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RESEARCH ARTICLE

Movement and function of the pectoral fins of the larval zebrafish (*Danio rerio*) during slow swimming

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SUMMARY

Pectoral fins are known to play important roles in swimming for many adult fish; however, their functions in fish larvae are unclear. We examined routine pectoral fin movement during rhythmic forward swimming and used genetic ablation to test hypotheses of fin function in larval zebrafish. Fins were active throughout bouts of slow swimming. Initiation was characterized by asymmetric fin abduction that transitioned to alternating rhythmic movement with first fin adduction. During subsequent swimming, fin beat amplitude decreased while tail beat amplitude increased over swimming speeds ranging from 1.47 to 4.56 body lengths per second. There was no change in fin or tail beat frequency with speed (means ± s.d.: 28.2±3.5 and 29.6±1.9 Hz, respectively). To examine potential roles of the pectoral fins in swimming, we compared the kinematics of finless larvae generated with a morpholino knockdown of the gene *fgf24* to those of normal fish. Pectoral fins were not required for initiation nor did they significantly impact forward rhythmic swimming. We investigated an alternative hypothesis that the fins function in respiration. Dye visualization demonstrated that pectoral fin beats bring distant fluid toward the body and move it caudally behind the fins, disrupting the boundary layer along the body's surface, a major site of oxygen absorption in larvae. Larval zebrafish also demonstrated more fin beating in low oxygen conditions. Our data reject the hypothesis that the pectoral fins of larval zebrafish have a locomotor function during slow, forward locomotion, but are consistent with the hypothesis that the fins have a respiratory function.

Supplementary material available online at http://jeb.biologists.org/cgi/content/full/214/18/3111/DC1

Key words: zebrafish, swimming, pectoral fin, genetic ablation, initiation.

INTRODUCTION

While many studies have examined pectoral fin movement and function in adult fish, less attention has been given to pectoral fins at the larval stage of development. For juveniles and adults of many fish species, pectoral fins act as primary propulsors during rhythmic swimming (e.g. Webb, 1973; Blake, 1979; Drucker and Jensen, 1996a; Drucker and Jensen, 1996b; Walker and Westneat, 1997; Hale et al., 2006), and in arrhythmic movements such as braking (e.g. Drucker and Lauder, 2003; Higham et al., 2005) and maneuvering (e.g. Drucker and Lauder, 2001; Drucker and Lauder, 2003). For larval fish, the pectoral fins move actively during rhythmic swimming (Batty, 1981; Müller and van Leeuwen, 2004; Thorsen et al., 2004), routine turning (Danos and Lauder, 2007) and feeding (Budick and O'Malley, 2000) behaviors, yet striking differences from adults in size and other aspects of morphology may result in different functional demands on the pectoral fins.

Much of the work examining the behavior of fish at early developmental stages has been on the larval zebrafish, a genetic model system that has been used broadly to examine motor control and movement (e.g. Fuiman and Webb, 1988; Müller et al., 2000; Thorsen et al., 2004; Danos and Lauder, 2007; McLean et al., 2007). Larval zebrafish beat their pectoral fins, alternating them rhythmically in combination with body undulation during swimming at low speed [~1–6 total body lengths (TL) s^{-1}]. This distinct movement pattern has been broadly referred to as 'slow swimming' or 'slow start' (Budick and O'Malley, 2000; Müller and van Leeuwen, 2004; Thorsen et al., 2004). During faster swimming, the body undulates but the pectoral fins remain positioned close to the body. While the basic patterns of undulatory movement and the coordination of body undulations with the pectoral fins have been described, variation of pectoral fin and body kinematics through the duration of the swim bout and with speed have not been examined in larvae. More broadly, the potential locomotor functions of rhythmic pectoral fin movements during forward swimming have not been been tested.

The first goal of this work was to measure basic kinematic variables of the slow swim gait and to determine how these kinematic variables change with swimming speed. Studies on adult and juvenile pectoral fin swimmers have demonstrated that these animals increase swimming speed by increasing fin beat frequency and amplitude (e.g. Gibb et al., 1994; Mussi et al., 2002; Hale et al., 2006), in some cases switching between pectoral fin-based gaits (Hale et al., 2006) before reaching a critical speed at which they switch to body undulations with no pectoral fin movement. Undulatory swimming speed is also frequency dependent while amplitude may be a factor at slower speeds (e.g. Bainbridge, 1958). While larval fish are known to have a discrete transition from swimming with both pectoral fins and body undulations to swimming with body undulations alone, the relationship of kinematics to swimming speed up to that point is unknown but

important for understanding motor control in larvae and for comparisons of motor systems among larvae, juveniles and adults.

Our second goal was to describe the kinematics in the initial phase of swimming movement. Initiation of swimming that involves pectoral fins and body undulations has not previously been investigated in fish [but see the paper of Dubuc and colleagues (Dubuc et al., 2008) for a review of lamprey swim initiation]. In particular, we examined whether pectoral fin movement during initiation demonstrates a distinct kinematic pattern and how the movement coordination between the pectoral fins and between the pectoral fins and the body is established. In humans, the initiation of movement has been studied in depth and found to be remarkably stereotyped (e.g. Carlsöö, 1966; Elble et al., 1994) and we were particularly interested in whether such consistent patterns of movement extended broadly to aquatic vertebrate swimming. In addition to providing a better picture of fin usage in routine slow swimming and the first examination of larval swim initiation, these behavioral investigations provide necessary kinematic and performance information for assessment of fin function.

Our last goal was to evaluate two general hypotheses that have been put forth for the role of larval pectoral fin beating: that it functions in locomotion (e.g. Batty, 1981) or that the behavior helps to shed oxygen-depleted water surrounding the body (e.g. Hunter, 1972; Weihs, 1980; Osse and van der Boogart, 1999). Towards this goal, we first compared the major components of routine swimming between normal fish and age-matched finless zebrafish in which the pectoral fins were genetically ablated through a morpholino knockdown of the gene *fgf24*. We hypothesized that if the fins function in locomotion, we would see decreased performance and/or stability in finless fish or finless fish would have altered body movements to compensate for the loss of the fins. Second, we visualized flow patterns around the pectoral fins with dye to test the hypothesis that the pectoral fins generate fluid movement that might aid respiration by displacing the boundary layer along the sides of the fish. Third, we examined swimming behavior of normal fish in high and low oxygen environments to test the hypothesis that decreased environmental oxygen results in increased pectoral fin movement.

This research broadens our understanding of larval fish locomotion by detailing the kinematics of the pectoral fins and the body during the slow swimming bout and by addressing possible functions of the pectoral fins in the movement of larvae. As the zebrafish larva has become a model system for studying vertebrate motor control and movement, these data provide a foundation for work on the pectoral fin system of this model. Fish go through remarkable post-hatching developmental changes in body morphology, including size, and in physiology that significantly alter how they interact with their physical environments. By addressing hypotheses on the roles of larval pectoral fins, this work provides an important step in understanding the functional development of the pectoral fins.

MATERIALS AND METHODS

Animals

Embryos of wild-type zebrafish [*Danio rerio* (Hamilton 1822)] were obtained from a laboratory breeding population. Embryos and larvae were raised at 28.2°C in 10% Hank's solution on a 14 h:10 h light:dark cycle until filming at 5 days post-fertilization (d.p.f.).

To generate finless fish, a morpholino to the gene *fgf24* was injected into wild-type zebrafish embryos at the one- or two-cell stage of development as previously described (Ahn et al., 2002; Fischer et al., 2003). Phenol Red was co-injected to make it possible to visualize the injection. At 48–72 h post-fertilization, embryos that had not yet hatched were dechorionated and fish without fins were sorted from the rest of clutch. These fish continued to be raised separately but under the same conditions as the un-injected fish. There was no significant difference in total length (TL) between normal and *fgf24* morpholino-injected (finless) fish in our samples (normal: 3.95±0.16 cm; finless: 4.01±0.15 cm; $P>0.35$) and the lack of fins was the only difference between the groups that we could discern through visual inspection of the animals.

