

COMPARISON OF THE FAST-START PERFORMANCES OF CLOSELY RELATED, MORPHOLOGICALLY DISTINCT THREESPINE STICKLEBACKS (*GASTEROSTEUS* SPP.)

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Summary

Fast-start escape performances for two species of threespine stickleback, *Gasterosteus* spp., were investigated using high-speed cinematography (400 Hz). The two fishes (not yet formally described, referred to here as benthic and limnetic) inhabit different niches within Paxton Lake, British Columbia, Canada, and are recent, morphologically distinct species. All escape responses observed for both species were double-bend C-type fast-starts. There were no significant differences between the species for any linear or angular parameter (pooled averages, both species: duration 0.048 s, distance 0.033 m, maximum velocity 1.10 m s^{-1} , maximum acceleration 137 m s^{-2} , maximum horizontal angular velocity 473.6 rad s^{-1} and maximum overall angular velocity 511.1 rad s^{-1}). Benthics and limnetics have the greatest added mass (M_a) at 0.3 and 0.6 body lengths, respectively. The maximum M_a does not include the fins for benthics, but for limnetics the dorsal and anal fins contribute greatly

to the maximum M_a . The deep, posteriorly placed fins of limnetics enable them to have a fast-start performance equivalent to that of the deeper-bodied benthics.

Both the limnetic and benthic fishes have significantly higher escape fast-start velocities than their ancestral form, the anadromous threespine stickleback *Gasterosteus aculeatus*, suggesting that the high performance of the Paxton Lake sticklebacks is an evolutionarily derived trait. In this biomechanical study of functional morphology, we demonstrate that similar high fast-start performance can be achieved by different suites of morphological characteristics and suggest that predation might be the selective force for the high escape performance in these two fishes.

Key words: stickleback, *Gasterosteus* spp., escape performance, fast-start, swimming performance, functional morphology.

Introduction

Fast-starts are used by many fish to catch evasive prey or to escape from predators (Webb and Skadsen, 1980; Rand and Lauder, 1981; Webb, 1984; Harper and Blake, 1988). Success in these behaviours, particularly predator evasion, has a direct impact on fitness. Characteristics that enhance fast-start performance are presumably under selective pressure in fishes that have a high risk of predation (Webb, 1975; Domenici and Blake, 1991; Kasapi *et al.* 1993).

The ability of fish to escape from predators may depend upon linear performance, (i.e. distance moved, velocity and acceleration; Webb, 1976; Vinyard, 1982; Weihs and Webb, 1984; Harper and Blake, 1988, 1990; Domenici and Blake, 1991), accurate timing (Eaton and Hackett, 1984) and turning ability (Howland, 1974; Webb, 1983; Domenici and Blake, 1993). Adaptations for optimal fast-start performance are thought to include a flexible body, a large muscle mass relative to total body mass and a large body depth caudally (Weihs,

1973; Webb, 1984; Blake, 1996). Thrust in the direction of motion during a fast-start is generated by the acceleration of the added mass, the mass of the volume of water influenced by a propulsive body section (Weihs, 1973; Frith and Blake, 1995). Deeper body sections move more water and thus produce greater thrust for accelerating the fish's mass.

Comparative studies of fast-start performance have included a wide variety of phylogenetically distinct fishes (see Blake, 1996, for a review). Comparison of the fast-start performances of two closely related, morphologically distinct, recent fish species provides an opportunity to use a biomechanical approach to study functional morphology at a finer phylogenetic scale. The sympatric sticklebacks (*Gasterosteus* spp.) of our study diverged recently (13 000 years ago; Schluter and McPhail, 1992). The two species, descended from the anadromous threespine stickleback *Gasterosteus aculeatus* (Bell, 1976; Schluter and McPhail,

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1992), are not yet formally described and are referred to as benthic and limnetic on the basis of their preferred feeding habitats. There is evidence that these are a species pair and not a single polymorphic species (McPhail, 1992; Schluter, 1993). For instance, morphological differences between the forms are retained when fish are bred for two generations in a common laboratory environment, and hybrids are morphologically intermediate when bred under the same conditions (McPhail 1984, 1992; Schluter, 1993). Morphologically intermediate individuals (hybrids) are rare in Paxton Lake, and there is strong assortative mating between the forms (Ridgway and McPhail, 1984). There are also stable allozyme (McPhail, 1992) and microsatellite DNA (E. B. Taylor, personal communication) differences between the species. The differences between these sympatric fishes are therefore genetically based, with little gene flow between the two populations (Schluter, 1993; McPhail, 1992).

The two stickleback species occupy different regions of Paxton Lake, British Columbia, Canada. Benthics live near the lake bottom in weedy areas, feeding primarily on insect larvae and other invertebrates, whereas limnetics form large schools in the pelagic region of the lake, foraging primarily on zooplankton (Larson, 1976; Schluter and McPhail, 1992; Schluter, 1993). Limnetics have a streamlined body (Fig. 1A), which may aid steady swimming (Law, 1994) but should lower thrust production for acceleration during fast-starts (Weihs, 1973; Webb, 1984). Benthics are deeper-bodied fish (Fig. 1A) and are thus expected to be able to produce greater thrust during fast-starts than limnetics. We originally predicted that morphological adaptations for steady swimming in limnetics would impair their fast-start performance and, therefore, that benthics would have higher fast-start performance than limnetics. We have found, however, that the escape fast-start performances of the two species were not significantly different, which we will suggest might be due to the selective force of predation.

Materials and methods

Fish collection and maintenance

Fish were collected from Paxton Lake, Texada Island, British Columbia, Canada (17 ha lake, 49°42'30" N, 124°31'30" W). Only juvenile and adult fish were used (i.e. no young from the year of the study). Fish were transported to the laboratory, where they were placed into four 1631 holding tanks in an environment chamber at a constant temperature of 15±1 °C. The tanks contained fresh, dechlorinated water and 0.3% salt. All fish were individually identified by colour patterns or slight size differences and were assigned a number. Fish were fed a mixture of live and previously frozen food once per day for 1 week prior to experimentation.

