

## INFLUENCE OF TAIL SHAPE ON TADPOLE SWIMMING PERFORMANCE

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### Summary

Many tadpoles respond to insect predators by developing deeper, and sometimes longer, tails. It has been assumed that the larger tail enhances aspects of swimming performance, because deep-tailed tadpoles survive well when confronted with hunting predators. We tested this hypothesis using both naturally occurring and surgically created variation in tail morphology of *Hyla versicolor* tadpoles. We measured swimming performance (maximum speed, time to reach a 2.5 cm radius, and angle of escape) and morphology (size and shape of the body and tail) in 288 tadpoles, of which half possessed the predator-induced morphology and the other half were from predator-free ponds. Large tadpoles swam faster than small ones, and shape was significantly correlated with size-corrected swimming performance. The fastest tadpoles had relatively shallow bodies and tail fins, and short tails; there was no difference in swimming performance between predator-induced and no-predator tadpoles. We performed an experiment to create independent variation in tail depth

and length by surgically manipulating tail shape in 270 tadpoles. Three tail-length treatments reduced the length of the tail fin by 21%, 34% and 55%; three tail-depth treatments reduced the maximum depth of the tail fin by 11%, 34% and 59%; two additional treatments controlled for the effects of anaesthesia and surgery. The angle of escape was unaffected by surgery. Maximum speed and minimum escape time were both significantly impaired by the high-removal treatments, but showed no evidence of decline until 30% of the tail (length or depth) was removed. These results suggest that the relatively deep tails in predator-induced tadpoles (approximately 10% deeper than in no-predator tadpoles) do not improve performance in burst swimming. Thus, predator-induced tadpoles are less vulnerable to predation for reasons other than enhanced swimming performance.

Key words: anuran, larva, *Hyla versicolor*, induced defence, locomotion, morphology, phenotypic plasticity, swimming, tadpole.

### Introduction

Simulation models and kinematic analyses of swimming in small aquatic vertebrates have revealed performance consequences of variation in body form. These analyses address the way in which swimming is affected by muscular activity, body shape and stiffness, and the configuration of fins on the tail or body (Webb, 1984; Wassersug and Hoff, 1985; Jordan, 1992; Jayne and Lauder, 1995; Long and Nipper, 1996; Walker and Westneat, 1997). Some studies suggest ways in which behaviour and body form may be adapted to challenges posed by feeding, predator escape or life history transitions. For example, Liu et al. (1996, 1997) concluded that the globose body shape of anuran larvae may have evolved because it enables tadpoles to grow hind legs prior to metamorphosis without loss of locomotor efficiency.

Kinematic studies generally do not explore the consequences of small gradations in body form, even though small differences among individuals may be of considerable evolutionary importance. Many amphibian larvae, for example, develop large tails when they are exposed to waterborne chemicals signalling the risk of predation (Smith

and Van Buskirk, 1995; Van Buskirk and Schmidt, 2000). Tail fin depth typically increases by approximately 5–15%; tail length can either increase or decrease, but usually does not change by more than 10%. Tadpoles possessing the predator-induced phenotype survive better when exposed to hunting predators than do tadpoles having the no-predator phenotype (McCollum and Van Buskirk, 1996). The mechanisms underlying the induced defence are not well understood, but the deeper tail fin has repeatedly been proposed to enhance swimming performance (McCollum and Van Buskirk, 1996; Van Buskirk et al., 1997; Doherty et al., 1998; Lardner, 2000; Van Buskirk and McCollum, 2000). Existing biomechanical analyses neither support nor refute this possibility because they do not address the consequences of subtle changes in shape.

Direct manipulation of the phenotype is an effective approach to studying the performance consequences of relatively small levels of phenotypic variation because it allows single traits to be altered while holding constant all other aspects of the phenotype (Benkman and Linholm, 1991; Lauder, 1996; Sinervo and Basolo, 1996). Here, we directly

manipulate the shape of the tail fin in tadpoles to test the hypothesis that the phenotypic response to predators improves swimming performance. Our surgical approach allows independent assessment of the consequences of tail fin length and depth, while all other aspects of shape, physiology and behaviour remain unchanged.

We chose the gray treefrog (Anura: Hylidae; *Hyla versicolor*) for this study because of its dramatic morphological response to predators and because tadpoles induced by exposure to predators are known to resist predation better than tadpoles from predator-free ponds. *H. versicolor* is abundant in most aquatic habitats at the University of Michigan's E. S. George Reserve in southern Michigan, USA, where we conducted this work. Compared with tadpoles from predator-free ponds, those in ponds with insect predators develop large tails and relatively small bodies, and decrease the time they spend feeding and swimming (Van Buskirk and McCollum, 1999). The tail fin of *H. versicolor* also becomes brightly coloured in the presence of predators, although this response is not found in most anuran larvae. The reaction is apparently triggered by chemical cues, since it can be induced by predators that are confined within cages. In short-term predation experiments, the predator-induced phenotype of *H. versicolor* has a lower risk of mortality than does the no-predator phenotype, at least under conditions in which encounters between tadpoles and predators are frequent (Van Buskirk and McCollum, 1999, 2000). Moreover, we know that predation is biased with respect to tadpole shape, because individuals with relatively shallow tail fins are killed disproportionately often even within groups of individuals having similar behaviour and sharing the same history of exposure to predators (Van Buskirk and Relyea, 1998; Van Buskirk and McCollum, 2000). These results suggest that deep tail fins enable tadpoles to accelerate, turn suddenly or swim rapidly during predator escape. Our study was designed to test this hypothesis.

### Materials and methods

We used two methods to assess the relationship between tail shape and swimming performance. First, we tested for a correlation between naturally occurring variation in morphology and swimming performance, and then we experimentally manipulated the shape of the tail and monitored the resulting change in performance. Tadpoles for both kinds of analyses came from artificial ponds in which we produced morphological variation similar to that observed in nature by manipulating the presence and absence of caged predators (Van Buskirk and McCollum, 1999).

Methods for rearing tadpoles of *Hyla versicolor* Le Conte are reported in detail elsewhere (e.g. McCollum and Van Buskirk, 1996). Briefly, 250 hatchling tadpoles were added to each of 10 outdoor plastic tanks (2.6 m<sup>2</sup> surface area, 1000 l volume), which were grouped into five blocks according to spatial proximity. Tanks were stocked with a standard recipe of leaf litter, pond water and zooplankton to establish semi-

natural aquatic communities. Within half the tanks, three plastic screen cages (1 l volume) enclosed *Anax longipes* dragonfly larvae (caged-*Anax* treatment); the remaining tanks contained three empty cages (no-predator treatment). The *A. longipes* were fed tadpoles every other day throughout the experiment to ensure that *H. versicolor* within the caged-*Anax* treatment were exposed to chemical evidence of predation.