For normal fish, our data set included 31 trials from 23 fish. So as not to bias the data toward individuals represented by multiple trials, we analyzed both the full data set and a more limited set that included one trial per fish. The trial included was determined with a random number generator. The data from finless fish include 22 trials from 13 fish and were analyzed in both full and culled sets. Because the conclusions of statistical tests on the full data set did not differ from those on the sets that included one trial per individual, results from analysis of only the latter, reduced, data set are presented unless otherwise noted.

High-speed video imaging of slow swimming

We used a Basler A500 (Basler Vision Technologies Inc., Exton, PA, USA) high-speed digital video camera (maximum spatial resolution of 512×1280 pixels) mounted on a dissection microscope (Leica Microsystems, Wetzlar, Germany) to film swimming bouts. To study the detailed kinematics of slow swimming bouts, we filmed groups of 5–6 normal or finless larval zebrafish in a rectangular glass tank (x–y–z dimensions 6.5×3×1.5 cm) at a frame rate of 1000 Hz. To ensure that we filmed slow swimming bouts that were not near the floor and sides of the tank, we recorded a focal plane in the center of the tank. Individual fish were identified following experiments using the unique skin pigmentation pattern on the head, which could be seen in the video frames using ImageJ (http://rsbweb.nih.gov/ij/). Trials in which fish identity was ambiguous were not included in the analyses.

Visualization of flow near the pectoral fins

To visualize fluid motion near the pectoral fins, we placed a single individual in a small Petri dish (3.5 cm diameter, 1 cm height) filled with Hank's solution and delivered a drop of Methylene Blue dye (VWR International, West Chester, PA, USA; 0.5% diluted in Hank's solution) approximately 1 mm anterior and 1–2 mm lateral to the head using a dulled syringe needle. The syringe was connected *via* tubing to a pneumatic transducer (Fluke Corporation, Everett, WA, USA), which allowed a very low pressure (~1 mmHg) to drive a slow leak of dye through the needle and into the water. For all flow visualization experiments, fish were near the bottom of the Petri dish and did not move forward during pectoral fin and body movement, possibly because of surface friction or floor boundary layer conditions. For a subset of flow visualization experiments, fish were partially embedded in agar following methods described previously (O'Malley et al., 1996) but with one pectoral fin free to move in the surrounding fluid. This later test was performed to address the possibility that fluid movement was induced by body bending.

Imaging behavior at different dissolved oxygen concentrations

To determine whether there was a change in pectoral fin or body movement in low *vs* high dissolved oxygen (DO) concentration Hank's solution, we prepared low DO concentration Hank's solution by boiling, 500 ml at a time, in a microwave for 20 min. Boiled

Hank's solution was placed in a clean glass bottle, which was filled to the top, closed off with a cap and sealed with Parafilm to prevent air from entering the bottle. Bottles were cooled to room temperature before experiments. The DO concentration was measured with a DO meter (model number MW600, Milwaukee Instruments, Gallarate, Italy) before use in any experiments. To increase DO concentration, boiled Hank's solution was transferred to another container with room for air, capped and shaken vigorously for ~5 min. DO concentration was then re-measured prior to experiments.

We filmed movements of larval zebrafish in Hank's solution with low or high DO concentrations for 111.24 s (1.85 min) using a frame rate of 150 Hz and a frame size of 510×504 pixels, which allowed 16,686 frames to be stored in computer memory. We imaged fish in a small, rectangular aquarium (2.2×2.2 cm in area, 1 cm in height; constructed from thin microscope slides) (Fisher Scientific, Pittsburgh, PA, USA) to allow visualization of movements near the edges. A single DO concentration measurement was taken from the Hank's solution placed in the aquarium following each filming. After all recordings were completed, a final DO concentration measurement was taken from the remaining Hank's solution. Based on these measurements, low DO Hank's solution had a DO concentration of 3.79±0.28 mg l^{-1} whereas high DO Hank's solution had a DO concentration of 7.12±0.18 mg l^{-1}. As a reference, the DO concentration in our fish facility is typically in the range 5.5–6.5 mg l^{-1}.

Behavior was recorded from 16 fish, eight per condition. Before filming, we allowed 1 min for each fish to acclimate to the aquarium and care was taken not to disturb fish during filming. All experiments were performed in a single day and used fish from a single clutch of eggs. Videos were analyzed with ImageJ. We measured the duration of each movement bout and classified movement bouts according to the usage of the pectoral fins as follows: (1) fin-only movement bouts that involved pectoral fin beats but no body undulation; (2) body-only movement bouts that involved periods of body undulation with no corresponding pectoral fin movement; and (3) fin–body movement bouts that involved both pectoral fin and body undulation. Movement bouts were classified as fin only or fin–body, only if the entire bout included pectoral fin movement. Other movement bouts that included both periods of body-only movement and periods of pectoral fin movement, such as when fish transitioned from fast swimming to slow swimming within a single bout, were classified as body-only movement bouts.

Digitization of points on the pectoral fins and body midline during slow swimming

Pectoral fin movement was digitized manually using ImageJ by tracking the position of a point located at the proximal base of the fin and a point at the distal tip of the fin. Frames in which the pectoral fin tip was not clearly visible were not used in our analysis. To analyze body movement, we tracked 51 uniformly distributed points along the midline of the body from the snout to the tail tip using a custom-written program in MATLAB 7.7.0 (The MathWorks, Natick, MA, USA), which makes use of functions from the MATLAB Spline Toolbox and the MATLAB Image Processing Toolbox (see Appendix).

Computing roll angle from the dorsal view

To measure the roll angle (i.e. the angle of rotation of the head about the rostrocaudal axis), we first measured the distance between the eyes in dorsal view video. When fish list, this distance decreases until the left and right eye overlap. We measured the distance, perpendicular to the midline of the head, between the proximal edges of the eyes at 6% of TL caudal to the snout. Measurements were made during the second tail beat cycle when the tail tip was at the body midline and at peak amplitudes (four measurements in total per trial). To convert these distance measurements to approximate roll angles, we took control data on distance between the eyes in fish that were positioned upright and then tipped laterally to a known angle. The correspondence between eye distance and roll angle was used to determine roll from our distance measurements taken from videos for which roll could not be measured directly.

To generate the control data, roll angles of 0, 5, 10, 15 and 24 deg were recorded in five fish that were embedded in agar to fix their position in the imaging tank (O'Malley et al., 1996). We performed a linear regression of roll angle *vs* the absolute change (relative to roll angle zero) in measured eye distance (R^2=0.77) to obtain the equation: roll angle=3.24+350.04×eye distance (intercept in units of deg and slope in units of deg mm^{-1}), which provides a roll angle estimate (dependent variable) given a measured eye distance change in the dorsal view (independent variable).

Analysis of slow swimming kinematics

Bout duration was measured as the time interval between the first and last quantifiable movement (fin or body). However, to compare normal and finless fish, we measured bout duration as the time interval between the onset and termination of body movement. The period of pectoral fin and body movement starting from bout onset to the end of the first tail beat cycle involved a pattern of pectoral fin movement distinct from pectoral fin movement during subsequent cycles. We therefore separately analyzed kinematics during initiation and during the next (second) tail beat cycle, which was more characteristic of a steady slow swimming movement pattern.

Because of asymmetric pectoral fin movement during initiation, fins were classified as 'leading' or 'trailing' depending on which fin was the first to end adduction (leading) during initiation. No clear refractory period was observed between fin strokes and thus fin beat cycle period was defined as the time interval between the start of abduction and the end of adduction, which coincided with the start of abduction of the next fin beat. We also measured the time of peak pectoral fin abduction, which defined the transition time between abduction and adduction.

Pectoral fin beat frequency was computed as the inverse of pectoral fin beat period. Pectoral fin beat amplitude was measured as the angle between the linear (inflexible) midline of the rostral 30% of the body and the line formed by a point at the fin base and a point at the fin tip at the time of peak pectoral fin abduction. The mean speed of pectoral fin abduction was computed as the pectoral fin beat amplitude divided by the time period between fin beat onset and peak abduction. The mean speed of pectoral fin adduction was computed similarly, as the pectoral fin beat amplitude divided by the time period between peak abduction and adduction offset.

