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BEHAVIORAL ECOLOGY – ORIGINAL RESEARCH

Meta-analysis of tadpole taste tests: consumption of anuran prey across development and predator strategies

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Abstract

The risk of predation and the costs and benefits of diverse anti-predator strategies can shift across the life stages of an organism. Yet, empirical examples of ontogenetic switches in defense mechanisms are scarce. Anurans represent an alleged exception; previous meta-analytic work suggests that unpalatability of developing anurans is "rare", whereas adult anurans in many lineages are well defended by toxic and/or unpalatable skin secretions. Here, we revisit the question of the unpalatability of anuran young in a meta-analysis of the relative proportion of prey consumed within 922 predation tests, including 135 anuran species. We tested the hypotheses that a predator's propensity to consume anuran young depends on (1) prey family, (2) predator manipulation strategy, and (3) prey ontogenetic stage. We used a binomial mixed model approach with considerations for multiple effect sizes within studies to evaluate the log odds ratio of the proportion of prey consumed by individual predators. Prey consumption was highly variable, but toads (Bufonidae) were consumed in lower proportions. Chewing invertebrates consumed more anuran prey than biting vertebrates. Late stage tadpoles were more vulnerable to predation than other stages of anuran ontogeny. However, more studies are needed to unravel the roles of development and evolutionary history in the chemical ecology of anuran young. This synthesis provides clear meta-analytic evidence that relative unpalatability is an important component in the anti-predator defenses of young in some anuran families, calling into question the degree to which chemically defended anuran families undergo ontogenetic switches in anti-predator strategies.

Keywords Bufonidae · Chemical defense · Granular glands · Toxins · Palatability

Introduction

Virtually all organisms are vulnerable to predation, and must use anti-predator strategies to survive. Such strategies can include behavioral mechanisms such as escape, thanatosis, or stotting as well as physical mechanisms such as cryptic or decoy coloration, exceptionally small or large size, sharp structures, or autotomy of extremities (Ruxton et al. 2019). Furthermore, some groups of organisms have undergone molecular and morphological evolution that facilitates the sequestration or synthesis of chemical defenses as a particularly effective secondary defense (Arbuckle and Speed 2015; Ruxton et al. 2019). The mosaic of defense mechanisms that make up the anti-predator strategy used by a species is thought to be determined by the relative costs and benefits of displaying defensive behaviors, developing defensive structures, and acquiring or producing chemical defenses, as well as the effectiveness of each mechanism against each of the predators in a given habitat (Higginson and Ruxton 2010; Caro et al. 2016). Across the life stages of an organism, the types of predators, the costs and benefits of different anti-predator strategies, and the developmental constraints on the use of different mechanisms can vary widely (Booth 1990; Hoso 2012). Thus, dramatic ontogenetic switches in the anti-predator strategy of an individual or species can be expected (Moran 1994; Caro et al. 2016; Medina et al. 2020). However, empirical examples of ontogenetic switches in anti-predator strategies are uncommon, and have typically been identified on a species-by-species basis, which hinders the generation of a broad understanding of how defense mechanisms shift across life stages (Hawlena

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et al. 2006; Grant 2007; Hoso 2012; Valkonen et al. 2014; Putman et al. 2015; but see Medina et al. 2020).

Many anurans undergo complex metamorphic change from aquatic larvae to terrestrial tetrapods. The order Anura may represent an exception to our poor understanding of ontogenetic switches in anti-predator strategies (Medina et al. 2020), because their pre- and post-metamorphic defense mechanisms—behavioral, physical, and in particular chemical—have been studied in great depth across numerous anuran families (Gunzburger and Travis 2005; Llewelyn et al. 2012; Arbuckle and Speed 2015; Hossie et al. 2017; Ferreira et al. 2019). For example, many anuran species are known to undergo ontogenetic shifts in color or pattern (reviewed in Hoffman and Blouin 2000), and tadpoles but not adults rely on inducible defenses of enlarged tail morphology and swimming behavior, especially among putatively more palatable clades (reviewed in Hossie et al. 2017).

Virtually all adult anurans produce or store some defensive compounds in their skin glands, but certain families (*e.g.*, Bufonidae, Dendrobatidae, Myobatrachidae) harbor particularly potent substances such as cardiotonic steroids, lipophilic and indole alkaloids, biogenic amines, and neuroactive peptides (König et al. 2015). Such groups are known to emphasize chemical defenses in their anti-predator repertoire, whereas other families are thought to rely more heavily on behavioral and/or physical mechanisms (Arbuckle and Speed 2015). Generally, predators learn to avoid eating chemically defended organisms because they are unpalatable (distasteful) and/or toxic (cause physiological harm) (Skelhorn and Rowe 2006; Hanlon and Parris 2013; Skelhorn et al. 2016; Rojas et al. 2017). Experimental tests of predator consumption involve trials in which each predator is presented with a single or small group of prey, together or separately, to determine the proportion of prey consumed relative to another clade or developmental stage (Lawler and Hero 1997). Such tests have been used extensively to assay the effectiveness of chemical defenses of anurans, especially in early developmental stages (see Gunzburger and Travis 2005). But, taken from a comparative perspective, these studies have not yet coalesced into a comprehensive understanding of the relative importance of chemical defenses in the anti-predator strategies of developing anurans, in large part because existing empirical and meta-analytic evidence is contradictory. Thus, it is unclear whether ontogenetic shifts in the use of chemical defenses are indeed common within some anuran lineages.

Some patterns have emerged from hundreds of studies with predator trials of young anurans despite variation in relative consumption due to differences in experimental design (Cabrera-Guzmán et al. 2012, but see Wilson and Williams 2014). For example, there is consensus that invertebrate predators of young anurans are less deterred by chemical defenses than vertebrate predators, perhaps due to mechanical differences in prey manipulation that affect the degree of sensory contact with unpalatable or toxic compounds (Wassersug 1973; Heyer et al. 1975; Brodie et al. 1978; Kruse and Stone 1984; Brodie and Formanowicz 1987; Kats et al. 1988; Peterson and Blaustein 1992; Wilbur 1997; Crossland 1998; Gunzburger and Travis 2005). Also, numerous studies have found that young of the true toads (Bufonidae) and some other families which are strongly chemically defended as adults are consumed in lower proportions as young than other families of anurans (Licht 1968; Kruse and Francis 1977; Brodie and Formanowicz 1987; Lawler and Hero 1997; Hayes et al. 2007; Karraker 2011). However, an extensive and highly cited review and meta-analysis of 142 studies on the unpalatability of amphibian eggs and tadpoles concluded that unpalatability is rare, and that young bufonids were no less palatable than other anuran clades (Gunzburger and Travis 2005). Another review focused on crayfish predators of amphibian young also found limited evidence for unpalatability (Wilson and Williams 2014). Both reviews suggest that other anti-predator mechanisms such as tail coloration, size, behavior, and jelly coats likely play a more important role than unpalatable or toxic substances in protecting amphibian young (Gunzburger and Travis 2005; Wilson and Williams 2014). This meta-analytic conclusion implies that there is, in fact, an ontogenetic shift in anti-predator strategy across numerous anuran families that rely heavily on chemical defenses as adults and on non-chemical mechanisms prior to metamorphosis.

