Morphology and Escape Performance of Tiger Salamander Larvae (*Ambystoma tigrinum mavortium*)

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ABSTRACT   The ability of an individual to escape predators is an important component of fitness. Several adaptive explanations of body shape variation in amphibians hypothesize relationships between swimming performance and morphology, but these ideas have rarely been tested. Here we investigate bivariate and multivariate relationships between natural variation in morphology and performance. We used high-speed video to examine fast-starts associated with escape responses in small tiger salamander larvae (*Ambystoma tigrinum*). Our results indicate that performance is influenced by interactions among aspects of morphology, physiology, and behavior. Relationships between morphometric variables and velocity could be detected with multivariate, but not bivariate statistical analyses. In particular, relationships between morphology and velocity depend on tail beat frequency (potentially a measure of effort or vigor). Relationships between morphology and acceleration were detected with bivariate analyses, but multivariate analysis suggests that acceleration performance, too, depends on interactions between morphology and tail beat frequency. We found a positive relationship between tail area and propulsive performance, which supports adaptive interpretations of variation in larval tail shape within and between amphibian species. *J. Exp. Zool. 297A:147–159, 2003.*   © 2003 Wiley-Liss, Inc.

INTRODUCTION

An individual's ability to escape enemies bears an intuitive relationship to the viability component of fitness. For example, fast-start swimming performance has been correlated with ability to avoid predation in sticklebacks (Swain, '92a, b) and tree frog larvae (Watkins, '96). Many features of animals' morphologies are thought to be specifically adapted for ecologically effective locomotor performance. The association between variation in morphology and variation in performance is what allows natural selection to operate on morphology (Arnold, '83; Pough, '89). Many of our ideas about functional morphology are based on interspecific associations between morphology and performance (e.g., Duellman and Trueb, '86; Gill, '90; Videler, '93; Feldhamer et al., '99). However, understanding the effects of morphology on performance variation within populations is important for understanding microevolutionary dynamics (Wainwright and Reilly, '94). Some variation may be due to phenotypic plasticity or ontogenetic changes, therefore control over rearing environment and age of experimental subjects is important in ecomorphological studies. Here we analyze variation in propulsive escape performance in a single-age cohort of lab-reared tiger salamander (*Ambystoma tigrinum*) larvae in order to (1) test the functional significance of morphological variation within a population, (2) analyze the influence of kinematics on the relationship between morphology and performance, and (3) compare propulsive performance of salamander larvae with previously published data on other aquatic vertebrates with disparate morphologies.

In amphibians with aquatic larvae, variation in the shape of larvae is thought to be functionally related to variation in the physical environments and ecological communities where the animals live. For example, "pond-type" salamander larvae

have large dorsal fins extending from the tip of the tail to the shoulder region, whereas "stream-type" larvae have smaller fins restricted to the tail (Noble, '27; Petranka, '98). These differences are thought to be adaptations to locomotion in still vs. flowing water (Valentine and Dennis, '64; Duellman and Trueb, '86). In other groups, such as fishes, macroevolutionary variation in body form is associated with ecological and behavioral variation to the extent that some authors have described a number of ecomorphological syndromes such as "accelerators," "manoeuvrers," and "sandswimmers" (Webb, '84; Videler, '93). Some frog (Smith and Van Buskirk, '95; Vorndran et al., 2002) and salamander (Van Buskirk and Schmidt, 2000) larvae develop large tails in response to chemical predator cues, although the functional link between tail size and fitness is not clear (Van Buskirk and McCollum, 2000). Morphology and plasticity appear to be locally adapted to different predation regimes in wood frog tadpoles, *Rana sylvatica* (Relyea, 2002). Irschick and Shaffer ('97) described extensive morphometric variation among larval populations of tiger salamanders (*Ambystoma* spp.), potentially reflecting local adaptation in body shape. Our analysis is the first to test the credibility of proposed functional links between morphological variation and performance variation within a population of pond-type salamander larvae.

Kinematic variables are usually considered components of performance. Variables such as response latency have seemingly clear consequences for the probability of a successful escape in nature. If an animal is slow to respond to a predator, it is more easily captured. However, other kinematic variables like tail beat frequency are part of what causes propulsion, and may reasonably be considered potential predictors of propulsive performance. Variation in kinematics may reflect variation in neurological or biochemical performance ability, or variation in motivation (Hertz et al., '88; Irschick and Losos, '98). For example, an animal that is slow to respond and beats its tail lackadaisically may either be physiologically impaired or it may lack the motivation to use maximal performance ability. Since we were specifically interested in propulsive performance (velocity and acceleration), we used our estimates of kinematic parameters as predictor variables. By considering the joint relationships of morphological and kinematic variables to fast-start propulsive performance, we were able to investigate interactions between kinematics and morphology as predictors of performance variation.

The aquatic escape response provides an opportunity to compare the performance of very different groups of animals during a similar series of behaviors. Fish and amphibian larvae use a relatively standard fast-start behavior, the C-start, to escape predators (Weihs, '73; Domenici and Blake, '97; Wilson and Franklin, 2000; Azizi and Landberg, 2002). The response to a startling stimulus is generally divided into three kinematic stages (Weihs, '73, Domenici and Blake, '97). Stage 1, the "C-start," is a preparatory stroke in which the animal usually jerks its entire body into a "C" or "L" shape with little or no movement of the center of mass. Stage 2 is a power stroke propelling the animal forward as it swings its tail rapidly from the coiled C-shape through a broad arc to the opposite side of its body. Stage 3 is the remainder of the escape response, usually including several more tail strokes and sustained high speed as the animal further distances itself from the predator. The relative importance of acceleration and velocity during an escape response probably depends on the nature of the threat, for example sit-and-wait vs. pursuit predators. Hoff et al. ('89) proposed that tiger salamander larvae may be adapted for high acceleration at the expense of high, sustained velocity relative to fish. This inference was based on morphology, ecology, and constant velocity swimming. We provide the acceleration data necessary to test their prediction.

