

FORM, FUNCTION, AND FITNESS: PATHWAYS TO SURVIVAL

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Two hypotheses have been considered in the literature regarding how anuran morphology reduces predation risk: by (1) improving escape swimming performance, or (2) using the tail as a lure to draw predator strikes away from the body of the tadpole. We investigated these hypotheses using a modification of the morphology, performance, and fitness path analysis of Arnold (1983, *Am. Zool.* 23:347–361). Indirect effects of morphology on fitness, as mediated by burst swimming speed, as well as direct paths from morphology to survival with dragonfly larvae were included in the path model. Tadpole morphology did affect burst swimming speed, however, burst swimming speed did not influence survival. Fast tadpoles were larger overall, had long tails, deep tail muscles, and proportionally small bodies. In addition, a shape trait similar to published descriptions of the tail lure morphology had a direct relationship with survival. Thus, only the tail lure effect was supported. This study documents the utility of analyzing multiple trait effects and demonstrates that including direct paths between traits and fitness in the morphology, performance, and fitness path model allows evaluation of alternative hypothesis of selection.

KEY WORDS: Fitness gradient, geometric morphometrics, morphology, natural selection, path analysis, performance gradient, tadpole.

Identifying which traits contribute to fitness is an important and challenging task in evolutionary biology (Mitchell-Olds and Shaw 1987; Wade and Kalisz 1990; Kingsolver and Schemske 1991; Rausher 1992; Conner 1996; Stinchcombe et al. 2002). Correlating traits to fitness by itself may not provide the resolution needed to ascertain how fitness is influenced by traits. Identifying what traits are under selection and determining how those traits influence fitness requires a broad approach in which the evolutionary biologist uses several analytical techniques and lines of evidence to support or reject a given hypothesis. In some cases multiple hypotheses must be considered at once. Thus, a premium is placed on diversifying the methodological tools available. This article demonstrates one such under-utilized tool, the use of the morphology, performance, and fitness paradigm de-

finied by Arnold (1983; Fig. 1A) with modifications suggested by Garland and Losos (1994; Fig. 1B) to test multiple hypotheses of selection.

Path analysis is frequently employed to quantify natural selection (Crespi and Bookstein 1989; Kingsolver and Schemske 1991; Mitchell 1992; Weis and Kapelinski 1994; Conner 1996; Conner et al. 1996; Sinervo and DeNardo 1996; Shipley 1997; Sheldon and Ellegren 1999; Scheiner et al. 2000; Alcalá and Domínguez 2005; Kaplan and Phillips 2006). In particular, path analysis can be used to incorporate intermediate variables between traits and fitness (Kingsolver and Schemske 1991; Scheiner et al. 2000). The value of intermediary factors is that they clarify functional relationships by providing logical transitions between traits and fitness (Kingsolver and Schemske 1991). Arnold (1983)

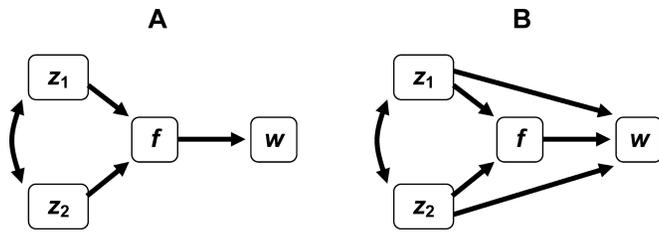


Figure 1. (A) The Arnold paradigm illustrated with two traits (Z_1 , Z_2) performance (f) and fitness (w). The double-headed arrow indicates covariance between traits. (B) Direct paths from traits to fitness can be added and may indicate whether other performance measures are involved in determining fitness.

provides a philosophical and methodological approach that highlights performance as an intermediate component between traits and fitness (Fig. 1A). This view of selection implies that traits in the classical sense (e.g., morphology, physiology) do not directly determine fitness. Fitness is determined by various aspects of organismal performance to which primary traits contribute (Brodie and Ridenhour 2003). Traits evolve, but performance is selected. Performance is a critical component in determining the outcome of species interactions (Grant 1986; Losos 1990; McPeck et al. 1996; Miles 2004; Walker et al. 2005).