The contradiction between comprehensive reviews and empirical consensus about the importance of chemical defenses as a major defense mechanism in some families of developing anurans may be rooted in a very conservative definition in previous reviews of “absolute” rather than “relative” unpalatability, in which only those prey that were always and completely rejected by a predator were considered unpalatable. Gunzburger and Travis (2005) themselves noted that such conservative criteria led to classification of some prey as palatable even when the authors of the original empirical studies reported very low levels of consumption and concluded prey were unpalatable. These criteria were applied intentionally in the interest of decoupling unpalatability from other factors that could influence the proxy measurement of the proportion of prey consumed such as prey behavior or color and predator hunger level or experience. Some authors have argued that absolute criteria are not a fair assessment of unpalatability (Portheault et al. 2007; Wilson and Williams 2014), because even a partial reduction of predator consumption could be evolutionarily relevant. Moreover, since the cutoff of the year 2000 of the papers included in the 2005 review, numerous additional palatability studies have become available, particularly in more tropical taxa outside of the genera *Rana* and *Bufo* (sensu lato) that dominated the 2005 review.

In this study, we revisit the topic of predator consumption of young anurans using a meta-analysis with a less restrictive approach based on effect sizes of the relative proportion of prey consumed rather than an absolute binary response of all-or-none rejection. We acknowledge that this approach incorporates an array of differences in experimental design that could add significant variation to the response variable, such as duration of predator starvation prior to a trial, whether prey were reared in the laboratory or collected from pristine or polluted habitats (Sievers et al. 2019), the number of prey items presented simultaneously, and whether prey were presented simultaneously (choice) or sequentially (no choice). However, we argue that patterns that emerge in spite of the substantial variation among more than 900 trials likely point to biologically relevant information that is worthy of further study. Also, the use of mixed effect models weighted by the relative magnitude of experiments should enhance upon the methodology employed in previous meta-analyses in which yes/no palatability responses for each study were compared with Pearson χ^2 contingency analyses or rank-sum tests without consideration for relative sample sizes (Gunzburger and Travis 2005; Wilson and Williams 2014).

We tested three long-standing hypotheses (Crossland 1998) about the variation in predator consumption of young anurans based on a meta-analysis of the effect size of proportion of prey consumed by a predator in 100 studies. First, due to the biochemical nature of their skin secretions (Peterson and Blaustein 1992; Hayes et al. 2007; Adams et al. 2011), we hypothesized that developing toads in the family Bufonidae are less palatable than developing anurans in other families. Second, due to differences in the degree of sensory transmission during prey manipulation (Peterson and Blaustein 1992; Gunzburger and Travis 2005) or receptor physiology (e.g., Okamura et al. 2003), we hypothesized that skin biting predators such as vertebrates will be more likely to avoid unpalatable prey than chewing invertebrates. Third, because eggs are often bestowed with maternal defenses and granular glands generally emerge during or after metamorphosis (Brodie et al. 1978; Lawler and Hero 1997; Hayes et al. 2009; Jara and Perotti 2009; Llewelyn et al. 2012; Regueira et al. 2016; Stynoski and O’Connell 2017), we hypothesized that larval anurans will be consumed more than eggs or metamorphic anurans.

Materials and methods

Literature search and study exclusion

We conducted systematic literature reviews in three databases (EBSCO, Web of Science, ScienceDirect) using the terms (frog OR anura* OR toad OR tadpole OR metamorph*) AND (unpalatab* OR toxic* OR distasteful OR palatab* OR predat*) from 1900 to 2018. We complemented the initial systematic searches with (1) revision of references within selected papers, (2) revision of papers included in the review by Gunzburger and Travis (2005), and (3) Google Scholar searches with combinations of the listed terms.

We excluded studies that did not fit the following retention criteria: (1) empirical and experimental data rather than a review or meta-analysis, (2) used live predator consumption as a response variable rather than prey responses to predator cues or human taste, (3) explicitly stated prey developmental stage as opposed to only reporting developmental time, weight, or length, (4) used individual predator tests that lasted less than 72 h, as opposed to mesocosm studies, long-term trials, or trials with multiple predators at once, (5) included at least two comparative treatment groups within the same experiment that directly address the hypotheses of this meta-analysis (e.g., egg vs. metamorph, bufonid vs. non-bufonid, chewing predator vs. biting predator), (6) clearly indicated a sample size larger than four predators per treatment group (i.e. not “a few predators”), and (7) used an enclosure that did not allow prey to escape from predators.

Data indexing

To standardize sampling effort across studies, we considered each individual predator as an experimental unit, which allowed for the inclusion of both studies that reported the proportion of a group of prey that was predated by a given predator (0–1 for each unit) and those that reported the proportion of trials in which separate predators did or did not consume a single prey item (0 or 1 for each unit). Each set of replicates of the same species of predator with a particular stage and species of prey was treated as an “observation” within a given experiment. When a single study reported multiple independent experiments, treatment groups in separate experiments were treated as separate observations and were only compared to other observations within the same experiment. In a few cases, the response values of multiple groups in a given study were combined using averages weighted by sample size, for example, if results were reported for Stage 29 and Stage 32 tadpoles that both fit into the category “early tadpole.” In some cases, when the data were not directly reported in the text, we carefully extrapolated from figures the proportions of prey consumed. To improve indexing reliability and consistency, exclusion of papers from search results and indexing of data from included studies were conducted separately by JLS and KPB and then cross-checked and corroborated.

