedings to allow *L. clamitans* tadpoles to grow to a sufficient size. The procedure was then repeated in the exact same manner for both the *L. clamitans* and *L. catesbeianus* species (e.g., 1 wk of bloodworm-only pellets fed three times, then skin-containing pellets the following week fed three times). Finally, bloodworm-only pellets were fed to fish for a final week, after all the anuran skin feedings were completed.

to each fish to give the amount of organic material consumed. This protocol was approved by the Brock University Research Committee on Animal Care and Use (AUPP 07-08-01, 07-04-01, and 07-09-06).

**Statistical analyses.**—Data were analyzed using SAS 9.1 (SAS Institute Inc., 2003). To determine whether the addition of skin samples from larval anurans changed the amount consumed, a repeated-measures MANOVA was performed on the change in consumption (i.e., consumption of bloodworms only was subtracted from the bloodworms + skin). Change in consumption was calculated separately for each fish at each feeding. The “proc glm” statement in SAS was used with both feeding and anuran species as repeated factors and individual fish as replicates.

To test the willingness of fish to consume bloodworms at the end of the experiment (after all tadpole skin feedings were completed), a paired *t*-test was used to compare the amount of bloodworm-only pellets consumed before the addition of any skin to the pellets to the amount consumed after all the larval anuran skin feedings were completed. Subsequently, a power test was conducted in order to validate the results of the paired *t*-test. The “proc power” statement in SAS was used, using a within-individual correlation of 0.5 between the two treatments and the observed SD of the difference between the treatments.

## RESULTS

Food consumed by fish depended on both the skin of anuran species added to the pellets

and the feeding number (i.e., the first, second, or third feeding with skin; species  $\times$  feeding number interaction, Wilks'  $\lambda = 0.0212$ ,  $F_{2,4} = 23.03$ ,  $P = 0.042$ ; Fig. 2). The greatest decrease in consumption occurred with the addition of *L. catesbeianus* skin, and is especially evident by the third feeding, where fish consumed very little (Fig. 2). The addition of *L. sylvaticus* skin had little impact on consumption; in fact, by the third feeding, consumption rate was equal to that of the prior week when no skin was added to the pellet (Fig. 2). Finally, the addition of *L. clamitans* skin elicited an intermediate response; consumption rate decreased relative to the addition of *L. sylvaticus* skin but was greater than the consumption rate of pellets containing *L. catesbeianus* skin (Fig. 2). Therefore, the relatively large decrease in consumption with feeding number for *L. catesbeianus* is what generates the significant interaction term.

There was no difference between the amount of bloodworm-only pellets consumed before the addition of any anuran skin compared to the amount consumed after the experimental trials ( $\bar{X}$  difference in consumption =  $-0.06$ ; paired *t*-test:  $t_5 = 1.54$ ,  $P = 0.185$ ). The power of this test to detect a difference in consumption rate of 0.10 g (the decrease in consumption observed when fish were fed *L. catesbeianus* skin for the third time) was 45%. However, one fish consumed considerably less after the experiment compared to other fish ( $-0.26$  g compared to an average of  $-0.03$  g for the other five fish). This one value likely inflated our estimate of variance used in the power analysis, so we reran the power analysis using SD calculated