Our analysis provides an assessment of the degree to which variation in commonly measured body proportions is associated with variation in locomotor performance in tiger salamander larvae, the influence of kinematics on those associations, and the credibility of functional morphological explanations for differences in body form among populations, species, and higher taxa.

METHODS

Salamander collection and care

We collected 45 naturally deposited eggs of *Ambystoma tigrinum mavortium* from an introduced population near Clear Lake, Lake County, California in February, 2001. These tiger salamanders are native to the Rocky Mountains, Great Plains, and arid Southwest (Shaffer and McKnight, '96; Petranka, '98) but were introduced extensively in California by a group of bait dealers

in the 1950s (S. P. D. Riley et al. unpublished data).

Upon hatching, we placed each salamander larva in its own 15cm × 45cm × 10cm deep plastic container with 4L of 10% Holtfretter's Solution (Asashima et al., '89). We maintained the rearing temperature at approximately 18°C (range 13–23°C) in an animal care facility at the University of California, Davis with a light:dark cycle that followed the natural local photoperiod. We fed the animals *ad libitum* from a mixed plankton culture comprised primarily of copepods and cladocera. Water was replaced every 5–7 days or as necessary. Escape response trials were conducted when the larvae reached 20 mm to 30 mm total length (∼60 days post hatching). Trials were conducted in random order, all within a 10–day period.

Data acquisition

Recording escape responses

We performed fast-start trials in a circular glass dish 15 cm in diameter and 2 cm deep. This dish was placed over a 1 cm grid so that we could measure distance on the same scale for each trial. For each larva, we filled the dish to 1.5 cm with the animal's own tank water. We used a small, shallow arena (similar to Van Buskirk and McCollum, 2000) primarily to limit vertical movements, which would have been unmeasurable in our recordings. Surface effects and ground effects can be large in such a small amount of water, suggesting that velocity and acceleration of salamander larvae away from such effects would tend to be greater than the values reported here. In nature, tiger salamanders rest on the substrate (Petranka, '98; personal observation), therefore ground effects are likely important for propulsive performance in the wild.

Animals were startled by sharply tapping the edge of the dish (Law and Blake, '96; Guderly et al., 2001). After many pilot trials, we found this to be the most reliable way to elicit an escape response. The stimulus was applied only after the animal had been motionless near the center of the dish for 5 s. The tapping stimulus we used was not as standardized as automated stimuli such as electric shock (Wilson and Franklin, 2000), but is no less standard than the tactile stimuli commonly employed in other studies (e.g., Hale, '99; Wakeling et al., '99; Azizi and Landberg, 2002). Variation in stimulus strength is not biased with respect to morphology. It may contribute to unexplained variance in performance (thereby reducing statistical power but not introducing a bias) and/or variance in response latency and other timing variables if those variables are influenced by motivation.

Escape sequences were recorded at 500 Hz (frames per second) and 1 × magnification with a NAC Memrecam ci high speed digital video system with lighting from two Fiber-Lite illuminators at high intensity. These fiber optic light sources did not cause the temperature in the experimental arena to rise measurably during recording. We recorded water temperature in the dish immediately prior to each experiment because temperature can have a significant effect on swimming performance in tadpoles (Watkins, 2000; Wilson and Franklin, 2000) and fish (reviewed in Domenici and Blake, '97).

We analyzed one escape sequence for each experimental animal. Since only a single fast-start event per animal was used, we do not account for within-individual variation in realized performance. Failure to account for within-individual variation reduces statistical power as such variation could swamp between-individual variation. However, there is no reason to expect within-individual variation in realized performance to be biased with respect to morphology.

Body measurements

After recording escape responses, we measured several external dimensions of each larva to the nearest 0.1 mm using digital calipers (Fig. 1). When placed on a damp sheet of Rite-in-the-Rain all-weather paper, the larvae were docile enough to measure without anesthesia. Head width (*HW*) was the maximum lateral width of the head. Snout-vent length (*SVL*) was measured from the
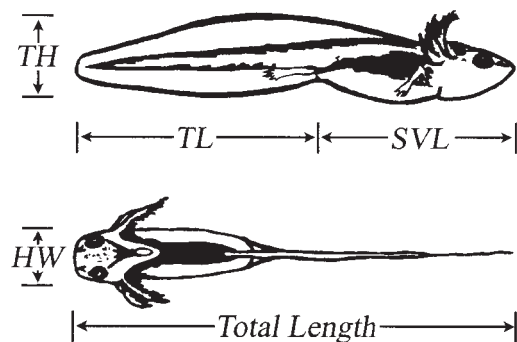

Fig. 1. External morphometrics of a tiger salamander (Ambystoma tigrinum) larva. TH is the maximum tail height, TL is tail length, SVL is snout-vent length, and HW is the maximum width of the head.

tip of the rostrum to the cloaca. Tail length (TL) was the distance from the cloaca to tip of the tail. Tail height (TH) was the maximum dorsoventral height of the tail fin. Tail area was approximated as $(TH \times TL)^{2/3}$ (the area of a parabola circumscribed by a rectangle with base TH and height TL). The square root of tail area ($TA^{1/2}$) was used in statistical analyses to maintain a linear scale. After measurement, we removed any droplets of water from the surface of the animal and weighed each larva to the nearest 0.001 g in an independently tared cup containing that salamander's tank water. We used the cube root of mass ($M^{1/3}$) to maintain a linear scale.