### Measurements of morphology

We measured morphology and swimming performance, and surgically manipulated tail shape, on tadpoles collected from the tanks on 16 days between 19 July and 22 August 1996, beginning when the tadpoles were 28 days old. Morphology was measured from photographs taken both before and after surgery. Tadpoles were placed into a water-filled Plexiglas chamber and photographed in side view using a 35 mm camera equipped with a macro lens and Kodak TMAX black-and-white film. We used image-analysis software to measure six traits from images of negatives projected onto a computer screen (Fig. 1A). The traits were body length and maximum body depth, tail fin length and maximum tail depth, tail fin depth halfway between the base and tip of the tail, and tail muscle depth where it meets the body. Traits were chosen to reflect the overall size and shape of the body and tail.

### Measurements of swimming performance

We measured swimming performance immediately after

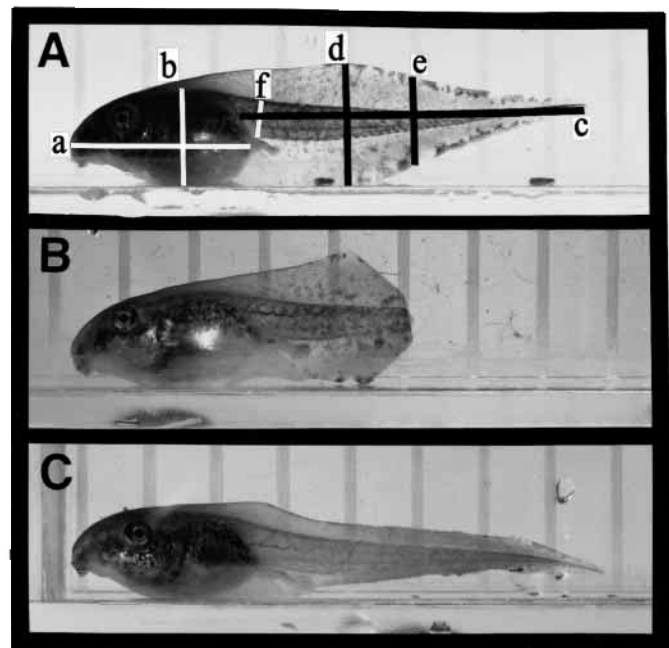


Fig. 1. Photographs of *Hyla versicolor* tadpoles illustrating the locations of measurements (A) and the results of the high-length (B) and high-depth (C) surgery treatments. The vertical bars behind the tadpole are 5 mm apart. The six measurements are body length (a), maximum body depth (b), tail length (c), maximum tail depth (d), tail depth at half-length (e) and tail muscle depth at the base (f).

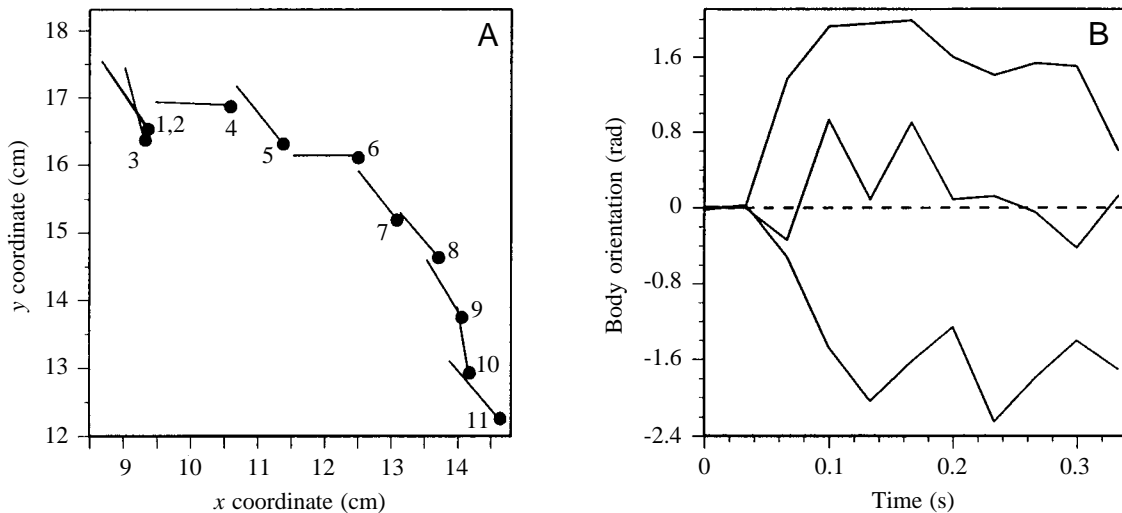


Fig. 2. Examples of swimming records for *Hyla versicolor* tadpoles. (A) The path traced by a tadpole during a fast start. Lines connect the base of the tail and the tip of the nose (represented by a filled circle), and numbers refer to the 11 frames sampled at 33 ms intervals. (B) The change in body orientation during fast starts of three representative tadpoles. Orientation is the angle of the body axis relative to the initial angle. The upper and lower trajectories represent tadpoles that turned sharply within 60–100 ms of starting, while the tadpole with the middle trajectory (the same record depicted in A) swam forward in an almost straight line.

tadpoles had been taken from the tanks by videotaping at  $30 \text{ frames s}^{-1}$  in a glass-bottomed tray (28 cm $\times$ 28 cm, filled to a depth of 1.8 cm). Sampling at 30 Hz would be insufficient for high-speed kinematic analysis, but is well suited for measuring direction and speed of travel, which are the focus of our interest here. The camera viewed tadpoles from below, through a glass shelf, to eliminate the distorting influence of waves at the water surface. We waited for tadpoles to come to rest, then motivated them to swim by prodding them near the base of the tail with a wire. Every tadpole was filmed until it swam rapidly at least three times; in most cases, this occurred within 15–20 s, and subsequent swims were slower as the tadpole tired.

Analyses were restricted to the three fastest swimming records for each tadpole, including the first 11 frames of each record (333 ms) beginning when the animal was completely immobile. During the 11 frames, the tadpole usually executed a C-start (Webb, 1986), reached maximum velocity and began to slow down. We digitized the locations of the base of the tail and tip of the snout in each frame and calculated the direction in which the tadpole was facing and the mid-body position from these two landmarks. To reduce measurement error, we used lowess smoothing (TriMetrix, 1993) on the raw position data and estimated all subsequent positions from the smoothed results. Fig. 2 illustrates an example of digitized locations during an escape sequence, together with changes in orientation that occur during escape.