Fast-start experiments

Fish were habituated to the bright lights (three Berkey Coloran Halide 650 W bulbs) needed for filming by turning the lights on during feeding for the week prior to testing. Feeding

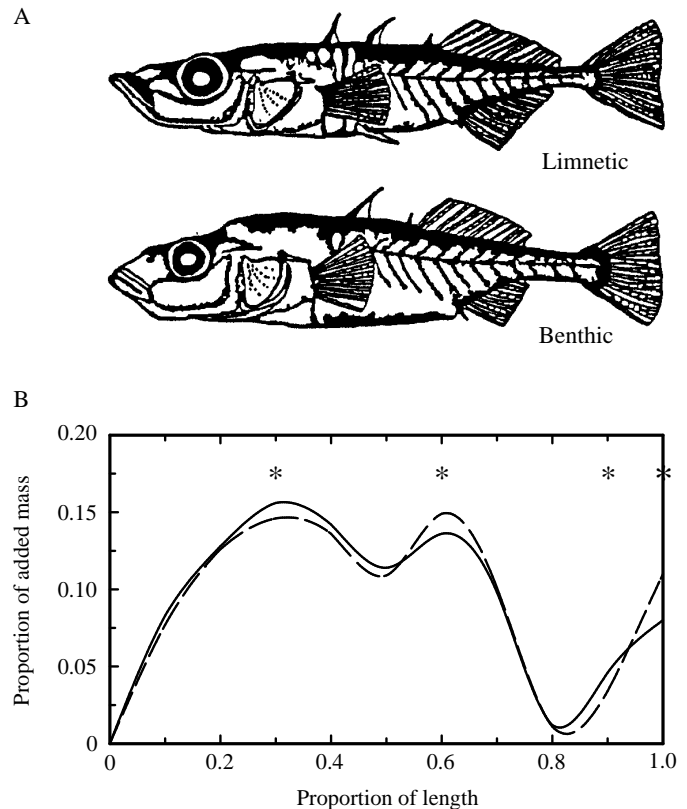


Fig. 1. (A) Drawings of Paxton Lake limnetic (top) and benthic (bottom) threespine sticklebacks (modified from Schluter, 1993). (B) Comparison of the distributions of mean added mass along the length of the body for benthics (solid line) and limnetics (dashed line). Asterisks indicate significant differences ($P < 0.05$) between the two species.

was stopped the day before experimentation. 91 of water (pretreated for a minimum of 3 days with limestone, 0.3% salt and aeration) was replaced daily in the experimental tank (described below). Experiments were conducted at 15±1 °C.

Single fish were transferred to the experimental glass tank (24 cm×29 cm×14.4 cm) and allowed to acclimate for 1 h prior to being startled. Attached to the back and floor of the tank were 1 cm square reference grids. Black paper covered the sides of the experimental tank so that the fish could not see the approaching stimulus. A mirror angled at 45° over the tank allowed the top view of the fish to be filmed. A profile view through the front of the tank and a top view from the mirror were filmed simultaneously at 400 frames s⁻¹ (Redlake Locam, camera model 51 with Sun-Dionar 16 zoom lens using Kodak 7250 colour 400 ASA tungsten high-speed reversal film). A 1 m pole with a rubber ball on the end was struck against the side of the tank to elicit the escape response. In none of the escapes analysed did fish touch the walls of the tank. An escape response was elicited for all fish tested.

Film analysis

One fast-start sequence was analysed for each fish tested. Sequences were projected (photo optical data analyser,

Photographic Analysis Ltd, model 224A) frame by frame onto paper, with the resulting image magnified 2.3–3 times. This procedure reduced the total error (measurement and sampling) to approximately $\pm 13\%$ for maximum acceleration and made negligible the errors for average acceleration, velocity and distance (Harper and Blake, 1989).

Escape responses have been shown to be a fixed action pattern consisting of two stages. Stage 1 (S1) consists of a unilateral contraction of the axial muscles, bending the fish into a C-shape, followed by stage 2 (S2), a strong propulsive stroke of the tail in the opposite direction (Gillette, 1987). Although a third stage in which the fish forms another curve or coasts is possible, this study included only S1 and S2 for analysis. The durations of S1 and S2 were delimited by the change in direction of movement of the head that occurred at the end of each stage.

For each escape response, the number of frames analysed included one frame before movement (time=0, start of S1) to five frames after the end of S2. The position of the centre of mass (CM, based on measurements described below) was marked on the paper, along with the position of the head and the end of the caudal peduncle in the top and profile views for each frame. These points were later analysed using a digitising pad (GTCO type, 0.61 m \times 0.91 m) connected to a personal computer.

Instantaneous velocities and accelerations during the two stages were determined from the first and second derivatives, respectively, of a five-point moving second-order regression (Lanczos, 1956) on the distance–time data. Displacement of the CM in the Z direction (*z*-axis=vertical), X direction (*x*-axis=parallel with the front glass of the tank) and Y direction (*y*-axis=parallel with the side glass of the tank) were combined using vector sums to express displacement in three dimensions (Law, 1994).

The pitch angle was measured in the profile view as the angle between a line through the longitudinal axis of the fish, corresponding to the fish's lateral line, and the horizontal grid on the back of the tank, parallel to the floor of the tank. The pitch measured from profile views (apparent pitch, γ) is affected by the orientation of the fish along the *y*-axis and may not be the true pitch angle. Therefore, the instantaneous orientation angle, τ , defined as the angle between the longitudinal axis of the fish and the X-direction of the grid on the floor of the tank, parallel to the front of the tank, was also measured. The actual pitch angle, p , was then determined by:

$$p = \arctan(\tan \gamma \cos \tau), \quad (1)$$

(Kasapi *et al.* 1993). Pitch angles were defined as positive if the head of the fish pointed up and negative if the head pointed down.