The original Arnold model does not include direct paths from traits to fitness (Fig. 1B). Yet as suggested by Garland and Losos (1994), allowing for alternative (direct) pathways may be useful to determine if other traits are correlated with fitness. Direct pathways from traits to fitness may have two interpretations. First, significant direct path coefficients between traits and fitness may arise from correlations between the measured traits and unmeasured traits that are functionally involved with fitness (see discussion in Conner et al. 1996). In this circumstance, a direct relationship between traits and fitness would be spurious and not meaningful for understanding how natural selection operates. Second, direct path coefficients may stem from unmeasured performance traits (Garland and Losos 1994). In this circumstance, a direct path between traits and fitness may suggest new functional hypotheses or support established adaptive hypotheses.

ANURAN MORPHOLOGICAL VARIATION AND SURVIVAL WITH PREDATORS

Our study system is the predator–prey interactions between a larval anuran (*Rana sphenoccephala*) and its common invertebrate predator (*Anax junius*). Morphology is strongly correlated with anuran larva survival in the presence of predators (Van Buskirk and Relyea 1998; Van Buskirk and McCollum 1999). To date, two mechanisms have been suggested as to how morphology influences survival with predators. First, tadpole morphology may influence swimming performance (e.g., burst speed; McCollum and Leimberger 1997; Richardson 2001, 2002; Trembath and Anholt

2001; Teplitsky et al. 2004; Dayton et al. 2005; Wilson et al. 2005), which can influence predator escape (Feder 1983; Watkins 1996; Kaplan and Phillips 2006). Second, a deep tailfin at mid-length acts as a lure attracting predators away from the more vulnerable body (Doherty et al. 1998; Blair and Wassersug 2000; Hoff and Wassersug 2000; Van Buskirk et al. 2003; Johnson and Eidietis 2005). Experimental manipulation using model tadpoles has convincingly demonstrated the nature and efficacy of the tail lure effect (Van Buskirk et al. 2003). Tail damage is a ubiquitous phenomenon in natural anuran populations and appears to be more common in ponds with high densities of invertebrate predators (Wilbur and Semlitsch 1990; Blair and Wassersug 2000; J. B. Johnson, pers. obs.). We performed predation trials for 190 *R. sphenoccephala* larvae for which burst speed and morphology had been measured. Data were then analyzed using path analysis to evaluate indirect effects of morphology on survival as mediated by burst swimming speed and by including direct paths from morphology to fitness to evaluate other potential functions, including that of tail lure.

Materials and Methods

SPECIMEN COLLECTION

Rana sphenoccephala larvae (Gosner stages 26–36; Gosner 1960; total length 2.35–7.90 cm) were collected from six ponds in the Stephen F. Austin Experimental Forest (Nacogdoches County, Texas, USA) from March through May of 2004. All six sites had invertebrate predators (e.g., *A. junius*, *Dytiscus* sp., *Procambarus* sp.) and no fish. Late instar *A. junius* naiads (dragonfly larvae) were also collected from these ponds for use as the experimental predator. *Anax* larvae are a common predator of tadpoles and have been used in previous experimental studies (Van Buskirk and Relyea 1998; Wilson et al. 2005). All tadpoles and naiads were brought to the Stephen F. Austin State University campus and housed in the Department of Biology's animal care facility. Animals were housed in water from their native pond and were kept on a light cycle representative of east Texas at that time of year. Specimens were allowed 12 h to acclimate before swimming performance trials were initiated.

SWIMMING PERFORMANCE TRIALS

Burst swimming performance was measured by placing a single tadpole in a plastic aquarium (34 × 25.6 × 5 cm), which contained 0.5 L of water. Tadpoles were allowed 5 min to acclimate to the aquarium before swimming trials began. Water temperature was recorded each time a new tadpole was added to the swimming aquarium (range = 20.0–24.5°C). Each tadpole was prodded with a dissecting probe (total length = 14 cm, width at tip = 1.2 mm) to elicit a burst start. Each tadpole was prodded in the area just

