

Effects of metamorphosis on the aquatic escape response of the two-lined salamander (*Eurycea bislineata*)

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Summary

Although numerous studies have described the escape kinematics of fishes, little is known about the aquatic escape responses of salamanders. We compare the escape kinematics of larval and adult *Eurycea bislineata*, the two-lined salamander, to examine the effects of metamorphosis on aquatic escape performance. We hypothesize that shape changes associated with resorption of the larval tail fin at metamorphosis will affect aquatic locomotor performance. Escape responses were recorded using high-speed video, and the effects of life stage and total length on escape kinematics were analyzed statistically using analysis of covariance. Our results show that both larval and adult *E. bislineata* use a two-stage escape response (similar to the C-starts of fishes) that consists of a preparatory (stage 1) and a propulsive (stage 2) stroke. The duration of both kinematic stages and the distance traveled during stage 2 increased with total length. Both larval and adult *E. bislineata* had final escape trajectories that were directed away from the stimulus. The main kinematic difference between larvae and adults is that

adults exhibit significantly greater maximum curvature during stage 1. Total escape duration and the distance traveled during stage 2 did not differ significantly between larvae and adults. Despite the significantly lower tail aspect ratio of adults, we found no significant decrease in the overall escape performance of adult *E. bislineata*. Our results suggest that adults may compensate for the decrease in tail aspect ratio by increasing their maximum curvature. These findings do not support the hypothesis that larvae exhibit better locomotor performance than adults as a result of stronger selective pressures on early life stages.

Movie available on-line:

<http://www.biologists.com/JEB/movies/jeb3978.html>.

Key words: amphibian metamorphosis, salamander, escape response, kinematics, C-start, tail morphology, locomotion, swimming, *Eurycea bislineata*, Plethodontidae, Caudata.

Introduction

Amphibian metamorphosis has been used as a model system for investigating the performance consequences of rapid changes in morphology (Lauder and Shaffer, 1986; Shaffer and Lauder, 1988). Previous studies have shown that the aquatic feeding (Lauder and Shaffer, 1986; Reilly and Lauder, 1988) and locomotor (Shaffer et al., 1991) performance of ambystomatid salamanders decrease after metamorphic restructuring of the feeding apparatus and resorption of the larval tail fin. These ontogenetic comparisons of performance between pre- and post-metamorphic individuals within salamander species provide insight into the evolution of life-history strategies through heterochronic shifts. While ontogenetic changes such as the growth and ossification of various vertebral elements and intervertebral articulations may have a significant effect on undulatory locomotion, these changes are independent of metamorphosis (Babcock and Blais, 2001). Morphological studies have shown that the development of many post-cranial features of salamanders

occurs continuously throughout ontogeny and may not be directly linked to size (Wake and Lawson, 1973) or metamorphosis (Worthington, 1971; Babcock and Blais, 2001). In this study, we will focus on discrete morphological changes associated with metamorphosis. Specifically, we will examine the effects of tail fin resorption on the aquatic escape performance of *Eurycea bislineata* (Plethodontidae), the two-lined salamander.

While there has been some interest in the terrestrial escape responses of salamanders (e.g. Dowdey and Brodie, 1989; Whiteman and Wissinger, 1991) and a few investigators have studied the aquatic escape behavior of anuran tadpoles (Will, 1991; von Seckendorf Hoff, 1989; von Seckendorf Hoff and Wassersug, 2000), the aquatic escape responses of salamanders remain largely unexplored. The escape response literature is dominated by studies of fishes. These focus on kinematics (for a review, see Domenici and Blake, 1997), motor pattern (Jayne and Lauder, 1993;

Westneat et al., 1998) and neural control (Eaton et al., 1977; Eaton and Bombardieri, 1978; Fetcho, 1991; Zottoli, 1978, 1995). Often referred to as a C-start or fast-start, the escape response of most fishes is a rapid movement consisting of two distinct stages. A variety of different criteria have been used to define the two stages, including acceleration profiles and muscle activation patterns, but, in common with many previous studies, we will use a kinematic definition (e.g. Domenici and Blake, 1993b; Hale, 1999). Stage 1 represents the preparatory stroke, characterized by a high degree of axial bending with little movement of the center of mass. Stage 2 represents the propulsive stroke in which a large-amplitude propulsive wave is passed down the body, moving the animal's center of mass away from the stimulus (Weihs, 1973).

Eurycea bislineata was chosen for this study because it is a common local species whose propensity for aquatic escape behavior was well known to us from previous experience in the field. Both adults and larvae spend their lives in close proximity to streams. The larvae are entirely aquatic, while the adults inhabit the edges and splash zone of streams (Petranka, 1998). In addition, adults may spend up to 4 months of the year in a stream during the breeding season (Petranka, 1998). Predator avoidance in streams is therefore assumed to be a critical aspect of the ecology of both larval and adult *E. bislineata*.