Tail position was measured using a tracking point located at 94% of TL caudal to the snout on the midline. We chose this point because it was the most caudal point that was reliably visible in our videos and was not located on the caudal fin, which may move differently from the muscular portion of the tail. A tail beat cycle was defined as the movement of the tail tracking point away from the center, to maximal lateral positions on each side of the body, and then back to the center. Tail beat frequency was computed as the inverse of the tail beat cycle period. Tail beat amplitude was defined as the maximum lateral displacement of the tail tracking point.

Body bending was measured by computing the curvature (κ) of the body midline (e.g. Long et al., 1994) using a standard formula:

$$\kappa = \frac{\left|x'y'' - y'x''\right|}{\left[(x')^2 + (y')^2\right]^{3/2}}, \quad (1)$$

where x and y denote the spatial coordinates of midline points and first and second derivatives are with respect to midline arc length. Curvature can be interpreted as the inverse of the radius of a circle tangent to the midline at a point. Curvature at a midline point oscillates in value during swimming at the tail beat frequency. As we were interested in peak bending, we measured the maximum curvature over the second tail beat cycle.

We tracked the position of the body using a point on the body midline located 36% of TL caudal to the snout. We chose this point because it was closest to the center of area of the body. The total distance traveled by fish over a bout was measured as the linear distance traveled by the body tracking point over the bout. The mean bout speed was computed as the total linear distance traveled by the body tracking point divided by the bout duration.

Instantaneous swimming velocity, U, was computed in two steps. First, the time derivative of the position of the body tracking point was computed using a central difference derivative approximation, yielding a time series of velocity vectors that incorporated both forward and lateral body motion. Second, in order to measure the forward component of velocity, we computed the dot products of body tracking point velocities with the vector defined by a linear fit to the path of motion of this point (i.e. the mean path of motion), which yielded the final U estimates. Because of the finite spatial resolution of the digital videos, we could not reliably compute U values below $0.5\,\mathrm{TL\,s^{-1}}$ (i.e. less than $2\,\mathrm{mm\,s^{-1}}$). Reduced measurement precision in this range was not a major limitation because U values stayed above $0.5\,\mathrm{TL\,s^{-1}}$ following the initiation stage.

Reynolds number (Re), a measure of the relative importance of inertial and viscous forces in the movement of an object through a fluid, was computed as:

$$Re = \frac{UL}{\nu}, \quad (2)$$

where $\nu = 0.95231\times10^{-6}\,\mathrm{m^2\,s^{-1}}$ is the kinematic viscosity of freshwater at room temperature, U is the instantaneous velocity of the body and L=TL is the total length of the body.

Statistics presented in the Results were performed with JMP version 8.0.2 (2009, SAS Institute Inc., Cary, NC, USA) and with MATLAB (version 7.7.0). For all tests, the Shapiro–Wilk W-test was used to test for normality. One-way analysis of variance (ANOVA) and Student's t-tests were used for normally distributed data; otherwise, Wilcoxon rank sums test was used. Trends of data with speed were fitted using ordinary least squares regressions. The significance level for all tests performed was set to α=0.05 and all P-values are reported in the text.

RESULTS

Slow swimming

Fish performed slow swimming in short bouts of body and fin activity (Fig. 1). In our experiments, bouts lasted on average 147±26 ms (mean ± s.d.) and during that time fish swam a distance of 0.28±0.13 TL (1.09±0.54 mm). Occasionally, fish swam out of the field of view during filming and so overall bout parameters could not be measured for these trials. Because of this, it is possible that we missed longer swim bouts, although the preponderance of short bouts suggests that such behaviors would be atypical in our filming conditions. The mean speed ($\bar{U}$) calculated across the entire burst, including initiation and deceleration at the end of the burst was $1.84\pm0.69\,\mathrm{TL\,s^{-1}}$ ($7.24\pm2.79\,\mathrm{mm\,s^{-1}}$). Mean speed calculated for a representative portion of the rhythmic swimming component of the bout was $2.73\pm1.04\,\mathrm{TL\,s^{-1}}$ ($10.76\pm4.15\,\mathrm{mm\,s^{-1}}$).

We examined changes in fin and tail beat frequency and amplitude with swim speed for the fin–body slow swim gait. As a representation of fin movement during swimming, we examined the amplitude and frequency of the first trailing fin beat after initiation. There was no significant difference in either amplitude or frequency when comparing this fin beat with the subsequent one (P=0.57), an indication that the rhythmic swim fin movement pattern was established by the second trailing fin beat. We found that fin beat frequency (28.2±3.5 Hz) did not change with mean speed during the rhythmic portion of the bout (Fig. 2A); however, our data show

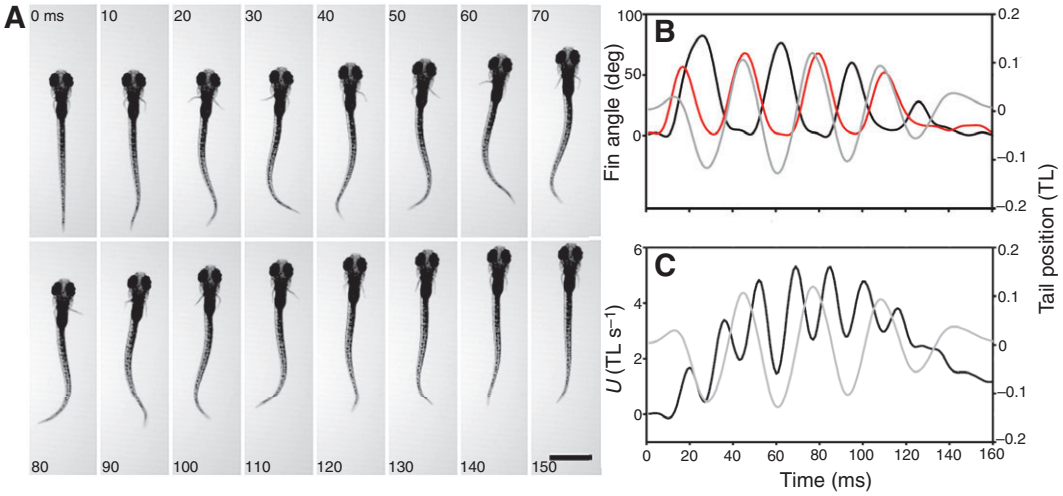

Fig. 1. A typical slow swim bout of larval zebrafish. (A) Images through the entire swim bout demonstrating the co-activation of fins and body through much of the behavior. Except at initiation and as the animal ends the swim bout, the fins alternate rhythmically. (B) Fin angles (data from the leading fin are in black, data from the trailing fin are in red) and tail position in total lengths (TL, gray) through the swim bout shown in A, illustrating coordination between the fins and frequency matching between the fins and tail. (C) Instantaneous speed (U) of the body (black) demonstrates the unsteady nature of slow swimming in the larval zebrafish studied. Tail position is in gray. Scale bar, 1 mm.