The turning angle (θ) for two-dimensional movement in the horizontal plane (X and Y directions) was calculated using dot products between one vector created by the change in position of the CM [e.g. position (x_2, y_2) to position (x_3, y_3)] and the previous vector [e.g. (x_1, y_1) to (x_2, y_2)]. The initial turn direction was considered positive, with subsequent turn angles

being signed positive if in the same direction and negative if in the opposite direction as the initial direction of turn (Law, 1994). The horizontal angular velocity of the CM was determined using a five-point moving regression (Lanczos, 1956) on turning angle–time data. The turning radius (TR) was calculated using the mean instantaneous distance moved (d) and the mean θ of the CM throughout S1. The turning radius is given by:

$$TR = 2d \cos(\theta/2). \quad (2)$$

Overall angular velocity in three-dimensional space was also determined. Angles between vectors (changes in positions of the CM in three-dimensional space, X, Y and Z directions) were calculated using dot products with the rotational directions assigned as described above for turning angles. The overall angular velocity was determined using the same smoothing technique used for the horizontal angular velocity.

Morphometrics

Fish were killed with an overdose of MS222, blotted dry with a paper towel, weighed, and measured for standard lengths (tip of rostrum to end of caudal peduncle) and total lengths (tip of rostrum to end of caudal fin). The CM of each fish was determined by suspending the fish from the mouth and marking the vertical line of gravity, then repeating this procedure for suspension from the cloaca. The CM was the point where the two lines crossed. The CM as a proportion of standard length was the distance along the straight body from the tip of the rostrum to the CM divided by standard length, and the distance from the fish's dorsal surface to the CM divided by the body depth at that position was the CM as a proportion of body depth.

The cross-sectional depth of the body was measured using Vernier callipers (± 0.005 cm) at intervals of 10% of total length (0.1L), while the fish was submerged in water in a Petri dish to ensure full extension of the fins. The added mass (M_a) of each section along the fish's body was calculated using:

$$M_a = 0.25\rho\pi D^2\beta l, \quad (3)$$

(modified from Lighthill, 1971), where ρ is the density of water, D is the depth of the section, l is the length of the section and β is a shape-dependent constant. For most fish cross sections, β is close to 1 (Lighthill, 1971); therefore, $\beta=1$ was used in calculations.

The sex of the fish and the presence of any external or internal parasites were recorded. Fish that were gravid or parasitised were excluded from the final data set.

Statistical tests

Performance parameters for the two species over the total fast-start (S1+S2) duration, distance, average velocity, maximum velocity, average acceleration and maximum acceleration were compared using Student's *t*-tests followed by sequential Bonferroni correction for multiple tests at $\alpha=0.05$, number of tests (k)=6. Sequential Bonferroni correction is

Table 1. Distance-derived parameters of the centre of mass for the total fast-start response (S1+S2) of benthics and limnetics

| | Benthic | Limnetic | Pooled |
|---|---------------|---------------|---------------|
| Duration (s) | 0.0487±0.0052 | 0.0475±0.0040 | 0.0481±0.0032 |
| Distance (m) | 0.032±0.004 | 0.035±0.006 | 0.033±0.003 |
| Average velocity (m s ⁻¹) | 0.66±0.07 | 0.71±0.11 | 0.68±0.06 |
| Maximum velocity (m s ⁻¹) | 1.10±0.17 | 1.09±0.16 | 1.10±0.12 |
| Average acceleration (m s ⁻²) | 13.9±4.5 | 15.5±4.2 | 14.7±3.0 |
| Maximum acceleration (m s ⁻²) | 129.9±19.4 | 143.8±20.6 | 137.1±14.2 |

Values are means ± 2 S.E.M.; benthic, $N=11$; limnetic, $N=12$.

Average velocity and acceleration were determined by averaging all values within each stage.

Species means were compared using Student's t -tests with *post hoc* sequential Bonferroni correction at $\alpha=0.05$, $k=6$. All means compared were not significantly different ($P>0.0083$).

more powerful than the Bonferroni correction in detecting significant differences (Rice, 1989).

There are differences in body size between these two species; therefore, to test the effect of size on performance, the total performance parameters were adjusted using a least-squares regression method for adjusting treatment means (Steel and Torrie, 1980; Weisberg, 1985). This adjustment uses linear regressions of the variable on standard length but, unlike analysis of covariance (ANCOVA), does not assume that there is no true population difference in the covariate (Steel and Torrie, 1980). In *Gasterosteus* spp., it is conventional to use 0.050 m as a standardising size for comparison (Hagen and Gilbertson, 1973; McPhail, 1984; Lavin and McPhail, 1985). In addition, 0.050 m is close to half-way between the mean benthic length (mean 0.0542 m) and the mean limnetic length (mean 0.0444 m). The size-corrected means with associated standard errors were then compared using Student's t -tests and *post hoc* sequential Bonferroni correction at $\alpha=0.05$, $k=6$.

The linear parameters between S1 and S2 and between species were compared using two-way repeated-measures analysis of variance (ANOVA) for each parameter followed by sequential Bonferroni corrections ($\alpha=0.05$, $k=6$) of the between-species comparisons, between-stages comparisons and interaction (species × stage) comparisons. A two-way ANOVA with Student–Newman–Keuls pairwise comparisons was used to compare the distances travelled in each of the three planes (X , Y and Z directions) for the two species. Two-way repeated-measures ANOVA with Student–Newman–Keuls pairwise comparisons were used for comparisons of pitch angles over the escape response, for M_a along the length, for S1 and S2 average and maximum horizontal angular velocities and for S1 and S2 average and maximum overall angular velocities. Student's t -tests separately compared maximum negative horizontal angular velocity, maximum negative overall angular velocity and TR/L. Least-squares linear regression was used to determine the relationship between final pitch angle and starting S2 pitch angle. The correlation between TR/L and S1 average velocity was determined using the Pearson product moment method. Morphological characteristics of the two species were

compared using Student's t -tests with sequential Bonferroni correction for multiple tests at $\alpha=0.05$, $k=5$. Proportion data (CM as a percentage of standard length, CM as a percentage of depth) were arcsine square-root-transformed prior to the t -tests (Zar, 1984). The positions of maximum added mass along the length of the body are from discontinuous data and were therefore compared using Mann–Whitney rank sum tests (Zar, 1984).