The two main goals of this study are to provide a kinematic description of the escape response of *Eurycea bislineata* and to examine the effects of metamorphosis on aquatic escape performance. We quantify and compare the following three aspects of escape performance: the duration of both stages of the escape, the final escape trajectory (at the end of stage 2) and the distance traveled during the propulsive phase of the escape (stage 2). On the basis of general scaling models (e.g. Schmidt-Nielsen, 1993), we predict that total escape duration will increase with body length. However, this scaling trend is not likely to shift significantly as a result of metamorphosis. On the basis of both empirical data (Eaton and Emberley, 1991; Domenici and Blake, 1993a; Hale, 1999) and theoretical predictions (Weihs and Webb, 1984) of optimal escape trajectory, we predict that both life stages of *E. bislineata* will have a final escape angle that is directed away from the stimulus. This escape trajectory places the most vulnerable parts of the body farthest from a predator. We will also test the hypothesis that the distance traveled during the propulsive phase of the escape will be a function of the relative depth of the thrust-producing segment (Weihs, 1973). Because of changes in tail shape associated with metamorphosis, we predict that, for a given size, adults will travel significantly shorter distances than larvae during stage 2 of the escape response. Finally, by comparing escape performance between larvae and adults, we will test the hypothesis (Carrier, 1996) that higher levels of predation on the early life stages of a species result in young individuals exhibiting better locomotor performance for their size than adults.

Materials and methods

Experimental animals

Seven adult and 14 larval *Eurycea b. bislineata* (Green) were collected from streams in Hampshire County, Massachusetts, USA. Larvae were found under rocks in the shallow edges of streams and were captured using a dip net, and adults were found under rocky substrata in the splash zone. Only salamanders with fully intact tails were kept for laboratory experiments. All salamanders were housed individually in plastic containers (approximately 500 ml) and kept in a refrigerator that maintained a constant temperature of 9 °C. This corresponds to the temperature at which they were collected and is well within the range of published values for the natural habitat of aquatic plethodontid salamanders (mean temperature 11.3±6 °C) (Hutchison and Dupré, 1992). Each container was partially filled with ion-balanced reverse-osmosis (RO) water and contained structure for salamanders to hide under and climb on. Salamanders were fed tubifex worms, small earthworms and wax worms. The life stage of each salamander was determined using three external characters: (i) the presence of external gills, (ii) the presence of a tail fin (also known as a median fin-fold) and (iii) the presence of either the dark, mottled coloration of the larval stage or the pattern of bright yellow coloration and characteristic pigmented dorso-lateral stripes of the adult stage. In this study, the 'adult' life stage refers only to the post-metamorphic morphology, with no reference to sexual maturity.

Morphometric data were gathered from digital images captured on a Nikon Coolpix 990 digital camera, calibrated with a ruler and analyzed using NIH Image 1.62. Animal collection, care and experimental procedures were approved by the University of Massachusetts Amherst Institutional Animal Care and Use Committee. All specimens were killed in a buffered solution of 2 g l⁻¹ tricaine methanesulfonate (MS222) in water prior to being photographed for morphometric measurements and later deposited in the Massachusetts Museum of Natural History at UMASS Amherst.

High-speed videography

Escape responses were filmed in dorsal view in a 40 cm×50 cm×10 cm plastic tank half-filled with RO water. To eliminate interference from the walls (such as the animals pushing off or fluid boundary effects), the field of view was limited to the center area (30 cm×30 cm) of the tank. Prior to eliciting escape responses, salamanders were placed near the center of the field of view to maintain a minimum of 10 cm (at least 1 body length) from the closest wall. High-speed video of escape responses was recorded at 250 frames s⁻¹ and 1/250 s shutter speed using a Kodak Ektapro EM processor and was downloaded to SVHS videotapes. Video sequences were converted to TIFF files using Adobe Premiere 5.1 and analyzed using NIH image 1.62. Distances in the video sequences were calibrated by filming a 16 cm ruler before each salamander was placed in the tank and after any adjustments to the camera. Water temperature in the filming tank was maintained at 10±1 °C to match the temperature of

the holding tanks and that of the salamander's natural environment.

Filming trials were limited to a maximum of 15 min and five escapes per individual per day to avoid fatigue and acclimation to the stimulus. After each filming trial, salamanders were allowed a minimum of 3 days of recovery prior to the subsequent filming trial. To test whether the salamanders were fatiguing during the 15 min filming period, we performed a one-factor analysis of covariance (ANCOVA) comparing the distance and duration of the first and last escape response recorded on each day by each salamander. We found no significant difference in escape distance ($P=0.984$) or duration ($P=0.843$) between the initial and final escape responses recorded on a given day, indicating that the salamanders did not fatigue during the course of the 15 min filming trial. To look for possible long-term acclimation of the salamanders to our experimental protocol, we compared the initial filming trials of each salamander with all subsequent days of data collection using a one-factor analysis of variance (ANOVA). We found that previous exposure to our experimental protocol did not significantly affect the distance ($P=0.216$) or duration ($P=0.392$) of the escape response. This suggests that long-term acclimation to both captivity and our experimental protocol did not have a significant effect on escape behavior.

Stimulus

To elicit escape responses, we oriented a glass stirring rod (4 mm in diameter) perpendicular to the long axis of the body and delivered a tactile stimulus to the forelimb or the pectoral region of the salamanders. We were occasionally able to elicit escape responses by directing a tactile stimulus to the hind limb or trunk region, but this was not consistently effective. Prior to the adoption of our experimental protocol, we tested the efficacy of various stimulus types used in previous studies to elicit escape responses. Pressure waves created by tapping the side or bottom of a tank have been effective as a method of inducing escape responses in fishes (e.g. Eaton and Emberley, 1991) and anuran tadpoles (Yamashita et al., 2000). In contrast, we were unable consistently to induce an escape response in *Eurycea bislineata* using this stimulus. However, since immobility has been shown to be a defensive response in amphibians, it cannot be considered a lack of response (Brodie et al., 1974; Caldwell et al., 1980). The use of immobility in combination with cryptic coloration may be an important predator avoidance strategy, but in this study we will focus on escape behavior induced by tactile stimuli. While it is consistent with our observations that a vibratory (pressure wave) stimulus induces immobility in *E. bislineata*, we have performed no specific tests of this behavior. Responses to all the various types of stimuli described above were largely consistent across both life stages and all individuals.