ventral to the lateral midline of the tail musculature, just above the developing rear limbs. This region of the tail musculature was chosen because it moves less during swimming compared to areas farther anterior or posterior, which limited the chance of interfering with the tadpoles' swimming while withdrawing the probe. In addition, Johnson and Eidietis (2005) found that this region of the tadpole was commonly struck by naiads. Care was taken to ensure that all tadpoles were given an equal stimulus. The probe was placed in the observer's right hand whereas the left hand steadied the right by grasping the mid-forearm. A single observer (JBJ) conducted all burst start trials. Video was captured at 240 frames/sec using a video camera (JVC GR-DVL 9800, JVC of America, Wayne, NJ) mounted 1 m above the aquarium. Three successful performance trials were obtained for each individual. A given trial was deemed acceptable if it reflected the behavior observed during actual predation strikes. During attempted predation events *R. sphenoccephala* have been observed contracting the tail toward the body in a deep stroke and producing a large forward thrust (JBJ pers. obs.; D. Saenz, unpubl. data). This swimming behavior has been referred to as a C-start by others (Wilson et al. 2005). Each tadpole was allowed 1 min to rest between runs. The first 48 frames (0.2 sec) of movement were used to estimate burst speed (following Dayton et al. 2005; Wilson et al. 2005). APAS software (Ariel Dynamics 2002) was used for kinematic analysis. Each frame was digitized using five points: the tadpole (center of the body), and four control points (corners of the tank to provide scale). APAS calculates velocity from the first derivative of displacement over time. Burst speed data were then smoothed using a cubic-spline function (Ariel Dynamics 2002). The fastest burst speed of the three runs was retained for further analysis. Repeatability of burst starts for each individual was assessed by calculating the intraclass (hierarchical, within individual) correlation for each individual's three burst starts. The observed intraclass correlation of $r = 0.62$ suggests that an individual's burst starts were repeatable. The tadpole was then removed, sedated (buffered MS-222 0.2g/L), placed briefly on a copy-stand and a lateral photograph (for morphometric analysis) was taken with a digital camera (Olympus Camedia C-2500L, Olympus Imaging America, Center Valley, PA).

PREDATION TRIALS

Predation trials began the day following performance and morphometric assays. Tadpoles were arranged in groups of 10 irrespective of their population of origin. Each group of 10 was assigned to a 38-L aquarium that had been filled with de-chlorinated water, 20 g of soil, 5 g of leaf litter, and 15 g of sticks (to provide perches for predators). Two *A. junius* larvae were placed in each aquarium. The design (10 tadpoles, 2 naiads in an aquarium) was replicated 20 times ($N = 200$ tadpoles exposed to predators). Tadpoles were placed in a protective cage within the predator

tank and allowed 30 min to acclimate to the scent of the predator before trials began. After 30 min, tadpoles were released into the aquarium with the *A. junius* larvae. Trials were conducted in a greenhouse, so predators and prey were exposed to a normal daylight/night cycle (14:10) and temperature variation (21–30°C). After 24 h, surviving tadpoles were removed, sedated, and photographed a second time for identification. Identification was accomplished by examining morphological features such as spotting patterns and blood vessels on the surface of the tail musculature. For *R. sphenoccephala*, which tend to be lighter in color than many other anuran larvae in east Texas, blood vessel patterns were extremely diagnostic. Images and raw data are available online for inspection and download with the understanding that the authors will be appropriately cited (<http://www.faculty.sfasu.edu/burtdonal/Johnsonetal/photosdata.htm>). In addition, we had an additional naïve observer classify surviving tadpoles using the diagnostic features mentioned above. Our identification was 99% congruent with that of the naïve observer.

MORPHOMETRICS

Geometric morphometric methods were used to quantify size and shape (Bookstein 1991; Rohlf and Marcus 1993; Marcus et al. 1996). Images taken of each individual before the predation trial were loaded into MakeFan6 (Sheets 2003) to create a standardized template for digitizing semilandmarks (Fig. 2). Semilandmarks are landmarks that are arranged along a featureless curve (e.g., an outline, Bookstein 1997). Thirteen landmarks were digitized in TpsDig (Rohlf 2004a). Landmarks 1–4 were traditional landmarks and represent (1) the tip of the snout, (2) the eye, (3) the proximal and ventral-most point of the tail muscle, and (4) the tip of the tail (Fig. 2). Landmarks 5–13 were semilandmarks and were digitized on the body and tail, respectively. The body chord consisted of a line between landmarks 1 and 3 and a second perpendicular line halfway between both landmarks. The tail chord was a line between landmarks 3 and 4 with three equidistant perpendicular lines starting at the third landmark. Semilandmarks were placed where each line crossed the body outline. Ten individuals were removed from

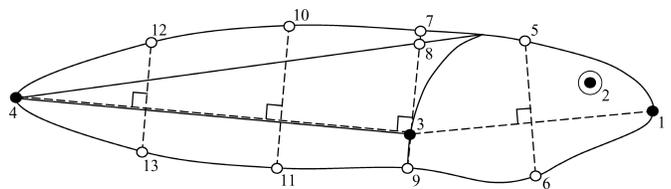


Figure 2. Diagram of a *R. sphenoccephala* larva illustrating placement of landmarks for the current study. Dotted lines represent triangulations used in placement of semilandmarks. Traditional (homologous) landmarks are shown filled; semilandmarks are unfilled.

the analysis because of poor positioning in the photos (thus final $N = 190$).