Results

Escape performance

All fast-starts were double-bend type (Domenici and Blake, 1991) involving the formation of a C-shape by the body of the fish (S1), followed by a contralateral bend (S2), turning the head in the opposite direction. Data for both sexes within a species were pooled because comparisons between males (benthic $N=4$, limnetic $N=9$) and females (benthic $N=7$, limnetic $N=3$) within a species for all parameters in S1 and S2 showed no significant differences (all $P>0.05$).

No significant differences (all $P>0.083$) between limnetics ($N=12$) and benthics ($N=11$) were found for any of the linear

Table 2. Size-corrected distance-derived parameters of the centre of mass for the total fast-start response (S1+S2) of benthics and limnetics

| | Benthic | Limnetic |
|---|---------------|---------------|
| Duration (s) | 0.0488±0.0204 | 0.0457±0.0188 |
| Distance (m) | 0.030±0.016 | 0.052±0.019 |
| Average velocity (m s ⁻¹) | 0.62±0.27 | 1.08±0.32 |
| Maximum velocity (m s ⁻¹) | 1.12±0.66 | 1.65±0.51 |
| Average acceleration (m s ⁻²) | 18.7±15.3 | 26.0±16.6 |
| Maximum acceleration (m s ⁻²) | 137.8±74.8 | 203.4±75.4 |

Values are means ± 2 S.E.M.; benthic, $N=11$; limnetic, $N=12$.

Average velocity and acceleration were determined by averaging all values within each stage.

Species means were compared using Student's t -tests with *post hoc* sequential Bonferroni correction at $\alpha=0.05$, $k=6$. All means compared were not significantly different ($P>0.0083$).

Table 3. Distance-derived parameters of the centre of mass for stage 1 (S1) and stage 2 (S2) fast-start response of benthics and limnetics

| | Benthic | Limnetic |
|--|---------------|---------------|
| S1 duration (s) }* | 0.0296±0.0046 | 0.0269±0.0031 |
| S2 duration (s) }* | 0.0184±0.0030 | 0.0206±0.0030 |
| S1 distance (m) | 0.018±0.003 | 0.018±0.003 |
| S2 distance (m) | 0.015±0.003 | 0.017±0.004 |
| S1 average velocity (m s ⁻¹) }* | 0.55±0.06 | 0.61±0.06 |
| S2 average velocity (m s ⁻¹) }* | 0.81±0.15 | 0.86±0.18 |
| S1 maximum velocity (m s ⁻¹) }* | 0.79±0.11 | 0.90±0.09 |
| S2 maximum velocity (m s ⁻¹) }* | 1.07±0.18 | 1.06±0.18 |
| S1 average acceleration (m s ⁻²) | 12.4±4.3 | 15.0±5.8 |
| S2 average acceleration (m s ⁻²) | 15.5±12.6 | 17.3±10.5 |
| S1 maximum acceleration (m s ⁻²) | 99.5±11.6 | 118.7±11.5 |
| S2 maximum acceleration (m s ⁻²) | 122.5±23.3 | 127.4±29.0 |

Values are means ± 2 s.e.m.; benthic, N=11; limnetic, N=12.

Parameters were compared using two-way repeated measures ANOVA and *post hoc* sequential Bonferroni correction at $\alpha=0.05$, $k=6$ on species comparisons, stages comparisons and interaction (species × stage) comparisons. All species comparisons and interaction comparisons are not significantly different (all $P>0.0083$).

*Stages comparisons that are significantly different. Significant pairwise comparisons are described in the text.

parameters measured over the total fast-start (Table 1). In order to minimise type I error, the sequential Bonferroni correction produces a very low P value for rejection; however, the minimum P value observed was 0.34. When corrected for possible influences of body size, there were still no significant differences (all $P>0.0083$) in any of the linear parameters between limnetics and benthics (Table 2). For the linear parameters in S1 and S2, there were no significant differences (all $P>0.0083$) for the species and interactions comparisons (Table 3). Both species had longer durations for S1 than for S2, and higher average and maximum velocities during S2 than S1 ($P<0.05$). The highest recorded total distance for a fast-start

Table 4. Pitch angles for stage 1 (S1) and stage 2 (S2) of escape responses of benthics and limnetics

| | Pitch angle (degrees) | |
|------------|-----------------------|-----------|
| | Benthic | Limnetic |
| Initial | 0.2±8.2 | 3.3±5.8 |
| S1 maximum | -12.2±11.4 | 1.3±10.1 |
| S2 maximum | -23.7±12.1 | -5.5±13.8 |
| Final | -20.9±11.8 | -3.8±11.4 |

Species $P<0.05$ * Stages $P<0.001$ * Interaction $P>0.05$

Values are means ± 2 s.e.m.; benthic, N=11; limnetic, N=12. Species means were compared using a two-way repeated-measures ANOVA.
*Significant difference. Significant pairwise comparisons are described in the text.

was 0.063 m, the highest velocity was 1.59 m s⁻¹ and the highest acceleration was 215.6 m s⁻².

Although the distances travelled during escape responses were not different for the two species (Tables 1, 2), benthics travelled in the X and Y directions significantly more than in the Z direction ($P<0.05$), whereas there were no significant differences ($P>0.05$) between the distances travelled in the three planes by limnetics (Fig. 2).