Kinematic analysis

We analyzed 7–11 escape responses per individual for a total of 159 escapes. All escape responses that included obvious movement in the vertical direction or did not include both

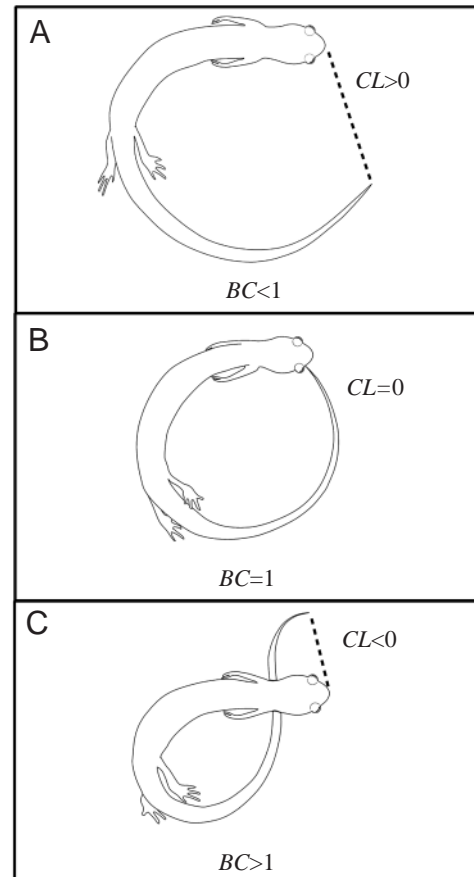


Fig. 1. Bending coefficient. Dorsal view of salamanders at maximum curvature demonstrating three escapes (A–C) with varying bending coefficients. Bending coefficient (BC) is defined as the ratio of head-to-tail chord length (CL ; dashed line) to total length (TL) subtracted from 1: $BC=1-(CL/TL)$. To view video clips of escape responses, refer to the supplemental information section of the *Journal of Experimental Biology* website (<http://jeb.biologists.com>).

kinematic stages were removed from our sample. In this study, stage 1 is defined as beginning at the first detectable movement and ending when the body reaches maximum curvature. Stage 2 is defined as beginning at maximum body curvature and ending at the frame in which the first propulsive wave has been propagated off the body. The duration of each kinematic stage was calculated by multiplying the total number of frames by the time interval (4 ms) between adjacent frames.

Body curvature was quantified using a variation of curvature coefficient as defined by Webb (1978). Curvature coefficient has previously been defined as the linear distance from the center of mass to the trailing edge of the tail at maximum curvature divided by that same distance at rest. Thus, curvature coefficient decreases with higher body curvature. In an attempt to use a more intuitive measure of curvature, we define a variable that increases with increasing body curvature. To avoid confusion, we call this parameter 'bending coefficient' (Fig. 1) and define it as the linear distance (chord length) from the tip of the snout to the tip of the tail divided by total length and subtracted from 1. The

escape kinematics of *Eurycea bislineata* is unique when compared with published results from fishes performing C-starts because the salamander's head can pass over its tail during high-curvature responses. Similar high-curvature movements have also been observed during the air-breathing maneuvers of *Xenopus laevis* tadpoles (Wassersug, 2001). In escapes in which the head passes over the tail, the distance between the snout and the tip of the tail is measured as negative, resulting in a bending coefficient greater than 1.

Escape angles are defined here as the number of degrees through which a salamander rotates its head during each kinematic stage. To calculate this, we measured the angular rotation of a digitized line drawn from the tip of the snout to the pectoral girdle. This region of the body is easily visualized in the video sequences and remained essentially straight for the duration of the escape. The angles reported are standardized relative to the initial position of the salamander, which is defined as 0° . In calculating escape angles, previous studies of C-start kinematics in fishes often used a line drawn from the rostral tip to the 'stretched-straight center of mass' (Eaton and Emberley, 1991). Although this approach may accurately measure the escape trajectories of many fishes, it does not accurately describe the high-curvature escape kinematics of salamanders with an elongate body form because significant axial curvature is often observed in the region between the snout and the 'stretched-straight center of mass'.

The distance that salamanders traveled during stage 2 was measured as the linear displacement of a digitized landmark on the pectoral girdle between the time at maximum curvature and the end of stage 2.

Statistical analyses

To compare the duration, distance and curvature parameters between the two ontogenetic stages, overall differences in total length were accounted for using a one-factor ANCOVA. Life stage was set as the factor, total length was set as the covariate and escape parameters were treated as the dependent variables. Mean values for each individual were used in the ANCOVA test. Significance levels are reported after the non-significant interaction terms have been removed. SuperAnova 1.11 was used for all non-polar statistical tests.