Landmark coordinates were imported into TpsRelw (Rohlf 2004b) to create relative warps. TpsRelw adjusts semilandmarks by sliding them into a position that minimizes bending energies and superimposes all individual landmark conformations by the method of generalized least squares (Bookstein 1991; Marcus et al. 1996). After superimposition is accomplished each specimen is compared to the consensus conformation and shape variables termed partial warps are generated. Eigendecomposition (principal components analysis) is then performed on the partial warp scores, which renders relative warps. Relative warps 1, 2, and 3 explained 82.5% of shape variation (RW1 = 60.6%, RW2 = 13.6%, RW3 = 8.3%). Thus further analysis was conducted with relative warps 1, 2, and 3. Centroid size was quantified as the square root of the summed squared distances of each landmark from the geometric center of each individual. We had originally intended to collect data on tadpole tail coloration that has been shown to be involved in the lure effect in addition to morphology (Van Buskirk et al. 2003, 2004). This approach was abandoned once it was apparent that coloration changed during the trials. Such variation violates an assumption of studies of natural selection that traits are constant over the period in which selection is measured (Arnold 1983; Lande and Arnold 1983).

STATISTICAL ANALYSIS

A path model was constructed using AMOS 16 software (SPSS, Chicago, IL). This model included the fitness gradient (influence of burst speed on survival), performance gradients (affects of size and shape on burst speed), and direct effects of morphology on fitness, as well as relevant correlations between morphological variables (size and shape; Fig. 3). Correlations (double-headed arrows in Fig. 3) between size and each relative warp were included in the model but correlations between relative warps were not because relative warps are uncorrelated by definition. The performance and fitness gradients taken together comprise the effect of morphology on fitness as a function of burst swimming performance, that is, indirect effects. Direct effects of morphology on fitness examine the possibility that morphology influences fitness in ways other than swimming performance. If direct effects of morphology on fitness occur, then other unmeasured performance variables may be involved in determining fitness (Garland and Losos 1994).

Because survival is categorical, least-square methods may violate assumptions of path analysis (i.e., non-normal error, multivariate normality; Tabachnick and Fidell 2001) which can bias parameter estimates and significance tests (Mitchell-Olds and Shaw 1987; Janzen and Stern 1998). Therefore, parameter estimation was performed using Bayesian parameter estimation methods that are appropriate for nonnormal and binary data (Arbuckle 2006).

Bayesian parameter estimation applies a Markov Chain Monte Carlo (MCMC) iterative algorithm to generate a marginal posterior distribution (MPD) of parameter estimates based on a uniform prior distribution and a likelihood function using Bayes Theorem (Scheines et al. 1999). The parameter estimates (θ) reported here are means of the MPD for a given relationship (Arbuckle 2006). Each parameter estimate is the mean of 60,500 iterations. Two-tailed significance tests of each mean parameter estimate (θ) were calculated as double the proportion of the MPD that exceeded zero for a respective parameter estimate (Arbuckle 2006). Additionally, to check for consistency across methods, an OLS path analysis was computed. Results were nearly identical with the Bayesian approach.

The influence of temperature on swimming speed was included as a directional effect on burst swimming speed but was not significant and was removed from the model. Quadratic terms for relative warps 1, 2, and 3 and size were added to both path models to test for nonlinear selection (Lande and Arnold 1983; Scheiner et al. 2000). Nonlinear relationships were not significant so the model reported here contains only linear terms.

The nature of relative warp 3 was suggestive of tail lure morphology. We investigated the degree of similarity between relative warp 3 and the tadpole models used to test the tail lure hypothesis by Van Buskirk et al. (2003). We digitized only the six tailfin landmarks that differed between models (i.e., landmarks 7 and 9–13 from Fig. 2). We calculated the eigenvector for the shape difference between the two tail lure models based on the six landmarks. We then postmultiplied this eigenvector by the superimposed set of six landmarks of our experimental tadpoles to yield scores for our tadpoles on the tail lure axis. To assess similarity between our relative warp 3 and tail lure morphology, we tested for a correlation between tail lure score and relative warp 3 scores. Finally, using photographs of the surviving animals we determined if tadpoles had incurred tail damage. If relative warp 3 is indicative of a tail lure then there should be correspondence between positive scores for relative warp 3 and incurring tail damage among the survivors. We tested this hypothesis by performing a logistic regression with relative warp 3 and centroid size (to account for allometric affects) as the independent variables and tail damage (damage = 1, no damage = 0) as the dependent variable.