For benthics, the initial pitch angle was significantly ($P<0.05$) more upward than the maximum pitch angle during S2, and the initial and final pitch angles were significantly different ($P<0.05$, Table 4). The greatest change in pitch occurred during S1, where benthic fish changed from a positive to a negative pitch angle (Fig. 3). Limnetics showed little change in pitch over the fast-start (Table 4). Benthics had a significantly ($P<0.05$) more downward pitch at the end of S2 than limnetics (Table 4), and all benthics had a downward final escape path, whereas three limnetics had an upward escape path (all others went down). Pitch angle at the start of S2 was significantly related to final pitch angle for benthics ($r^2=0.54$, $P<0.05$) and limnetics ($r^2=0.81$, $P<0.05$), indicating

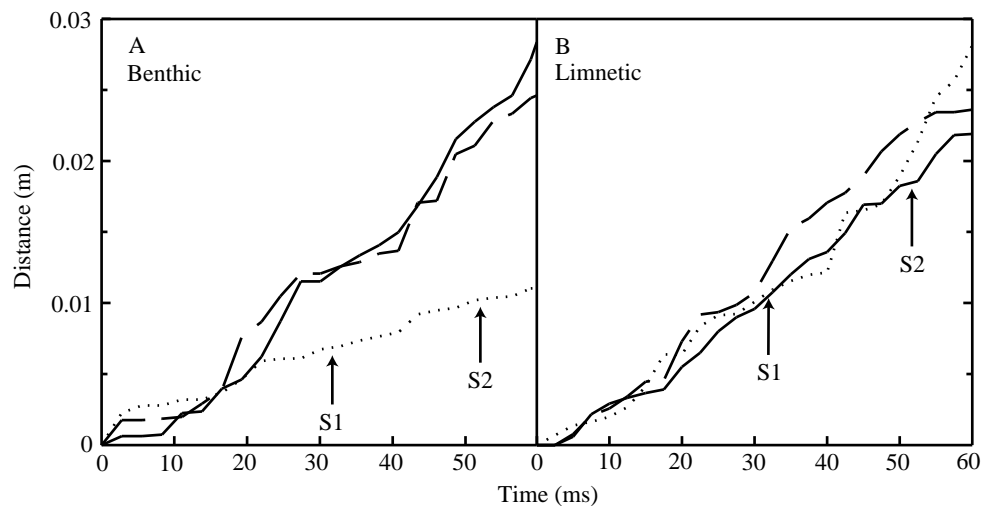


Fig. 2. Distance-time curves of the centre of mass of a benthic (A) and a limnetic (B) during a fast-start. The X-direction (solid line), Y-direction (dashed line) and Z-direction (dotted line) distance components are shown. Arrows indicate the ends of stage 1 (S1) and stage 2 (S2).

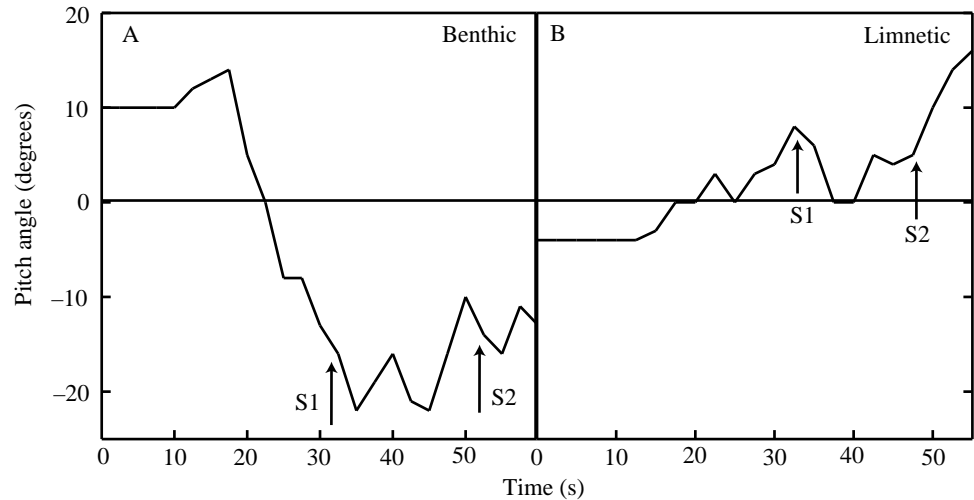


Fig. 3. Pitch angles over the duration of an escape response of a benthic (A) and a limnetic (B). Arrows indicate the ends of stage 1 (S1) and stage 2 (S2).

that the extent of final pitch was largely determined during S1 (Fig. 4).

There were no significant differences ($P>0.05$) between the two fish species for horizontal angular velocities (Table 5) or for overall angular velocities (Table 6) during the escape response. For both species, the maximum horizontal and overall angular velocities were higher ($P<0.05$) during S1 than during S2 (Tables 5, 6). Horizontal angular velocity contributed greatly to overall angular velocity, with S1 maximum horizontal angular velocity making up 88% and 93% of S1 maximum overall angular velocity for benthics and limnetics, respectively, and S2 maximum horizontal angular velocity making up 84% of S2 maximum overall angular velocity for both species. The maximum negative horizontal angular velocity (benthics $-134.1\pm 49.8\text{ rad s}^{-1}$, limnetics $-202.0\pm 110.4\text{ rad s}^{-1}$) and maximum negative overall angular velocity (benthics $-162.1\pm 73.8\text{ rad s}^{-1}$, limnetics $-272.0\pm 110.6\text{ rad s}^{-1}$) occurred

during S2 for both species and did not differ significantly ($P>0.05$) between the two fish species.

Benthics had a significantly ($P<0.05$) tighter turning radius for their length ($TR/L=0.046\pm 0.006$) than did limnetics ($TR/L=0.058\pm 0.010$). There was a positive correlation between S1 average velocity and TR/L (Fig. 5, $r=0.53$, $P<0.05$).