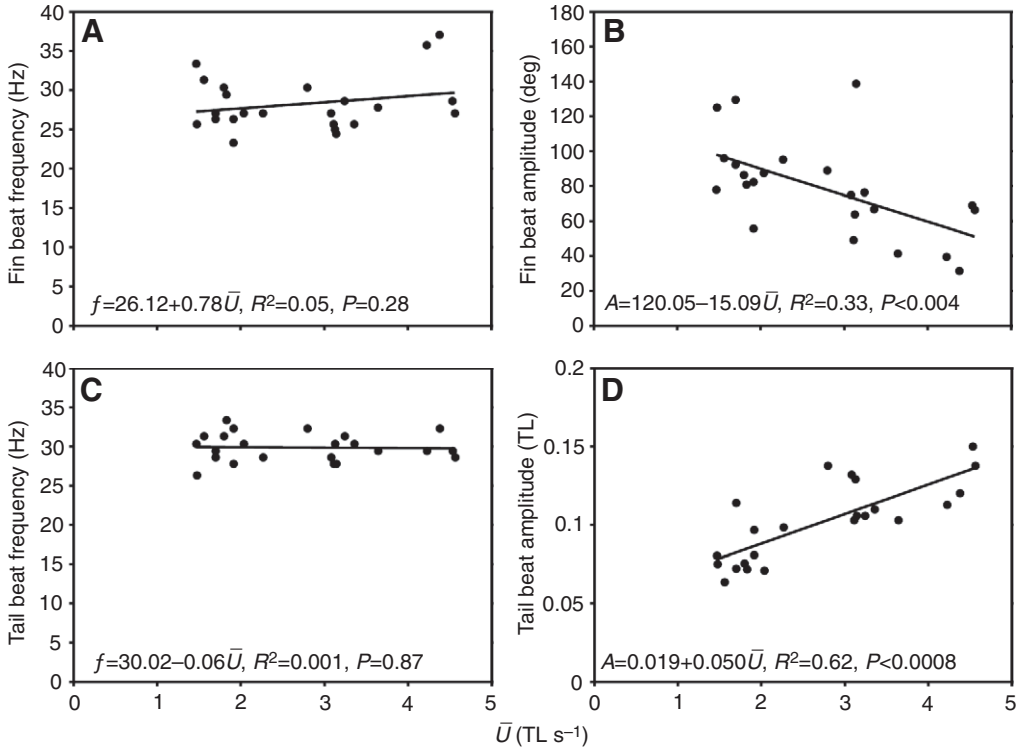

Fig. 2. The relationship between kinematic parameters and swimming speed. Variations in (A) fin beat frequency and (C) tail beat frequency were not correlated with mean speed of movement ($\bar{U}$). (B) Fin beat amplitude showed a negative correlation while (D) tail beat amplitude was positively correlated over the range of speeds examined. *f*, frequency; *A*, amplitude.

a significant decline in fin beat amplitude with mean speed (Fig. 2B, fin beat amplitude ranged from 31.4 to 138.5 deg, with a mean amplitude of 78.8±27.4 deg). To summarize, we found no significant modulation of fin beat frequency with speed, but there was a decline in fin beat amplitude with speed.

We found that there was no correlation of tail beat frequency with swimming mean speed during the rhythmic portion of the bout, with frequency remaining steady at 29.6±1.9 Hz through the mean speed range of 1.47 to 4.56 TL s^{-1} (Fig. 2C). In contrast, tail beat amplitude increased with increasing mean speed during the rhythmic portion of the bout (Fig. 2D), more than doubling over the speed range, with measured amplitudes ranging from 0.05 to 0.15 TL s^{-1} (averaging 0.10±0.03 TL among trials). In summary, we found that tail beat amplitude increased with speed but tail beat frequency did not vary significantly with speed.

Slow swimming bouts were marked by periodic increases and decreases in speed (Fig. 1C), demonstrating that slow swimming in larval fish is not at a steady velocity but varies considerably within each cycle of rhythmic swimming. Velocity oscillations may be pronounced because of the *Re* conditions experienced by the fish. *Re* calculated for both mean bout speed and mean speed during the second tail beat were, respectively, 30±12 and 45±18, falling into the intermediate *Re* range [1<*Re*<1000 (Jordan, 1992)] where viscous as well as inertial forces are significant. At intermediate *Re*, momentum generated during the power stroke of the tail will dissipate more quickly than it would at high *Re*, where inertial forces dominate. Instantaneous *Re* calculated at the extremes of the oscillations in velocity for the second tail stroke also fell into the intermediate *Re* range. At the velocity minimum *Re*=23±10 and at the velocity maximum *Re*=67±28.

Initiation of slow swimming

We used the period of time from the first visible pectoral fin or body movement to the end of the first tail beat cycle to define the initiation stage of a slow swim bout (Fig. 3A). We observed both pectoral fin and body movement during all initiation events. The pectoral fins, positioned close to the body at rest, moved asynchronously and asymmetrically. Unlike the first discernable movement of the pectoral fins, which sometimes occurs simultaneously (in three of the 23 trials analyzed a difference in timing was not measurable on our 1000 Hz video recordings), there was uniformly a clear difference in the timing of the end of the first fin beat. We therefore classified the pectoral fin beats as leading or trailing according to whether they ended the initiation fin beat first (defined as leading) and second (defined as trailing). During the initiating movement, there was no significant difference between the onset times of the two fins but the leading fin had a significantly lower amplitude fin beat than the trailing fin (76±28 *vs* 94±29 deg, $P<0.01$) and a shorter fin beat duration (28.6±2.6 *vs* 40.3±4.2 ms, $P<0.0001$). In addition, the speed of adduction of the leading fin was significantly greater than the speed of abduction (adduction: 5.83±1.47 deg ms^{-1}; abduction: 4.38±1.52 deg ms^{-1}; $P<0.004$). The differences in movement duration and amplitude between the fins resulted in establishment of the pattern of alternation characteristic of rhythmic swimming by the end of the initiating fin strokes (Fig. 1B, Fig. 3A).

The speeds of adduction and abduction were correlated for both the leading and trailing fin as were the speeds of these movement phases when compared between fins. Overall, the mean speed of adduction or abduction for one fin correlated with increasing mean speed for the other phase of movement of that fin and for the

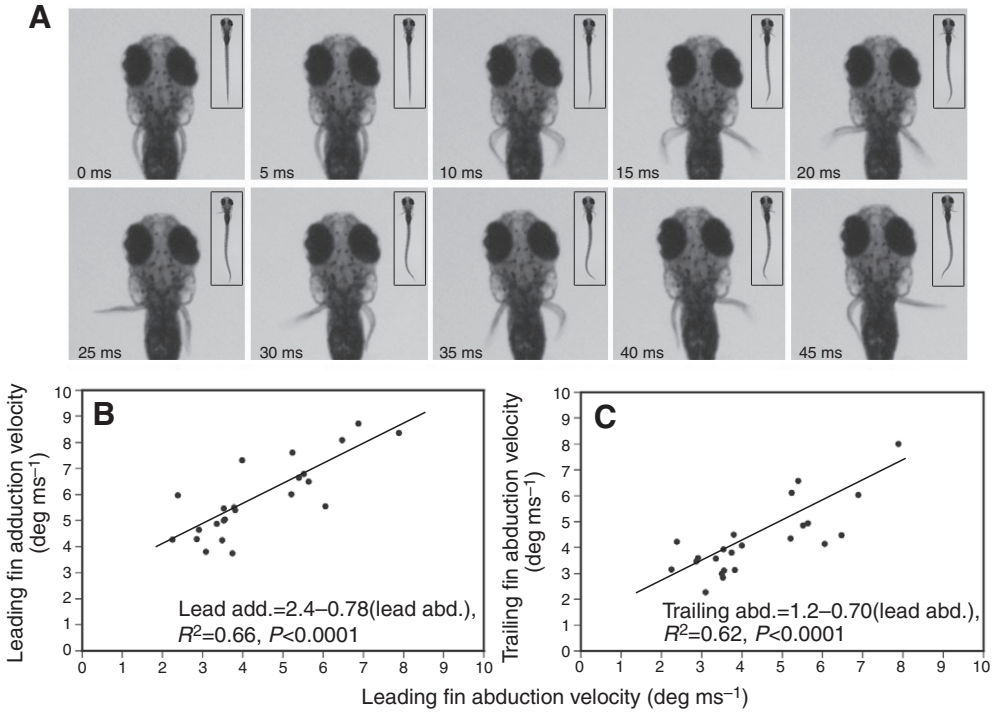

Fig. 3. Initiation of fin movement during slow swim bouts. (A) An example of fin movement during initiation. While the first abduction is variable, a consistent alternating pattern is established during first adduction of the fins. Body movement (inset) during this period is variably coordinated with fin movement. (B,C) Despite the variation in coordination during abduction, there is consistency in the speed of movement both between the phases of the leading fin beat (B) and between the two fins (C).

movement of the other fin. These trends are represented with regressions of leading fin speed in adduction and trailing fin speed in abduction regressed against leading fin abduction (respective R^2 values=0.66 and 0.62, P<0.0001; Fig. 3B,C). These data indicate that there is consistency in the timing of slow swim initiation despite variability in initiation speed.

