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Burst swim speed in tadpoles inhabiting ponds with different top predators

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ABSTRACT

Selection is likely to favour anti-predator strategies that are effective against predators encountered frequently. Larval anuran communities fall along a gradient of pond permanency and pond permanency also affects the type of top predator present. These environmental factors combine to create distinct categories of pond types. In this study, I quantified the predator avoidance trait burst swim speed and related traits for 14 anuran species found in three pond types and within three taxonomic families. Absolute swim speed differed significantly among species and among taxonomic families. Inclusion of size in the model revealed a three-way interaction between size, habitat and taxonomic family. Tail beat frequency and body shape differed significantly among species, but with no pattern by family or habitat. Functional relationships among traits also did not differ among family by habitat groups. The evolution of swim speed was significantly correlated with the evolution of increased size. In general, these results suggest that anurans have invaded new pond types using multiple mechanisms to cope with the predators that are present.

Keywords: anti-predator traits, anurans, burst swim speed, community ecology, comparative study, evolutionary correlations, functional relationships, tadpoles.

INTRODUCTION

Ecological community structure is determined through the combined effects of abiotic and biotic factors (e.g. predation, competition, disease, mutualism). The biotic factors of predation and competition have been extensively considered as mechanisms driving the structure of community assemblages; these factors can play a substantial role in determining community structure (Hairston *et al.*, 1960; MacArthur, 1972; Menge and Sutherland, 1976; Connell, 1983; Schoener, 1983; Sih *et al.*, 1985). Interactions between biotic factors and abiotic factors can further modify patterns in community structure observed in nature (Dunson and Travis, 1991). One well-understood example of this is the change in abundance and type of predators found in aquatic communities along a gradient of pond permanency (Wellborn *et al.*, 1996; Skelly, 1997). Differences in pond permanency lead to concomitant changes in the type and abundances of predators inhabiting the pond.

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Predators, in turn, can be a major force structuring communities. In aquatic communities, many studies have shown that, for a variety of taxa (e.g. zooplankton: Brooks and Dodson, 1965; Zaret, 1980; amphipods: Wellborn, 1994; odonates: McPeek, 1990a,b; anurans: Heyer *et al.*, 1975; Collins and Wilbur, 1979; Werner and McPeek, 1994), species assemblages change drastically with the addition of a different predator (reviewed by Wellborn *et al.*, 1996). This pattern arises because species differ in vulnerability to predators. Furthermore, those defence strategies that are effective for avoiding predation against one predator may be ineffective against a different predator (McPeek, 1990b; Wellborn *et al.*, 1996; Skelly, 1997). For example, a few lineages of damselflies in the genus *Enallagma* have made evolutionary shifts from occupying fish lakes to occupying fishless lakes where larval dragonflies are top predators (McPeek, 1995). Damselfly larvae in fish lakes avoid predation best by escaping detection; once detected by a fish (the top predators in this system), larvae cannot escape predation through fleeing the much faster fish predators. However, damselflies in fishless lakes have better success escaping dragonfly predation if they swim away from approaching predators (McPeek, 1990b; McPeek *et al.*, 1996). For the fishless habitat, McPeek (1990b, 1995; McPeek *et al.*, 1996) has shown that selection favours those traits that increase burst swimming speed.

Larval anurans inhabit a wide variety of ponds and predators can have a substantial effect on the species composition of tadpole communities (Morin, 1983; Woodward, 1983; Werner and McPeek, 1994; Azevedo-Ramos *et al.*, 1999). For example, the species composition of tadpole communities undergoes remarkable change with increases in the number of predatory newts (Morin, 1983). Also, predation is the major factor affecting species assemblages in natural tropical tadpole communities (Azevedo-Ramos *et al.*, 1999). These predator effects on species assemblages reflect species differences in vulnerability to predators (Azevedo-Ramos *et al.*, 1999). Werner and McPeek (1994) found that tadpoles of two ranid sister species differed in vulnerability to fish, salamander and dragonfly larvae predation in a manner consistent with the distribution of ranids in natural ponds.

Work on the biomechanics of swimming in tadpoles (reviewed by Wassersug, 1989) demonstrates that tadpoles can be effective swimmers. Liu *et al.* (1996, 1997) have used computational fluid dynamics to model the mechanics of tadpole swimming. These studies showed that tadpole bodies are well designed for swimming given the constraints imposed by their complex life cycle (namely, the presence of growing hind limbs in the larval stage). Selection for fast burst swimming speed has been shown in *Hyla regilla*; Watkins (1996) found that tadpoles with faster burst swim speeds were more likely to survive in the presence of garter snake predators. Feder (1983) found escape from turtle predation in *Rana berlandieri* larvae depended on burst swim speed.

These results suggest that predators may create selection pressure in tadpoles that is dependent on the pond type a species inhabits. If anuran lineages have been able to diversify into new habitats by responding to this selection, we expect a relationship between phenotype and habitat type. Alternatively, evolutionary history may be a stronger influence on tadpole phenotype. If this is true, we expect a relationship between taxonomic family and phenotype. Anurans from three different families have diversified into more than one habitat type. This makes them ideal for testing the relative roles of selection and ancestry on the anti-predator trait of burst swimming speed. I quantify burst swim speed in tadpoles spanning the complete range of developmental stages and sizes in each of 14 anuran species and test the relative roles of lineage and pond type used in determining burst swim speed.