Morphometrics

There were no significant differences between males and females within either species for any of the morphological measurements (all $P>0.05$); therefore, the data for a given species were pooled. Benthics are heavier, longer and deeper-bodied than limnetics (Table 7). The position of the centre of mass, however, does not differ. The distribution of M_a along the length of the fish differs significantly ($P<0.05$) between the two species (Fig. 1). Benthics have the greatest M_a at $0.3L$, whereas the position of maximum M_a for limnetics is at $0.6L$ (Fig. 1). The

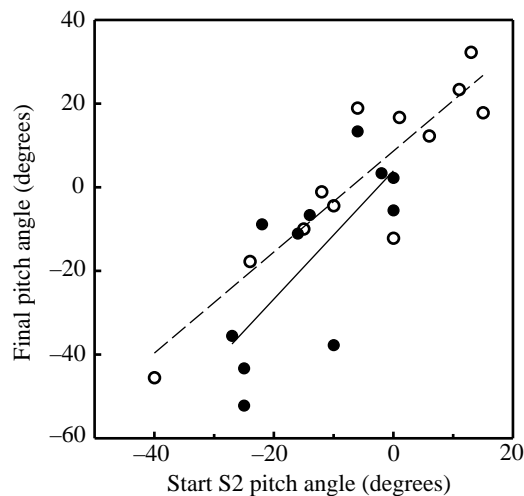


Fig. 4. Relationship between pitch angle at the start of stage 2 (S2) (x) and final pitch angle (y) for benthics (filled circles and solid line, $y=-2.47+1.38x$, $r^2=0.54$, $P<0.05$) and limnetics (open circles and dashed line, $y=1.77+1.09x$, $r^2=0.81$, $P<0.05$).

Table 5. Horizontal angular velocity of the centre of mass for stage 1 (S1) and stage 2 (S2) of the escape responses of benthics and limnetics

| | Horizontal angular velocity (rad s^{-1}) | |
|------------|---|--|
| | Benthic | Limnetic |
| S1 average | 227.8 \pm 73.8 | 111.9 \pm 75.9 |
| S2 average | 20.8 \pm 62.8 | 36.6 \pm 51.7 |
| | Species $P>0.05$ | Stages $P<0.05^*$ Interaction $P>0.05$ |
| S1 maximum | 481.5 \pm 129.0 | 412.1 \pm 123.1 |
| S2 maximum | 182.0 \pm 96.7 | 198.6 \pm 78.8 |
| | Species $P>0.05$ | Stages $P<0.05^*$ Interaction $P>0.05$ |

Values are means ± 2 s.e.m.; benthic, $N=11$; limnetic, $N=12$.

Averages were determined by averaging all values within each stage.

Species means were compared using two-way repeated-measures ANOVA with Student–Newman–Keuls pairwise comparisons.

*Significant difference. Significant pairwise comparisons are described in the text.

Table 6. Overall angular velocity of the centre of mass in three dimensions for stage 1 (S1) and stage 2 (S2) of the escape responses of benthics and limnetics

| | Overall angular velocity (rad s ⁻¹) | |
|------------|---|-------------|
| | Benthic | Limnetic |
| S1 average | 264.7±62.2 | 140.6±77.6 |
| S2 average | 4.30±66.3 | 10.4±62.8 |
| | Species $P>0.05$ Stages $P<0.05^*$ Interaction $P>0.05$ | |
| S1 maximum | 544.4±92.3 | 444.0±137.5 |
| S2 maximum | 217.1±94.2 | 235.9±79.8 |
| | Species $P>0.05$ Stages $P<0.05^*$ Interaction $P>0.05$ | |

Values are means ± 2 S.E.M.; benthic, $N=11$; limnetic, $N=12$.

Averages were determined by averaging all values within each stage.

Species means were compared using two-way repeated-measures ANOVA with Student–Newman–Keuls pairwise comparisons.

*Significant difference. Significant pairwise comparisons are described in the text.

maximum M_a does not include fins for benthics, but for limnetics the dorsal and anal fins contribute greatly to the maximum M_a .

Discussion

Most teleosts, including benthic sticklebacks (Fig. 2), move their CM primarily in a horizontal plane during S1 and S2 (Eaton *et al.* 1977; Webb, 1983). In contrast, escapes of marbled hatchet fish *Carnegiella strigata* (Eaton *et al.* 1977), knifefish *Xenomystus nigri* (Kasapi *et al.* 1993) and limnetic sticklebacks (Fig. 2) involve acceleration of the CM in all three dimensions. During the escape response of limnetics, the body moves in all three dimensions and the escape direction is variable. The CM of benthics moves primarily in the horizontal

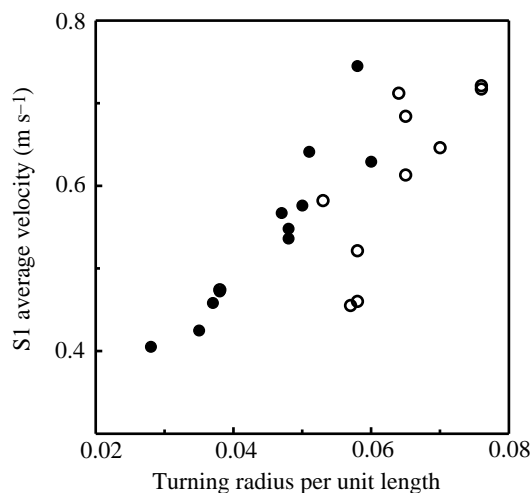


Fig. 5. Correlation between turning radius per unit length and average S1 velocity ($r=0.53$, $P<0.05$) for benthics (filled circles) and limnetics (open circles).

Table 7. Morphological characteristics of benthics and limnetics

| | Benthic | Limnetic |
|------------------------------|-------------|--------------|
| Mass (g) | 2.264±0.223 | 0.858±0.091* |
| Length (cm) | 5.42±0.32 | 4.44±0.21* |
| Depth at CM (cm) | 1.17±0.10 | 0.83±0.06* |
| CM as a proportion of length | 0.43±0.01 | 0.43±0.003 |
| CM as a proportion of depth | 0.49±0.002 | 0.55±0.002 |

Values are means ± 2 S.E.M.; benthic, $N=11$; limnetic, $N=12$.