Coordination between fin and body movements during initiation was variable; however, the absolute delay between the end of the trailing fin beat and the end of the first tail beat cycle was 5.48±4.31 ms, which is small compared with the duration of the first tail beat cycle – 33.81±4.14 ms (these delays were 16% of the duration of the first tail beat cycle on average). From the onset time of body movement during initiation to the end of the first tail beat cycle, fish traveled 0.033±0.016 TL.

Comparison of slow swimming with and without pectoral fins

We compared the overall bout kinematics of finless, *fgf24* morpholino-injected, fish (Fig. 4) and normal fish. Bout duration was not significantly different between finless and normal fish [169±32 ms for finless fish, P=0.05, lowest significance level set to α=0.003 with a sequential Bonferroni correction (Rice, 1989) for the number of tests (N=19) comparing finless and normal fish]. The distance traveled per bout was not significantly different (0.30±0.17 TL for finless fish, P=0.69). In addition, there was no significant difference in either the mean bout speed or the mean speed in the second tail stroke between normal and finless fish (respective means for finless fish and P-values for comparison with normal fish: 1.69±0.78 TL s^{-1}, P=0.57, and 2.13±0.86 TL s^{-1}, P=0.07). The frequency of the second tail stroke was not significantly different between normal and finless fish (finless: 28.97±1.63 Hz, P=0.14) and not correlated with speed (R^2=0.21, P=0.11, regression of tail beat frequency *vs* speed for finless fish). The performance of finless fish during the initiation of swimming was assessed by comparing the first tail beat of the swim bout between normal and finless trials. During the first tail beat, finless fish traveled a distance of 0.025±0.011 TL, which was not significantly different from normal fish (P=0.08).

To determine whether finless fish compensated for the lack of pectoral fins by modifying the bending of the body midline, we compared the maximum curvature and amplitude of the midlines of finless and normal fish at 10% length intervals from 40% TL to 90% TL (curvature at 30% TL and more rostral was near zero because the midline was relatively inflexible in this region). At some positions along the body (Fig. 5A,B) regressions indicated a trend of increasing curvature with increasing speed; therefore, we used ANCOVA to compare curvature values of normal and finless fish. We found no significant differences between the slopes or intercepts of the regression lines of curvature *vs* speed for finless and normal fish at each of the sites 40–90% TL along the body (the smallest P-value for curvature comparisons was P=0.0431 for regression slopes at 80% TL, which did not meet the α=0.003 significance level), indicating no significant difference in body bending between finless and normal fish.

The amplitude of lateral midline movement increased with swimming speed at all examined midline sites (40–90% TL) among both finless and normal fish ($0.59 \leq R^2 \leq 0.89$). We found no significant differences in the slopes or intercept values of regression lines of amplitude *vs* speed for finless and normal fish (Fig. 5C,D) at all examined midline sites (the smallest P-value for amplitude comparisons was P=0.0437 for regression intercepts at 60% TL).

Another potential effect of the lack of pectoral fins is a change in stability. We compared the amplitude of lateral movement at the snout (0% TL) between finless and normal fish to determine whether head yawing movement changed as a result of the absence of the fins. Amplitude at the snout increased with swimming speed, but there was no significant difference between the intercepts or slopes of the regression lines of snout amplitude *vs* swimming speed for finless and normal fish (Fig. 5E). Finless and normal fish did not move out of the focal plane of the camera during slow swimming bouts, suggesting that pitch angle remained approximately constant

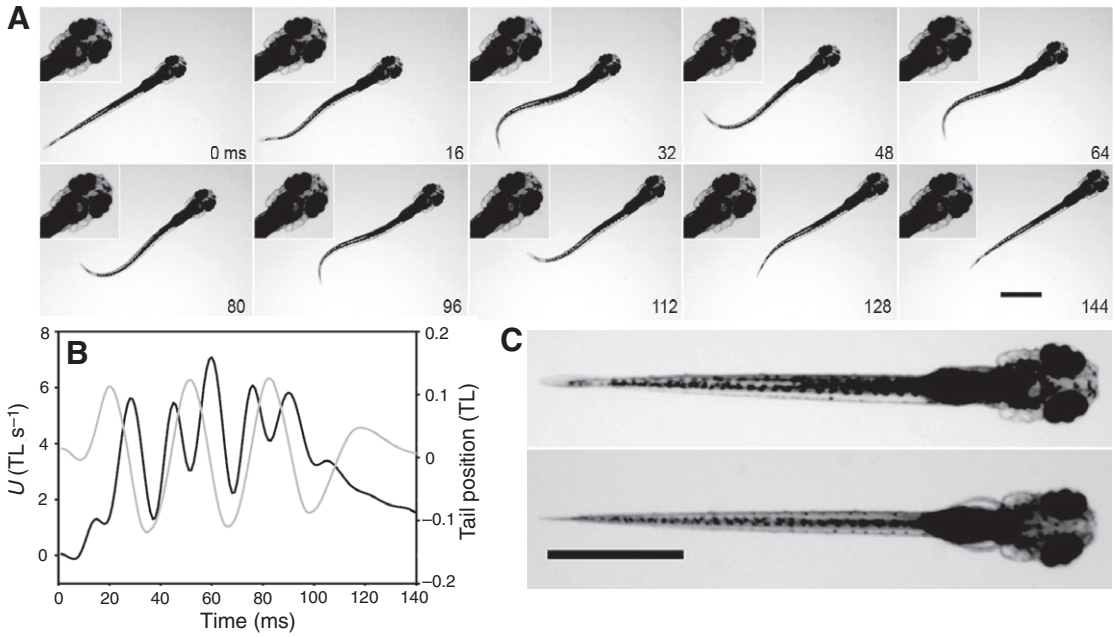

Fig. 4. A typical slow swim bout of a finless larval zebrafish. (A) Frames through the entire bout showing body movement and forward progression without pectoral fins. Head rolling and yawing movement (inset images, at twice the magnification and centered on the head) was barely perceptible for this bout. Scale bar, 1 mm; time (lower right) is in ms. (B) Instantaneous speed (*U*; black, left axis) and tail position (gray, right axis) for the bout shown in A. (C) Magnified images of a finless (top) and normal (bottom) larval zebrafish. Finless fish in A and C are the same individual. Scale bar, 1 mm.

and parallel to the tank bottom over the course of a bout. Among finless fish, the distance between the inner edges of the eyes (at 6% TL) did not change by more than 0.02 mm (corresponding to a maximum roll angle of 10.2 deg as given by the regression equation derived from roll calibration methods, with a mean eye distance change across bouts of 0.013±0.005 mm) when measured at the start and end of the second tail stroke, as well as when the tail tip had maximal lateral positions during the second tail stroke. Preliminary frontal and lateral view high-speed video data showed no obvious roll or pitch instabilities among finless and normal fish (see supplementary material Movies 1–5).

Flow pattern near the moving pectoral fin

We used dye visualization to look qualitatively at the effect of pectoral fin movement on the surrounding fluid. Pectoral fin beats resulted in movement of dye-marked fluid from a position anterior and lateral to the head to a position posterior to the fin base and adjacent to the surface of the body (Fig. 6A). Near the fin, dye-marked fluid appeared to be stretched, folded and pushed backwards by each fin beat.