For each observation in included studies, we recorded the predator species, predator’s prey manipulation type, prey species, prey family, developmental stage of prey, number of predators offered prey, and proportion consumed. We indicated the prey manipulation type of each predator species as biting vertebrate, chewing invertebrate, or piercing invertebrate, following Cabrera-Guzmán et al. (2012) and Szuroczki and Richardson (2011) with the assistance of source articles and peer-reviewed literature about particular species. We grouped developmental stages of prey based on the descriptions in the Methods section of each study into five categories following Gosner (1960): egg (< stage 22), hatchling (stages 22–25), early tadpole (stages 26–36), late tadpole (stages 37–41), and metamorph (stages 42–46). We reconstructed phylogenetic relationships of the predators used in selected studies with the taxize, ggtree, and phylopic (Chamberlain and Szocs 2013; Yu 2020; Chamberlain 2020) packages in R using NCBI Taxonomy ID information. To show the proportion of prey consumed by predators on a phylogenetic basis, we pruned the tree from Jetz and Pyron (2018) to those anuran species from the studies that met inclusion criteria.

Data analysis

To analyze proportional effect sizes of consumption responses with values from zero to one, we compared log odds ratio (rather than Cohen's *d* or *r*) among pairs of observations in studies, weighted by the sample size of each observation. To take into account the psuedoreplicative effect of correlated error structure among multiple effect sizes in a single paper, as optimized for hierarchical odds ratio meta-analyses, we employed Simmonds and Higgins' binomial models with both random study specific and observation effects using the glmer function from the lme4 package in R (Version 3.6.3) (see Model type 3 in Jackson et al. 2018; script of all analyses and figures available in Online Resources 1 and 2). We also attempted Simmonds and Higgins models with only random study effects and Van Houwelingen bivariate models (Model types 2 and 6 in Jackson et al. 2018, respectively), but chose not to use them in this meta-analysis due to much higher Akaike Information Criterion (AIC) values (ΔAIC > 25) and in some cases non-convergence of models. Many studies included pairs of observations for some but not all combinations of predictor variables (*i.e.,* compared eggs and late tadpoles of toads and non-toads by fish, but did not examine hatchlings or invertebrate predators). Therefore, we ran five separate models, each of which included only the data available from studies with pairs of observations directly relevant to a particular hypothesis: (1) predator strategy bite versus chew, (2) life stage egg versus early tadpole, (3) hatchling versus late tadpole, (4) late tadpole versus metamorph, and (4) family toad versus not toad. For each of the five subsets of data, we used a full model with Predator strategy, Prey stage, and Toad/not toad as fixed factors and Study and Observation as random factors. We attempted model selection, but removal of fixed factors did not result in any reductions of AIC by a minimum ΔAIC of 2 (Online Resource 1), so we report results of the full models. For all models, effect sizes with confidence intervals that did not overlap zero for the predictor variable of the hypothesis associated with a given subset of data were considered significant.

We acknowledge that accounting for evolutionary relationships in effect size analyses would reduce the risk of non-independence, and thus we attempted but decided against testing for phylogenetic independence in this meta-analysis for multiple reasons. First, common tools for testing phylogenetic signals in meta-analyses (phylometa (Lajeunesse 2011), ape::binaryPGLMM (Paradis and Schliep 2019), phylolm::phyloglm (Ho and Ane 2014), metafor::rma.mv (Viechtbauer 2010), phyr:pglmm (Ives et al. 2019)) cannot be used for the current study due to the combination of odds ratio effect sizes and hierarchical random effects of multiple experiments within studies. Also, such phylogenetic covariance matrices would require collapsing data for each species from multiple life stages (*i.e.,* some species represented only by one trial with eggs and others by multiple studies with hatchlings, late tadpoles and metamorphs), which would misrepresent the true influence of phylogenetic history on effect sizes (see explanation of odds ratio data loss in Chang and Hoaglin 2017; Park and Beretvas 2019). Lastly, a specific objective of this study was to compare the consumption of bufonid toads to other taxa as a fixed factor, which would induce multicollinearity with the inclusion of phylogenetic correlation matrices in mixed models. Therefore, we include visualizations of the effect of evolutionary history on prey consumption in lieu of statistical model corrections (see Figs. 3, 4, 5).

Results

Literature search

We identified 64 articles in EBSCO, 73 in ScienceDirect, and 22 in Web of Science, which were corroborated with 142 articles cited in Gunzburger and Travis (2005) and other articles identified in the reference section of selected articles and on Google Scholar, for a total of 205 unique articles. After applying exclusion criteria as outlined above, 100 articles remained, of which 60 have been published since the year 2000 and 32 were also considered in the review by Gunzburger and Travis (2005). They included 922 observations of treatment groups with unique predator species, prey species, and prey stage combinations in each study, with a mean of 9.22 ± standard deviation 16.74 (range 2–145) observations per article. The mean number of individual predators (n) per observation was 14.1 ± 25.97 (range 2–420) for a grand total of 12,990 predators across all trials in all studies. Subsets of data used in separate mixed effect models that provided paired experimental designs directly relevant to one of five main hypotheses included the following number of pairs of observations and average sample size per observation: bite versus chew predator strategy (98 pairs, average $n = 11.7$), egg versus early tadpole life stage (53, 12.4), hatchling versus late tadpole life stage (32, 19.1), late tadpole versus metamorph life stage (10, 45.3), and toad versus not toad family (166, 14.7).

Descriptive statistics

The articles selected for this meta-analysis comprised 182 species from 129 genera and 72 families of predators in 922 observations (most common genera in Fig. 1a). Predators in 882 (95.7%) observations were aquatic, whereas 40 (4.3%) were terrestrial. Of the observations, 465 (50.4%) included predators that manipulate prey by biting (e.g., fish), 350 (38.0%) included predators that chew prey (e.g., odonate larvae), 77 (8.3%) included predators that manipulate prey by piercing (e.g., giant water bugs), and 28 (3.0%) and 2 (0.2%) included predators that manipulate prey by scraping (snails) or sucking (leeches), respectively (Fig. 2).

Anuran prey in selected studies consisted of 135 species from 57 genera in 15 families (common genera in Fig. 1b). Of the 922 observations mentioned above, there were 243 (26.4%) of predation of anuran eggs, 209 (22.7%) of hatchlings, 321 (34.8%) of early tadpoles, 115 (12.5%) of late tadpoles, and 34 (3.7%) of young metamorphic anurans. Consumption by predators varied by prey species (Fig. 3) as well as life stage and family (Fig. 4).