We measured three components of swimming performance from each record: maximum speed, escape time and angle of escape. These were chosen to reflect different components of swimming performance that might function during predator escape (Feder, 1983; Weihs and Webb, 1984; Watkins, 1996; Hoff and Wassersug, 2000). We estimated velocity for each pair of video frames and saved the fastest speed from each

record; swimming velocity may be important during pursuit by visual predators. We also estimated the elapsed time until the tadpole moved 2.5 cm radially from its point of origin, which we consider to be of ecological importance because it reflects the time required to evade a strike from a sit-and-wait predator. This measure is related to velocity and linear acceleration, but it differentiates between a tadpole that swims in a circle, and thus gets nowhere, and one that swims in a straight line, thus putting distance between itself and the predator. Our third measure of swimming performance was the angle of escape, measured as the difference between the direction in which the tadpole was facing after 100 ms and the direction in which it had been facing prior to executing the C-start. Preliminary inspection of the videotapes indicated that most tadpoles adopted their angle of escape within approximately 100 ms (Fig. 2B). This measure may reflect a tadpole's ability to manoeuvre quickly to escape a predator at close range.

The three performance measures were moderately repeatable: 0.71 for velocity and 0.64 for time to 2.5 cm (both  $P < 0.0001$ ) and 0.37 for escape angle ( $P = 0.19$ ) (Lessells and Boag, 1987). Nevertheless, we restricted our analyses to the maximum velocity, minimum time to travel 2.5 cm and maximum escape angle for every tadpole, under the assumption that less extreme records reflected events in which the tadpole was not performing at full capacity.

#### Surgical treatments

The experiment included eight treatments designed to distinguish the contributions of tail depth and tail length to swimming performance. In all cases, tadpoles were lightly anaesthetized in benzocaine (ethyl *p*-aminobenzoate). Three treatments reduced the length of the tail by approximately 10%, 30% and 50% (Fig. 1B), and three treatments reduced

tail depth by approximately 10%, 30% and 50% (Fig. 1C). These levels were chosen to include and exceed the natural phenotypic response to predators. Ideally, we would bracket natural phenotypic values by adding length or depth to tails, but we had no way to augment tail proportions. Two additional treatments served as controls. To check for the effects of benzocaine, tadpoles in an 'anaesthesia control' treatment underwent anaesthesia but were not cut. To control for the effects of benzocaine and surgery, we anaesthetized tadpoles in the 'sham' treatment and made incisions in the tail fin immediately above and below the muscle. These cuts did not extend to the perimeter of the tail or affect its surface area. We sampled tadpoles from both tanks in a block at the same time, removing them in groups of eight from each tank and assigning them to treatments at random.

We trimmed tissue using a scalpel in a fashion that maintained normal tail shape as far as possible. For the length treatments, we shortened the tail by the desired amount and then restored the tail's tapered profile (see Fig. 1B). For the depth treatments, we removed tissue from both above and below the muscle to reduce the maximum depth of the tail (Fig. 1C). Most tadpoles recovered from anaesthesia within a few minutes, and the missing portions of the tail regenerated within approximately 10 days.

After surgery, tadpoles were placed individually into plastic cups containing 150 ml of water. We fed the tadpoles with rabbit chow and allowed them to recover until the following day, when we photographed them and measured their swimming performance again.

#### Statistical analyses

We collected 336 tadpoles for the surgery experiment (42 in each treatment), but some had to be discarded because of mortality, lost swimming data or photographic accidents either before or after surgery. In the end, 288 tadpoles had complete records prior to surgery, and 270 of these had complete data after surgery as well. Missing records were distributed fairly evenly among the eight treatments, so that every treatment contained 32–37 tadpoles.

Swimming speed and all measures of morphology increased strongly with body size, and time to 2.5 cm decreased with size, so our first step was to standardise traits to remove the effects of size. Body size was defined as the first component from a principal components analysis (PCA) on the covariance matrix of the three morphological measurements that were unaffected by surgery (body length, body depth and tail muscle depth). The first axis explained 94% of the variance in the original data and was positively correlated with all three traits (the coefficients of the first eigenvector were 0.85, 0.50 and 0.18 for body length, body depth and tail muscle depth). Size-corrected swimming performance, and body and tail shape, were defined as the residuals of original variables after regression on body size. All regressions were highly significant ( $r^2$  ranging from 0.50 to 0.98 for morphology and from 0.13 to 0.18 for swimming performance). Transformation was unnecessary because all variables showed linear relationships

with size. We did not correct escape angle for size because it was unrelated to body size ( $r^2=0.005$ ), and we always analysed the absolute value of escape angle under the assumption that turns to the right and left were equivalent.

We used canonical correlation analysis to test whether swimming performance was associated with morphology in the sample of unmanipulated tadpoles prior to surgery. Canonical analysis, a multivariate extension of the standard bivariate correlation analysis, tests whether two sets of variables are associated with each other. The analysis finds the linear combination of each set of variables, called a canonical variate, that maximizes the correlation between the two canonical variates (SAS Institute, 1990). Here, we asked whether there was a significant correlation between a canonical variate representing swimming performance (estimated from three variables: the absolute value of escape angle and size-corrected maximum speed and time to travel 2.5 cm) and a variate representing morphology (estimated from four variables: size-corrected body length, body depth, tail length and tail fin depth). This analysis did not include tail muscle depth, because all shape measurements were defined as linear combinations of body size and the three traits employed in the PCA. Muscle depth was chosen for elimination because it has twice as much measurement error as the other four traits, perhaps because of the small size of the muscle and the difficulty of identifying its lower edge (J. Van Buskirk, unpublished data).

Morphological changes in response to caged *Anax longipes* were analysed using analysis of variance (ANOVA) on tank means from the rearing experiment, since the tanks were independent experimental units. Blocks were treated as a random factor, and treatment was tested over its interaction with block. We used the Bonferroni method to adjust the acceptable  $\alpha$ -value for the five traits upon which the analysis was performed (Morrison, 1976). For the surgical experiment, tadpoles were treated as independent experimental units, and we analysed changes in swimming performance due to surgical treatment using multivariate analysis of variance (MANOVA). For each tadpole, we calculated the difference in its tail shape and swimming performance measures (value after surgery minus value before surgery) and asked whether changes in tail length or depth could explain changes in swimming speed, escape time and escape angle.

## Results

### Effects of experimental treatments on tadpole morphology

*Hyla versicolor* tadpoles responded to caged dragonflies by growing relatively short bodies and long and deep tails (Table 1; Fig. 3). The body was approximately 2% deeper in the presence of *Anax longipes*, the tail muscle was approximately 5% deeper and the tail fin was 10% deeper and longer. Thus, the phenotypic response to predators increased the scope of morphological variation within our sample of tadpoles prior to manipulation.