Behavior and performance

During C-starts, all larvae appeared to pivot around a stationary point at the anterior edge of the gut (clearly visible through their translucent bodies—see Fig. 2). We used this pivot point as the landmark to record position over time. One author (BMF) digitized the pivot point frame by frame in NIH Image 1.6.2. for Macintosh (developed at the U.S. National Institutes of Health and available free on the Internet at http://rsb.info.nih.gov/nih-image/), yielding one x-y coordinate for every 2 ms.

Sequences of position over time were smoothed by fitting locally weighted polynomials with the *loess* function in S-Plus 4.0 (see Venables and Ripley, '97). The smoothing parameter (''span'' or ''neighborhood'') was chosen so as to produce a mean squared error matching that of repeated attempts to digitize a single known stationary point (Wakeling and Johnston, '98; Walker, '98). Cumulative displacement of each larva over time was calculated from the smoothed estimates of position. We estimated velocity and acceleration as the first and second differentials, respectively, of displacement. We used maximum velocity V_{max} and maximum acceleration A_{max} as performance variables. These are numerical maxima within a trial that we used as metrics of realized performance. They do not necessarily reflect maximum performance ability.

Smoothing the x-y data prior to calculating displacement is necessary to minimize the systematic bias created by the accumulation of digitization error from frame to frame (Appendix). Appropriate smoothing of the raw x and y data makes the variances from digitization error approach zero without oversmoothing true variation in displacement over time.
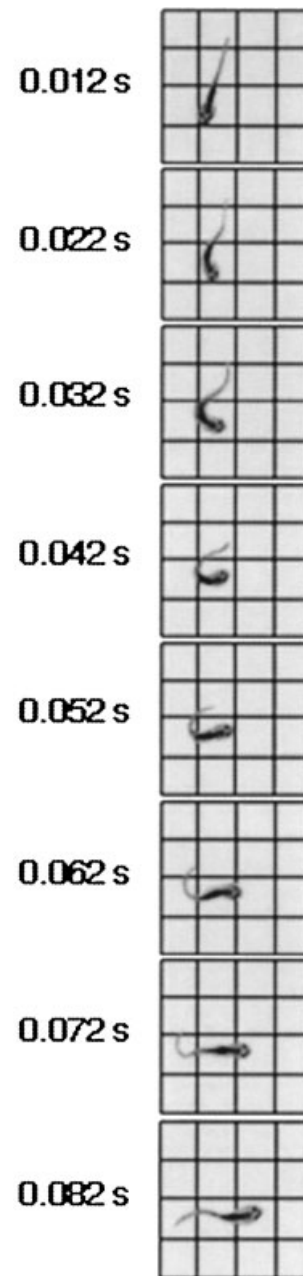

Fig. 2.   Typical escape response of a tiger salamander larva. Frames are 0.010s apart and the clock was started at the time of the stimulus. The first frame shown (0.012s after the stimulus) is the frame just prior to the first movement of the animal. The first four frames are the preparatory stage, or C-start. The following frames are the propulsive stage. Each frame was digitally retouched and should be considered an illustration rather than a source of data.

We analyzed three kinematic variables representing ''timing'' or ''quickness.'' Latency to respond (LTR) was the time between the stimulus and first movement of the larva. The time of the stimulus was recorded as the beginning of the

visible ripple produced on the surface of the water by the stimulus. C-start duration (C_{dur}) was the time from this first movement until the pivot point began to move. This appeared to correspond to the change in the direction of movement of the head, as used to delimit the C-start by Hale ('99) and others, however, in many cases the head did not so much change direction as simply stop rotating and begin to move forward. Therefore, the onset of forward motion was a more reliable way to define the C-start for our study animals. Stride frequency (SF) was the inverse of the duration of the first power stroke (time from end of the C-start to maximal extension of the tail on the opposite side of the body).

Data analysis

We inspected univariate and bivariate plots in order to screen for departures from normal distributions and linear relationships. LTR and SF needed logarithmic transformations to satisfy normality. V_{max} and the response time variables (logLTR, $Cdur$, logSF) had significant quadratic regressions on temperature, so we included T^2 in subsequent analyses. Bivariate correlation coefficients were calculated in order to indicate the strength and direction of net associations between performance and other variables.

Multivariate relationships among variables were assessed using canonical correlation analysis (CCA). CCA is an extension of regression analysis with multiple "X" and multiple "Y" variables. CCA finds a pair of linear functions, one including X variables and another including Y variables, whose values are maximally correlated, then finds a second pair, orthogonal to the first, that maximally account for any residual associations, and so on for as many pairs of canonical functions as the minimum of the number of X and the number of Y variables (for more detailed explanation, see Arnold and Bennett, '88; Afifi and Clark, '96; Fitzpatrick and Dunk, '99). We partitioned our variables into a set of performance variables (Ys) and a set of predictors (Xs). The two measures of performance were maximum acceleration (A_{max}) and maximum velocity (V_{max}). The set of nine potential explanatory variables included morphometrics ($M^{1/3}$, SVL, TL, TH, $TA^{1/2}$, HW), kinematics (lnLTR, $Cdur$, lnSF), and temperature (T, T^2). Interaction terms between morphometrics and timing variables were also considered as potential explanatory variables. Thus, we statistically controlled for correlations between kine-

matics and morphology while estimating the links between these predictors and performance. With so many variables relative to experimental animals, we were forced to utilize a stepwise variable selection algorithm for multivariate data (implemented in NCSS 6.0) in order to reduce the dimensions of the CCA. We imposed the constraints that at least one function of temperature be included and that if an interaction term was to be included its individual constituents must also be included (i.e., the model must be hierarchical).