Hierarchical meta-analysis

In the first of five meta-analytic models, we included 196 observations within 18 studies that compared biting and chewing predators of the same stage and species of prey. We found that chewing predators were 2.8 times more likely to consume prey than biting predators, with a significant difference in the proportion of prey consumed between manipulation strategies of predators (estimate = 1.02, $z = 2.34$, $p = 0.019$; Fig. 5; see full ANOVA tables of meta-analyses in Online Resource 1).

Then, we used three more models to understand the effects of life stage on anuran prey consumption. In the second model, we included 106 observations from nine studies that compared egg and early tadpole life stages in the same prey and predator species. We found no significant difference between eggs and early tadpoles at Stages 26–36

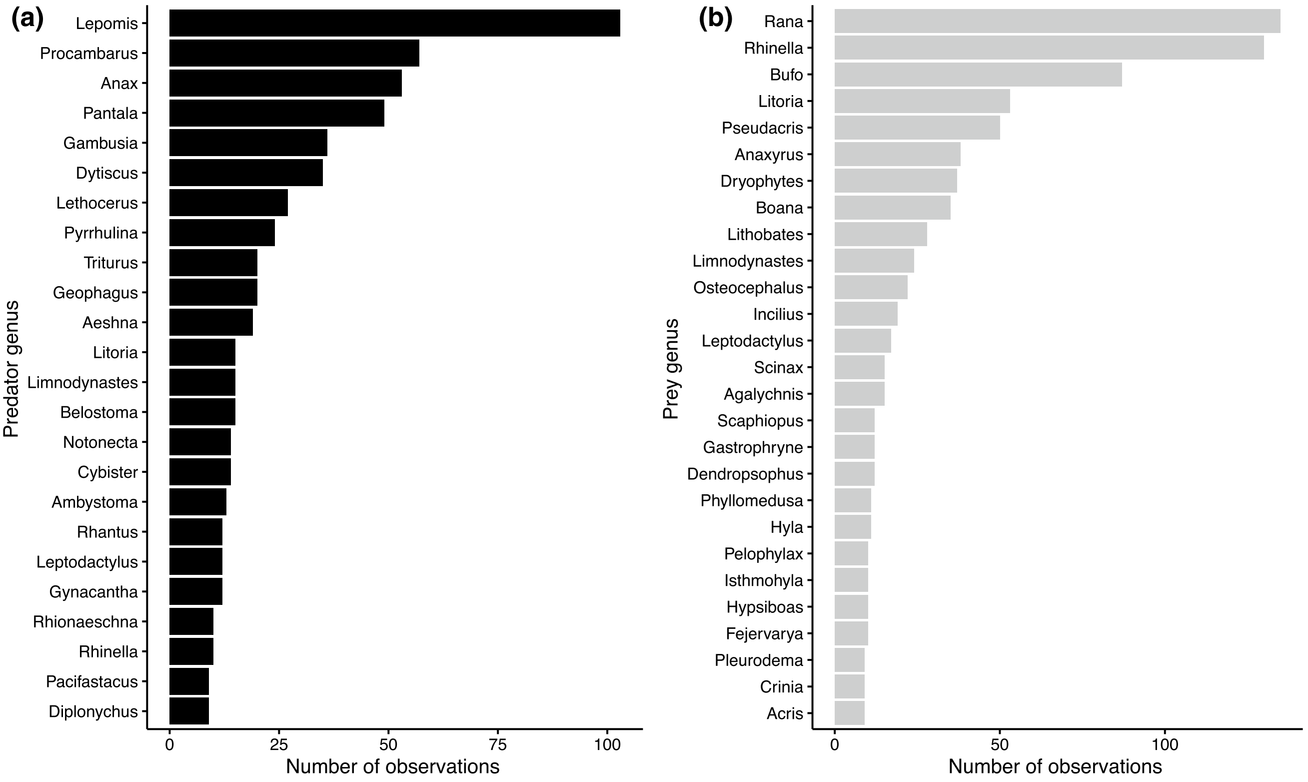


Fig. 1 Histograms of the most commonly represented genera of **a** predators ($N = 603$ observations in 24 genera) and **b** prey ($N = 830$ observations in 27 anuran genera) among 100 studies selected for a meta-analysis of anuran prey consumption