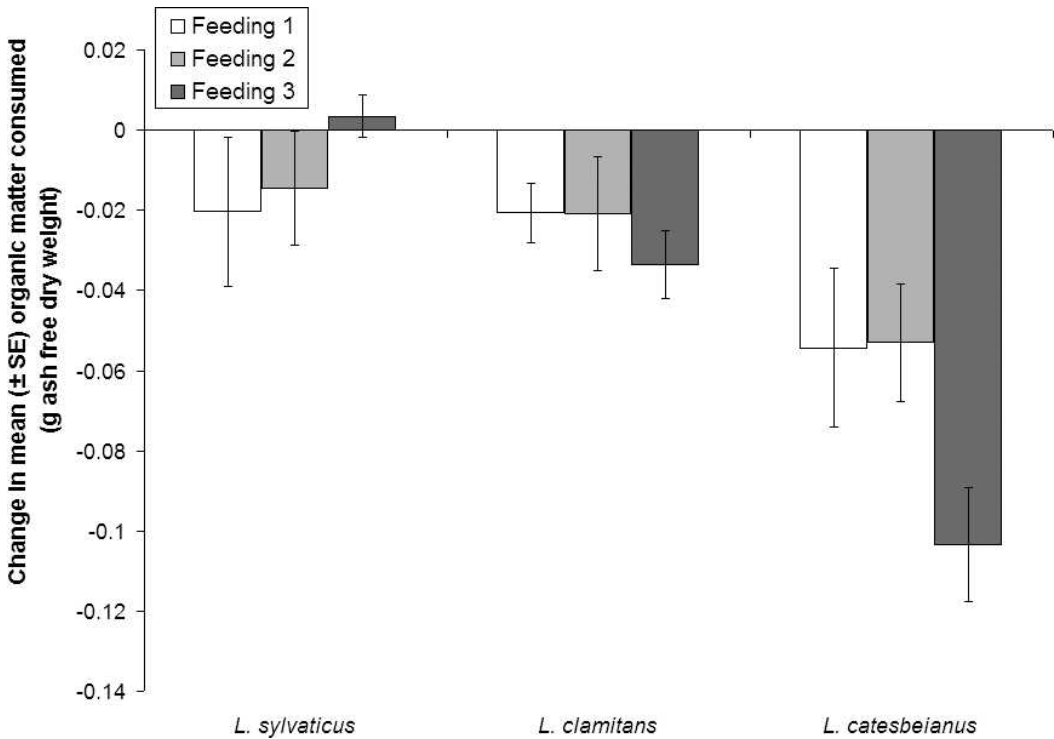


FIG. 2.—Change in mean organic matter consumed (skin-containing pellets – bloodworm-only pellets) by the larvae of three species of *Lithobates*, averaged across the six fish. Values below the horizontal line indicate that, on average, fish consumed less food. Feedings refers to each of the 3 feeding days in which fish were given pellets containing tadpole skin. There is a significant species  $\times$  feeding effect (Wilks'  $\lambda = 0.0212$ ,  $F_{2,4} = 23.03$ ,  $P = 0.04$ ).

with this one fish removed. That power analysis gave a power of 92% for a difference of 0.10 g.

#### DISCUSSION

To the best of our knowledge, this is the first study to show that *L. catesbeianus* tadpoles are unpalatable to sunfish based on the taste of skin alone. Although previous studies have suggested unpalatability of these tadpoles (Kats et al., 1988; Werner and McPeck, 1994), none of them clearly isolated the taste of skin from confounding factors such as predator hunger level and prey behavior. By measuring the amount of organic matter available for digestion in uniform pellets of food, we demonstrate that the taste of *L. catesbeianus* skin reduces predation rate, thereby potentially acting as an effective antipredator mechanism (Gunzburger and Travis, 2005).

We have also been able to show that a range of palatability exists, with *L. clamitans* tadpoles being relatively unpalatable; consumption of pellets containing *L. clamitans* skin decreased significantly by the third feeding when compared to the consumption of pellets containing *L. sylvaticus* (the most palatable species; Fig. 2). This result demonstrates a clear range in palatability among the three anuran species. This is in keeping with previous work that suggested palatability for any predator-prey combination depends on predator hunger levels and the presence of alternative prey. Kruse and Stone (1984) found that the willingness of Largemouth Bass (*Micropterus salmoides*) to consume *Bufo* tadpoles was positively correlated with fish hunger levels. Blouin (1990) showed that Bluegill Sunfish (*Lepomis macrochirus*) consumed more *H. gratiosa* tadpoles than *H. cinerea* tadpoles for a given time period, but he also noted that fish would eventually

consume all *H. cinerea* tadpoles. He, therefore, argued that *H. cinerea* was a less preferred prey (Blouin, 1990). We propose that this is similar to our finding that *L. clamitans* skin is somewhat distasteful, as opposed to *L. catesbeianus*, which sunfish refuse to eat even after 10 d of no other food available (D. Szuroczi and J.M.L. Richardson, personal observation). Our methodology provides an objective method of quantifying palatability that can be used to robustly compare palatability of species tested at different times or in different studies.

The fact that *L. clamitans* is somewhat unpalatable raises the question as to why it is maintained in a species that either occurs at lower densities with fish or is found in ponds lacking fish altogether (Collins and Wilbur, 1979; Morin, 1983; Richardson, 2001; Werner and McPeck, 1994). It is unclear whether unpalatability or distastefulness is passively maintained because no negative selection acts on the trait (meaning that there is no real cost associated with producing a chemical repellent or toxin) or is maintained (even if there is a cost associated with unpalatability) because it allows *L. clamitans* larvae to exist in permanent ponds with fish if required. Regardless, our result should be interpreted with caution, because we collected *L. clamitans* from a site with no predatory fish, and it is likely that our tadpoles represented a single clutch of eggs.