Comparison with other aquatic vertebrates

We calculated summary statistics for comparison with adult fish data reviewed by Domenici and Blake ('97), data on fish larvae (Hale, '99; Wakeling et al., '99), and frog data (*Limnodynastes peronii*) presented by Wilson and Franklin (2000). We made several plots in order to qualitatively compare locomotor performance in *A. tigrinum* with some other aquatic vertebrates. Performance variables were transformed to match the units reported in Domenici and Blake ('97) (lengths s^{-1} for velocity and m s^{-1} for acceleration).

RESULTS

All larvae tested responded with a C-start followed by a strong stage 2 power stroke and continued swimming rapidly for many more strokes (Fig. 2). Displacement, velocity, and acceleration during a single randomly chosen sequence are graphed in Figure 3. The onset of forward motion from standing still was abrupt, with maximum acceleration taking place within the first 2 ms of the propulsive stroke. Most of our experimental larvae attained maximum velocity near the end of stage 2, followed by a more modest second peak in velocity near the end of the next tail stroke (Fig. 3). Eight (of 43) larvae attained both maximum velocity and maximum acceleration within the first 2ms of the power stroke, with velocity falling off through the remainder of stage 2. More rarely (2 of 43 animals), maximum velocity was attained later, during the second propulsive stroke. These variations on the pattern of velocity over time were not statistically associated with variation in any other variables measured (not shown).

The sharp peak of acceleration within the first video frame of the power stroke indicates that the temporal resolution of the system was too coarse to accurately capture the acceleration profiles of
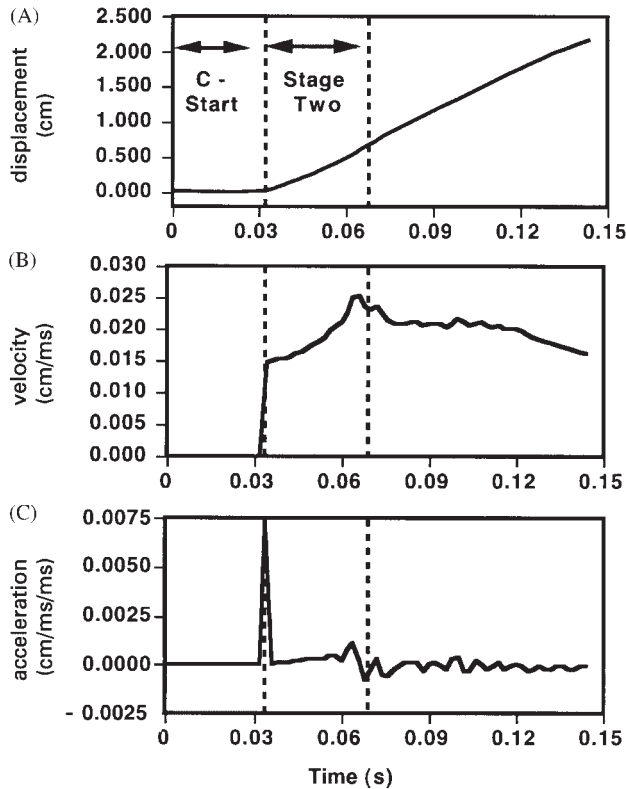
Fig. 3. Examples of a smoothed displacement profile (**A**) of a single randomly chosen tiger salamander larva, and the velocity (**B**) and acceleration (**C**) profiles derived from it.

these *Ambystoma* larvae (unfortunately, 500 frames per second is close to state of the art). It is impossible to tell whether our data deviate substantially from the norm for small fish because few studies have reported graphs similar to Figure

3 using similar methods. Spikes of acceleration are suggested by the distance-time curves depicted for stickleback fast starts by Law and Blake ('96) and velocity-time curves for sculpins in James and Johnston ('98). Also, dramatic acceleration peaks are not unusual during ballistic tongue protrusion by frogs and chameleons (Wainwright et al., '91; Nishikawa, '99). High acceleration relative to video sampling rate causes high sampling variance and a negative bias for estimates of maximum acceleration (Harper and Blake, '89; Walker, '98). However, it is unlikely to produce spurious associations with morphometrics. Thus, while the average A_{max} reported in Table 1 is probably an underestimate, the sources of error considered here only make our analyses conservative with respect to statistical tests of association among variables.

Correlates of performance variation

Descriptive statistics for all variables are given in Table 1. Bivariate correlation coefficients are presented in Table 2. Maximum acceleration (A_{max}) and maximum velocity (V_{max}) were positively correlated, although the association is not considered significant after a sequential Bonferoni adjustment (Table 2). Bivariate correlations between morphological measurements and performance were not statistically significant (Table 2). Both measures of performance were weakly associated with timing variables; in general, the sooner an animal responded (low *LTR*) and the more quickly it executed the response (low *Cdur* and high *SF*), the higher its A_{max} and V_{max}

TABLE 1. *Summary statistics for the variables studied in relation to escape performance of tiger salamander larvae[1]*

Measure	Abbr.	Units	Mean	CV	Min	Max
Head width	*HW*	mm	5.43	0.06	4.60	6.20
Total length	*HW*	mm	25.98	0.07	21.90	30.30
Snout-vent length	*SVL*	mm	14.43	0.09	11.60	17.60
Tail length	*TL*	mm	11.55	0.10	9.20	14.20
Tail height	*TH*	mm	4.76	0.12	3.60	6.10
Tail area	*TA*	mm2	36.84	0.18	23.31	50.43
Mass	*Mass*	g	0.1390	0.2315	0.0860	0.2210
Latency to respond	*LTR*	ms	20.76	0.86	6.00	94.00
Duration of C-start	C_{dur}	ms	28.00	0.34	18.00	76.00
Duration of stage 2	2_{dur}	ms	34.10	0.21	22.00	60.00
Stride Frequency	*SF*	1/ms	0.0304	0.1779	0.0167	0.0455
Maximum velocity	*Vmax*	cm/ms	0.0295	0.2499	0.0144	0.0547
Maximum velocity	*Vmax*	SVL/s	2.06	0.26	0.99	3.60
Max. acceleration	*Amax*	cm/ms/ms	0.0110	0.3713	0.0044	0.0274
Temperature	*T*	°C	20.27	0.09	14.50	22.10

CV is the coefficient of variation.