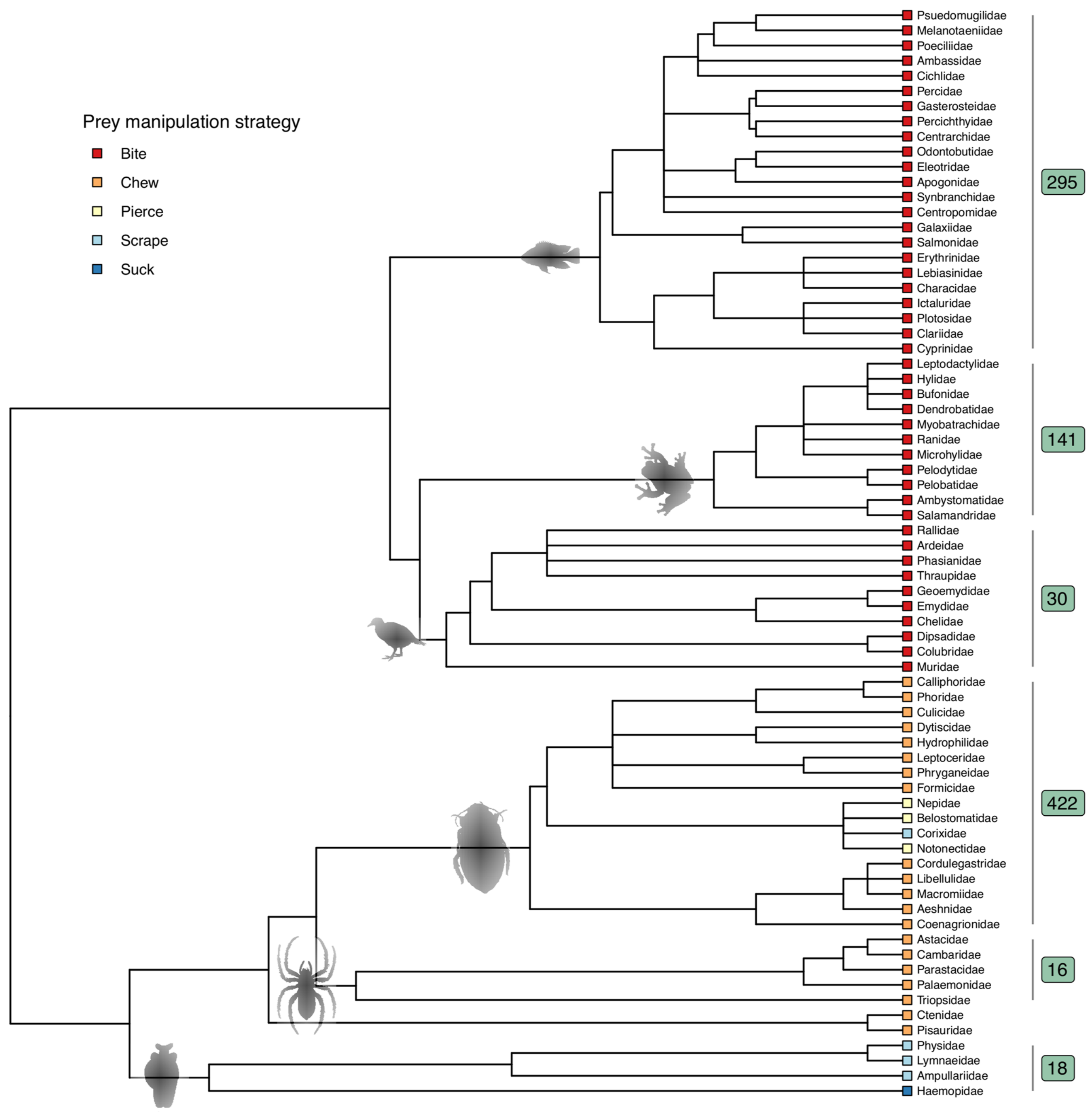


Fig. 2 Phylogenetic relationships of predators used across 922 observation groups in 100 studies in a meta-analysis of anuran prey consumption. Tree constructed with taxize package in R using NCBI classification ID. Silhouettes indicate predator clades (top to bottom) of fish, amphibians, amniotes, insects, non-insect arthropods, and non-arthropod invertebrates. Numbers indicate the number of observation groups from each clade within studies in this meta-analysis. Colored tips indicate the prey manipulation strategy used by each predator clade

(estimate = 0.50, $z = 1.09$, $p = 0.27$; Fig. 5). The third model compared hatchling and late tadpole stages in 64 observations among ten studies, and found a significant difference between those stages (estimate = − 1.16, $z = -2.47$, $p = 0.014$) such that predators were 3.19 times less likely to eat hatchlings (Stage 22–25) than late stage tadpoles (Stages 37–41; Fig. 5). In the fourth model, we compared consumption of the same prey species by the same predator species among 20 observations of late tadpoles and metamorphs (Stages 42–46) from seven studies, and did not detect significant differences in prey consumption among these two life stages (estimate = − 0.46, z = − 0.49, $p = 0.62$; Fig. 5).

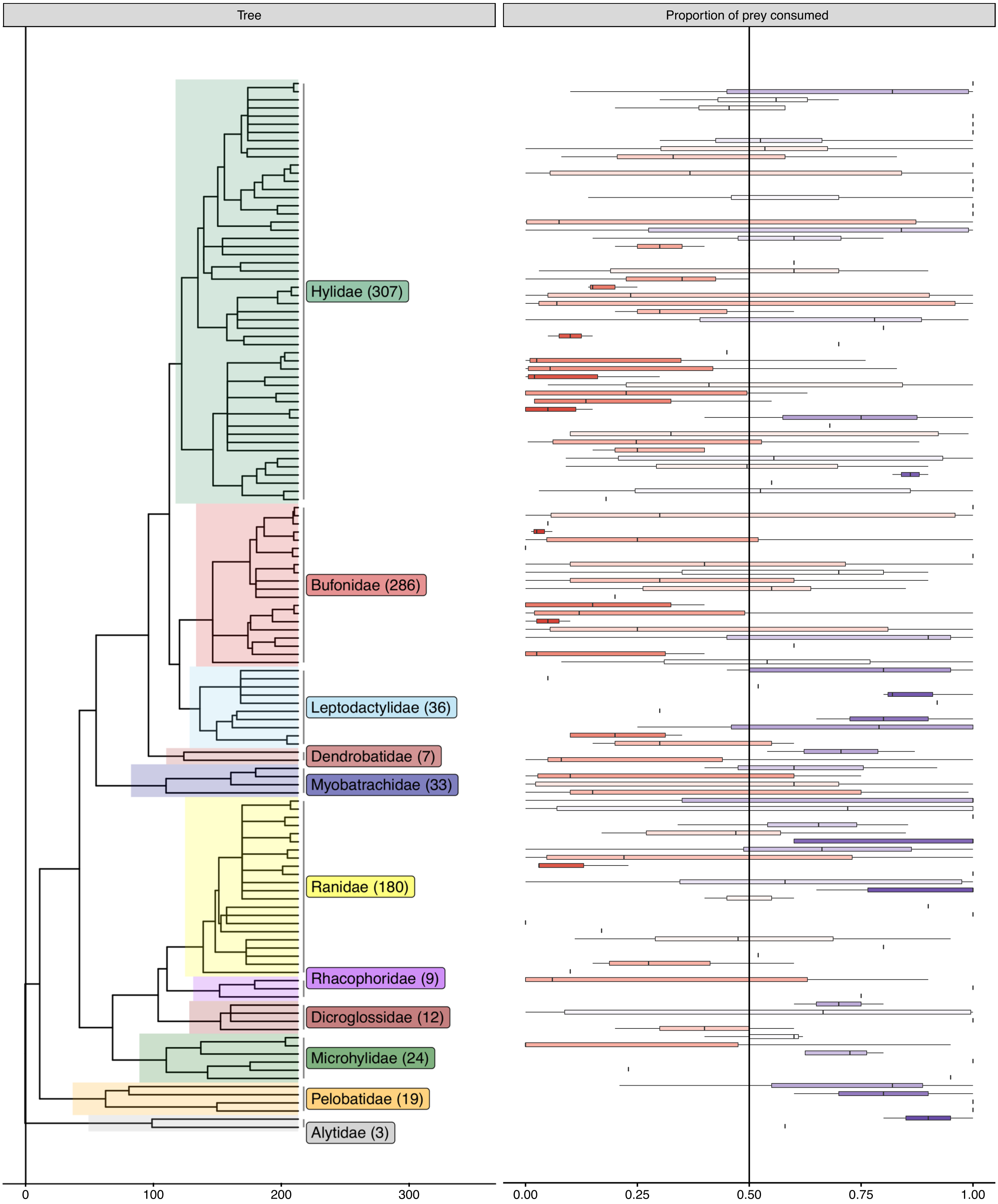
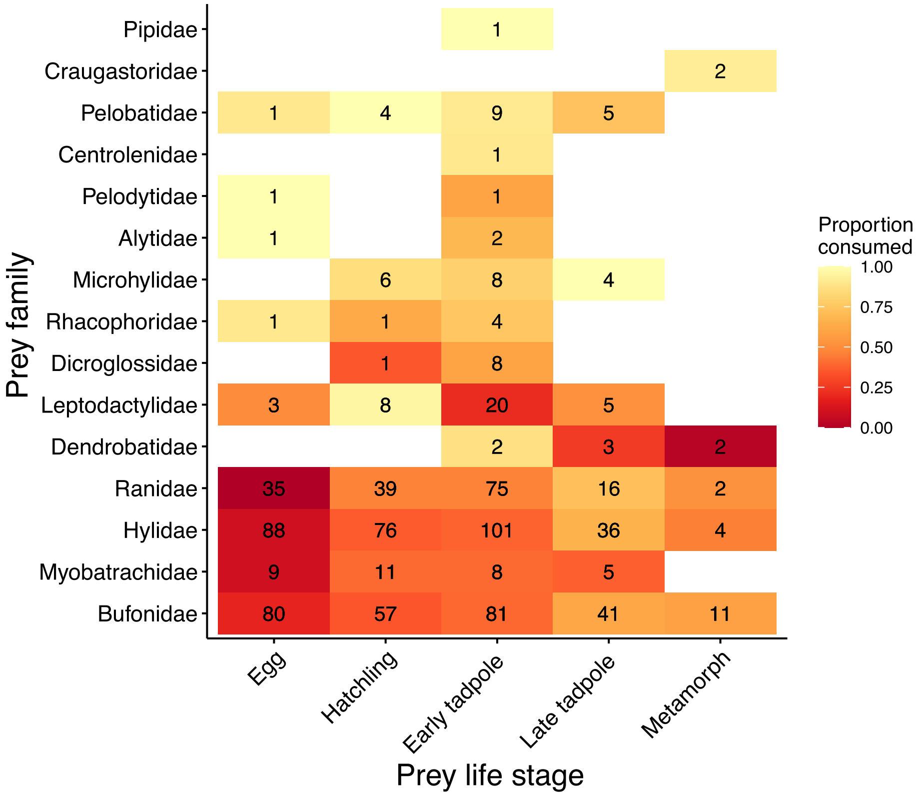