The surgery experiment successfully manipulated the intended traits (Fig. 4). The low-, medium- and high-depth

Table 1. Mean values of morphometric traits and performance measurements for predator-induced and no-predator *Hyla versicolor* tadpoles sampled from the tank experiment

	No-predator treatment		Caged- <i>Anax</i> treatment	
	Uncorrected	Size-corrected	Uncorrected	Size-corrected
<b>Morphological responses</b>				
Body size (PC1 score)	2.933±0.066	–	3.299±0.035	–
Body length (cm)	1.104±0.025	–0.004±0.001	1.238±0.014	–0.011±0.002
Body depth (cm)	0.584±0.013	0.009±0.002	0.678±0.004	0.019±0.004
Tail length (cm)	1.770±0.017	0.020±0.040	2.208±0.038	0.168±0.014
Tail fin depth (cm)	0.639±0.014	–0.031±0.005	0.812±0.018	0.034±0.009
Tail muscle depth (cm)	0.206±0.005	–0.009±0.001	0.246±0.005	0.001±0.002
<b>Swimming performance responses</b>				
Maximum speed (cm s <sup>-1</sup> )	26.42±1.226	–0.429±0.863	27.49±1.682	–2.034±1.616
Time to 2.5 cm (s)	0.140±0.005	0.001±0.004	0.139±0.006	0.010±0.005
Escape angle at 100 ms (rad)	1.631±0.045	–	1.594±0.076	–

Values are treatment means ± 1 S.E.M. based on five replicate plastic tanks for the original measures and residuals after regression against body size (size-corrected measures) ( $N=288$  tadpoles).  
 Escape angle was not corrected for body size.  
 Body size is defined as the first axis from a principal component (PC) analysis.

treatments reduced tail fin depth by 11 %, 34 % and 59 %, respectively; no other trait was affected by surgery. The three length treatments reduced tail length by 21 %, 34 % and 55 % and did not significantly alter any other traits. These results illustrate that the surgery treatments successfully altered

the target traits and produced a broad range of phenotypic variation.

#### Swimming performance in unmanipulated tadpoles

Swimming performance was highly variable. Maximum speed ranged from 10 to 40 cm s<sup>-1</sup>, averaging approximately 27 cm s<sup>-1</sup> (Fig. 5A; Table 1). Minimum time to reach 2.5 cm varied from approximately 100 to 300 ms and averaged 140 ms (Fig. 5B; Table 1). The distribution of escape angles indicated that most tadpoles turned sharply either left or right while executing a fast start (Fig. 6). There were no differences between the predator-induced and no-predator phenotypes in any of the three measures of swimming performance (maximum speed,  $F_{1,4}=1.15$ ,  $P=0.34$ ; time to 2.5 cm,  $F_{1,4}=3.81$ ,  $P=0.12$ ; escape angle,  $F_{1,4}=0.29$ ,  $P=0.62$ ; ANOVAs on tank means).

Swimming performance, corrected for body size, was significantly related to body and tail shape in our sample of unmanipulated tadpoles (Table 2; Fig. 5C). The coefficients for maximum speed and time to 2.5 cm in the first canonical variate of swimming were much greater than the coefficient for escape angle, indicating that this axis was related primarily to linear velocity. The canonical variate of morphology that was most closely related to swimming performance was dominated by body depth and the size of the tail: tadpoles with large scores on this axis had relatively deep bodies with long and deep tails. The relationship between morphology and swimming performance was imprecise but highly significant: tadpoles with deep bodies, deep tail fins and long tails swam relatively slowly, and this relationship was similar for both the predator-induced and no-predator phenotypes (Fig. 5C). In a covariance analysis testing whether morphology affected swimming similarly for the two phenotypes, the canonical variable representing swimming performance varied

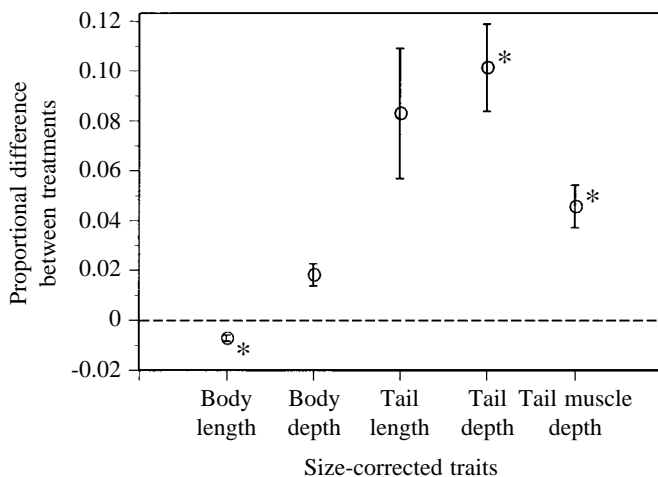


Fig. 3. Responses of *Hyla versicolor* tadpoles to caged dragonflies, for five measures of body and tail shape (residuals after regression against body size). The proportional change is the difference between predator-induced tadpoles and tadpoles from predator-free ponds divided by the value in the no-predator ponds. The dashed line represents the case in which the two phenotypes do not differ in shape. Tadpoles developed deeper and more muscular tails in the presence of caged *Anax longipes* dragonfly larvae, and somewhat shorter bodies. Values are means ± 1 S.E.M. of five replicate tanks; 38–42 tadpoles were sampled from each tank. Traits indicated by an asterisk were significant at  $P < 0.01$ , which is corrected for multiple comparisons.