In our analysis of escape angles, we used a one-factor ANOVA to test for individual effects within each life stage. No significant difference in escape angle was detected among individuals. This allowed us to pool all escapes within each life stage prior to running polar statistics. A Watson-Williams test was used to compare stage 1 and final escape angles between the two life stages (Zar, 1996). Mean values and 95% confidence intervals for stage 1 angles and final escape angles were also calculated for both life stages (Zar, 1996).

Results

The most common startle behavior observed was a two-stage escape response consisting of an initial bend away from the

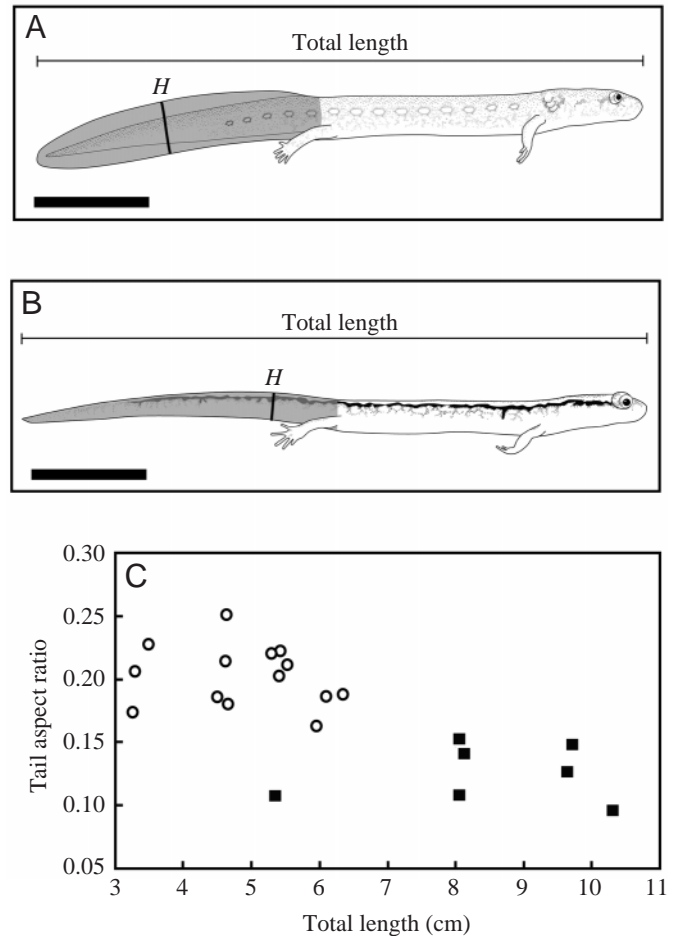


Fig. 2. Lateral view of larval (A) and adult (B) *Eurycea bislineata*. Tail aspect ratio (\mathcal{A}) is defined as the ratio of the square of maximum tail height (H) to lateral surface area (LSA) of the tail (shaded); $\mathcal{A} = H^2/LSA$. (C) Aspect ratio versus total length. Aspect ratio differs significantly ($P=0.0007$) between larvae (\circ) and adults (\blacksquare) but is not significantly correlated with total length ($P=0.4593$; one-factor ANCOVA). Scale bar, 1 cm.

stimulus followed by a traveling propulsive wave. Despite considerable variability in behavior following the end of stage 2, most escapes ended with the animal either gliding to a stop or continuing into a bout of steady swimming.

Tail morphology

The change in tail shape of salamanders at metamorphosis is a morphological transition likely to affect aquatic locomotor performance. Tail aspect ratio, defined as the ratio of maximum tail height squared to tail area, is an important morphological determinant of swimming hydrodynamics and performance and is used in this study as our measure of tail shape (Fig. 2) (Vogel, 1994). A significant difference in aspect ratio was found between the two life stages using a one-factor ANCOVA ($P=0.0007$). Aspect ratio ranged from 0.16 to 0.25 for larvae and from 0.09 to 0.16 for adults. Tail aspect ratio was not found to be significantly correlated with total length ($P=0.4593$). See Table 1 for a summary of morphometric data.

Table 1. Summary of morphological variables for 14 larval and seven adult *Eurycea bislineata*

Variable	Mean ± S.D.	Range
Total length (mm)		
Larvae	50.04±9.71	32.50–63.52
Adults	81.42±17.83	53.43–103.07
Tail length (mm)		
Larvae	24.68±5.64	15.02–33.56
Adults	45.51±12.10	27.14–59.32
Maximum tail height (mm)		
Larvae	4.21±0.92	2.51–5.44
Adults	4.28±0.96	2.34–5.39
Tail area (mm ²)		
Larvae	94.02±36.80	36.01–155.12
Adults	150.21±64.13	49.13–259.45
Tail aspect ratio		
Larvae	0.20±0.02	0.16–0.25
Adults	0.13±0.03	0.09–0.16
Mass (g)		
Larvae	0.49±0.23	0.13–0.88
Adults	1.18±0.51	0.35–1.80

Tail aspect ratio=(maximum tail height)²/tail area.

Maximum curvature

To compare differences between the two life stages and to assess the effect of total length on bending coefficient, we used a one-factor ANCOVA with life stage as the factor and total length as the covariate. Bending coefficient differs significantly between life stages ($P=0.0007$), with adults using an escape response of greater curvature (Fig. 3; Table 2). In contrast to most escapes performed by adults, larvae rarely performed escape responses with bending coefficients greater than 1. We found no significant correlation between bending coefficient and total length (Table 2).