Fig. 3 Taxonomic relationships of anuran prey species ($N=135$) represented in 922 observation groups across 100 studies in a meta-analysis of anuran prey consumption. Boxplots show the average proportion of prey consumed as reported for each species in each study (0/red = none eaten, 1/blue = all eaten). Species represented by a single experiment appear as a dash rather than a box. Tree adapted from Jetz and Pyron (2018) and information on prey consumption compiled from references (Online Resource 1)

Fig. 4 Heatmap showing the proportion of prey consumed by predators in studies included in this meta-analysis. Data are separated by five life stages and ordered by average consumption (0 = none eaten, 1 = all eaten) across 15 anuran families. Colors are based on raw data averaged across all observations per cell, and do not account for relative quantities of observations (indicated with numbers; $N = 879$ total observations in heatmap), sample sizes within each observation, or methodological differences among studies. Blank spaces indicate family-stage combinations with no observations in selected studies



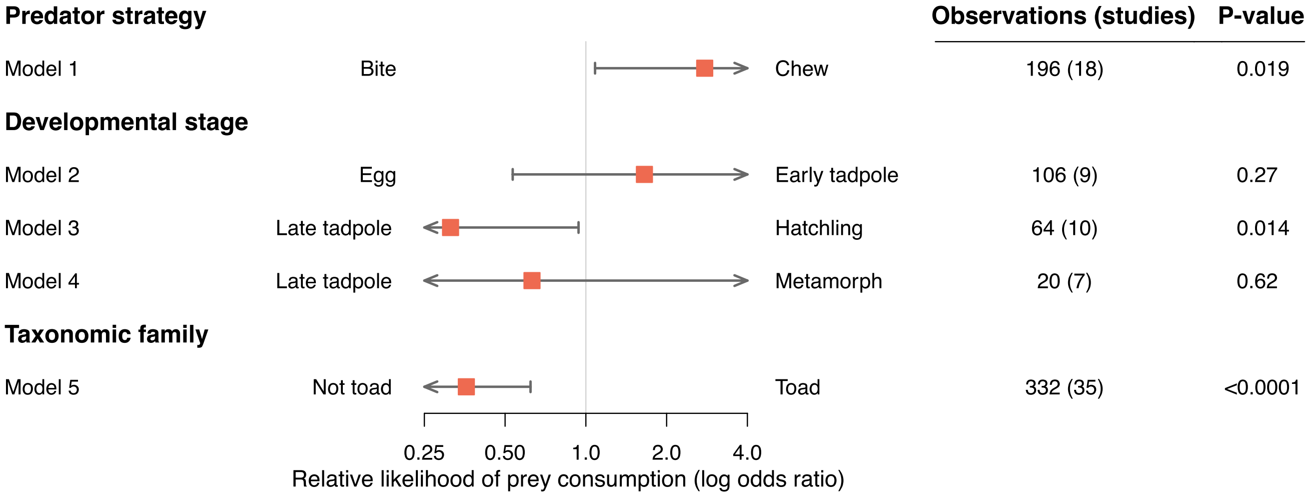


Fig. 5 Forest plot with results of five hierarchical meta-analyses of the consumption of anuran prey: one of prey manipulation strategies of predators ($N = 196$), three of different anuran life stages ($N = 106$, 64, and 20 respectively), and one of family-level comparisons ($N = 332$). The five models were comprised of the same fixed and random factors, but the subset of data used in each model varied depending on the direct relevance of pairs of observations to each hypothesis. Arrows indicate confidence intervals that extend beyond the range labeled on the axis

The fifth model was based on 332 observations among 35 studies in which the same life stages of a toad and non-toad prey species were tested with the same predator species. In this model we detected a significant difference among prey family groups (estimate = -1.99, $z = -8.12$,

$p < 0.001$), such that bufonid toad prey were 7.3 times less likely to be consumed than non-toad prey (Fig. 5).