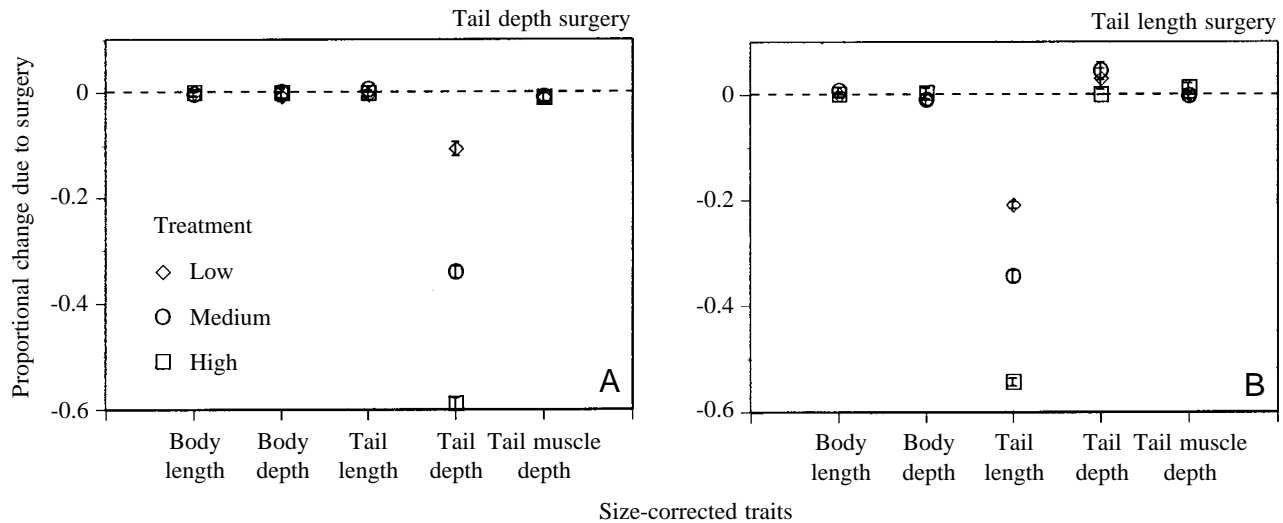


Fig. 4. Changes in body and tail shape due to the surgery treatments expressed as proportions of the value prior to surgery. The depth treatments affected only tail depth (A) and the length treatments affected only tail length (B). Values are means  $\pm$  1 s.e.m. for 38–42 individual tadpoles for each treatment; the dashed line represents the case in which a trait did not change.

significantly with the morphology variable ( $F_{1,284}=26.65$ ,  $P<0.001$ ) and between phenotypes ( $F_{1,284}=8.42$ ,  $P=0.004$ ), while the morphology-by-phenotype interaction was not significant ( $F_{1,284}=0.05$ ,  $P=0.829$ ). The prediction emerging from this comparative analysis is that small surgical reductions in tail length and depth should, in contrast to expectations derived from other sources (e.g. Wassersug, 1989; Van Buskirk et al., 1997).

#### Swimming performance in surgically manipulated tadpoles

Manipulation of *H. versicolor* tail shape dramatically affected swimming performance, but only when large amounts

of the tail fin were removed (Fig. 7). Swimming speed decreased strongly and time to 2.5 cm increased in the high-depth and high-length treatments. The medium and low treatments had no significant impact on swimming performance, and escape angle showed no response to any treatment. Multivariate analysis of all three performance measures revealed a significant treatment effect, indicating that there was substantial variation among the eight surgery treatments in performance (Table 3). The effect of tadpole phenotype was not significant in the MANOVA, but the significant univariate responses of maximum speed and time to travel 2.5 cm suggest that predator-induced and no-predator tadpoles were somewhat different in these performance

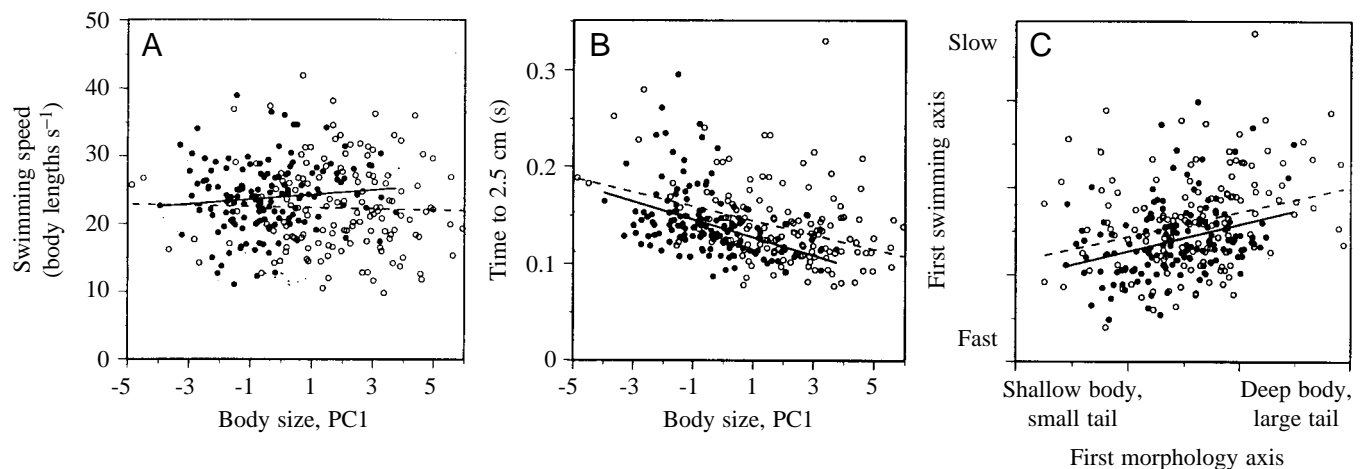


Fig. 5. Swimming speed (A) and time to swim 2.5 cm (B) for 288 individual *Hyla versicolor* tadpoles and the relationship between canonical variables for swimming performance and morphology (C). In A and B, the x axis is a body size variable defined as the first morphology axis. Both measures of swimming performance were highly variable in this sample of unmanipulated individuals. Tadpoles from the no-predator treatment were smaller and had slightly better size-specific swimming performance than those from the ponds with caged predators (open circles, broken lines). Canonical analysis showed that those with relatively deep bodies and tails swam poorly, and the relationship between morphology and swimming did not differ between the two phenotypes. PC1, principal component 1.

Table 2. Canonical correlation analysis testing the relationship between three measures of swimming performance and four measures of shape in *Hyla versicolor* tadpoles

Canonical variate	Canonical correlation	$r^2$	$F$	$P$	Standardized coefficients of the variate						
					Swimming variate			Morphology variate			
					Time to 2.5 cm	Maximum velocity	Escape angle	Body length	Body depth	Tail length	Tail fin depth
1	0.321	0.103	2.95	0.0005	0.570	-0.484	0.058	0.298	1.340	0.787	0.662
2	0.108	0.012	0.67	0.6781	0.605	0.632	-0.949	1.193	0.229	0.649	1.255
3	0.049	0.002	0.33	0.3347	1.487	1.511	0.324	0.468	0.154	1.054	-0.237

Body size was not included because both morphology and swimming performance (except escape angle) were residuals after regression on body size.

The significance test is a maximum likelihood estimate of the probability that the correlation between that pair of canonical variates, and between all subsequent pairs of variates, is equal to zero.  $N=288$  tadpoles for which the full set of variables was available.

measures. On average, predator-induced tadpoles had a slightly lower maximum speed and a longer time to 2.5 cm. The non-significant surgery-by-phenotype interaction shows that the two phenotypes responded similarly to surgery. Planned *a priori* contrasts tested for effects of specific changes in tail shape, in each case comparing a surgery treatment with the two controls (Table 3). Only the high-depth and high-length treatments differed from controls in the MANOVA. Univariate analyses indicated that this effect arose from a decline in

maximum swimming velocity and an increase in minimum time to 2.5 cm, while escape angle showed no change from the controls.