Results

Positive scores for relative warps 1 and 2, taken collectively describe tadpoles with small bodies, deep tail muscles, long tails, and anteriorly shallow tailfins (Fig. 3). Negative scores for the shape axes of relative warp one describe tadpoles with large bodies, shallow tail muscles, short tails, and anteriorly deep tailfins (Fig. 3). Tadpoles with positive scores for relative warp 2 were

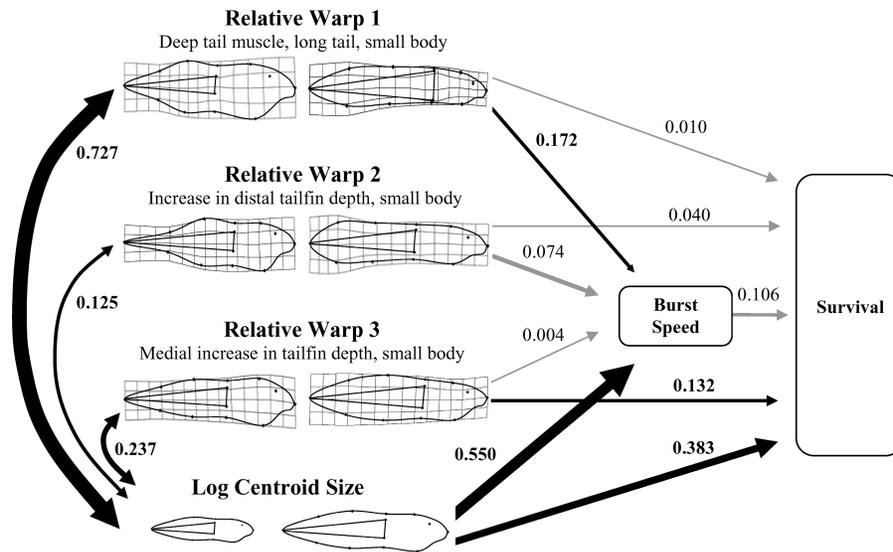


Figure 3. Path model illustrating the relationship of tadpole morphology, swimming performance and fitness, and including direct effects of morphology on fitness. Graphical representations of each relative warp indicate the direction of shape change associated with increasingly positive scores. These representations (transformation grids) have been amplified ($\times 2.5$) to make clear the morphological variation described by each axis. Single-headed arrows represent unidirectional relationships between variables. Double-headed arrows represent correlations between relative warps and size. Values near each arrow represent the mean Bayesian parameter estimate and the weight of the arrows is proportional to the strength of the relationships. Significance of a given relationship of $0.001 < P < 0.05$ is denoted by black arrows and nonsignificant relationships are indicated by gray arrows.

small bodied, with large tail muscles, and distally broadened tailfins (Fig. 3). Individuals with low relative warp 2 scores had large bodies, small tail muscles, and narrow distal tailfins (Fig. 3). High relative warp 3 scores had small bodies and medially deep tailfins (Fig. 3). Tadpoles with low values for relative warp 3 had large bodies and narrow tailfins (Fig. 3).

The fitness gradient connecting burst speed to survival was not significant ($\theta_{\text{Speed} \rightarrow \text{Fitness}} = 0.106$, $P = 0.218$; Fig. 3). Performance gradient for relative warp 1 was significantly related to burst speed ($\theta_{\text{RW1} \rightarrow \text{Speed}} = 0.172$, $P = 0.029$). The fastest tadpoles were those with long tails, small bodies, and relatively large tail muscles (Fig. 3). Relative warps 2 and 3 did not have a significant association with speed ($\theta_{\text{RW2} \rightarrow \text{Speed}} = 0.074$, $P = 0.170$; $\theta_{\text{RW3} \rightarrow \text{Speed}} = 0.004$, $P = 0.934$; Fig. 3). Larger tadpoles were significantly faster than smaller ones ($\theta_{\text{Cent Size} \rightarrow \text{Speed}} = 0.550$, $P \leq 0.001$; Fig. 3). Direct paths from relative warps 1 and 2 to survival were not significant ($\theta_{\text{RW1} \rightarrow \text{Survival}} = 0.010$, $P = 0.910$; $\theta_{\text{RW2} \rightarrow \text{Survival}} = 0.040$, $P = 0.525$; Fig. 3). The direct path from relative warp 3 to survival was significant ($\theta_{\text{RW3} \rightarrow \text{Survival}} = 0.132$, $P = 0.05$; Fig. 3). Larger tadpoles had a higher frequency of survival ($\theta_{\text{Cent Size} \rightarrow \text{Survival}} = 0.383$, $P \leq 0.001$; Fig. 3). The influence of morphology in determining survival as a function of burst swimming speed (i.e., indirect effect) for relative warps 1, 2, and 3 and log centroid size were 0.018, 0.008, 0.0004, and 0.059, respectively. Strong allometric effects were present as indicated by correlations between the shape variables and centroid