Discussion

The results of this systematic review and meta-analysis support the idea that unpalatability plays an important role in predation avoidance for some families of young anurans. Thus, our findings call into question whether it is accurate to generalize anurans as a group that exhibits widespread ontogenetic switches in anti-predator strategies, specifically in the relative importance of chemical defenses across life stages (Gunzburger and Travis 2005; Medina et al. 2020). In general, we found that, across empirical studies, predators were more likely to avoid anuran young if they were from families that use chemical defenses as adults (see Fig. 4; Bufonidae, Myobatrachidae, Dendrobatidae, Hylidae, Ranidae, Leptodactylidae; reviewed in König et al. 2015, Arbuckle and Speed 2015) and/or were at the ontogenetic stages most likely to contain maternal chemical defenses (egg, hatchling) or store their own chemical defenses (metamorph) (Hayes et al. 2009; Llewelyn et al. 2012; Stynoski and O'Connell 2017). Thus, there may not be dramatic ontogenetic switches in the relative importance of chemical defenses within the anti-predator strategies of those anuran families, which refutes previous meta-analytic results concluding that unpalatability is rare in young anurans (Gunzburger and Travis 2005)—particularly in bufonid toads, which rely heavily on chemical defenses as adults.

Moreover, we provide some of the first strong meta-analytic evidence to support three classic hypotheses regarding the palatability of developing anurans (Crossland 1998): biting vertebrates are more likely to avoid young anurans than chewing invertebrates, recently hatched tadpoles are less palatable than late stage tadpoles, and young bufonid toads are less palatable than non-bufonid anuran young. Carefully designed experiments are needed to directly test the primary hypothesis arising from this meta-analysis, which is that palatability and/or toxicity is the causal mechanism underlying the preferences of predators for particular anuran life stages and clades. Palatability is one of a suite of traits including nutritional value, size, and ease of capture that could drive patterns in predator preferences, all of which vary concomitantly during feeding experiments with live predators and prey like those included in this meta-analysis. Ideally, this synthesis of data from 100 studies of predator consumption of anuran young will stimulate further research to fill in gaps and enrich our understanding of the comparative chemical ecology of anurans and of ontogenetic switches in anti-predator strategies.

Prey manipulation strategies

Based on meta-analytic models, chewing invertebrates were almost three times more likely to consume young anurans than biting vertebrates, concurring with previous studies suggesting that vertebrates avoid amphibian prey more than aquatic insects do (Brodie et al. 1978; Kats et al. 1988; Gunzburger and Travis 2005; Adams et al. 2011). Numerous proximate and ultimate differences between vertebrate and invertebrate predators could form the basis of the causal mechanisms that shape this pattern of prey consumption. However, one classic hypothesis is that such phylum-level variability in predator deterrence could be due to differences in prey manipulation strategies because such strategies determine the degree of contact between prey tissues containing distasteful substances and predator tissues capable of detecting those substances (Brodie et al. 1978; Gunzburger and Travis 2005). Specifically, it has been suggested that invertebrate predators would be less deterred by chemical defenses because they use piercing mouthparts and thus are not in direct contact with chemically defended skin tissues (Wassersug 1973; Crossland 1998; Jara and Perotti 2009). In fact, many aquatic insects—such as the dytiscid beetles and odonate larvae that are major predators of young anurans—do not pierce but rather chew prey with mandibles (Szuroczki and Richardson 2011; Cabrera-Guzmán et al. 2012).

In the current meta-analysis, we did not include data from studies with piercing invertebrates such as belastomatids, and still detected lower consumption of anuran young by biting vertebrates than by chewing invertebrates, suggesting that piercing as a prey manipulation strategy does not explain this variability in prey avoidance. Rather, the reason for differences in predation responses in palatability tests among biting vertebrates and chewing invertebrates could be that vertebrate predators have more specialized and therefore more sensitive chemosensory physiology (Cabrera-Guzmán et al. 2012).

Important differences in the evolutionary history of toxin resistance could also explain variation in prey consumption by different predator groups (Wilson and Williams 2014; Arbuckle et al. 2017). Some convergent molecular mechanisms underlie the evolutionary history of sensitivity to toxic inhibitors like bufonid bufadienolides and related cardiotonic steroids among vertebrates and invertebrates (Mohammadi et al. 2016). However, key functional differences in the biochemical binding sites for bufonid chemical defenses known as Na,K-ATPases arose during the evolutionary separation of vertebrates and invertebrates—specifically via both retained absence and secondary loss of ouabain binding sites on the catalytic α-subunit of some invertebrate isoforms of Na,K-ATPase, as seen in *Drosophila melanogaster* and *Caenorhabditis elegans* (Okamura et al. 2003). So far they appear to be a rare exception among invertebrates, but a subfamily of fireflies (Lampyrinae) and the heteropteran milkweed bug can even synthesize or sequester some types of bufadienolides (Berger et al. 2021). Investigation of the roles of palatability and toxicity in defending bufonid young from vertebrate and invertebrate predators would benefit from consideration of the diverse physiological and molecular mechanisms that shape a given predator's sensory range and toxin resistance.

Prey developmental stages

Meta-analytic models show that predators were about three times as likely to eat late-stage tadpoles than hatchlings, but they did not detect significant differences between predator consumption of egg and early tadpole stages. Together these findings suggest that prometamorphic tadpoles (Stages 37–41) are particularly vulnerable to predators, which in some taxa could be attributed to the gradual loss of maternal chemical defenses deposited in eggs at oviposition (Hayes et al. 2009; Llewelyn et al. 2012). However, this ontogenetic pattern could also be explained by factors unrelated to chemical defenses, as other studies found prometamorphic and metamorphic tadpole stages to be the most vulnerable to snake predation and attributed that vulnerability to reduced swimming agility due to a transitional body form (i.e., coordination of four legs and a long tail) (Wassersug and Sperry 1977; Arnold and Wassersug 1978). One family, Dendrobatidae, showed a different pattern in which predator consumption declined over developmental time, which could be attributed to the fact that all developmental palatability studies to date in this family are based on species in which mother frogs feed tadpoles with eggs that contain chemical defenses and those defenses accumulate in tadpoles over time (Stynoski et al. 2014; Saporito et al. 2019; Fischer et al. 2019).