### Discussion

These results demonstrate that some components of swimming performance in *Hyla versicolor* tadpoles are related to body and tail shape, and that a reduction in the surface area of the tail fin causes a decline in performance. At some level, these findings were expected. However, the details of both results were surprising. Tadpoles that naturally possessed large tails relative to their body size, and those that exhibited predator-induced morphology, did not swim better than tadpoles with smaller tail fins. Similarly, experimental reduction in tail length or depth by up to approximately 30% had no significant impact on swimming speed or the angle of escape. These results have important implications for interpreting the adaptive basis of phenotypic plasticity in anuran larvae and highlight the importance of functional studies to test evolutionary hypotheses.

Experiments convincingly show that predators impose natural selection for increasing tail fin depth in several species of larval amphibian (Van Buskirk et al., 1997; Van Buskirk and Relyea, 1998; Van Buskirk and Schmidt, 2000). In every case, survival when exposed to predators is higher for tadpoles with relatively deep tail fins, irrespective of other differences in morphology and behaviour. At least three functional interpretations are consistent with these results. First, a relatively large tail may enhance swimming speed, acceleration or manoeuvrability, thereby improving a tadpole's ability to escape an attacking predator. Second, a relatively large tail may improve escape capabilities for reasons other than swimming performance. Finally, the tail fin may not influence predator escape directly, but may be phenotypically correlated with some other unmeasured trait that is itself a target of selection. This last possibility is difficult to evaluate on the basis of available information, but seems unlikely given that the plastic response in tail fin depth exceeds that of any other trait.

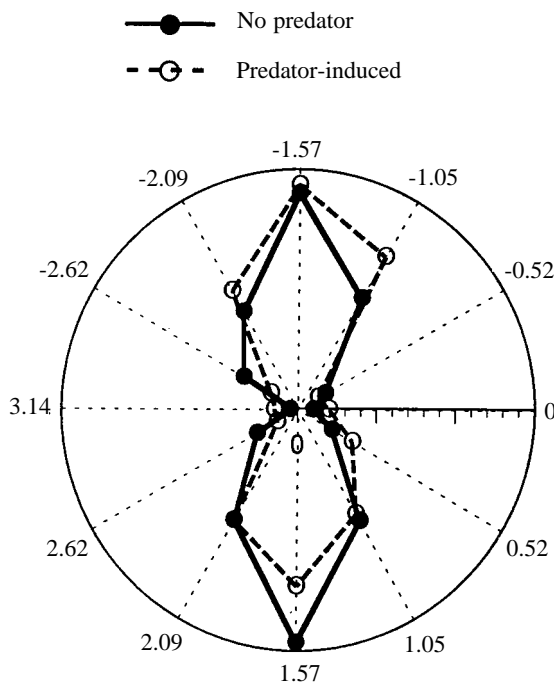


Fig. 6. Distribution of escape angles (in rad) during fast-starts by 288 *Hyla versicolor* tadpoles sampled from artificial ponds with or without caged dragonfly predators. Escape angle was measured 100 ms after the tadpole began to swim and is scored relative to the initial orientation of the tadpole ( $=0$  rad). The scale on the radial axis is the number of tadpoles with angles falling within categories of width  $\pi/6$  rad. Most tadpoles of both phenotypes turned sharply to the left or right.

Table 3. *Multivariate and univariate analyses of variance testing for effects of changes in tail shape on swimming performance in 270 Hyla versicolor tadpoles*

Comparison	Multivariate test			Univariate tests					
				Maximum speed		Time to 2.5 cm		Turning angle	
	d.f.	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Main effects									
Tadpole phenotype	3,252	1.91	0.1287	5.37	0.0213	4.41	0.0366	0.00	0.9910
Surgery treatment	21,724	3.24	0.0001	7.06	0.0001	7.51	0.0001	1.11	0.3600
Surgery × phenotype	21,724	0.43	0.9817	0.55	0.7960	0.50	0.8323	0.25	0.9732
Contrasts with controls									
Depth-low	3,252	0.88	0.4542	0.11	0.7441	0.01	0.9265	2.19	0.1402
Depth-medium	3,252	0.08	0.9724	0.07	0.7976	0.01	0.9218	0.00	0.9752
Depth-high	3,252	4.90	0.0025	11.99	0.0006	13.39	0.0003	0.06	0.8067
Length-low	3,252	0.72	0.5424	0.15	0.6994	0.10	0.7575	2.03	0.1552
Length-medium	3,252	2.23	0.0849	5.69	0.0178	1.74	0.1882	0.73	0.3926
Length-high	3,252	13.67	0.0001	33.07	0.0001	36.21	0.0001	0.40	0.5259

The response vector in the MANOVA is defined by the change due to surgery in maximum swimming speed, minimum time to travel 2.5 cm and angle of escape measured after 100 ms.

Contrasts compare the six surgery treatments with the sham and anaesthesia controls.

Kinematic data and comparisons among species support the first interpretation for the tail depth response, but perhaps not for tail length. These studies suggest that increasing tail fin depth should improve performance in fast-start situations and sudden turns, but increasing tail length may not improve velocity or acceleration. Fish species with deep fins and large lateral surface areas accelerate and turn well, but generally have poor endurance (Webb, 1984; Weihs, 1989). The extensive literature on the kinematics of swimming in fish is of uncertain relevance for tadpoles, however, since tadpoles lack stiff fins and have a different body shape from that of fish (Hoff and Wassersug, 2000). In tadpoles, the kinematics of the deepest part of the tail during steady swimming are most appropriate for generating thrust (Wassersug and Hoff, 1985; Weihs, 1989; Liu et al., 1996), suggesting that the mid-tail provides a substantial fraction of the power for swimming. The same region of the tail probably contributes to linear and angular acceleration as well, since increasing the depth of the deepest part of the tail fin increases the surface area available for producing thrust. These studies also suggest that the long tapered portion of the tail is not sufficiently stiff to contribute to forward propulsion. Two possible functions of the tail tip, as a hydrofoil to reduce turbulence and as ballast to dampen the lateral excursion of the mid-tail region (Wassersug and Hoff, 1985; Wassersug, 1989), would probably not contribute to the sudden swimming responses involved in predator escape. To summarize, kinematic data for larval anurans support the interpretation that the morphological response to predators (at least for tail depth) may decrease tadpole vulnerability by improving fast-start performance and sudden turns.