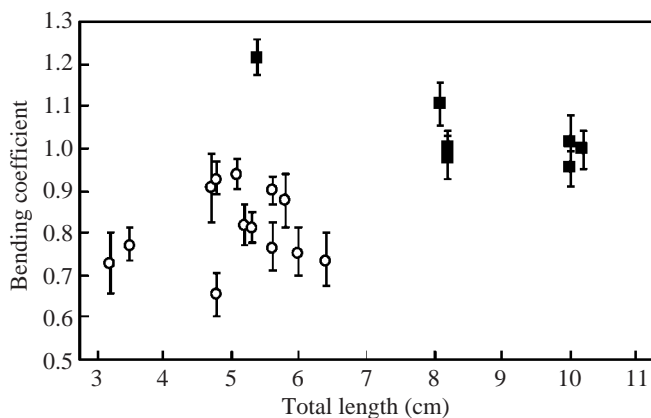


Fig. 3. Mean bending coefficient (\pm S.E.M., $N=7-11$ escapes) for each individual plotted against total length. Bending coefficient differs significantly ($P=0.0007$) between larvae (○) and adults (■) but is not significantly correlated with total length ($P=0.1767$; one-factor ANCOVA).

Table 2. ANCOVA results of comparisons between the two life stages of *Eurycea bislineata* and correlations with total length

Variable	Life stage		Total length	
	F-ratio	P-value	F-ratio	P-value
Stage 1 duration	7.368	0.0142*	9.421	0.0066**
Stage 2 duration	0.175	0.6810	9.018	0.0076**
Total escape duration	1.358	0.2590	11.093	0.0037**
Bending coefficient	16.581	0.0007***	1.977	0.1767
Stage 2 distance	1.803	0.1960	52.734	<0.0001***

* $P<0.05$; ** $P<0.01$; *** $P<0.001$.

Escape duration

Stage 1, stage 2 and total escape durations were analyzed with a one-factor ANCOVA with life stage as the factor and total length as the covariate, and these results are summarized in Table 2. There is a significant difference in the duration of stage 1 between the two life stages ($P=0.0142$), with adults taking longer to reach maximum curvature. Stage 2 duration and total escape duration were statistically indistinguishable between the two life stages. We found significant positive correlations between total length and stage 1, stage 2 and total escape durations (Fig. 4). For all three parameters, the data were best fitted with a least-square linear regression (Fig. 4).

Distance traveled in stage 2

A one-factor ANCOVA with distance as the factor and total length as the covariate was used to compare the distance traveled by larvae and adults during stage 2 (Fig. 5). While there was no significant difference between larvae and adults, there was a significant positive correlation between distance traveled during stage 2 and total length (Table 2).

Escape trajectories

We quantified escape angles by tracking the rotation of a digitized line drawn from the tip of the snout to the pectoral girdle. Escape trajectories generally consisted of a large initial rotation of the head away from the stimulus during stage 1, followed by a smaller-magnitude rotation in the opposite direction during stage 2, resulting in a final escape angle that was directed away from the stimulus (Fig. 6). The escape angles were standardized for analysis so that the initial position of the head is at the 0° position, the stimuli come from 270° and initial rotation is clockwise.

A one-factor ANOVA revealed that neither stage 1 angle ($P=0.1820$; $P=0.1822$) nor final escape angle ($P=0.3315$; $P=0.921$) had a significant individual effect for the escapes of larvae and adults respectively. Therefore, all escape angles for a given life stage were pooled prior to being analyzed using polar statistics. For each life stage, mean values and 95% confidence intervals were calculated for both stage 1 and final escape angle. Larvae have a mean stage 1 angle of $158.14 \pm 9.11^\circ$ ($N=108$ escapes) (Fig. 6A) and a final escape angle of $80.27 \pm 11.14^\circ$ (Fig. 6C). Adults ($N=51$ escapes) have a mean