We analyzed the movement of fluid by the pectoral fin during five slow swimming bouts, each bout performed by a different individual. The mean number of pectoral fin beats per bout was 10.2±3.1. The mean tail beat frequency of swim bouts during flow visualization was significantly lower than the mean tail beat frequency during slow swimming bouts recorded in our non-flow visualization experiments (26.43±1.96 Hz, $P<0.02$, compared with data in Fig. 2C). The mean pectoral fin beat frequency during flow visualization (26.42±2.93 Hz, measured from the fin that was moving dye) was not significantly different from the mean fin beat frequency in our non-flow visualization experiments ($P=0.26$, compared with data in Fig. 2A). The distance traveled in the posterior direction by the edge of the dye cloud moved by the fin, parallel to the rostrocaudal axis, was 0.055±0.007 mm per fin beat, or about 0.56 mm over the course of an average bout. Over the course of a bout, dye clouds had maximal width, perpendicular to the rostrocaudal axis, of 0.42±0.07 mm.

To determine whether fluid motion was produced by the pectoral fins and not by body movement, we immobilized three fish in soft agar, leaving one pectoral fin free to move, and repeated the flow visualization experiment. For all three fish, we observed a qualitative pattern of dye-marked fluid motion in response to pectoral fin beats that was similar to the pattern observed in unrestrained fish (Fig. 6A, last frame). The distance traveled by the edge of the dye cloud in the posterior direction was 0.040±0.014 mm per fin beat in agar-restrained fish, which was not significantly different from dye movement in unrestrained fish ($P=0.19$), but slightly smaller in mean value possibly as a result of the presence of agar surfaces that constrained fluid movement along the caudal half of the tail, or the lack of body-generated flows. Maximal dye cloud width over the bout (0.39±0.06 mm, for agar-restrained fish) was not significantly different between agar-restrained and unrestrained fish ($P=0.53$). We concluded that the majority of the observed fluid movement near the fins of unrestrained fish was due to the pectoral fins and not due body movement.

Fig. 6B shows an example of forward swimming with no pectoral fin movement through a region of dye-marked fluid. Upstream, dye-marked fluid remains separated from the body by a clear region of fluid caudal to the head. This demonstrates that forward swimming without pectoral fin beats is not always sufficient to bring upstream fluid to a position near the surface of the body.

Pectoral fin and body movement at different DO concentrations

To study the role of pectoral fin movement in respiration, we compared fin movement between high and low oxygen conditions.

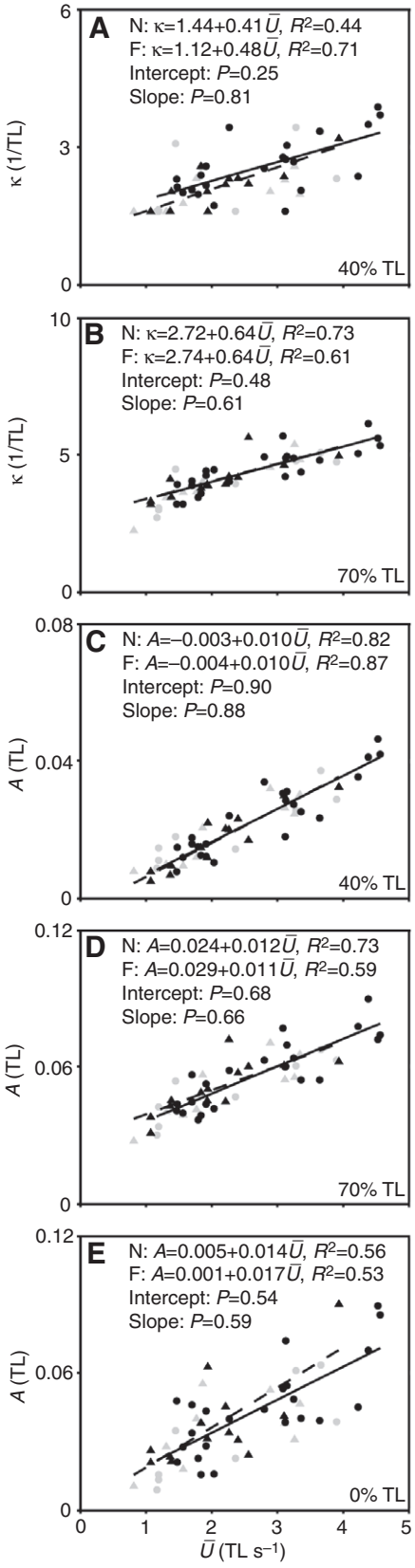

Fig. 5. Comparison of body kinematics between normal and finless fish. Regressions of body curvature (κ) and body amplitude (*A*) *vs* mean speed ($\bar{U}$) for normal (N, circles, solid regression line) and finless (F, triangles, dashed regression line) fish were not significantly different (*P*-values are derived from ANCOVA comparisons of the regression lines). Data plotted in black are from the reduced data set of trials from different individuals on which the regressions are based, and data in gray are from the remaining trials. (A,B) Curvature at 40% and 70% TL increased with mean speed among normal and finless fish. (C,D) Tail amplitude at 40% and 70% TL also increased with mean speed among normal and finless fish. (E) Amplitude at the snout (0% TL) showed no significant difference in yawing movement between normal and finless fish, and increased with mean speed.

These experiments tested the hypothesis that more fin movement would be observed in low oxygen conditions, which would support the putative function of the fins in respiration. Data from these experiments are summarized in Fig. 7.

We found a significant increase in the number of fin-only movement bouts performed by fish in low compared with high DO concentration Hank's solution (low: 100.38±56.90 bouts *vs* high: 16.5±0.18 bouts; $P<0.004$), but no significant difference in the number of fin–body movement bouts (low: 45.25±48.56 bouts *vs* high: 59.88±45.22 bouts; $P=0.54$) or body-only movement bouts (low: 3.13±6.51 bouts *vs* high: 1.63±2.13 bouts; $P=0.55$) of fish in low *vs* high DO conditions. The mean duration of each bout type was not significantly different between high and low oxygen conditions (fin only: $P=0.81$, mean duration 143±31 ms high DO, 147±29 ms low DO; fin–body: $P=0.16$, mean duration 163±62 ms high DO, 212±68 ms low DO; body only: $P=0.19$, mean duration 149±36 ms high DO, 184±32 ms low DO).

We determined the proportions of total trial time that the fish spent performing each of these behaviors and at rest (Fig. 7C,D). The percentage of the total recording time that fish engaged in fin-only movements increased in the low DO condition (fin only: $P<0.007$, mean percentage of total time 2.1±2.5% high DO, 13.7±8.7% low DO). The percentage of the total recording time that fish engaged in fin–body or body-only movements was not significantly different between high and low DO conditions (fin–body: $P=0.95$, mean percentage of total time 7.3±5.5% high DO, 7.6±8.8% low DO; body only: $P=0.47$, mean percentage of total time 0.2±0.3% high DO, 0.5±1.0% low DO).

For a subset of the movement bouts (nine fin–body bouts per condition, nine fin-only bouts in the low DO condition and three fin-only bouts in the high DO condition), we estimated the mean fin beat frequency over the duration of the bout by counting the number of fin beats per bout and dividing this number by the bout duration. We found no significant difference across conditions between mean fin beat frequency during fin-only ($P=0.64$) or fin–body ($P=0.08$) movement (fin–body: mean fin frequency 23±4 Hz high DO, 19±4 Hz low DO; fin only: mean fin frequency 17±2 Hz high DO, 18±3 Hz low DO). In summary, we found a significant increase the total time fish engaged in fin-only movement in the low DO condition, with no significant difference in mean fin beat frequency across high and low DO conditions.

DISCUSSION

From our high-speed video observations of the slow swimming bouts of larval zebrafish, we found consistent patterns of pectoral fin movement during both the initiation and the rhythmic periods of the bout. Patterned limb movement is used by many animals to power locomotion, yet our data reject the hypothesis that pectoral fin movement in larval zebrafish has a locomotor function during forward swimming. Our primary test of locomotor function was to compare swimming of normal larval zebrafish with swimming of larval zebrafish in which the pectoral fins were genetically ablated.