size ($r_{\text{Cent Size} \leftrightarrow \text{RW1}} = 0.727$, $P = \leq 0.001$; $r_{\text{Cent Size} \leftrightarrow \text{RW2}} = 0.125$, $P = 0.012$; $r_{\text{Cent Size} \leftrightarrow \text{RW3}} = 0.237$, $P = \leq 0.001$; Fig. 3). The association between relative warp 3 and the tail lure axis of Van Buskirk et al. (2003) was significant ($r_{\text{TL} \leftrightarrow \text{RW3}} = 0.270$; $P \leq 0.0001$; $N = 190$). The logistic regression for relative warp 3 and centroid size on tail damage indicated marginal significance for high scores of relative warp 3 being associated with tail damage ($\chi^2_{\text{Model}} = 4.7$, $P = 0.100$, $R^2_{\text{Cox \& Snell}} = 0.04$, $b_{\text{RW3}} = 31.76$, $\chi^2 = 2.81$, $P = 0.09$; $N = 124$). But centroid size was not correlated with tail damage ($b_{\text{Cent Size}} = 0.53$, $\chi^2 = 1.37$, $P = 0.242$; $N = 124$).

Discussion

The relationship between morphology and burst speed has been suggested as an important component of predator evasion for many vertebrates (tadpoles: Dayton et al. 2005; Wilson et al. 2005; Kaplan and Philips 2006; fish: Langerhans et al. 2004; Walker et al. 2005; lizards: reviewed by Garland and Losos 1994; Miles 2004). But, the morphology, burst speed, predator evasion hypothesis has rarely been tested in total (Kaplan and Philips 2006; this study). Our findings do not support the hypothesis that burst speed is a mechanism for evading *Anax* predation by *R. sphenocéphala* as burst speed was not significantly associated with survival (Fig. 3). Previous work indirectly supports this finding. Several studies have found that tadpoles reared with nonlethal

exposure to odonate larvae do not swim faster than tadpoles reared in a no predator control (McCullum and Limberger 1997; Van Buskirk and McCollum 2000; Teplitsky et al. 2005; Wilson et al. 2005). This suggests that induced morphological changes in anuran larvae as a result of exposure to chemical cues from odonate predators do not function to increase burst swimming speed. This is a logical conclusion if survival with odonate predators does not hinge on swimming fast, as our study suggests. Burst speed is likely relevant for evasion of fish. Tadpoles reared with predacious fish have been shown to develop morphologies similar to higher scores on our relative warp 1 and swim significantly faster than naiad-induced or control (no-predator) tadpoles (Teplitsky et al. 2005; Wilson et al. 2005).

Causal arguments for the role of morphology in affecting performance are strengthened if the morphology observed to produce a given performance outcome is compatible with what might be expected based on first principles (Losos 1990). Although biomechanical models of swimming in tadpoles have been performed (Wassersug and Hoff 1985; Liu et al. 1997) we lack models detailing the influence of morphological variation on burst starts specifically. The biomechanical basis of burst speed, however, has been well explored in fish. Models derived for fish predict that an animal with long propulsion appendages, a smaller anterior region, and larger thrust-generating muscle relative to overall size should perform faster burst starts, all else being equal (Webb 1982, 1986; Webb and Blake 1985). These predictions for fish have been supported by empirical work (Langerhans et al. 2004), and faster swimming fish more successfully evade their predators (Walker et al. 2005). Tadpoles with high relative warp 1 scores had relatively long tails, small bodies, and large tail muscles and performed fast burst starts (Fig. 3). These findings are similar to what would be expected based on the empirical and theoretical work done with fish. Similar findings were also made by Dayton et al. (2005), who also worked with *R. sphenoccephala*. While drawing parallels between fish and tadpoles is likely sound in a general sense, tadpoles are morphologically distinct from fish (Wassersug 1989). It remains an open avenue to explore the detailed theoretical basis of morphology's influence on burst speed for anuran larvae, to demonstrate the biomechanical basis for the empirical results on morphology and burst speed.