METHODS

I collected tadpoles of 14 anuran species from eastern North America; 6 species were studied in New Hampshire in 1996 and 8 species were studied in Florida in 1997 (species listed in Fig. 1). Tadpoles were caught by dipnet and returned to the laboratory, where they were housed in 38-litre plastic tubs (14 × 21 × 8.5 cm). Early-stage tadpoles were housed in 100% filtered pond water (from a pond in which that species was found); later-stage tadpoles (Gosner stage 30 and later; Gosner, 1960) were kept in 50% filtered pond water and 50% aged tap or well water. I fed animals 'tadpole food' (pellets of processed grain and plant products supplemented with vitamins) purchased from Carolina Biological Supply Company (Burlington, NC) and did partial or complete water changes whenever water became fouled (typically every 4–7 days). I found that all species of tadpoles thrived under this regime.

I classified anuran species into categories based on the pond types in which their tadpoles are found. Tadpoles inhabit ponds that vary along a gradient of pond permanency; this gradient has been well described and, although it is continuous in nature, two clear transitions are seen along the gradient (Semlitsch *et al.*, 1996; Wellborn *et al.*, 1996; Skelly, 1997). Wellborn *et al.* (1996) termed these the 'permanence transition' and the 'predator transition' and these two transitions lead to three categories of ponds: 'temporary habitats', 'permanent, fishless habitats' and 'habitats with fish' (see fig. 2 of Wellborn *et al.*, 1996). The difference in prey species' assemblage between permanent fishless and fish ponds has been

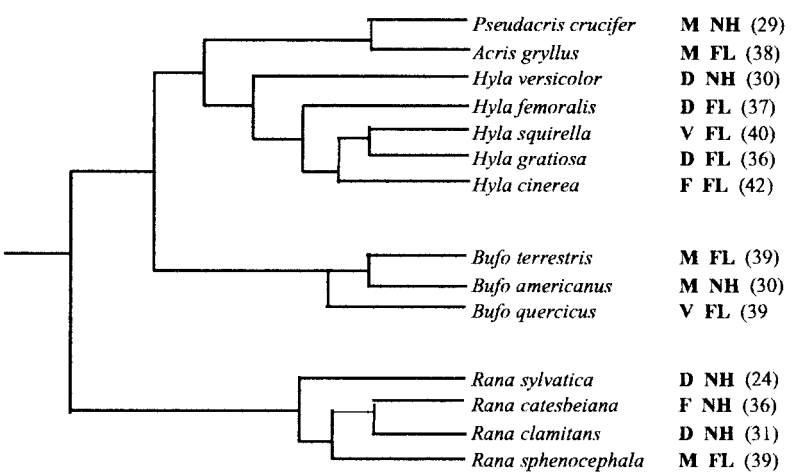


Fig. 1. Phylogenetic relationships among North American species in the families Hylidae, Ranidae and Bufonidae. Lengths of tree branches do not reflect true branch lengths. The tree is based on electrophoretic (Hylidae) and DNA (Ranidae, Bufonidae) data from Hedges (1986), Hillis and Davis (1986) and Graybeal (1997), respectively. Family level relationships are based on morphological characters (Duellman and Trueb, 1994). **Bold** letters indicate habitat type where larvae are found (**V** = vernal, **D** = dragonfly, **F** = fish, **M** = multiple) and the state in which the species was studied (**FL** = Florida, **NH** = New Hampshire). The numbers of individuals tested per species are shown in parentheses.

well established for many different taxonomic groups (e.g. cladocerans: Brook and Dodson, 1965; odonates: McPeek, 1990a,b; anurans: Collins and Wilbur, 1979; Morin, 1983; Werner and McPeek, 1994; Skelly, 1995). Evidence suggests that it is the change in top predators that leads to differential species' assemblages among these two types of permanent ponds (reviewed by Wellborn *et al.*, 1996) and thus I will refer to habitats with fish as 'fish ponds' and permanent habitats without fish as 'dragonfly ponds' to emphasize the role of large aquatic insects, such as dragonfly larvae, as the top predators in permanent fishless ponds (McPeek, 1990a,b; Werner and McPeek, 1994; Skelly, 1996). Although relatively permanent, dragonfly ponds may dry on occasion (i.e. during drought conditions). The 'permanence transition' separates dragonfly ponds from those habitats that dry predictably every year; I will subsequently call these 'vernal ponds'.