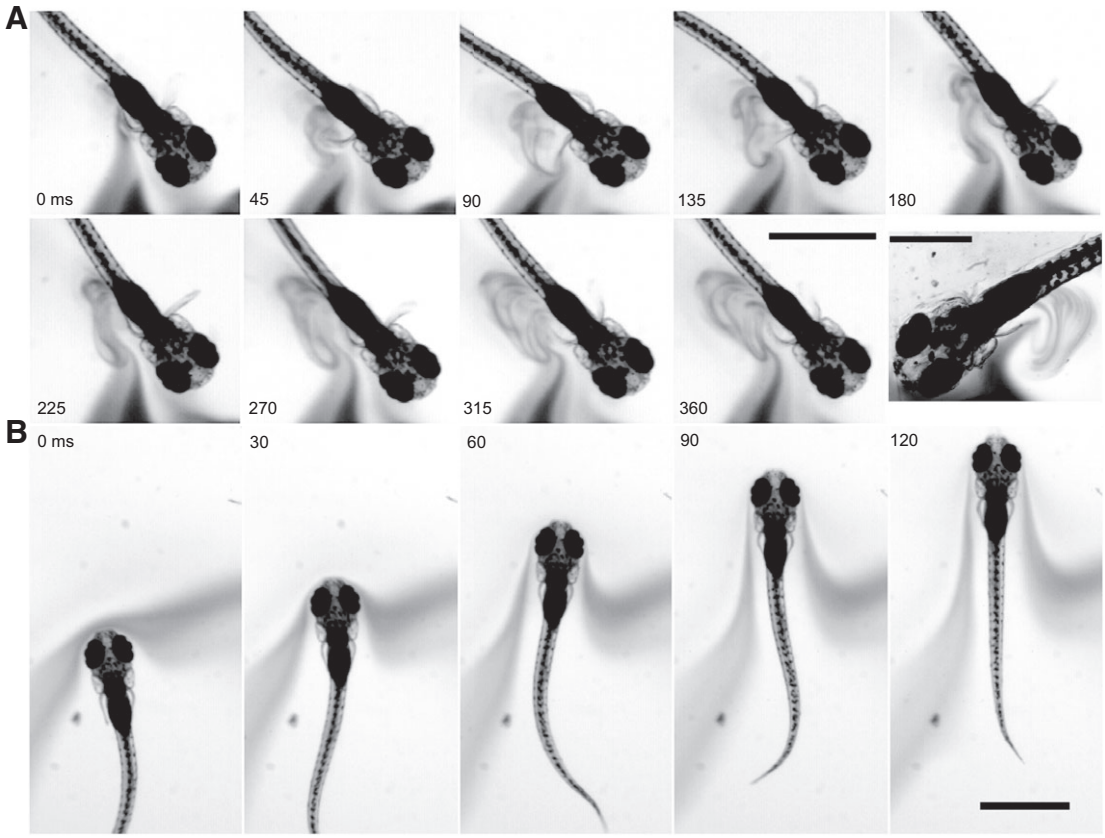

Fig. 6. Flow visualization during pectoral fin movement and during swimming without pectoral fin movement. (A) Pectoral fins moved dye-marked fluid from a position lateral and rostral to the fin base to a position caudal to the fin base and close to the side of the body. Frames (with time shown, lower left, in ms) depict dye-marked fluid movement over the course of a slow swim bout. Scale bar, 1 mm. The lower right frame shows a qualitatively similar flow pattern following a bout of pectoral fin movement of a partially agar-embedded fish, for which body movement was prevented. Scale bar, 0.5 mm. (B) Forward swimming through an upstream dye-marked fluid region without pectoral fin beats shows that upstream, dye-marked fluid remains separated from the body behind the head. Time is shown, top left, in ms. Scale bar, 1 mm.

This comparison showed no change in the speed or stability of swimming when the fins were not present. Consistent with these data, we found no change in pectoral fin beat frequency with increasing speed, and a decline in pectoral fin beat amplitude with increasing speed, which differs markedly from the increases in both fin beat amplitude and frequency with increasing speed observed in fish that power forward locomotion using the pectoral fins (see 'Trends in movements with swimming speed' below for further discussion and references). The results of our test of the locomotor role of the fins are limited to forward swimming, as we did not test potential locomotor roles of the fins in other behaviors such as maneuvering or escape.

One alternative hypothesis for a function of the fins during forward swimming is that their movement aids respiration by inducing fluid flows that transport oxygenated water close to the lateral surface of the body where cutaneous respiration occurs. We found support for this hypothesis based on simple flow visualization experiments and also by observing pectoral fin movement in low and high oxygen conditions.

Trends in movements with swimming speed

We found that pectoral fin and body movement variation with speed differ from what would be predicted by previous work on adult fish. For pectoral fin swimmers, both fin beat frequency and fin beat amplitude have been shown to increase with increasing swimming speed in synchronous (e.g. Webb, 1973; Gibb et al., 1994; Drucker and Jensen, 1996a; Drucker and Jensen, 1996b; Mussi et al., 2002; Walker and Westneat, 1997; Hale et al., 2006) and alternating (e.g. Arreola and Westneat, 1996; Hale et al., 2006) gaits. In contrast, larval zebrafish swimming in similar ranges of length-specific speed showed no significant change in the frequency of the pectoral fin beats with increasing speed, and a significant decline in fin beat amplitude with increasing speed. We suspect the decline in fin beat amplitude with increasing speed is due to an inability of the fins to resist fluid forces, such as drag, at higher speeds [see Vogel (Vogel, 1994) for a discussion of the dependence of drag on speed]. As expected, larval zebrafish differ markedly from adult fish in their fin beat frequencies, with the fin beat frequencies recorded [similar to those previously reported by others (Müller and van Leeuwen, 2004; Thorsen et al., 2004)] being much higher in larvae, of the order of 30 Hz, than in adult fish, which were generally well below 10 Hz (e.g. Gibb et al., 1994; Walker and Westneat, 1997; Mussi et al., 2002).

Since Bainbridge's seminal work (Bainbridge, 1958), adult fish have been shown in many studies to increase tail beat frequency with increasing swimming speed (e.g. Hunter and Zweifel, 1971; Long et al., 1996; Steinhausen et al., 2005), with modulation of tail beat amplitude having a lesser effect and varying mainly at slower swimming speeds. In contrast, for the slow swim gait studied here, we found there was no significant change in tail beat frequency but

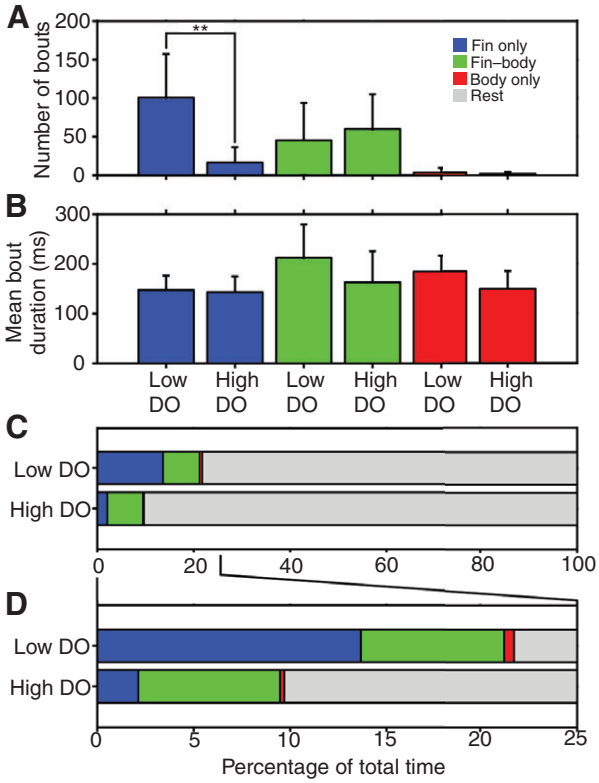

Fig. 7. Pectoral fin and body movement in low and high oxygen environments. Fin-only movement bouts increased in number in low oxygen conditions. The percentage of the total recording time fish were engaged in fin-only movement bouts increased in the low oxygen condition. $^{**}P<0.005$. (A) Mean number (+s.d.) of each bout category in low and high dissolved oxygen (DO) conditions. (B) Mean durations (+s.d.) of each bout category in low and high DO conditions. (C) Percentage of the total recording time that fish were engaged in fin-only, fin–body and body-only movement bouts or were at rest for low and high DO conditions. (D) Magnified view of the plot in C.