Size is another aspect of morphology, which first principles predict should increase speed (Webb 1982, 1986; Webb and Blake 1985). Body size is a major factor influencing community structure and food web dynamics in addition to predator-prey interaction in many systems (Layman et al. 2005; Woodward et al. 2005). Thus, it is not surprising that size has the largest effect in our data (Fig. 3). Larger tadpoles swam faster than smaller tadpoles in our trials (Fig. 3). The role of size in influencing speed is fairly ubiquitous in the anuran performance literature. Larger tadpoles are consistently faster in most studies that tested for the

relationship (Richards and Bull 1990; McCollum and Leimberger 1997; Van Buskirk and McCollum 2000a,b; Wilson and Franklin 2000; Richardson 2001; Trembath and Anholt 2001; Richardson 2002; Arendt 2003; Arendt and Hoang 2005; Dayton 2005; Eiditis 2005; Teplitsky 2005; Wilson et al. 2005; but see discussion in Watkins 2000 for a specific exception). Once grasped by naiads, larger tadpoles are more difficult to kill (Caldwell et al. 1980) and our data support this; larger *R. sphenoccephala* tadpoles were most likely to survive with *Anax* (Fig. 3). Size may serve as both a primary antipredator defense against predators such as fish where speed is effective in preventing capture and a secondary antipredator defense by exceeding the gape of the predator. The capacity to disentangle the influence of size on burst speed and other determinants of survival shows the strength of path-analytic methods to separate indirect and direct effects of traits on fitness.

To date, few generalities have emerged for morphology and burst speed performance gradients in anuran larvae, with the exception of overall size (see above). The inability to define common aspects of body shape, per se, and burst speed among studies of anuran larvae may result from at least two factors. First, disparate morphometric and performance assay methods can make comparisons between studies difficult. Second, body shape may be under different constraints in different systems. Selection on morphology for burst speed performance may be constrained by selection on morphology from other factors, and as a result other features such as physiological adaptations (Watkins 2000) may evolve to provide the speed to compensate (i.e., trait compensation; DeWitt et al. 1999; Rundle and Brönmark 2001). In different environmental contexts such constraints may vary in their importance. Thus, fast tadpoles from divergent environments may look quite different morphologically. This is a likely scenario considering that on a landscape level (among ponds) tadpoles experience a diverse range of predators, hydroperiods, and food types (Pfennig 1990; Wellborn et al. 1996; Richardson 2001; Relyea 2004; Relyea and Auld 2005). When these disparate selection pressures conflict they result in trade-offs prohibiting maximization of phenotype-environment matching in any single direction (Schluter 2000; Richardson 2001; DeWitt and Langerhans 2003; Relyea and Auld 2005).

Tadpole morphology has also been linked with the tail lure antipredator tactic (Doherty et al. 1998; Blair and Wassersug 2000; Hoff and Wassersug 2000; Van Buskirk et al. 2003). This morphology, verbalized as a deep mid-tailfin, is thought to provide a target (the tail) to draw an attack away from the more vulnerable body (Van Buskirk et al. 2003). Van Buskirk et al. (2003) created models based on measurements made of predator induced and noninduced tadpoles. These models were used to investigate the effect of tailfin shape and size on naiad strikes and found that models with deep mid-tailfins drew fewer attacks to the body and more to the tail. Relative warp 3 in our study is suggestive