I used these broad categories to classify the tadpole species I collected according to pond type. Extensive previous studies have observed consistent patterns in the segregation of several different anuran species among pond types defined by the predation–permanence transitions (Heyer *et al.*, 1975; Collins and Wilbur, 1979; Morin, 1983; Woodward, 1983; Kats *et al.*, 1988; Skelly, 1996; Alford, 1999). Although I did not quantify predators in the ponds from which I collected tadpoles, I sampled these 28 ponds (16 in Florida and 12 in New Hampshire) extensively throughout the 9 months I spent in Florida and two breeding seasons in New Hampshire. I had strict and repeatable criteria for the classification of species; I used only species I had observed in at least three different ponds and I collected individuals from at least two ponds (greater than 10 miles apart) for experiments. In the course of my sampling, I never observed species in ponds inconsistent with their classification in this paper. A pond was only considered vernal if it dried predictably every year. In Florida, these were mostly low-lying areas that filled with water during the rainy season. In New Hampshire, these included rock pools that filled in spring when rivers overflowed the bank and deep tyre tracks that filled with rain or snow melt. I classify *Hyla squirella* as a vernal pond species because I found it only in such ponds. In New Hampshire, the only species I found in such vernal ponds was *Bufo americanus* (which is also found in fish ponds). Dragonfly ponds were those ponds that held water both before and after the breeding season, had not dried for several years previously, and had no predatory fish (personal observation; M. McPeek, personal communication for New Hampshire ponds and J. Travis, personal communication for Florida ponds). Fish ponds are the most permanent ponds and all contained predatory fish (personal observation; M. McPeek and J. Travis, personal communications). Species that I classified as dragonfly pond species were found in many different dragonfly ponds, but were never found in fish ponds. Similarly, fish pond species were found in several different fish ponds, but never in dragonfly ponds. Some species were consistently found in two of these pond types. For example, *B. americanus* was found both in vernal ponds and in fish ponds throughout my sampling area in New Hampshire. *Pseudacris crucifer* was found in both dragonfly and fish ponds that I sampled in both New Hampshire and Florida. Species found consistently in two different pond types were labelled 'multiple pond' species. Thus, there were four levels of habitat type: vernal, dragonfly, fish and multiple pond species. My classification of species is consistent with classifications used in previously published work (e.g. Werner and McPeek, 1994: *Rana clamitans*, *R. catesbeiana*; Skelly, 1996: *P. crucifer*).

The three most speciose anuran families in North America – the Hylidae, Ranidae and Bufonidae – have representative species in two or more of the four habitat categories. Immunological data and fossil record evidence suggest that progenitors in the Bufonidae

and Hylidae families invaded North America from the south during the Palaeocene and subsequently differentiated in isolation from South America (Savage, 1973; Duellman and Trueb, 1994). Phylogenetic data confirm North American bufonids as a monophyletic group (Maxson, 1984; Graybeal, 1997). North American ranids invaded from Eurasia during the Miocene. Molecular data suggest the North American *Rana catesbeiana* and *R. pipiens* groups are each monophyletic, but that the *R. sylvatica* group is more closely related to the European *R. temporia* group than to the North American *R. catesbeiana* or *R. pipiens* groups (Post and Uzzell, 1981; Hillis and Davis, 1986). The monophyly of anuran clades suggests that diversification into the three pond types has occurred during radiation of the lineages within North America (with the possible exception of *R. sylvatica*). Furthermore, a consideration of phylogenetic relationships hypothesized from molecular data suggests that, within hylids and ranids, replicate invasions into the same pond types has occurred (Fig. 1).

Burst swim speeds were quantified by placing individual tadpoles in a shallow Plexiglas container ($34 \times 25.6 \times 5$ cm) filled to a depth of 2.5 cm with filtered pond water from the appropriate pond type. All trials were done between 10:00 and 14:00 h. Trials were done at the same temperature tadpoles were housed at; I recorded water temperature before testing animals to allow correction for effects due to temperature. The average temperature was 22.13°C and 26.25°C for the experiments in New Hampshire and Florida, respectively. I induced tadpoles to swim by drawing a small metal spatula quickly across the tail, simulating a predator attack. A camera positioned directly above the swimming chamber recorded swims onto S-VHS videotape at 60 frames per second. At least three good (i.e. reasonably long and straight and not too near the edge of the container) swims were recorded for each individual. After the swim trial, tadpoles were preserved individually in 10% formalin to allow for morphometric measurements (Dartmouth College IACUC Protocol 98-03-10; Florida State University ACUC Protocol 9701). Swims were quantified in 24–42 individuals for the 14 species (see Fig. 1 for species and number of individuals tested for each species); I took care to use individuals from as broad a size and developmental stage range as possible for each species studied.

I calculated swim speeds using Optimas® imaging software to mark the head position of the tadpole every 5 frames (6 frames for 1997 data) to determine total distance moved and time elapsed (as determined by the number of frames). I recorded maximum tail amplitude for each swim as the length of a line drawn from the tip of the tail to the point where this line intersected the body axis line (extended past the body, if necessary) at a 90° angle. The number of tail beats per swim were also counted and converted into tail beat frequency. For each individual, the first three swims videotaped were quantified. The fastest of these three swims was used in all subsequent analyses.

I made six morphometric measurements from preserved tadpoles using Optimas®: total length, tail length, tail musculature height (at widest point), tail fin height (at widest point), tail area (the area inside an outline of the tail fin) and body area (the area inside an outline of the dorsal view of the body). A principal components analysis of the covariance matrix, for all species combined, revealed that these morphological measures all loaded strongly and positively on the first principal component (PC1); I used PC1 scores as an estimate of 'size' for tadpoles. I found that PC2 loaded positively with body length (0.4142) and tail length (0.6020), but negatively with tail fin height (−0.5293) and tail musculature height (−0.4296). I used PC2 scores as an estimate of 'shape'; individuals with high PC2 scores had relatively narrower tails and longer bodies.