TABLE 2. *Bivariate correlation coefficients between pairs of variables show their net realized statistical relationships[1]*

	HW	Len	SVL	TL	TH	TA$^{1/2}$	Mass$^{1/3}$	T^2	lnLTR	C$_{dur}$	lnSF	V$_{max}$
Head width												
Total length	0.691***											
Snout-vent length	0.740***	0.816***										
Tail length	0.366*	0.796***	0.300									
Tail height	0.313*	0.520***	0.425**	0.413**								
Tail area$^{1/2}$	0.401*	0.768***	0.439**	0.808***	0.870***							
Mass$^{1/3}$	0.789***	0.786***	0.823***	0.435**	0.612***	0.633***						
Temperature^2	−0.15	−0.18	−0.001	−0.302	0.145	−0.065	−0.058					
Log$_e$(Latency to respond)	−0.05	−0.05	−0.2	0.119	−0.288	−0.124	−0.189	−0.430**				
Duration of C-start	−0.08	−0.05	−0.11	0.029	−0.203	−0.12	−0.147	−0.675***	0.405**			
Log$_e$(Stride frequency)	−0.04	−0.131	0.09	−0.311†	0.003	−0.163	0.036	0.314*	−0.1231	−0.269		
Maximum velocity	−0.064	−0.177	0.048	−0.243	0.197	−0.003	0.048	0.440**	−0.457**	−0.422**	0.667***	
Max. acceleration	0.326	0.344*	0.312*	0.233	0.277	0.310	0.333*	0.137	−0.240	−0.278	0.332*	0.409**

*$P<0.005$; **$P<0.01$; ***$P<0.001$; †Correlation between tail length and untransformed stride frequency was $r=−0.330$, $P=0.038$.
[1]P-values < 0.001 (***) are considered statistically significant after a sequential Bonferonni adjustment.

(Table 2). All morphometrics were significantly positively intercorrelated ($\alpha=0.05$), which may be due to variation in overall size (size-adjustment is accomplished by the inclusion of *SVL* and $M^{1/3}$ in the CCA). *Cdur* and ln*LTR* were weakly positively correlated, but neither was significantly correlated with ln*SF*. *Cdur* was correlated with T^2. Stride frequency was not significantly associated with any morphometric.

Our final CCA model included T^2, ln*SF*, four morphometrics (*HW*, *SVL*, TA$^{1/2}$, $M^{1/3}$), and their interactions with ln*SF* (Table 3). Both pairs of canonical variates were statistically significant ($\alpha=0.05$). The proportions of the standardized variance of the performance variates explained by the predictor variates were 0.41 and 0.15 for the first and second variates, respectively (Canonical Redundancy Analysis from Proc CANCORR, SAS Institute, '99). The CCA results match the results of separate multiple regressions on A_{max} and V_{max} (not shown). The standardized coefficients in Table 3 are proportional to the strength

TABLE 3. *Canonical Correlation Analysis (CCA) of morphology and kinematics vs. propulsive performance in Ambystoma tigrinum larvae*

	Standardized Coefficients		Variable-variate correlations			
Variable	X_1	X_2	X_1	X_2	Y_1	Y_2
HW	13.37	1.65	−0.02	0.61	−0.01	0.39
SVL	6.86	−17.04	0.08	0.48	0.07	0.31
TA$^{1/2}$	5.71	34.28	0.06	0.53	0.04	0.34
Mass$^{1/3}$	−22.07	−11.70	0.10	0.51	0.08	0.33
T^2	0.36	−0.15	0.52	−0.18	0.42	−0.11
ln(SF)	−2.44	−1.73	0.84	−0.05	0.68	−0.03
HWXln(SF)	17.08	1.36	0.52	−0.49	0.42	−0.32
SVLXln(SF)	7.76	−19.59	0.36	−0.46	0.29	−0.29
TA$^{1/2}$Xln(SF)	6.74	41.43	0.33	−0.45	0.26	−0.29
Mass$^{1/3}$Xln(SF)	−25.88	−13.56	0.36	−0.46	0.29	−0.29
	Y_1	Y_2				
V$_{max}$	0.92	−0.59	0.79	−0.09	0.99	−0.14
A$_{max}$	0.16	1.09	0.43	0.54	0.54	0.84
R^2	0.65	0.41				
Wilks' λ	0.21	0.59				
P	0.0001	0.0401				

of the contribution of each variable to variation in the canonical variate (i.e., they are analogous to partial regression coefficients). Variable-variate correlations are also sometimes used as aids to interpretation (Manly, '86). Contrasts between standardized coefficients and variable-variate correlations reflect covariances among variables, which are accounted for in the coefficients but not in the correlations. In cases like this, where the canonical scores depend on interactions between variables that are themselves intercorrelated, differences between coefficients and correlations are to be expected. We prefer to interpret the fitted multivariate model. The first performance variate (Y_1) was dominated by V_{max} and the second (Y_2) by A_{max} with some negative influence of V_{max}. The first explanatory variate was dominated by $M^{1/3}$, HW, and their interactions with lnSF. The second explanatory variate was dominated by $TA^{1/2}$, SVL, and their interactions with lnSF. Temperature did not come out as a major factor, probably due to its correlation with lnSF (Table 2).