of a tail lure morphology as it too describes increased depth of the tailfin at mid-length (Fig. 3). The highly significant association between the models published by Van Buskirk et al. (2003) and relative warp 3 supports this suggestion. The effect of relative warp 3 on speed was not significant but its direct effect on fitness was significant (Fig. 3). In addition, the direct effect of relative warp 3 on survival ($\theta_{RW3 \rightarrow Survival} = 0.132$) was considerably greater than its indirect effect on survival via burst speed (i.e., $\theta_{RW3 \rightarrow Speed} \times \theta_{Speed \rightarrow Fitness} = 0.0004$). Whereas the direct versus indirect comparison for the other morphological variables was considerably smaller (RW1: direct 0.01, indirect 0.018; RW2: direct 0.04, indirect 0.008; cent. size: direct 0.383, indirect 0.059). If this direct effect represents tail lure performance then we can expect that among survivors, those with higher tail lure scores would exhibit the marks of unsuccessful predation (i.e., damaged tails indicating failed strikes). The logistic regression of relative warp 3 and size on tail damage for surviving tadpoles was not significant at an alpha of 0.05 but was close ($P = 0.09$). The predicted association between relative warp 3 and tail damage can be viewed as a one-tailed hypotheses, in which case the association is significant ($P = 0.045$). Also, tail coloration would have likely had a strong interaction with tail depth and presumably with high values of relative warp 3 but could not be reliably measured because it changed during the predation trials. Accounting for the effect of tail color and its interaction with relative warp 3 may have strengthened the association between relative warp 3 and tail damage. In conclusion, we suggest that the significant direct path of relative warp 3, relative warp 3's similarity to the Van Buskirk et al. (2003) models, and the positive correlation between relative warp 3 and tail damage supports the tail lure hypothesis.

Without the work of Van Buskirk and colleagues (Van Buskirk et al. 2003) we would have less support for our interpretation that relative warp 3 functions as the tail lure morphology. This is a limitation of incorporating direct effects of traits on fitness. Direct paths between traits and fitness are less informative and their interpretation more subjective than those whose effects pass through measured aspects of performance en route to fitness. In our case we were fortunate to find a high degree of similarity between the induced and noninduced models of Van Buskirk et al. (2003) and relative warp 3 and a reasonable correlation between relative warp 3 and tail damage. Thus, ancillary information can be invaluable in interpreting direct paths. But lacking a clear interpretation of function for direct paths however would still perform the valuable service of suggesting directions to take future research. Garland and Losos (1994) suggest that "a significant path" from morphology to a component of fitness would indicate either a direct effect of morphology on fitness or of the presence of some unmeasured performance variable. We find it difficult to imagine a functional relationship between traits

and fitness without having some performance intermediary. We suggest that direct paths between traits and fitness either represent some unmeasured performance variable (e.g., attracting predators to the tail) or stem from the trait being correlated with another unmeasured trait-performance pair. Ultimately, direct paths are open to interpretation and will depend on one's definition of "performance."

Our results suggest that the tail lure is an effective antipredator strategy employed by *R. sphencephala* tadpoles against *A. junius*, that morphology strongly determines burst swimming performance, but that burst swimming does not affect survivorship with this lie-in-wait predator. Certainly other antipredator traits are likely also involved in affecting performance aspects of predator evasion in this system (e.g., myofibrillar ATPase, Watkins 2000; tail coloration, Van Buskirk et al. 2004). Or unmeasured traits may be involved in determining fitness (e.g., behavior, Van Buskirk and McCollum 2000a; Relyea 2001a,b; palatability, Wassersug 1971; Peterson and Blaustein 1992). No single study or experiment can evaluate the total phenotype under selection and the methods illustrated here are no different. Our study provides an empirical illustration of employing direct paths (Garland and Losos 1994) in a path analysis of morphology, performance, and fitness (Arnold 1983) to test multiple hypotheses of selection with the caveat that other traits may be involved in determining fitness, which may be correlated with the measured variables. We suggest that the methods presented here combined with experimental manipulations (Wade and Kalisz 1990) will be highly effective in identifying how traits affect fitness.

The outcome of species interactions (competition, predation, etc.) is determined not by traits directly but how traits affect performance in the whole organism (Arnold 1983). Performance, when integrated as a function of a trait's contribution to fitness, can clarify how selection operates. In addition, unmeasured aspects of performance may be inferred by including direct pathways to fitness. The methodology outlined by Arnold (1983) and Garland and Losos (1994) is a powerful tool that has been underused by evolutionary and functional ecologists. This approach has allowed us to demonstrate that *R. sphencephala* tadpoles use tail lure, but not burst speed, as a defense against predation by *Anax*. If our approach used only the traditional Arnold analysis (Fig. 1A) our conclusions would have been quite different. Excluding direct paths from morphology to survival completely ignores the tail lure effect and inflated the significance of speed on survival ($P = \leq 0.001$). This suggests that studies performing similar analyses must investigate direct paths to ensure that effects of performance on fitness are not exaggerated.

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