While the *L. catesbeianus* skin in our experiment came from slightly more developed, field-caught tadpoles (our attempts to rear bullfrogs from eggs in the lab were unsuccessful), this is unlikely to have affected our results. First, all tadpoles used were in early stages (less than Gosner stage 34) with differences detectable only through microscopic examination of toe development in the hind leg (Gosner, 1960). Second, previous studies using fish predators have used Gosner stages 25–40 and found all stages to be equally unpalatable to fish (Kruse and Francis, 1977; Kruse and Stone, 1984).

It seems unlikely that unpalatability of these tadpoles is an inducible defense based on our study. We maintained all tadpoles in the lab for 1 mo prior to use in experiments, so that in the unlikely event that palatability is

affected by environment, environment for all species was standardized prior to the experiment. While inducible chemical defenses are common in plants against herbivores (as reviewed by Chen, 2008; Karban and Myers, 1989), with the exception of marine sponges (Thoms and Schupp, 2008), we know of no animals that produce toxicity or unpalatability as an inducible defense against predators (but note that in many herbivorous insects palatability is affected by diet; e.g., Sword et al., 2000). In addition, previous work has presumed palatability of larval anurans to be constitutive (e.g., Jara and Perotti, 2009). Although tadpoles of various species are well-known to show inducible morphological changes that depend on the predator present during rearing, these inducible morphological defenses are unrelated to palatability, which depends only on the predator–prey combination (Relyea, 2001).

As in many herbivorous insects, in adult dendrobatid frogs the alkaloids responsible for the toxic secretions used as predator deterrent are obtained through the animal's diet (Daly et al., 1994, 2002). Although it is possible that *L. catesbeianus* tadpoles accumulate natural dietary sources of toxins, our methodology of maintaining tadpoles in the laboratory for a 1-mo period and feeding them a laboratory diet decreases the likelihood of tadpoles expressing toxins that were accumulated from natural dietary sources. Further, for tadpoles to accumulate toxins through their diet would require tadpoles to be selective foragers, which seems unlikely because tadpoles have been reported to be largely indiscriminate foragers (Dickman, 1968).

Our results showing experiential learning in sunfish is similar to other studies using fish predators. Kruse and Stone (1984) found that bass consumed all tadpoles and did not begin discriminating against *Bufo* tadpoles until the fifth trial. Experiential learning of unpalatability may further explain some of the contradictions in the literature regarding the palatability of different anuran species. For example, one of us (J.M.L. Richardson, personal observation) previously observed that all predators tested (*Lepomis gibbosus*, *Lepomis punctatus*, *Notophthalmus viridescens*, and *Anax junius*) would consume an *L. catesbeia-*



*nus* tadpole, but because this was based on using an independent predator individual for each feeding, it precluded learned aversion, which this study suggests is required. Likewise, if sunfish captured for experimental studies have already encountered *L. catesbeianus* tadpoles in the wild, then upon experimental presentation with a *L. catesbeianus* tadpole, fish would most likely refuse to consume the tadpole. We observed this in a prior study (D. Szuroczki and J.M.L. Richardson, personal observation) using sunfish collected from a pond that housed breeding *L. catesbeianus*. Three of the five fish collected, which we later hypothesized to have had prior experience, refused to consume any *L. catesbeianus* tadpoles even after 9 d of food deprivation, whereas the remaining two sunfish, which we later hypothesized to be naïve, initially consumed *L. catesbeianus* tadpoles on two separate presentations and then refused any subsequent tadpoles. Therefore, knowledge of fishes' prior experience is essential for accurate and interpretable results of larval anuran palatability to fish.

Although the fish in the current study were obtained from a pond that contained *L. catesbeianus* tadpoles, the fish were caught in the spring and only the smallest individuals were kept and used in the current study (approximately 5–6 cm in length), thus minimizing any prior experience with *L. catesbeianus* tadpoles. Adults of *L. catesbeianus* typically breed in late June to early July in our study locale (Harding, 2006), and the only tadpoles available for the fish in the spring would have been large tadpoles from the previous season that had overwintered. Therefore, the available tadpoles would have been too large for the small fish used in the current study to consume or even handle; the fish used in this study were likely naïve to *L. catesbeianus* tadpoles.