In order to help interpret the interaction terms involving lnSF and morphometrics, we plotted 3–dimensional cross-sections of the fitted multivariate surfaces represented by the canonical variates. Figure 4a-h shows 3-dimensional surfaces representing the estimated relationships of performance variates, lnSF, and each morphometric while holding all other variables constant at their respective means. These plots suggest that the morphometrics had very little association with performance when larvae moved their tails slowly (low stride frequency), but were quite strongly associated with performance at high stride frequencies. The only major discordance between the first and second components of performance was the role of snout-vent length (Table 3, Fig. 4g,h). At high stride frequencies, long SVL was associated with high values of Y_1 (Fig. 4g) but low values of Y_2 (Fig. 4h). Otherwise, both performance variates were positively associated with HW and $TA^{1/2}$ and negatively associated with $M^{1/3}$, given high stride frequencies.

Comparison with other aquatic vertebrates

Some comparisons with fish (data from Domenici and Blake, '97; Hale, '99; Wakeling et al., '99) and striped marsh frogs, *Limnodynastes peronii*, (Wilson and Franklin, 2000) are illustrated in Figure 5. Maximum velocities (in total body lengths s^{-1}) of salamanders were slower than
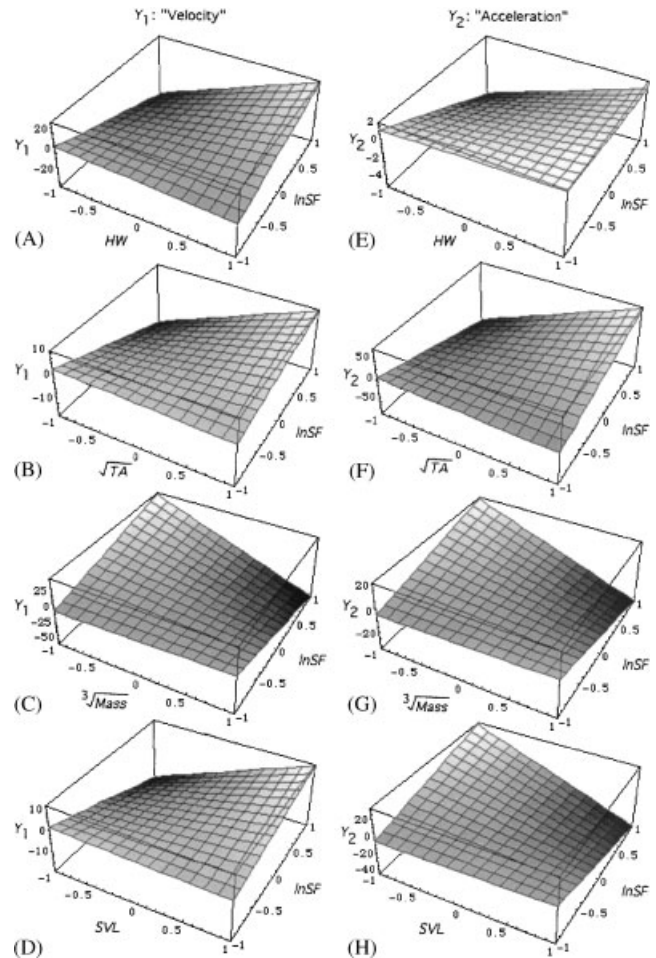

Fig. 4. Three dimensional cross-sections of the fitted canonical correlation model (Table 3) illustrate the interactions between stride frequency and morphometrics in influencing performance. See Table 1 for variable abbreviations. **A-D** illustrate the first canonical correlation which primarily describes relationships involving maximum velocity, and **E-H** illustrate the second canonical correlation which primarily describes relationships involving maximum acceleration.

what would be expected of tadpoles or adult fish with similar body lengths, but roughly equivalent to similar sized salmon larvae (Fig. 5a). Response duration (total time of stages 1 and 2) was also similar to that of salmon larvae, but slightly shorter than the average of the tadpoles (Fig. 5b; the nonlinearity of the salmon relationship is due to a developmental shift from the eleuthero-embryo to juvenile stages (Hale, '99). In contrast, salamander maximum acceleration (m s^{-2}) was comparable to the highest values reported for adult fish and far greater than frog and fish larvae of comparable size (Fig. 5c).
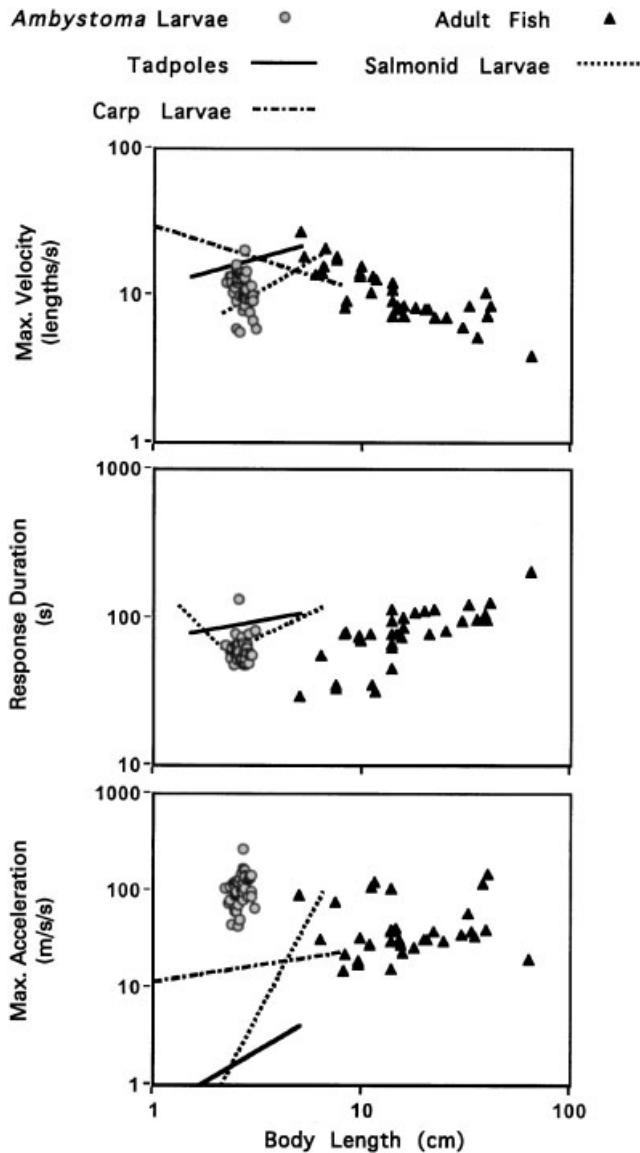
Fig. 5. Comparisons of fast start performance among aquatic vertebrates. Response duration (B) is the sum of the times taken to complete the C-start and the first power stroke of the tail (Stage 2). Data on adult fish were reviewed by Domenici and Blake ('97), and each data point generally represents an average of several fish from one study. The lines are fitted relationships reported by Wilson and Franklin (2000) on *Limnodynastes peronii* tadpoles, Hale ('99) on salmonid larvae, and Wakeling et al. ('99) on carp larvae. Salamander data are from the present study.