Our results firmly reject this interpretation. Morphological variation of the magnitude observed in nature had no detectable effect on swimming performance. This was not a consequence

of inadequate statistical power. The experiment had sufficient replication to detect a decrease in maximum speed of approximately  $3 \text{ cm s}^{-1}$  (11 % of pre-surgery swimming speed), but there was not even a trend in performance until reductions in tail depth or length exceeded 30 % of the original tail dimension (Fig. 7). This suggests that the morphological responses to insect predators shown by most anuran larvae, which are usually in the range 5–15 %, could not possibly affect burst swimming speed or turning. The natural responses are far below the level at which we first observed any impact on swimming.

These findings encourage us to explore other possible explanations for phenotypic plasticity in tadpole tail shape and the benefits of a deep tail fin. There are at least two ways in which a relatively large tail might facilitate predator escape without directly improving swimming performance. First, a large tail may allow a tadpole to lose much of the fin without affecting subsequent swimming performance. Loss of up to 30 % of the tail depth can be sustained without adverse consequences for performance. This implies that a predator-induced tadpole (with 15 % extra tail depth to begin with) may be able to sustain more tail damage without affecting swimming than can a no-predator tadpole. Our results may argue against this hypothesis, since the non-significant interaction between tadpole phenotype and the surgery treatment indicates that the impact of surgery on the swimming performance of predator-induced and no-predator tadpoles was not very different. However, a proper test of this idea would require trimming constant amounts of tissue from each tadpole, rather than constant proportions as we did here.

A second possible function of the large and conspicuous tail is that it may attract the predator's attention, thereby deflecting strikes at the last instant away from the vulnerable body region and onto the tail (Caldwell, 1982). Strikes to the body itself are



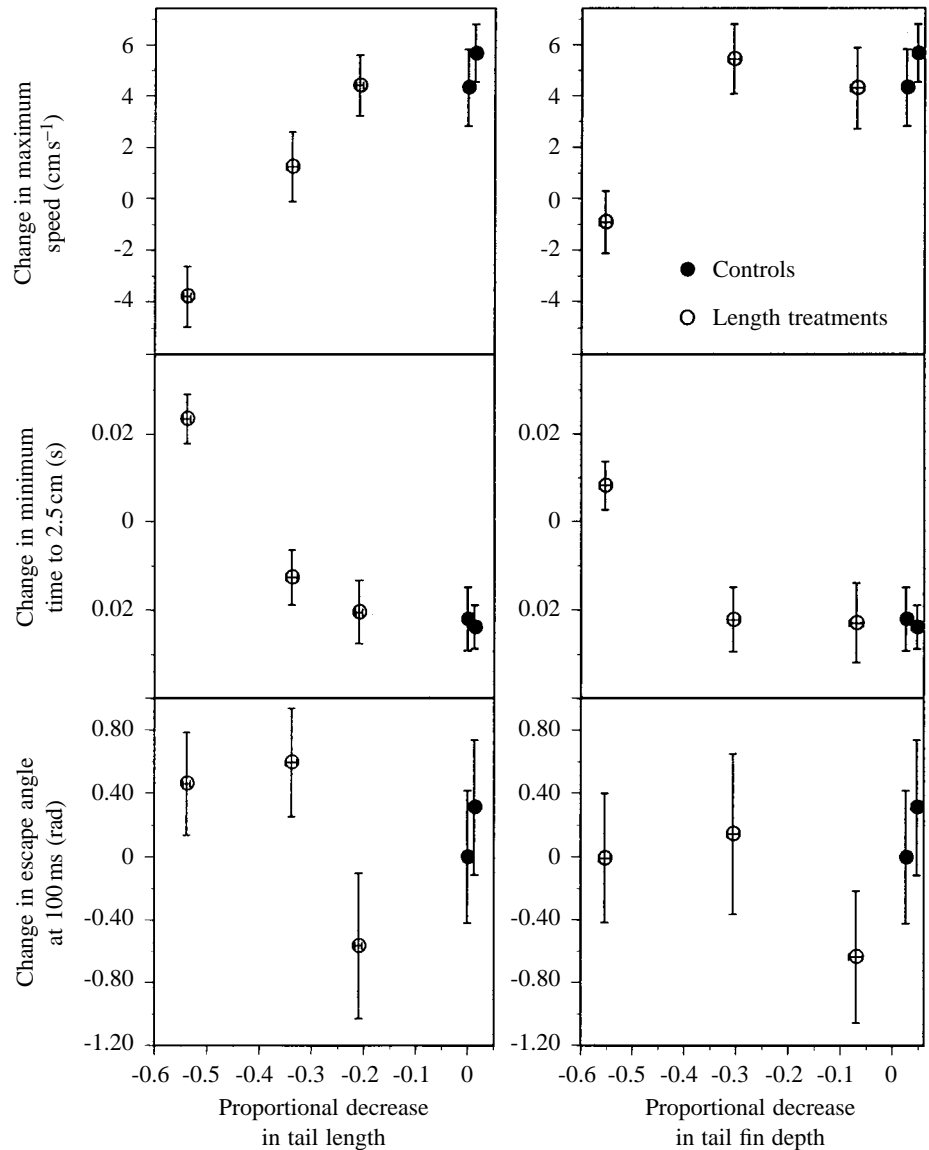


Fig. 7. Effects of changes in tail shape on the swimming performance of *Hyla versicolor* tadpoles. Performance and phenotype were measured for each tadpole before and after surgical treatment and are expressed as absolute differences (swimming performance) or proportional differences (tail shape). Swimming performance includes the maximum speed (top panels), the minimum time for the tadpole to travel 2.5 cm (middle) and escape angle relative to initial body angle measured after 100 ms (bottom). Tail shape includes tail length (left) and tail depth at the midpoint of the tail (right). Values are means  $\pm 1$  S.E.M. for 32–37 individual tadpoles from each treatment.

more deadly than those to the tail, because sections of the tail can be torn away to allow the tadpole to escape (Doherty et al., 1998), whereas a ruptured body wall is almost certainly lethal. That tadpoles are often attacked on the tail, but escape and survive, is supported by the frequency of tail damage in natural anuran populations (Caldwell, 1982; Blair and Wassersug, 2000). Also, the high levels of tail damage in predator-induced tadpoles after predation experiments suggest that relatively large tails may draw a disproportionate number of strikes (Van Buskirk and McCollum, 2000).

To summarize, although morphological variation in the tail fin is closely associated with survival in predation experiments, our data strongly suggest that the mechanism underlying the survival advantage does not involve fast-start performance or swimming speed. It is likely, but untested, that a large tail fin directly improves survival by other mechanisms. Alternatively, tail depth may increase because it is correlated with unknown and unmeasured traits that affect predator avoidance and escape, although a non-adaptive interpretation of the tail fin

response is difficult to reconcile with its taxonomic distribution (J. Van Buskirk and S. A. McCollum, unpublished data).