Statistical analyses

I quantified swim speed in millimetres per second. Predation risk depends on the absolute distance between predator and prey. Therefore, absolute speed, which determines how quickly a tadpole can place distance between itself and the potential predator, is the relevant performance measure of predator avoidance. However, since swim speed and related traits can be correlated with size, I also analysed the data using size (PC1) as a covariate.

Tadpole swims conducted in Florida were done at a temperature that averaged 4°C higher than swim speeds conducted in New Hampshire. Swim speed and tail amplitude were positively correlated with temperature. To correct for this effect, a regression of burst swim speed with temperature was performed (for all species grouped together) and the residuals from this regression were used in subsequent analyses. Tail amplitude was similarly corrected for temperature; no correction was done for number of tail beats, as this variable was not related to temperature. The transformed data did not differ between New Hampshire and Florida species ($F_{1,12} = 0.02$, $P > 0.895$, for geography main effect using species(geography) as the error term), allowing me to analyse species from both geographic regions together in a single analysis.

To consider habitat and taxonomic family effects, I ran analyses of variance using habitat and family as fixed factors and species nested within habitat × family cells as a random factor; thus species(habitat × family) was used as the error term to test for family, habitat and habitat × family effects. Since not every family has species found in every habitat type, this design contained three empty cells. To test for effects in an unbiased way, I divided the model into two subsets that were each completely crossed (Searle, 1987). One subset compared hylids and ranids found in dragonfly, fish and multiple habitats; the second subset compared bufonids and hylids found in vernal and multiple habitats. Although not completely independent, these two subsets had only two species in common. Where the full model and the subset models reached the same conclusions, I report only the results using the subset models. I use a conservative Bonferroni-corrected critical *P*-value of 0.013 because I analysed four related response variables separately.

I had an *a priori* expectation of how the variables were functionally related to one another. I used path analyses to test the hypothesized functional relationships among traits (Li, 1975; Hayduk, 1988). Relationships among the variables burst swim speed ($mm \cdot s^{-1}$), tail amplitude (mm), tail beat frequency ($beats \cdot s^{-1}$), size (= PC1) and shape (= PC2) were estimated for each species at each developmental stage using the hypothesized relationships outlined in Fig. 4. I used LISREL® 8.30 (Scientific Software International, 2000) to generate maximum likelihood estimates of path coefficients for a completely standardized solution. Analysis of variance (ANOVA) using path coefficients as data tested for habitat and family effects on functional relationships. As above, the results from the analyses of two balanced subsets of the model are presented (Searle, 1987). All analyses of variance were run in SAS® 8.01 (SAS Institute Inc., 1999).

I also calculated Felsenstein's independent contrasts using species trait means (Felsenstein, 1985; McPeek, 1995). I used this technique to estimate evolutionary change in the traits studied and looked for correlations among the evolutionary rates of different traits. Since phylogenetic relationships for each family had been done using different techniques, branch length estimates for the families were not in equivalent scales. However,

if the evolutionary rates of traits are correlated with one another, this should be reflected in the correlation coefficients between contrasts, independent of relative branch lengths. Therefore, I tested for correlations in evolutionary rates by calculating contrasts on trees with branch lengths assigned random values between 0 and 1. I calculated contrast values using this tree with randomly assigned branch lengths for two traits and then calculated the correlation coefficient between the contrasts of the two traits. This procedure was repeated 2000 times for each pair of trait values and 95% confidence intervals for each correlation were determined by ranking the resulting correlation coefficients and finding the two values that encompassed 95% of the values (Manly, 1991). Significant correlations were determined by comparing randomization *P*-values with Dunn-Šidák-corrected critical *P*-values (Sokal and Rohlf, 1995).

RESULTS

Absolute burst swim speed (not corrected for size) differed significantly among species ($F_{13,476} = 19.14$, $P < 0.001$) (Fig. 2A). *Post-hoc* Tukey comparisons revealed that this effect was not driven by any one species, but that several species differed from each other. In general, *Rana catesbeiana* and *R. sphenocephala* swam significantly faster than most other species, while *Bufo americanus* and *B. terrestris* swam significantly slower than most other species. An ANOVA (on the full, unbalanced dataset) of family and habitat effects on absolute burst swim speed (using species(family × habitat) as the error term) revealed a significant family effect, with ranids being faster swimmers than hylids, which, in turn, were faster than bufonids ($F_{2,5} = 10.16$, $P < 0.018$, all family comparisons $P < 0.05$ in *post-hoc* Tukey test) (Fig. 2A). This effect was not significant for either of the subset analyses of variance, presumably because of decreased power from smaller sample sizes in each of the subset models. Note that although some of the differences in absolute swim speed clearly reflect significant differences in mean size of species, mean size differences do not account for all differences in burst swim speed (Fig. 2B). For example, *Rana sphenocephala* and *Acris gryllus* both have fast burst swim speeds for their size, while *Bufo americanus* has a slow burst swim speed for its size (Fig. 2B).

Since burst swim speed within a species is typically correlated with body size, I also tested for differences in burst swim speed among species using size (PC1) as a covariate. Slopes of burst swim speed with size differed among species (interaction $F_{13,462} = 2.60$, $P < 0.002$). This reflected the fact that burst swim speed was correlated with size in some species, but not others (Fig. 3).