DISCUSSION

We used bivariate (Table 2) and multivariate (Table 3; Fig. 4) analyses to explore the relationships of morphology and kinematic timing to escape performance in *A. tigrinum* larvae. Our results support a functional link between body shape and locomotor performance in salamander larvae. In particular, the positive association between tail area and acceleration is consistent with the proposition that large tails of pond-type salamander larvae are adaptations for rapid acceleration in still water (Valentine and Dennis, '64; Duellman and Trueb, '86). This association is also consistent with improvement of escape performance ability as the explanation for predator-induced tail size polymorphisms in some amphibian larvae (Smith and Van Buskirk, '95; Hoff and Wassersug, 2000; Van Buskirk and Schmidt, 2000; Vorndran et al., 2002). Van Buskirk and McCollum (2000) found a negative relationship between natural variation in tail depth and velocity in *Hyla versicolor* tadpoles, however, they did not consider acceleration or tail beat frequency. Previous work on *Ambystoma* swimming performance focused on sustained swimming in a flow tank (Hoff et al., '89; Frolich and Biewener '92) or over relatively long distances (Shaffer et al., '91; Austin and Shaffer, '92). Hoff et al. ('89) analyzed film sequences in which animals swam at constant velocity for at least three tail beats. Shaffer and colleagues (Shaffer et al., '91; Austin and Shaffer, '92) measured velocity using the time taken to cross 10 cm increments. Here, we studied the dynamics of *A. tigrinum* larvae accelerating from rest over time periods shorter than 0.100 s and distances shorter than 2 cm, because these are the natural scales of their escape responses. Previous studies also used larger larvae; we focused on small larvae because they would be most vulnerable to predation in the wild. As *Ambystoma* larvae grow, they become the top predators within their ponds, regularly consuming insect species that prey on smaller salamander larvae (Dodson and Dodson, '71; Armentrout, '73; Collins and Holomuzki, '84).

Hoff et al. ('89) concluded that *Ambystoma* larvae are inefficient at constant velocity swimming relative to fish and tadpoles, and suggested that *Ambystoma* morphology is adapted to short-duration, high-acceleration bursts at the expense of sustained high velocity swimming. Fast-start performance is likely to be an important component of survival in natural populations of *Ambystoma* larvae. Tiger salamander larvae are exposed to a variety of predators (e.g., Collins and Holomuzki, '84; Holomuzki, '86a,b; Petranka, '98). The predominant predators in most *A. tigrinum* breeding ponds are sit-and-wait predators such as water bugs (Hemiptera: Belostomatidae), dragonfly nymphs (Odonata: Anisoptera),

and predaceous diving beetle larvae (Coleoptera: Dytiscidae) (personal observation; Wellborn et al., '96). None of these are capable of high speed pursuit, but their short-range predatory strikes can be fast. Dragonfly nymphs (*Aeschna* spp.) can extend their predatory labia roughly 16 mm in 16–25 ms (Pritchard, '65; Tanaka and Hisada, '80). With regard to avoiding these predators, instantaneous acceleration seems more important than maximum velocity; by the end of the stage 2 power stroke a salamander larva is probably either out of danger or already captured. The morphology and anguilliform swimming of salamander larvae appear to be quite well suited to extremely high acceleration (Fig. 5c) despite being inefficient for reaching and maintaining high velocities (Fig. 5a; Hoff et al., '89).

Naturally, the caveat not to mistake correlation for causation applies to this analysis. Phenotypic covariances among traits can produce non-causal associations between traits and performance, and likewise, can obscure true causal relationships (Lande and Arnold, '83; Price et al., '84). Multivariate analyses are designed to dissect such covariances, but can neither divine causation nor anticipate the influence of unmeasured variables.

Comparison of the bivariate correlation coefficients (Table 2) and the canonical coefficients (Table 3) reveals some interesting patterns. Bivariate associations include both direct and indirect effects, while the CCA attempts to control for some indirect effects by taking into account the covariances within each set of variables. SVL and $M^{1/3}$ had net positive correlations with acceleration, but had negative relationships to the second performance variate (Y_2 in Table 3) once multivariate relationships were taken into account. One interpretation of this is that underlying negative relationships of SVL and $M^{1/3}$ to acceleration are revealed in the multivariate analysis but overridden in bivariate analyses because larger larvae also have larger heads and tails, and therefore greater acceleration ability. However, a low score on Y_2 may reflect low acceleration, high velocity, or both. Long, heavy salamander larvae with relatively small heads and tails may attain relatively high V_{max} for a given A_{max}, reducing their Y_2—not necessarily because of poor acceleration, but because of changes in the relationship between A_{max} and V_{max} with changing size and shape.