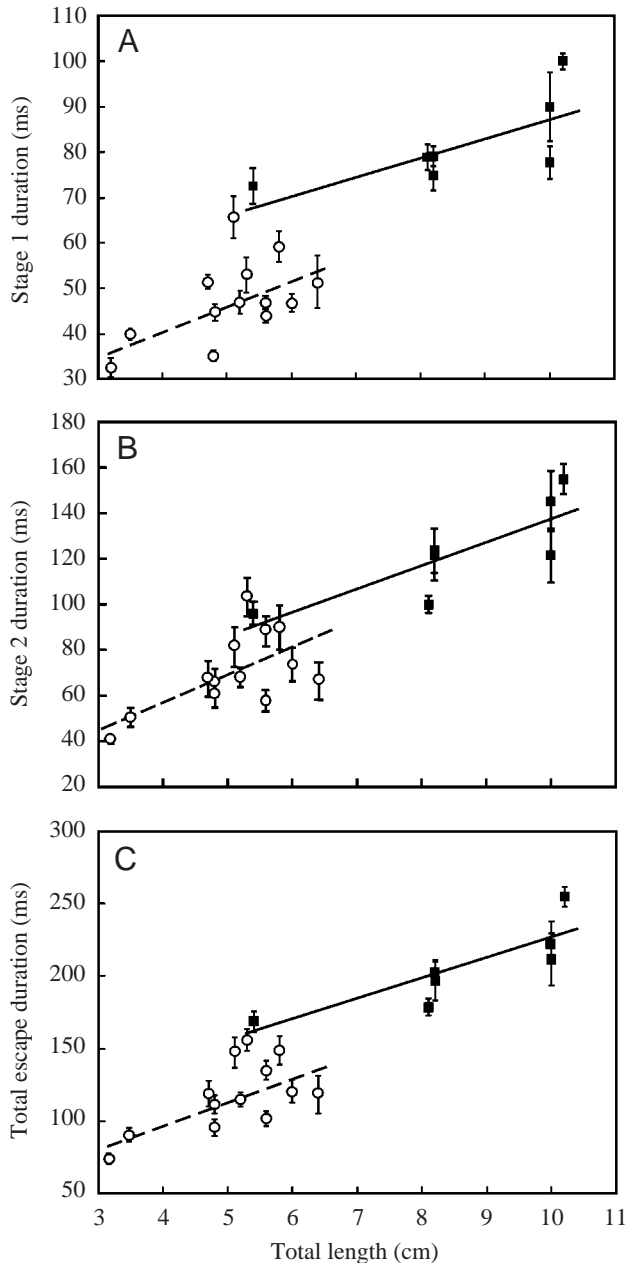


Fig. 4. Stage 1 duration (A), stage 2 duration (B) and total escape duration (C) versus total length. Mean values calculated for each individual are plotted \pm S.E.M. ($N=7-11$ escapes). Equations of linear regressions for stage 1 duration are $y=5.45x+19.8$ ($r^2=0.32$, $P=0.051$) for larvae (open circles and dashed lines) and $y=4.03x+47.2$ ($r^2=0.50$, $P=0.076$) for adults (filled squares and solid lines). Equations of linear regressions for stage 2 duration are $y=11.5x+12.1$ ($r^2=0.38$, $P=0.025$) for larvae and $y=10.3x+34.8$ ($r^2=0.663$, $P=0.026$) for adults. Equations of linear regressions for total escape duration are $y=16.9x+31.9$ ($r^2=0.40$, $P=0.02$) for larvae and $y=14.3x+82.1$ ($r^2=0.72$, $P=0.016$) for adults. For results of one-factor ANCOVA, refer to Table 2.

stage 1 angle of $175.08 \pm 12.19^\circ$ (Fig. 6B) and a final escape angle of $97.12 \pm 9.45^\circ$ (Fig. 6D). We tested for differences between the two life stages with the Watson–Williams test and

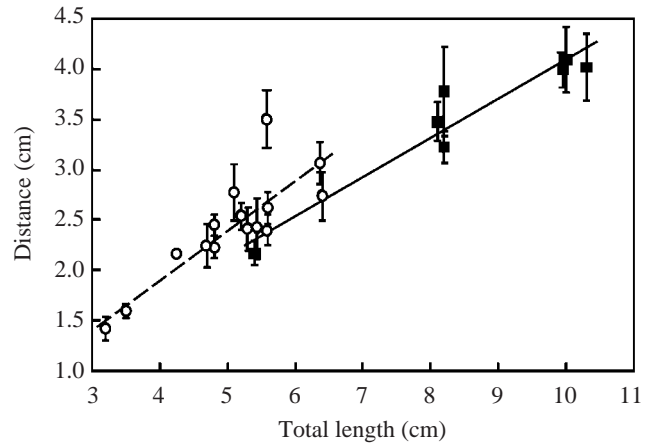


Fig. 5. Distance covered in stage 2 versus total length. Mean values calculated for each individual are plotted \pm S.E.M. ($N=7-11$ escapes). Equations of linear regressions for stage 2 distance are $y=0.482x+0.0097$ ($r^2=0.69$, $P=0.0002$) for larvae (open circles and dashed line) and $y=0.380x+0.274$ ($r^2=0.91$, $P=0.0008$) for adults (filled squares and solid line). Distance travelled in stage 2 does not differ significantly between the two life stages ($P=0.196$). Stage 2 distance is significantly positively correlated with total length.

found that adults had mean escape angles that were significantly higher than larvae for both stage 1 angle ($P=0.038$) and final escape angle ($P=0.001$). Despite this, we found that the 95% confidence intervals of the mean final escape angles of both life stages overlapped with 90° . This indicates that both life stages have final escape trajectories that, on average, are directed away from the stimulus (Fig. 6C,D).

Discussion

Resorption of the larval tail fin of salamanders at metamorphosis results in a significant change in tail aspect ratio. It is generally understood that such decreases in tail depth decrease the mass of water accelerated and therefore the thrust produced by a propulsive segment (Webb, 1977; Weihs, 1973). In agreement with theoretical predictions, Shaffer et al. (1991) showed that maximum burst swimming speed of larval *Ambystoma californiense* is significantly higher than that of metamorphosed individuals without a tail fin. On the basis of hydrodynamic theory and existing empirical data, the significantly lower tail aspect ratio of adult *Eurycea bislineata* (Fig. 2) should decrease the thrust produced and therefore the distance traveled in stage 2 of the escape.