The significant interaction between size and species made it impossible to simply correct swim speed for size, so I analysed lineage and habitat effects using size as a covariate. Burst swim speed was affected by a significant three-way interaction between size (PC1), habitat and family ($F_{3,467} = 4.35$, $P < 0.005$ for the full, unbalanced model). The relationship between size and burst swim speed differed by family and habitat, ranging from a near zero slope (e.g. *Pseudacris crucifer* and *Hyla gratiosa*) to a strongly positive slope (e.g. *Bufo americanus*; Fig. 3). For both subset analyses of variance, there was a strong effect of size on burst swimming speed (Table 1), and the significant effect of species within family × habitat group suggests differences among species overwhelm differences between family or habitat (Table 1).

Three traits related to burst swim speed – tail amplitude, tail beat frequency and body shape – were also analysed for species, family and habitat effects. Tail amplitude

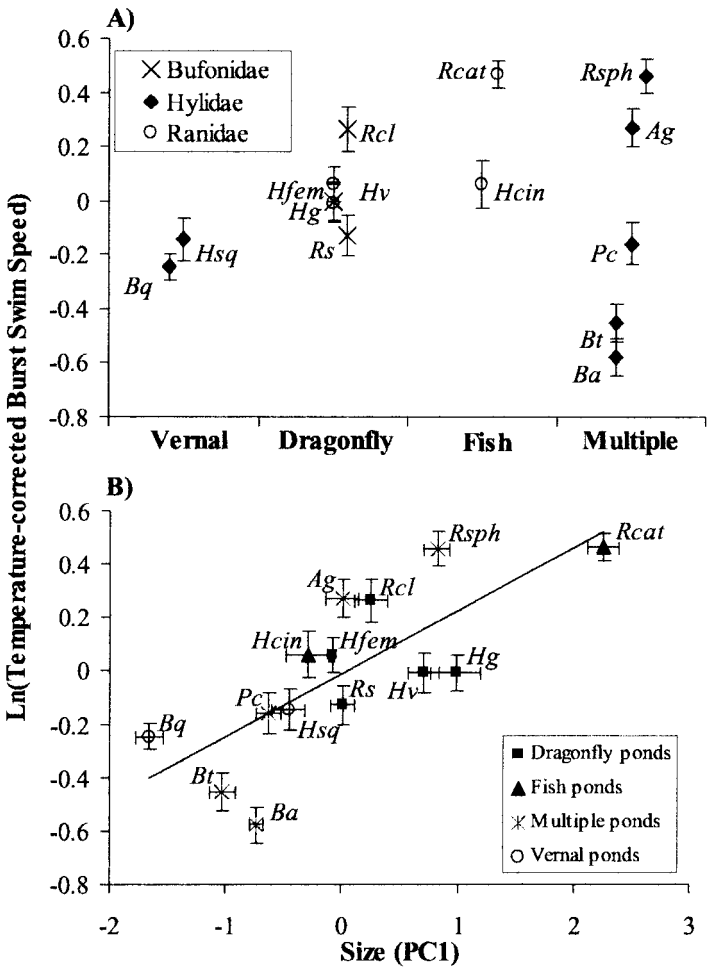


Fig. 2. (A) Mean absolute burst swim speed (after temperature effects were removed) for each species in each family by habitat combination. (B) Mean temperature-corrected swim speed plotted against the mean size (PC1 score) for each species. Note that differences in size of species only explains approximately half of the variance in swim speed (regression $r^2 = 0.52$). Species name abbreviations are: *Pc = Pseudacris crucifer, Ag = Acris gryllus, Hv = Hyla versicolor, Hfem = H. femoralis, Hsq = H. squirella, Hg = H. gratiosa, Hcin = H. cinerea, Bt = Bufo terrestris, Ba = B. americanus, Bq = B. quercicus, Rs = Rana sylvatica, Rcat = R. catesbeiana, Rcl = R. clamitans, Rsph = R. sphenocephala.*

(analysed using size as a covariate) did not differ among species ($F_{13,462} = 1.01$, $P < 0.45$). Tail beat frequency was also analysed using size as a covariate and the slope of tail beat frequency with size differed among species (interaction $F_{13,453} = 4.55$, $P < 0.001$). In analyses of family and habitat, tail beat frequency was found to differ significantly only for the species nested within the family × habitat term (Table 1). Effects of body shape were tested with an ANOVA using PC2 as the response variable; species also differed in this trait value ($F_{13,476} = 17.13$, $P < 0.001$), but differences were not related to family