Another model focused on differences in consumption between anuran life stages did not detect significant differences between consumption of late stage tadpoles and metamorphs, although statistical power to detect such differences was notably lower for this model ($n = 10$ pairs of observations). The granular (poison) glands which sequester and/or synthesize chemical defenses begin to form in prometamorphosis and then mature and grow during and after metamorphosis (Regueira et al. 2016; reviewed in Stynoski and O'Connell 2017), which in some empirical studies has been linked to reduced predation following metamorphosis (Garton and Mushinsky 1979; Formanowicz and Brodie 1982; Hayes et al. 2009; Llewelyn et al. 2012). However, our work suggests that more research is needed within and among different anuran groups to develop a more complete picture of the influence of the ontogeny of granular glands and accumulation of defensive secretions on predator deterrence, particularly during and following metamorphosis (Brodie et al. 1978; Hayes et al. 2009).

Prey phylogenetic history

Contrary to a previous meta-analysis by Gunzburger and Travis (2005), which classified young bufonid toads as no less palatable than young in other anuran families, here we provide strong meta-analytic evidence that predators were over 7 times less likely to consume young bufonid toads than young in other anuran families. The contrast in conclusions of the two meta-analyses could be a consequence of the number and diversity of included studies and organisms; more than half of the studies in the current meta-analysis have been published since the previous meta-analysis was published, and they include a wider diversity of anuran families, including many tropical taxa. The contrast is likely also due to a change from an absolute (all-or-none) definition of unpalatability in the previous meta-analysis (Gunzburger and Travis 2005) to a relative (proportional) definition in the current meta-analysis. Relative unpalatability is arguably a more relevant measure of the evolutionary influence of chemical defenses on predator–prey interactions, due to possible population level shifts in relative survival rates of palatable and unpalatable prey (Formanowicz and Brodie 1982; Brodie and Formanowicz 1987; Hossie et al. 2021).

Bufonid chemical defenses are comprised of a particularly potent chemical cocktail including bufadienolides, biogenic amines, alkaloids, and other proteins (Rodríguez et al. 2017), which are known for the effective and even dangerous effects they can have on predators (Shine 2010). Many comparative empirical studies have found that young bufonids are less favorable to predators than other anurans (Formanowicz and Brodie 1982; Brodie and Formanowicz 1987; Lawler and Hero 1997; Karraker 2011) and they are also more distasteful to humans (Wassersug 1971). Furthermore, a meta-analysis of tadpole anti-predator responses to predator cues found that anuran larvae from less palatable families like the Bufonidae exhibit fewer inducible defenses like increased tail depth or behavioral responses (Hossie et al. 2017), perhaps because their skin secretions constitute an ample anti-predator strategy as they do in adult bufonids. Thus, it is not surprising that this meta-analysis supports the hypothesis that young bufonids are, in fact, more protected from predator consumption than other anuran young overall.

This family-level analysis was focused on Bufonidae in part due to the number of studies available that have compared bufonids to other families, and it did not account for wide variation in predator consumption within families. However, on a qualitative level, we also observed relatively low consumption of young in some other families (e.g., Myobatrachidae) and in particular clades within some families (e.g., genera *Cyclorana* and *Litoria* within the family

Hylidae; see Fig. 3). Future studies that take into consideration the biochemical diversity of peptides, alkaloids, steroids, and other toxins that are synthesized or sequestered in the skin glands of particular anuran taxa are needed to further elucidate the evolutionary and ontogenetic patterns of chemical defenses both among and within clades.

Future research and conclusions

During this systematic review, it became apparent that there are a number of gaps in the study of the ontogeny of chemical defenses and their role in predator deterrence. First, there are hundreds of empirical and comprehensive studies about the behavioral responses of diverse predators to anuran prey at different life stages and in different taxonomic groups. However, such behavioral trials with live predators and prey are not the ideal experimental design with which to isolate the effect of chemical defenses on the behavior of predators and survival of prey, because they are innately confounded by other factors such as prey movement, size, experience, and color as well as predator motivation, size, and experience (Gunzburger and Travis 2005; Gosavi et al. 2014). Therefore, we suggest that future research in this area would benefit from more careful implementation of techniques such as standardized food pellets produced with prey tissues (Szuroczki and Richardson 2011) or bioassays with extracts of chemical defenses (Schulte et al. 2017; Bolton et al. 2017), and even better if they are used in combination with biochemical characterization of pellet or extract contents.

Second, distastefulness and toxicity cannot be easily decoupled when predator consumption is the response variable in "taste test" designs (Wilson and Williams 2014). For example, it cannot be assumed that predator avoidance of a dendrobatid metamorph is due specifically to lipophilic alkaloids or that avoidance of a toad egg is due to specifically to bufadienolide steroids. Unidentified, distasteful, and more soluble and/or volatile peptides that might accompany such family-specific toxic defenses could be the reason that a predator avoids those bad tasting prey long before they would be influenced by the toxic substances themselves (Gonzalez et al. 2021). However, the separation of gustatory signals from toxic effects in anuran prey has rarely been investigated.

Third, it seems that few studies of palatability have taken into consideration that if a given predator has not previously learned to avoid intoxication by a prey item, then the acute or chronic toxic effects of prey chemical defenses will not affect consumption rate (Darst et al. 2006). Apart from indirect assays based on predator behavior, toxicity can also be measured more directly with methods such as cytotoxicity assays (Halliday et al. 2009), minimum lethal dose, sleeplessness, or LD_{50} assays (Darst et al. 2006; but see Bolton et al. 2017; Weldon 2017), or irritant assays (Protti-Sánchez et al. 2019). It is important to carefully consider the design and interpretation of palatability and/or toxicity assays, to incorporate the natural history of the species in question when selecting predators or assay animals, and to carefully choose chemical delivery methods (injecting, feeding live prey, feeding extracts, skin or cuticle contact) based on the hypothesis of interest (Weldon 2017).

It is clear that much remains to be explored as techniques continue to improve in order to better isolate, quantify, and assay the wide variety of unpalatable and toxic chemical defenses that can be found across anuran ontogeny and taxa, as well as their ecological and evolutionary roles in avoiding predation. Future research should target the physiological mechanisms that underlie the diversity of defensive compounds found in the skin of both pre- and post-metamorphic individuals, such as the evolutionary development of granular glands and the molecular pathways and processes that allow the sequestration and synthesis of distasteful and harmful molecules in different lineages and life stages. Such work will bring into focus a more integrative perspective of the role of chemical defenses in anuran survival across life stages, and will better elucidate the degree to which anurans can serve as a model for the evolution of ontogenetic switches in anti-predator strategies.

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Code availability Custom R script of data analyses and visualizations are included in this published article and its supplementary information files.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article was based on an analysis of data that are available in previously published research articles and does not contain any studies with human participants or animals performed by any of the authors.

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