In contrast to the results predicted by this hypothesis, we found that the distance traveled in stage 2 did not differ significantly between larvae and adults (Table 2). Although the adults in our sample had tails with lower aspect ratio, they perform escapes with significantly higher maximum curvature (Fig. 3). With an increase in the maximum curvature, a corresponding increase in the wave amplitude and therefore the rearward thrust component of the propulsive wave should occur in stage 2. Support for this comes from Andraso (1997),

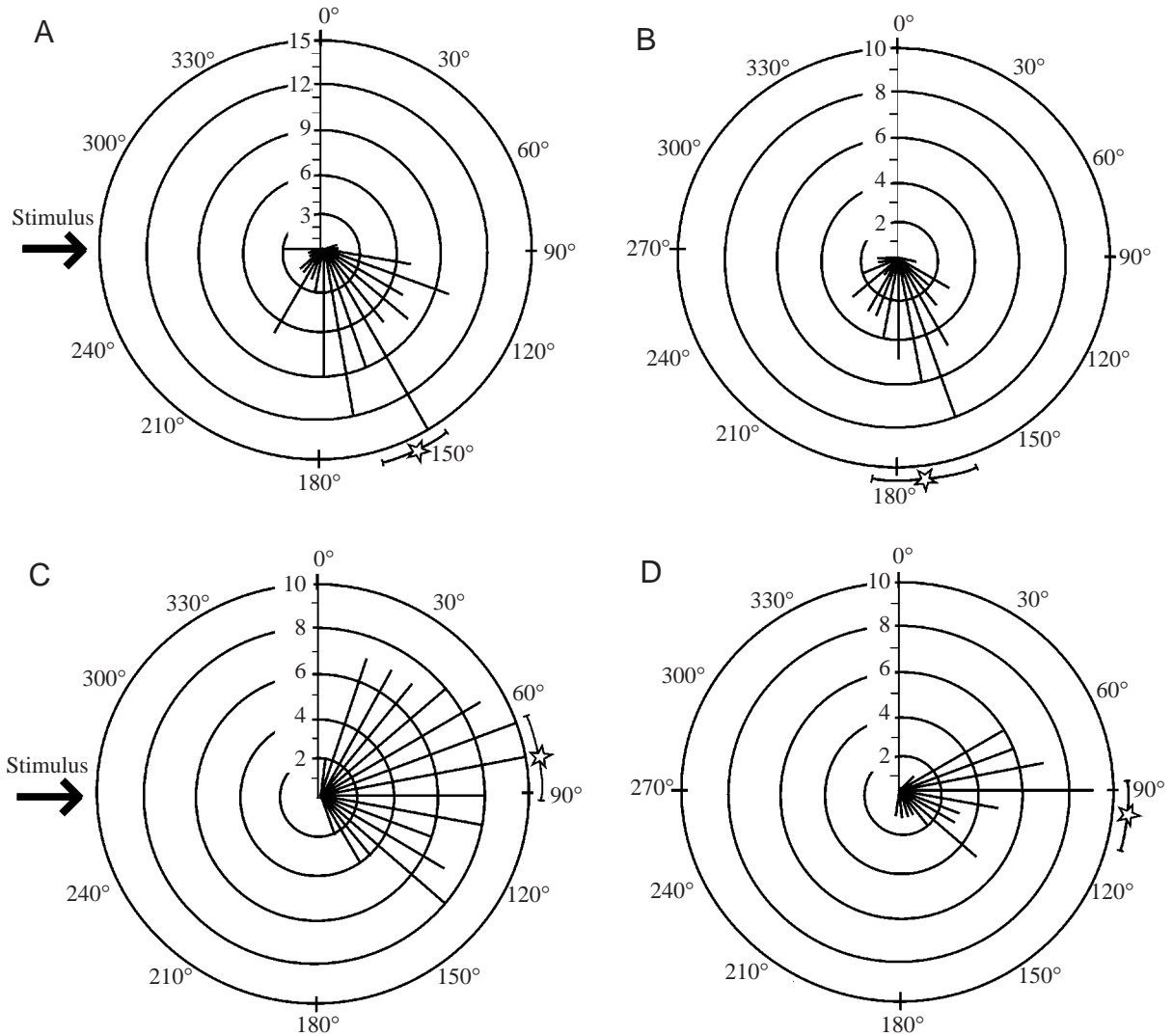


Fig. 6. Polar histograms showing the distribution of head angle at the end of stage 1 (stage 1 angle) (A,B) and stage 2 (final escape angle) (C,D) respectively for larvae (A,C) ($N=108$) and adults (B,D) ($N=51$). The number of escapes within 10° intervals increases linearly. The data are standardized so that the salamander's head is initially aligned at 0° , the stimulus is at 270° and all escapes begin in the clockwise direction. For each polar plot, all escapes from a given life stage are pooled. Stars represent mean values for each life stage and error bars are 95% confidence intervals. The 95% confidence intervals for the mean final escape angle of both larvae (C) and adults (D) overlap with 90° , indicating that neither is significantly different from 90° .

who found that higher maximum curvature increases the distance traveled during C-starts in the brook stickleback *Culaea inconstans*. It is possible that the decreased tail aspect ratio of adult *Eurycea bislineata* is compensated for by an increase in maximum curvature, thereby maintaining a mean stage 2 escape distance that is similar to that of larvae. Thus, our initial performance predictions based on morphological differences are complicated by kinematic variation in the behavior.