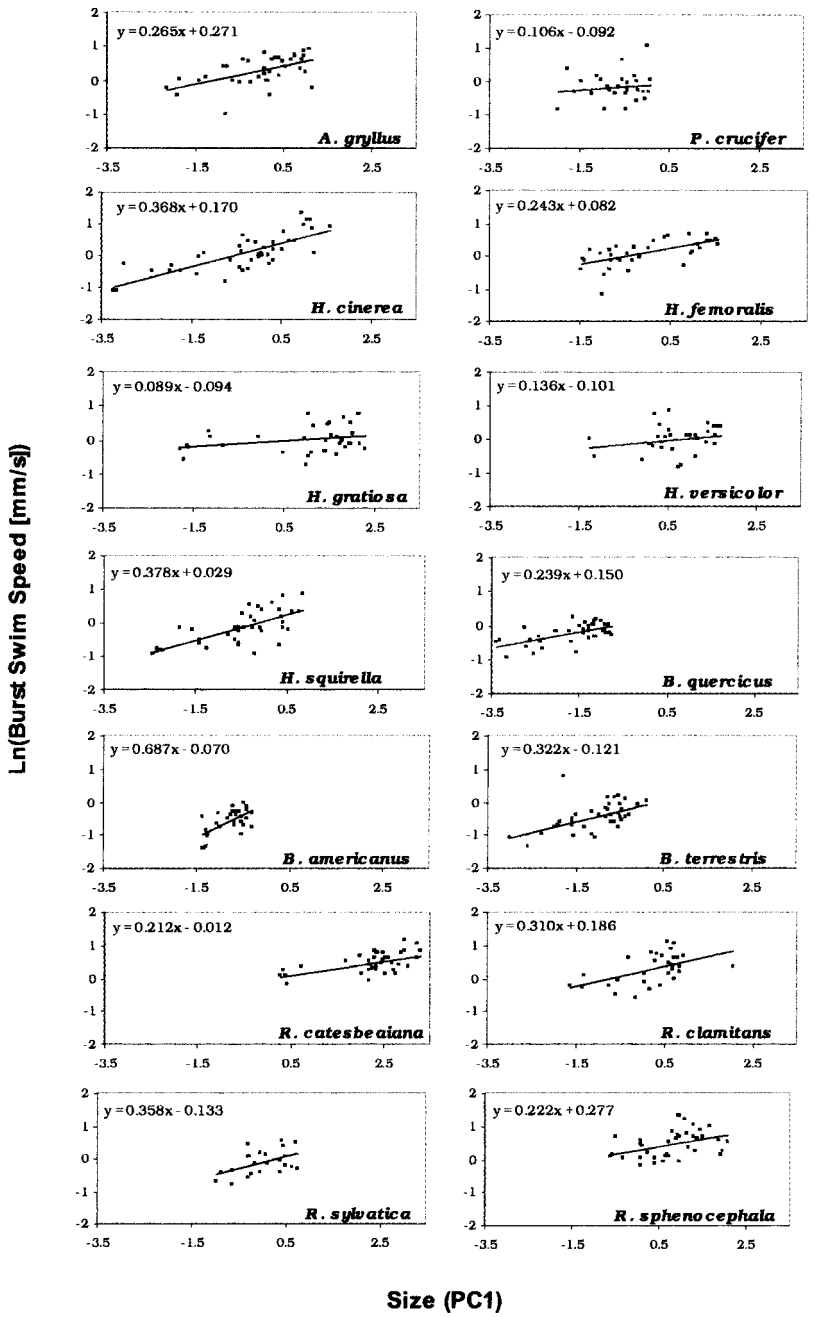


Fig. 3. The relationship between size and burst swim speed for tadpoles of 14 anuran species.

(hylid–ranid subset: $F_{1,4} = 2.05$, $P > 0.225$; hylid–bufonid subset: $F_{1,2} = 2.76$, $P > 0.239$) or habitat (hylid–ranid subset: $F_{2,4} = 1.98$, $P > 0.253$; hylid–bufonid subset: $F_{1,2} = 5.24$, $P > 0.149$).

Table 1. *F*-statistics and *P*-values for effects of taxonomic family, habitat type and their interaction from an ANOVA of the digestion variables tested.

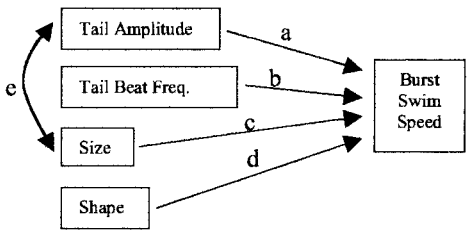
	ln(burst swim speed)	ln(tail amplitude)	ln(tail beat frequency)
ANOVA 1: hylids and ranids in dragonfly, fish and multiple ponds			
Family	$F_{1,4} = 0.03$, $P < 0.87$	$F_{1,4} = 0.02$, $P < 0.91$	$F_{1,4} = 1.72$, $P < 0.26$
Habitat	$F_{2,4} = 1.11$, $P < 0.42$	$F_{2,4} = 3.00$, $P < 0.16$	$F_{1,4} = 1.40$, $P < 0.35$
Size	$F_{1,326} = 11.80$, $P < 0.0001$	$F_{1,326} = 5.51$, $P < 0.02$	$F_{1,317} = 3.92$, $P < 0.05$
Family × habitat × size	$F_{2,326} = 3.85$, $P < 0.02$	$F_{2,326} = 0.84$, $P < 0.43$	$F_{2,317} = 1.74$, $P < 0.18$
Family × habitat	$F_{2,4} = 0.19$, $P < 0.83$	$F_{2,4} = 1.79$, $P < 0.28$	$F_{2,4} = 0.21$, $P < 0.82$
Species (family × habitat)	$F_{4,326} = 3.28$, $P < 0.0001$	$F_{2,4} = 0.93$, $P < 0.45$	$F_{4,317} = 5.69$, $P < 0.001$
ANOVA II: hylids and bufonids in vernal and multiple ponds			
Family	$F_{1,2} = 0.27$, $P < 0.66$	$F_{1,2} = 0.03$, $P < 0.88$	$F_{1,2} = 1.42$, $P < 0.36$
Habitat	$F_{1,2} = 0.20$, $P < 0.70$	$F_{1,2} = 0.01$, $P < 0.95$	$F_{1,2} = 0.28$, $P < 0.65$
Size	$F_{1,205} = 81.81$, $P < 0.001$	$F_{1,205} = 2.21$, $P < 0.14$	$F_{1,196} = 0.68$, $P < 0.41$
Family × habitat × size	$F_{1,205} = 4.58$, $P < 0.04$	$F_{1,205} = 1.22$, $P < 0.27$	$F_{1,196} = 0.81$, $P < 0.37$
Family × habitat	$F_{1,2} = 0.71$, $P < 0.49$	$F_{1,2} = 1.01$, $P < 0.42$	$F_{1,2} = 1.05$, $P < 0.41$
Species (family × habitat)	$F_{2,205} = 8.45$, $P < 0.001$	$F_{2,205} = 4.07$, $P < 0.02$	$F_{2,196} = 10.91$, $P < 0.001$