Head width was positively related to propulsive performance. This may indicate an association between head width and cross-sectional area of the lateral musculature. The head may also serve an important stabilizing function. It is, in *Ambystoma* larvae of this size, virtually the only part of the body anterior to the center of rotation. When the lateral musculature contracts, it should tend to pull the ends of the animal toward one another, as in the C-start. During forward motion, inertial forces between the water and the anterior part of the animal must prevent the head from wagging so that the oblique push of the tail against the water is transferred into forward motion (Daniel and Webb, '87; Videler, '93). An animal with a large tail and small head may tend to overcome the lateral resistance of the water against the head when moving its tail too vigorously, causing much of the force of the tail stroke to be wasted rotating the animal rather than accelerating it forward. While selection on head shape is probably dominated by the demands of feeding, the relationship between head size and the rest of the body may have important physical consequences for locomotion.

Our most broadly relevant result is the description of the interaction between tail beat frequency and morphology in influencing escape performance (Table 3, Fig. 4). The functional importance of morphological variation was apparent only when the animals beat their tails rapidly. Variation in tail beat frequency may reflect motivation (i.e., variation in how startled animals really were) and/or variation in the neurophysiological mechanisms underlying the speed of muscle contractions. In nature, motivation is likely to vary from situation to situation, leading to behavioral modulation of the effort put into an escape response (Hertz et al., '88; Irschick and Losos, '98; Irschick, 2000). This is interesting in light of recent discussions about the relationship of behavior and performance. Garland and Losos ('94; see also Domenici and Blake, 2000) suggested a modification of Arnold's ('83) morphology→performance→fitness scheme to include behavior as a "filter" between performance and fitness. They defined performance as "an animal's ability to do something when pushed to its morphological, physiological, or biochemical limits" (Garland and Losos, '94, p. 242). We prefer to call this "performance capacity" after Irschick and Garland (2001) in contrast to "realized performance," which is how much of one's performance capacity is actually used during a particular action. For example, Domenici and Blake (2000, p. 8) offer that "motivation may play an important role in determining the fast-start performance of

escaping prey.'' Presumably what is meant by ''performance'' here is how fast the animal actually moves during a particular escape response, i.e., realized performance. Thus, behavior is in fact a filter between performance capacity and realized performance, and realized performance provides the direct link to the fitness consequences of a particular action. Our results underscore the potential importance of behavior as a ''filter'' between physically determined performance capacity and realized performance (Garland and Losos, '94; Irschick and Garland, 2001).

The strength of selection on performance-related traits depends not only on the outcomes of escape responses, but also on how often animals are required to perform near the limits of their capacity (Biewener, 2002). Our results show that the vigor with which an animal beats its tail has a marked impact on the relationship between performance and morphology. For example, natural selection may only favor large tails if salamander larvae have both the physiological capacity and ecological/behavioral demand to beat their tails furiously. Our understanding of the functional morphology of locomotion stands to benefit substantially from multivariate analyses that include some measure of behavioral and/or neurophysiological vigor.

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APPENDIX

Here we offer a mathematical proof that error in estimates of position causes a positive bias in estimated displacement between video frames. Estimated x-y coordinates over time will have some degree of scatter around the true trajectory of the animal, so if we estimate the trajectory by connecting successive estimates of position, our estimated trajectory will tend to zigzag around the true trajectory. As a result, the distance between successive digitized points is greater, on average, than the true distance between successive positions. Let the coordinates of the ith digitized point be represented by the random variables X_i and Y_i. Assume these are unbiased estimators of the true ith x and y coordinates, μ_{Xi} and μ_{Yi}. That is, the expectations converge on the true values; $E[X_i]=\mu_{Xi}$ and $E[Y_i]=\mu_{Yi}$. We calculate D_i, the distance traveled between video frames i and $i-1$, using the Pythagorean theorem:

$$D_i^2 = (\mu_{Xi} - \mu_{Xi-1})^2 + (\mu_{Yi} - \mu_{Yi-1})^2$$
$$= (E[X_i] - E[X_{i-1}])^2 + (E[Y_i] - E[Y_{i-1}])^2. \quad (1)$$

The raw x-y coordinates give the following estimator:

$$d_i^2 = (X_i - X_{i-1})^2 + (Y_i - Y_{i-1})^2, \quad (2)$$

which has bias given by

$$\begin{aligned}
\text{bias}\{d_i^2\} =& E(d_i^2) - D_i^2 \\
=& E[(X_i - X_{i-1})^2 + (Y_i - Y_{i-1})^2] \\
& - [(E[X_i] - E[X_{i-1}])^2 + (E[Y_i] - E[Y_{i-1}])^2] \\
=& E[X_i^2] - E[X_i]^2 + E[X_{i-1}^2] - E[X_{i-1}]^2 \\
& + E[Y_i^2])^2 + (E[Y_i] - E[Y_{i-1}])^2] \\
=& Var[X_i] + Var[X_{i-1}] \\
& + Var[Y_i] + Var[Y_{i-1}] > 0. \quad (3)
\end{aligned}$$

Our proof demonstrates that digitization errors introduce a positive bias proportional to the variance of estimates of position around the true position. If we were to compute cumulative displacement by summing a series of biased distances, our estimates of displacement would also be biased and no amount of smoothing would correct the problem.

In contrast, many authors have remarked that errors are compounded by differentiation (e.g., Harper and Blake, '89; Biewener and Full, '92; Walker, '98). Displacement is a first differential of position. By smoothing the estimates of 2-dimensional position over time, we are attempting to filter out error closer to its source than if we smooth estimates of displacement calculated from raw position estimates.

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