The function of the amphibian tail fin as an effective thrust-generating surface remains somewhat contentious. Despite a lack of ossified support elements, the tail fin of bullfrog (*Rana catesbeiana*) tadpoles does not undergo passive lateral deflection during steady swimming and may therefore contribute to thrust production (Wassersug and von Seckendorf

Hoff, 1985; Doherty et al., 1998). However, experimental tail fin ablations of up to 30% in depth have no significant effect on maximum swimming velocity or the time needed to travel 2.5 cm in the gray treefrog *Hyla versicolor* (Van Buskirk and McCollum, 2000). Understanding the locomotor contributions of the larval tail fin in salamanders will require ablation experiments comparing locomotor performance within individuals.

The final escape trajectory (measured at the end of stage 2) of the prey relative to a stimulus has been considered an important measure of escape performance (Eaton and Emberley, 1991; Domenici and Blake, 1993a; Hale, 1999). Although values for the final trajectory of fast-starts in fishes are variable when the stimulus is directed at the cranial or caudal end of the animal, laterally directed stimuli often result

in an escape trajectory directly away from the stimulus (Eaton and Emberley, 1991; Domenici and Blake, 1993a; Hale, 1999). Theoretical predictions of predator avoidance strategies suggest that the optimal escape angle lies within 21° of a predator's approach angle (Weihs and Webb, 1984). Consistent with theoretical predictions and the observed behavior of fishes, we found that the mean final escape angle (end of stage 2) for both life stages did not differ significantly from 90° (which is directly away from the stimulus). This final escape angle positions the tail closest to and the head farthest from the stimulus direction. This escape trajectory is also consistent with observations that tadpoles and salamanders often suffer tail loss or damage during interactions with predators (e.g. Caldwell et al., 1980; Whiteman and Wissinger, 1991).

In one laboratory experiment, adult *Eurycea bislineata* utilized tail autotomy in 59% of encounters with a predatory snake (*Thamnophis sirtalis*), and 33% of individuals collected in the field had damaged, autotomized or regenerated tails (Whiteman and Wissinger, 1991). Wake and Dresner (1967) reported that 8.3% of museum specimens of *E. bislineata* were either missing or had regenerated more than half the length of their tails. Although adults in our study utilize higher curvature and greater net angular rotation during the escape, both life stages use an escape trajectory that is directly away from a laterally directed stimulus (Fig. 6).

Given the limited size range of our sample, we will only discuss the general scaling trends observed in our data, with no reference to various scaling models. We found that stage 1, stage 2 and total escape duration had significant positive correlations with total length. These results are consistent with previous studies investigating escape performance across a size range (Webb, 1977, 1978; Domenici and Blake, 1993b; Hale, 1999). Although the mean total escape durations for both life stages of *Eurycea bislineata* are considerably higher than for fish of similar body length, no comparable data are currently available for C-starts in fish with a similarly elongate body shape (Domenici and Blake, 1997). An elongate body shape may be correlated with increased overall body flexibility, allowing for higher maximum curvature during the escape response (Brainerd and Patek, 1998). In support of this, *E. bislineata* (with its elongate shape) utilizes higher-curvature escape responses than previously observed in fishes performing C-starts. Westneat et al. (1998) observed that, in high-curvature responses, the head and the caudal fin of *Polypterus palamas* made contact at maximum curvature. Lacking a caudal fin, the tapering tails of adult *E. bislineata* may allow for greater maximum curvature by permitting the head to pass over the tail during stage 1 of the escape. Given the high-curvature responses of adult *E. bislineata*, it is likely that relatively more time is required to reach maximum curvature, thereby increasing the total duration of the escape.

The distance that *Eurycea bislineata* travels during stage 2 of the escape is positively correlated with total length. Similar scaling trends for this performance parameter have been

observed in fishes (Webb, 1977, 1978; Domenici and Blake, 1993b). Despite the relatively shallow body of *E. bislineata*, the range of distances traveled in stage 2 is comparable with values reported for fish of similar body length (Webb, 1978; Hale, 1999; Domenici and Blake, 1993b). The decrease in potential thrust production due to a lower maximum body depth may be counteracted by the high-amplitude propulsive wave associated with the high-curvature responses of *E. bislineata*.

It is possible that the neural mechanism initiating an escape response in *Eurycea bislineata* differs from the Mauthner-initiated response described in fishes (Eaton et al., 1977; Eaton and Bombardieri, 1978; Fetcho, 1991; Zottoli, 1978, 1995). Although numerous studies have correlated C-start behavior with the presence and activity of Mauthner neurons (Zottoli, 1977), Eaton et al. (1982) showed that alternative neural mechanisms can also induce kinematically similar escape responses. Alternative neural mechanisms or variations in Mauthner cell morphology could affect both the latency of a response and the type of stimulus that induces a response. Although it is not known whether Mauthner cells are present in *E. bislineata*, they have been described in larvae and adults of numerous amphibian species, including several other plethodontid salamanders (Will, 1991; Zottoli, 1978).

Carrier (1996) hypothesized that, as a result of heavy predation on the early life stages of individuals, selection will favor relatively better locomotor performance early in ontogeny. In the case of *Eurycea bislineata*, the testing of such predictions is complicated by morphological and ecological changes at metamorphosis. We found no evidence to support the hypothesis that aquatic escape performance decreases after metamorphosis in *E. bislineata*. It is likely that kinematic modulation of the escape response allows adults to sustain an effective level of escape performance despite changes in morphology and ecology.

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