Note: See text for an explanation of the design. Because multiple tests were performed for each stage, a conservative critical value of $P < 0.017$ should be used to assess statistical significance.

Functional relationships

To consider functional relationships among variables related to burst swim speed, I used path analysis. Path coefficients for each species were estimated based on hypothesized functional relationships (Fig. 4). Because path coefficients are a species level variable, replication was limited (several family × habitat groups had only a single species). Therefore, to analyse this unbalanced data set in a more robust manner, I ran a one-way ANOVA in which I categorized species according to family × habitat group and tested for differences between groups. In this analysis, no significant differences were found among family × habitat groups (tail amplitude → speed: $F_{8,5} = 0.15$, $P > 0.989$; tail beat frequency → speed: $F_{8,5} = 0.88$, $P > 0.583$; size → speed: $F_{8,5} = 0.21$, $P > 0.974$; shape → speed: $F_{8,5} = 0.67$, $P > 0.708$). These non-significant results reflect large differences between species that swamp out family and habitat effects (Fig. 4).

Path coefficients from size to swim speed were significantly positive for all species except *Pseudacris crucifer* (where the coefficient was near zero), *Hyla versicolor* and *Rana sphenocephala* (the coefficient was positive for these two species, but not significantly different from zero). The functional relationship between shape (PC2) and swim speed was significantly positive for *R. sphenocephala*, *R. clamitans* and *Hyla cinerea* (multiple, dragonfly and fish pond species, respectively), implying these species have increased swim speeds with increased relative tail lengths. The functional relationship between tail beat frequency and swim speed was significantly negative in four species and significantly positive in four species, ranging from −0.49 in *Bufo quercicus* to 0.76 in *Rana sylvatica*; no pattern with taxonomic family or habitat was apparent (Fig. 4). The functional relationship between tail



Species	Family	Habitat	a	b	c	d	e
B. quercicus	B	V	0.186	-0.490^{++}	0.644^{++}	0.002	-0.219
H. squirella	H	V	0.291	-0.219	0.508**	-0.066	0.692^{++}
H. femoralis	H	D	0.247	-0.357*	0.560*	-0.216	0.814^{++}
H. gratiosa	H	D	-1.317^{++}	0.094	1.456^{++}	0.093	0.874^{++}
H. versicolor	H	D	0.204	0.541*	0.146	0.076	0.693^{++}
R .clamitans	R	D	-0.074	0.256	0.705^{++}	0.343*	0.776^{++}
R. sylvatica	R	D	0.125	0.760^{++}	0.44**	-0.058	0.618^{++}
H. cinerea	H	F	0.040	-0.243*	0.799^{++}	0.239*	0.837^{++}
R. catesbeiana	R	F	0.179	0.650**	0.948^{++}	-0.117	0.756^{++}
B. americanus	B	M	0.085	0.649^{++}	0.475^{++}	0.075	0.434**
B. terrestris	B	M	-0.140	-0.171	0.591*	-0.006	0.717^{++}
A. gryllus	H	M	-0.152	-0.340*	0.781**	-0.103	0.797^{++}
P. crucifer	H	M	0.176	0.105	-0.051	0.172	0.485**
R. sphenocephala	R	M	0.078	-0.084	0.291	0.341*	0.733^{++}

Fig. 4. Path diagram of hypothesized relationships between traits and path coefficients quantified for each species. Numbers given are standardized path coefficients; symbols indicate which unstandardized coefficients differed significantly from zero ($^{*}P < 0.05$; $^{**}P < 0.01$; $^{++}P < 0.001$). Species are grouped by habitat type and taxonomic family: B = Bufonidae, H = Hylidae, R = Ranidae, V = vernal ponds, D = dragonfly ponds, F = fish ponds, M = multiple ponds.

amplitude and burst swim speed was weak for all species except *Hyla gratiosa*, which had a strong negative relationship between these two traits.