CM, centre of mass of straight fish.

Species means were compared using Student's t -tests with *post hoc* sequential Bonferroni correction for multiple tests at $\alpha=0.05$, $k=5$.

*Significant difference between species.

plane during S1 and S2, but the head pitches forward during these stages (Table 4; Fig. 3), allowing the subsequent escape path to be in a downward direction (Fig. 4). Limnetics may use variability in escape path and three-dimensional movement to confuse predators in the open-water habitat of the pelagic zone. In contrast, benthics may use pitch to confuse predators and to escape downwards to hide in the mud on the bottom of the lake (T. C. Law, personal observation).

For these sticklebacks, there is a positive relationship between S1 average velocity and TR/L (Fig. 5). There may be physical and behavioural trade-offs between high linear performance and tight turns. At the very high velocities of escape fast-starts, the fish may be moving at their limit of centripetal acceleration. Thus, for every unit increase in velocity the fish must also increase its turning radius in proportion to the square of its velocity in order to stay within the limit of constant centripetal acceleration (Howland, 1974). A smaller turning radius corresponds to a greater change in direction for a given distance travelled, which may confuse a striking predator or allow the prey to avoid capture by a less manoeuvrable predator. High velocity and tight turns may be used at different times by sticklebacks for avoiding predators, depending on the predator's strike tactics. The timing of the escape turn becomes less critical the smaller the turning radius; thus, a lower velocity and a tighter turning radius may be advantageous when the distance and speed of the predator are unknown by the prey; however, when the predator's response is known, a high velocity and a larger turning radius might be a better strategy for the prey (Howland, 1974).

During the escape response, both sticklebacks had high positive angular velocity during S1 (Tables 5, 6) and high negative angular velocity in S2, indicating a reversal of turn direction between S1 and S2. This change in the turning direction of the CM is a distinguishing feature of double-bend C-starts (Domenici and Blake, 1991). The turning of the CM during S1 describes an arc of minimal turning radius (TR) for the fast-start (Webb, 1983). The TR for these sticklebacks (benthics 0.045L, limnetics 0.057L) is tight in comparison with that of fusiform fishes such as yellowfin tuna *Thunnus albacares* (0.47L; Blake *et al.* 1995), smallmouth bass

Micropterus dolomieu and rainbow trout *Salmo gairdneri* (0.11L and 0.18L, respectively; Webb, 1983) and comparable to that of a deep-bodied fish specialised for turning, the angelfish *Pterophyllum eimekei* (0.065L; Domenici and Blake, 1991). Our data suggest that the fusiform shape of these sticklebacks does not compromise their turning performance.

Webb (1984) suggests that specialisation for one swimming mode, such as manoeuvrability, should compromise performance in the other swimming modes, sustained steady swimming and fast-starts. Fish that are specialised for median- or paired-fin propulsion (manoeuvrability) should therefore show reduced fast-start performance compared with generalists or fast-start specialists (Webb, 1984). There does seem to be a compromise in swimming performance for fish employing the same propulsor for routine swimming and fast-starts. Webb (1988) showed that the steady swimming performance of northern pike *Esox lucius* (a fast-start specialist) is inferior to that of rainbow trout (a generalist). In addition, Harper and Blake (1990) found that during fast-starts northern pike achieve mean maximum accelerations of 120 m s^{-2} while rainbow trout only reach 60 m s^{-2} . Fast-start performance, however, does not seem to be compromised in fish that use different propulsors for different swimming modes. Some specialists for median- or paired-fin slow swimming attain accelerations during escape fast-starts as high as, or higher than, that of generalists. Angelfish are capable of accelerating at 79 m s^{-2} (Domenici and Blake, 1991) and knifefish at 128 m s^{-2} (Kasapi *et al.* 1993). The knifefish is now thought to be a specialist for both slow-speed manoeuvrability and high-speed fast-starts (Kasapi *et al.* 1993). Both angelfish and knifefish are laterally compressed, which provides a large body depth for thrust during fast-starts. The sticklebacks in our study are more fusiform in shape; therefore, one might expect that they should have poor fast-start performance compared with the deeper-bodied angelfish and knifefish. Nonetheless, they achieved a mean maximum acceleration of 137 m s^{-2} (Table 1), and the highest recorded acceleration was 216 m s^{-2} . Although sticklebacks are fusiform, paired-fin specialists, they attained accelerations comparable with those of fast-start specialists. Accelerating and manoeuvring employ axial locomotion and paired-fin locomotion, respectively. These systems are 'decoupled'; i.e. the pectoral muscles and fins used for paired-fin manoeuvring are separate from the myotomal muscles and body and caudal fin sections used for fast-starts (Blake, 1996). Therefore, there may be no compromise between performance in manoeuvrability and in burst swimming.

Contrary to our original prediction that benthics should have higher fast-start performance than limnetics, the performances of the two species were not different (Tables 1, 3). Despite a significant difference in body size between the two species (Table 7), there were still no differences in fast-start performances after the possible effects of size had been removed (Table 2). Acceleration and velocity over a given time during fast-starts have been shown to be independent of size (Webb, 1976; Domenici and Blake, 1993). Acceleration

rates should theoretically be independent of size since the inertia of body mass and added water mass constitute the major resistance to acceleration, and muscle mass is a relatively constant percentage of body mass (Webb, 1976). The typical independence of acceleration and body size is evident from the equivalent performance of the small stickleback with that of the much larger pike. Differences in fast-start performance can be seen, however, when comparing different body forms; thus, body morphology may be more important than body size in determining fast-start performance.