Our results suggest that fish can discriminate between pellets that look identical but differ in taste depending on the addition of tadpole skin. This discrimination occurred even though fish are generally regarded as visually oriented predators (Guthrie and Muntz, 1993). In the current study, fish did not taste each of the five pellets offered separately upon each of the

three feedings for all of the conditions. Therefore, the fish must have learned to associate a chemical cue from the skin detectable prior to ingestion with some kind of bad taste or postingestion consequence, and subsequently ignored pellets with the same chemical signature. It is likely that fish were using chemical cues to detect skin in the pellets, because fish have also been shown to possess sensitive chemosensory organs (Toshiaki, 1993). In other studies using whole tadpoles, fish were observed to generalize behavior to typically palatable species (Blouin, 1990; Kruse and Stone, 1984); it is possible that by using isolated skin chemicals, we made them more accessible to detection by the fish. Either way, this result suggests that fish possess a fine-tuned ability to detect food items that are offensive and then modify foraging to reflect a learned aversion.

In conclusion, this study is the first to show that a range of unpalatability exists in *Lithobates* tadpoles and that the compound leading to unpalatability is in the skin. As such, unpalatability maybe be an important defense mechanism against any predator that tends to consume its prey items whole (e.g., fish, birds, and mammals) as opposed to aquatic invertebrates that pierce the skin and suck the bodily fluids. Further work should be aimed at identifying the compound(s) in the skin that make *L. catesbeianus* and *L. clamitans* larvae unpalatable, and to ascertaining whether this compound is toxic (e.g., such as alkaloids) or simply distasteful, because this area of research has been largely ignored. The potential for alarm cues secreted by tadpoles in response to predation threat as a possible cause of unpalatability has also received little attention and should be investigated in future studies. Finally, it would be interesting to determine how palatability differences interact with characteristics such as coloration and behavior in determining predation rates, and to determine whether or not palatability varies across the geographic range of both *L. catesbeianus* and *L. clamitans*.