The present study underscores the important role of functional analyses in the study of adaptation. Selection experiments and estimates of quantitative genetic variables, widely used in evolutionary biology, can pinpoint agents of selection and predict evolutionary change in phenotypes, but they cannot identify mechanisms of selection. Manipulating a putative agent of selection may cause shifts in phenotypes, just as our manipulations of predators have caused selection on tadpole tail fins (e.g. Van Buskirk et al., 1997). Selection results can be combined with analyses of design and kinematics to build arguments about probable functions and mechanisms. However, none of these approaches can identify mechanisms of selection, which requires data on the functional consequences of phenotypic variation (Lauder, 1996). Our study provides an example in which the results of direct manipulation contradict the argument from design and kinematics.

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### References

- Benkman, C. W. and Lindholm, A. K.** (1991). The advantages and evolution of a morphological novelty. *Nature* **349**, 519–520.
- Blair, J. and Wassersug, R. J.** (2000). Variation in the pattern of predator-induced damage to tadpole tails. *Copeia* **2000** (in press).
- Caldwell, J. P.** (1982). Disruptive selection: a tail color polymorphism in *Acris* tadpoles in response to differential predation. *Can. J. Zool.* **60**, 2818–2827.
- Doherty, P. A., Wassersug, R. J. and Lee, J. M.** (1998). Mechanical properties of the tadpole tail fin. *J. Exp. Biol.* **201**, 2691–2699.
- Feder, M. E.** (1983). The relationship of air breathing and locomotion to predation on tadpoles. *Physiol. Zool.* **56**, 522–531.
- Hoff, K. V. S. and Wassersug, R. J.** (2000). Tadpole locomotion: axial movement and tail function in a largely vertebraless vertebrate. *Am. Zool.* (in press).
- Jayne, B. C. and Lauder, G. V.** (1995). Red muscle motor patterns during steady swimming in largemouth bass: effects of speed and correlations with axial kinematics. *J. Exp. Biol.* **198**, 1575–1587.
- Jordan, C. E.** (1992). A model of rapid-start swimming at intermediate Reynolds number: undulatory locomotion in the chaetognath *Sagitta elegans*. *J. Exp. Biol.* **163**, 119–137.
- Lardner, B.** (2000). Morphological and life history responses to predators in larvae of seven anurans. *Oikos* **88**, 169–179.
- Lauder, G. V.** (1996). The argument from design. In *Adaptation* (ed. M. R. Rose and G. V. Lauder), pp. 55–91. New York: Academic Press.
- Lessells, C. and Boag, P.** (1987). Unrepeatable repeatabilities: a common mistake. *Auk* **104**, 116–121.
- Liu, H., Wassersug, R. J. and Kawachi, K.** (1996). A computational fluid dynamics study of tadpole swimming. *J. Exp. Biol.* **199**, 1245–1260.
- Liu, H., Wassersug, R. and Kawachi, K.** (1997). The three-dimensional hydrodynamics of tadpole locomotion. *J. Exp. Biol.* **200**, 2807–2819.
- Long, J. H. and Nipper, K. S.** (1996). The importance of body stiffness in undulatory propulsion. *Am. Zool.* **36**, 678–694.
- McCollum, S. A. and Van Buskirk, J.** (1996). Costs and benefits of a predator-induced polyphenism in the gray treefrog *Hyla chrysoscelis*. *Evolution* **50**, 583–593.
- Morrison, D. F.** (1976). *Multivariate Statistical Methods*. New York: McGraw Hill.
- SAS Institute** (1990). *SAS/STAT User's Guide*, version 6. Cary, NC: SAS Institute Inc.
- Sinervo, B. and Basolo, A. L.** (1996). Testing adaptation using phenotypic manipulations. In *Adaptation* (ed. M. R. Rose and G. V. Lauder), pp. 149–185. New York: Academic Press.
- Smith, D. C. and Van Buskirk, J.** (1995). Phenotypic design, plasticity and ecological performance in two tadpole species. *Am. Nat.* **145**, 211–233.
- TriMetrix** (1993). *Axum Technical Graphics and Data Analysis, Reference Manual*. Seattle: TriMetrix Inc.
- Van Buskirk, J. and McCollum, S. A.** (1999). Plasticity and selection explain variation in tadpole phenotype between ponds with different predator composition. *Oikos* **85**, 35–39.
- Van Buskirk, J. and McCollum, S. A.** (2000). Mechanisms of an inducible defence in tadpoles: morphology and behaviour influence mortality risk from predation. *J. Evol. Biol.* **13**, 336–347.
- Van Buskirk, J., McCollum, S. A. and Werner, E. E.** (1997). Natural selection for environmentally induced phenotypes in tadpoles. *Evolution* **51**, 1983–1992.
- Van Buskirk, J. and Relyea, R. A.** (1998). Selection for phenotypic plasticity in *Rana sylvatica* tadpoles. *Biol. J. Linn. Soc.* **65**, 301–328.
- Van Buskirk, J. and Schmidt, B. R.** (2000). Predator-induced plasticity in larval newts: trade-offs, selection and variation in nature. *Ecology* (in press).
- Walker, J. A. and Westneat, M. W.** (1997). Labriform propulsion in fishes: kinematics of flapping aquatic flight in the bird wrasse *Gomphosus varius* (Labridae). *J. Exp. Biol.* **200**, 1549–1569.
- Wassersug, R. J.** (1989). Locomotion in amphibian larvae (or ‘Why aren’t tadpoles built like fishes?’). *Am. Zool.* **29**, 65–84.
- Wassersug, R. J. and Hoff, K. V. S.** (1985). Kinematics of swimming in anuran larvae. *J. Exp. Biol.* **119**, 1–30.
- Watkins, T. B.** (1996). Predator-mediated selection on burst swimming performance in tadpoles of the Pacific tree frog, *Pseudacris regilla*. *Physiol. Zool.* **69**, 154–167.
- Webb, P. W.** (1984). Body form, locomotion and foraging in aquatic vertebrates. *Am. Zool.* **24**, 107–120.
- Webb, P. W.** (1986). Locomotion and predator–prey relationships. In *Predator–Prey Relationships* (ed. M. E. Feder and G. V. Lauder), pp. 24–41. Chicago: University of Chicago Press.
- Weihls, D.** (1989). Design features and mechanics of axial locomotion of fish. *Am. Zool.* **29**, 151–160.
- Weihls, D. and Webb, P. W.** (1984). Optimal avoidance and evasion tactics in predator–prey interactions. *J. Theor. Biol.* **106**, 189–206.