Weihs (1973) hypothesised that body shape is a key determinant of maximum acceleration in fish. Northern pike, known for high acceleration during fast-starts (Harper and Blake, 1990), have morphological characteristics such as a large surface area caudally and a high percentage of body mass as muscle that are considered favourable for high thrust production during fast-starts (Webb, 1984). Intraspecific comparisons of fast-start performance for coho salmon *Oncorhynchus kisutch* (Taylor and McPhail, 1985) and threespine stickleback (Taylor and McPhail, 1986) showed that fish with greater body depth and caudal fin depth are capable of higher fast-start performance. In addition, Webb (1977) showed that amputation of the median fins reduces the fast-start performance of rainbow trout. The positioning and depth of the anal and dorsal fins can influence the fast-start performance of fishes because of their contribution to overall body depth (Frith and Blake, 1991). Thrust from the caudal fin and the body section that contains the dorsal and anal fins accounts for more than 90% of total thrust in northern pike (Frith and Blake, 1991). The posterior placement of the dorsal and anal fins contributes 26% to total thrust (Frith, 1990). These fins increase the depth of section and therefore the added mass (Frith and Blake, 1995; Weihs, 1973). Webb (1978) found that, for rainbow trout, caudally placed dorsal and anal fins contribute approximately 27% to total thrust. In addition, Webb *et al.* (1992) found that the low power produced by the caudal fin of the longnose gar *Lepisosteus osseus* was compensated for by the median fins, which were posteriorly positioned. Similarly, the dorsal fin of the limnetic stickleback is placed farther back on the body, directly above the anal fin, in comparison with the benthic fish (Fig. 1). This placement increases the body depth for limnetics and therefore increases their added mass. The position of greatest added mass for limnetics is at $0.6L$, where the depth is due primarily to the dorsal and anal fin heights (Fig. 1). This increases added mass where the majority of thrust is produced, which may account for their high fast-start performance.

The similarly high escape performance in limnetics and benthics is consistent with the likelihood that these sticklebacks encounter predators in the lake and are therefore under selection for high performance. The main predators of sticklebacks include piscivorous fish, diving birds, garter snakes and insects (Wootton, 1984; Larson and McIntire, 1993). Cutthroat trout *Oncorhynchus clarki* were indigenous to Paxton Lake but may have been extirpated in 1957 when lake water levels were reduced (Larson and McIntire, 1993). In 1969, Paxton Lake was

stocked with coho salmon fry that grew to adults by 1970 and were feeding extensively on sticklebacks, but the salmon population was not self-sustaining (Larson and McIntire, 1993). Despite these potential predators, the intensity of predation may not have been equal on the two species. Limnetics live in a more open habitat and might, therefore, be more visible and more susceptible to predators. Conversely, benthics may be preferentially selected by predators because of their larger body size and reduced body armour. Studies of predation on sticklebacks have shown predator selection for larger prey (Külling and Milinski, 1992) and for less armour (Hagen and Gilbertson 1973; Moodie *et al.* 1973). Although predation risk may not be equal in the two habitats, during the breeding season both species build nests in the littoral zone and may at that time have similar exposure to the same suite of predators. If most of the sticklebacks in this lake are experiencing predation, selection should be sufficiently strong to maintain high escape performance in both species because the consequences to fitness from even one possible encounter with these predators are substantial.

Inferential studies can correctly define selective processes if the investigation includes the study of incipient or recent species with knowledge of both the organism's ecology and a particular trait's functional significance (Schluter, 1995). The sticklebacks of this study are recently derived species, and their ecology is fairly well known (Larson, 1976; McPhail, 1992; Schluter and McPhail, 1992; Schluter, 1993). The choice of two recent (<13 000 years), sympatric species increases the likelihood that differences represent selection as one would expect little divergence by drift in such a short time (Endler, 1986; Garland and Adolph, 1994). Therefore, we would expect few morphological differences unless these differences were the result of selective pressures. The sticklebacks of the present study differ in characteristics that can be linked to thrust production and thus acceleration during fast-starts. Therefore, these fishes provide a good opportunity to study the functional significance of morphological differences in the context of escape performance and to infer possible mechanisms for selection on escape fast-start performance.

The ancestral form of the Paxton Lake sticklebacks is the anadromous threespine stickleback (Bell, 1976; Schluter and McPhail, 1992). By comparing the published startle fast-start performance of this species (Taylor and McPhail, 1986) with that of its descendants, we can determine whether the trait (high fast-start performance) is conserved (Harvey and Pagel, 1991). This comparison also allows us to have the added power of a three-species test (Garland and Adolph, 1994). Both the limnetics and benthics (Table 1) have significantly higher (one-way ANOVA; $P < 0.05$) escape fast-start velocities (acceleration was not reported) than the anadromous fish (average velocity $0.40 \pm 0.06 \text{ m s}^{-1}$, maximum velocity $0.66 \pm 0.08 \text{ m s}^{-1}$; Taylor and McPhail, 1986). This result suggests that the high performance of the Paxton Lake sticklebacks is not a conserved trait but is derived, indicating that there has probably been evolution in fast-start performance. The reason for the divergence in morphology, which nevertheless allows for

similarly high escape performance, may be related to other life-style differences, such as the need to overcome constraints for sustained swimming (Law, 1994).

In this biomechanical study of functional morphology, we have demonstrated that similarly high fast-start performance can be achieved with different suites of morphological characteristics. Although the influence of predation on the fast-start performance of these fishes is not yet known, piscivorous fish were at one time abundant in the lake and there are bird, snake and insect predators currently resident in the lake. The relatively lower performance of the ancestral form suggests that some evolution in performance has occurred in these fishes since their colonisation of fresh water in the last 13 000 years. Therefore, predation may be an important selective force for high escape fast-start performance in these sticklebacks. The direct link between morphology, fast-start performance and fitness can only be determined by collecting data on fast-start performance and survival of sticklebacks with varying morphologies along with assessments of predation risk.

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