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## LITERATURE CITED

- BLOUIN, M. S. 1990. Evolution of palatability differences between closely-related treefrogs. *Journal of Herpetology* 24:309–311.
- BOWERS, M. D. 1980. Unpalatability as a defense strategy of *Euphydryas phaeton* (Lepidoptera, Nymphalidae). *Evolution* 34:586–600.
- BOWERS, M. D. 1992. The evolution of unpalatability and the cost of chemical defense in insects. Pp. 216–245. *In* B. D. Roitberg and M. B. Isman (Eds.), *Insect Chemical Ecology: An Evolutionary Approach*. Chapman and Hall, New York, USA.
- BRODIE, E. D., AND D. R. FORMANOWICZ. 1987. Antipredator mechanisms of larval anurans: Protection of palatable individuals. *Herpetologica* 43:369–373.
- CHEN, M. S. 2008. Inducible direct plant defense against insect herbivores: A review. *Insect Science* 15:101–114.
- CHIVERS, D. P., AND R. S. MIRZA. 2001. Importance of predator diet cues in responses of larval wood frogs to fish and invertebrate predators. *Journal of Chemical Ecology* 27, 1, 45–51.
- COLLINS, J. P., AND H. M. WILBUR. 1979. Breeding habits and habitats of the amphibians of the Edwin S. George Reserve, Michigan, with notes on the local distributions of fishes. *Occasional Papers of the Museum of Zoology, University of Michigan* 686:1–34.
- CROSSLAND, M. R. 2001. Ability of predatory native Australian fishes to learn to avoid toxic larvae of the introduced toad *Bufo marinus*. *Journal of Fish Biology* 59:319–329.
- DALY, J. W., H. M. GARRAFFO, T. F. SPANDE, C. JARAMILLO, AND A. S. RAND. 1994. Dietary source for skin alkaloids of poison frogs (Dendrobatidae). *Journal of Chemical Ecology* 20:943–955.
- DALY, J. W., T. KANEKO, J. WILHAM, H. M. GARRAFFO, T. F. SPANDE, A. ESPINOSA, AND M. A. DONNELLY. 2002. Bioactive alkaloids of frog skin: Combinatorial bioprospecting reveals that pumiliotoxins have an arthropod source. *Proceedings of the National Academy of Science USA* 99:13996–14001.
- DALY, J. W., T. F. SPANDE, AND H. M. GARRAFFO. 2005. Alkaloids from amphibian skin: A tabulation of over eight-hundred compounds. *Journal of Natural Products* 68:1556–1575.
- DICKMAN, M. 1968. The effect of grazing by tadpoles on the structure of a periphyton community. *Ecology* 49:1188–1190.
- DUELLMAN, W. E., AND L. TRUEB. 1994. *Biology of amphibians*. The John Hopkins University Press, Baltimore, Maryland, USA.
- EKLOV, P., AND E. E. WERNER. 2000. Multiple predator effects on size-dependent behavior and mortality of two species of anuran larvae. *Oikos* 88:250–258.
- GOSNER, K. L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16:183–190.
- GUNZBURGER, M. S., AND J. TRAVIS. 2005. Critical literature review of the evidence for unpalatability of amphibian eggs and larvae. *Journal of Herpetology* 39:547–571.
- GUTHRIE, D. M., AND W. R. A. MUNTZ. 1993. The role of vision in fish. Pp. 89–121. *In* T. P. Pitcher (Ed.), *Behavior of Teleost Fishes*. Chapman and Hall, London, UK.
- HARDING, J. H. 2006. *Amphibians and Reptiles of the Great Lakes Region*. The University of Michigan Press, Ann Arbor, Michigan, USA.
- JARA, F. G., AND M. G. PEROTTI. 2009. Toad tadpole responses to predator risk: Ontogenetic change between constitutive and inducible defenses. *Journal of Herpetology* 43:82–88.
- KARBAN, R., AND J. H. MYERS. 1989. Induced plant responses to herbivory. *Annual Review of Ecology and Systematics* 20:331–348.
- KATS, L. B., J. W. PETRANKA, AND A. SIH. 1988. Antipredator defenses and the persistence of amphibian larvae with fish. *Ecology* 69:1865–1870.
- KRUSE, K. C., AND M. G. FRANCIS. 1977. Predation deterrent in larvae of bullfrog, *Rana catesbeiana*. *Transactions of the American Fisheries Society* 106:248–252.
- KRUSE, K. C., AND B. M. STONE. 1984. Largemouth bass (*Micropterus salmoides*) learn to avoid feeding on toad (*Bufo*). *Animal Behaviour* 32:1035–1039.
- LICHT, L. E. 1968. Unpalatability and toxicity of toad eggs. *Herpetologica* 24:93–98.
- LIEM, K. F. 1961. On the taxonomic status of the granular patches of the Javanese frog *Rana chalconota* Schlegel. *Herpetologica* 17:69–71.
- MORIN, P. J. 1983. Predation, competition, and the composition of larval anuran guilds. *Ecological Monographs* 53:119–138.
- PALO, R. T., AND C. T. ROBBINS. 1991. *Plant defenses against mammalian herbivory*. CRC Press, Inc., Boca Raton, Florida, USA.
- RELYEA, R. A. 2001. The relationship between predation risk and antipredator responses in larval anurans. *Ecology* 82:541–554.
- RICHARDSON, J. M. L. 2001. The relative roles of adaptation and phylogeny in determination of larval traits in diversifying anuran lineages. *American Naturalist* 157:282–299.
- SAS INSTITUTE INC. 2003. SAS 9.1. SAS Institute Inc., Cary, North Carolina, USA.
- SWORD, G. A., S. J. SIMPSON, O. T. M. EL HADI, AND H. WILPS. 2000. Density-dependent aposematism in the desert locust. *Proceedings of the Royal Society B: Biological Sciences* 267:63–68.
- THIEMANN, G. W., AND R. J. WASSERSUG. 2000. Patterns and consequences of behavioural responses to predators and parasites in *Rana* tadpoles. *Biological Journal of the Linnean Society* 71:513–528.
- THOMS, C., AND P. J. SCHUPP. 2008. Activated chemical defense in marine sponges: A case study on *Aplysinella rhax*. *Journal of Chemical Ecology* 34:1242–1252.
- TOSHIKAKI, H. J. 1993. Role of olfaction in fish behaviour. Pp. 171–195. *In* T. P. Pitcher (Ed.), *Behaviour in Teleost Fishes*. Chapman and Hall, London, UK.
- WALTERS, B. 1975. Studies of interspecific predation within an amphibian community. *Journal of Herpetology* 9:267–279.
- WERNER, E. E., AND M. A. MCPEEK. 1994. Direct and indirect effects of predators on two anuran species along an environmental gradient. *Ecology* 75:1368–1382.

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