Evolutionary contrasts

The evolution of size was positively correlated with the evolution of increased burst swimming speed (Table 2; randomization $P < 0.001$, Bonferroni-corrected critical

Table 2. Confidence intervals for correlations between evolutionary contrast values

Contrasts	Confidence intervals
Tail amplitude and swim speed	−0.465 to 0.136
Tail beat frequency and swim speed	−0.560 to 0.040
Size and swim speed	0.336 to 0.666
Shape and swim speed	−0.321 to 0.300
Tail amplitude and tail beat frequency	−0.653 to 0.068
Tail amplitude and size	−0.498 to 0.016
Tail amplitude and shape (tail height *vs* length)	−0.357 to 0.166
Tail beat frequency and size	−0.661 to 0.097
Tail beat frequency and shape (tail height *vs* length)	−0.071 to 0.373
Size and shape (tail height *vs* length)	−0.389 to 0.040

$P = 0.002$). This suggests that increasing body size may be one mechanism by which tadpoles can increase burst swim speed. No other trait pairs displayed significant correlations in evolution (Table 2).

DISCUSSION

Anuran species differed in burst swim speed capabilities, but differences could not be attributed to either habitat or family effects alone. Family, habitat and body size interact in determining swim speed capabilities; within a family × habitat group, species vary greatly in maximum burst swim speed capabilities. Note that this does not necessarily mean that no species have responded to selection from predators with increased burst swim speed abilities. However, the large variance among species within a habitat type suggests that there is not one single response in burst swimming speed required for anurans to persist in a particular habitat type. This may reflect the presence of alternative strategies available for persistence in a habitat type (Richardson, 2001).

Differences among species in the allometric relationship of burst swim speed with body size suggest that some species are undergoing (or have undergone) more intense selection for increased burst swimming speed. In those animals in which fast swimming is favoured, individuals should maximize swim speed for their size; as individuals grow, the maximum swim speed possible will also increase. Thus, in tadpoles for which there is selection to maximize swim speed, we expect a strong and positive relationship between size and burst swim speed. However, in tadpoles where swim speed has not responded to selection pressures, we do not expect this relationship because, as animals grow, they will not necessarily realize their increased potential for swim speed (Fig. 3). This phenomenon has also been observed in damselfly larvae that are known to be under selection for increased swimming speed (McPeek *et al.*, 1996). Significantly faster burst swim speeds observed in three of the four ranid species studied suggest that species of this family have responded to selection for increased swimming speed.

Slower burst swim speeds observed in bufonid species may reflect the ability of bufonids to use traits other than fast swim speed to avoid predation. Most bufonid tadpoles are

thought to be unpalatable and may avoid predation using this trait instead (Formanowicz and Brodie, 1982; Brodie and Formanowicz, 1987; Peterson and Blaustein, 1991, 1992). Similarly, slower swim speeds observed in some hylids suggest they may not use increased burst swim speed to avoid predation. Some hylids using dragonfly ponds are known to be phenotypically plastic, increasing tail fin height in the presence of *Anax* predators (Smith and Van Buskirk, 1995; McCollum and Van Buskirk, 1996; Van Buskirk *et al.*, 1997). These hylids generally have dramatic tail fin sizes (personal observation) and, when developing in the presence of *Anax*, also acquire dramatic red coloration of the tail fin (McCollum and Van Buskirk, 1996; Skelly, 1997). Coloration of the tail fin may act as an anti-predator mechanism by deflecting predator attacks away from the body to the tail where strikes are less likely to be lethal (Caldwell, 1982; Wilbur and Semlitsch, 1990; McCollum and Van Buskirk, 1996; Skelly, 1997). Several other anti-predator mechanisms may succeed for tadpoles. Larvae of the dragonfly hylid *Hyla gratiosa* grow to a very large size, outgrowing gape-limited predators in the pond.

The differences in allometric relationships among species calls into question the common practice of reporting tadpole swimming speeds in units of body length per second (Wassersug and Hoff, 1985; Wassersug, 1989). This ratio measurement assumes that all animals have the same relationship between body size and absolute swimming speed. The results presented here show this to be untrue. Thus, comparisons of swim speeds between species when burst swim speed is presented in units of body length per second are difficult to interpret in a meaningful ecological or evolutionary context. Consideration of differences in allometric relationships for different species is vital for a complete understanding of how species have responded to selection pressures (McPeek *et al.*, 1996).

Tadpole body shape may partially reflect differences in microhabitat use across species. Some species (e.g. *Hyla gratiosa*, *H. versicolor*) tend to be found higher in the water column, often sitting stationary in the middle of the water, whereas other species (e.g. *H. cinerea*, *Pseudacris crucifer*) are found at the bottom of the pond (personal observation). Species found mid-water appear generally to have broader tail fins and this may reflect the need for increased stability (Vogel, 1994). Thus, tadpole shape may reflect selection pressures for swim speed and microhabitat use. A more thorough analysis of tadpole shape and the trade-offs between changes in shape, increased speed, stability and manoeuvrability in the water is needed to test this idea.

The results of this study demonstrate that, for some lineages at least, multiple mechanisms for dealing with the challenges of new predators as diversification into new habitats occurs are viable. This is not to say that generalities regarding biotic interactions within a community and evolutionary responses to these interactions are not to be found. Rather, the results emphasize the need to consider the underlying mechanisms that lead to observed responses. Understanding these mechanisms will allow us to predict outcomes based on potential mechanisms we hypothesize to be functioning. However, it is also clear from this study that understanding community organization requires us to consider suites of traits and not each trait in isolation. Differential susceptibility to predators drives the segregation of many aquatic species into different community types (Wellborn *et al.*, 1996); in anurans, this segregation is driven not by a single key trait, but rather by a suite of differences among traits.

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