that tail beat amplitude increased strongly with increasing speed. Hunter presents scaling of speed with length in anchovy larvae (*Engraulus mordax*) and found increasing tail beat frequency with speed (Hunter, 1972); however, a wider range of behaviors (and thus speeds) was included in the sample. Adding fast burst swimming (above the speed when the pectoral fins are actuated) would no doubt also result in an increase in tail beat frequency in larval zebrafish. The ability of zebrafish larvae to modulate tail beat frequency is demonstrated by physiology data on motor neuron activity during fictive swimming (McLean et al., 2007), and tail beat frequencies during swimming that follows the startle response are also higher than those recorded here (Müller and van Leeuwen, 2004). Thus, while we suggest that tail beat frequency is not modulated during the slow swim gait described in this paper (which combines pectoral fin movement and body undulation), it is clear that tail beat frequency is associated with a variation in speed through the broader range of rhythmic swimming behaviors. These data suggest that with increasing speed through the entire range of swimming, larval zebrafish increase tail beat amplitude at a constant frequency and then further increase speed by increasing tail beat frequency. These data also imply that the motor control of slow swimming (combined fin and body movement) and fast swimming (body undulation only) may be modulated in very different ways; the former by increasing motor neuron recruitment to increase tail beat amplitude with increasing speed, and the latter instead, or in addition, by varying the timing of motor neuron bursts.

Differences between adult and larval swimming may result from a number of factors including the experimental context in which swimming is studied as well as the difference in length scale between larvae and adults. Studies of larger fish are often conducted in flow tanks with fish swimming continuously and prompted by the fluid movement or other stimuli. Because of their small size and because steady continuous swimming is unnatural for them, the slow swimming bouts we recorded in larval zebrafish were self-initiated and occurred in a short, intermittent burst of movement. It is possible that fish employ different movement strategies in different behavioral contexts. A more striking set of differences is the size and absolute speed of larval *versus* adult animals. These differences are reflected in the *Re*, which is large for adult fish swimming [$Re>300$, sometimes reaching $Re>1000$ during burst swimming and coasting in adult zebrafish (Fuiman and Webb, 1988; McHenry and Lauder, 2006)] but low/intermediate ($Re<100$) for the swimming we recorded here. The differences in hydrodynamic regime have strong effects on the generation of thrust during movement. While intermediate *Re* regimes are not well understood, Jordan's (Jordan, 1992) paper examining intermediate *Re* swimming in a chaetognath (*Sagitta elegans*) finds similar trends with speed to those we found with larval zebrafish swimming at intermediate *Re* regimes: increasing amplitude of movements with increasing swimming speed but no change in frequency [of tail beats in this study, of undulatory propulsive waves in Jordan's study (Jordan, 1992)]. These similarities suggest that amplitude modulation of an undulating system may be a common approach to varying speed in these *Re* conditions. A third possible difference for trends in movement with speed may be that larvae and adults have different functional demands on fin and body morphology. We examined this possibility for pectoral fin movements and discuss the results below (see 'Functions of the larval pectoral fins').

Initiation

While initiation of swimming by many fish, including larval-stage zebrafish, has been studied in the context of startle (for a review, see Domenici and Blake, 1997) (Liu and Fetcho, 1999), the initiation of routine swimming has received little attention [an exception being neural control of lamprey swim initiation (for review, see Dubuc et al., 2008)]. We focused on describing the pattern of swim initiation both to begin to address this relatively unexplored behavior and to provide a baseline for examining possible functional roles of the pectoral fins in initiation.

In human walking, the best-studied system for rhythmic locomotion initiation, movement of the legs follows a relatively consistent pattern of muscle activity, force generation and movement (e.g. Carlsöö, 1966; Elble et al., 1994) when performed in a consistent behavioral context. In the larval zebrafish, we saw unexpected variability in the first part of the initiation movement. In fact, we classified the fins as leading and trailing by, respectively, the first and last fin to complete the first fin stroke because the initiation of the two fins in some trials was effectively simultaneous at our frame rate (1000 Hz) and image resolution. Despite variability in movement onset, due to the velocity of movement and the lower amplitude of the leading fin stroke, by the end of the first fin stroke a clear coordination pattern was established. In addition, the velocity of adduction and abduction were correlated for each fin as were the velocity of adduction or abduction in one fin with the same phase

of movement in the other, suggesting a broad level of coordination of motor control among the movements that compose an initiation event. The change from symmetric fin posture prior to initiation to alternating coordination by the end of the first pectoral fin beat is reminiscent of the gait transition between synchronous and alternating movement that occurs within a single beat in steady swimming juvenile reef fish (Hale et al., 2006) in that they both involve switching between two fin coordination patterns, synchronous and alternating, and that switch occurs in a single fin beat; however, variability in the timing of movement during the gait transition has not been assessed and the neural control of either change in coordination pattern is not known.

Functions of the larval pectoral fins

We addressed hypotheses of fin function with a multi-pronged approach. We were particularly interested in investigating two hypotheses that have been proposed in the literature: (1) that the fins function in forward locomotion, either by generating thrust or by stabilizing the body; and (2) that the fins are respiratory, circulating fluid near the body surface, a main site of oxygen absorption in larvae (e.g. Rombough, 2002). Our main test of locomotor function was to genetically ablate the fins and assess the effect of the absence of fins on locomotor performance. There was no statistically significant difference in the overall performance of the normal and finless animals or in their patterns of movement.

A concern with studies that ablate anatomy to examine function is that the animal may adapt its behavior to maintain performance. In this case, our concern was that body movements might change and compensate for fin force production lost through the ablation. However, our analysis of curvature and body amplitude did not indicate any significant changes in body kinematics in fish without pectoral fins. A second concern is the possibility of non-pectoral fin morphological changes, or detrimental health effects associated with *fgf24* morpholino injections, although this concern is lessened by our inability to find significant differences in the slow swimming behavior or TL between finless and normal larvae. A previous study that utilized *fgf24* morpholino injections (Fischer et al., 2003) did not find morphological changes associated with these injections other than the absence of pectoral fins. In addition, *fgf24* morpholino-injected larvae were raised to adulthood in our fish facility (M.H.G. and M.E.H., personal observations), which suggests that these fish did not have major health problems. A third concern is the stress of an ablation procedure on the animal. A major motivation for us to use a genetic ablation approach rather than surgically removing the pectoral fins was to avoid stress on the animal, which could alter swimming behavior in a way unrelated to the absence of fins. We suggest that genetic ablations may be useful for studies of function in other movement systems.

Flow visualization experiments using dye demonstrated that the pectoral fins move fluid from a distance lateral to the fin to the side of the body and that this fluid is moved from rostral to caudal along the side of the fish. Moreover, it is clear, even from these simple visualizations, that the dye-marked fluid originally located lateral to the fish is displacing fluid at the surface of the animal. This is the pattern that would be expected if the fins are involved in respiration: active transport of fluid captured far from the surface of the body to a position near the body surface, where DO can be absorbed. Dye experiments also made it possible to see that forward swimming without pectoral fin movement is not always sufficient to transport fluid to a position near the skin on the body: when fish swam forward without pectoral fin movement, upstream, dye-marked fluid remained well-separated from the body surface posterior to the head.

A role for pectoral fin movement in respiration was further supported by experiments in which DO was manipulated. We found that larval zebrafish increased the number of, as well as the total time spent engaged in, bouts of fin-only movement when placed in low oxygen conditions. This result is comparable to that of the study by Jonz and Nurse, who also reported an increased number of respiratory movements (which included pectoral fin movements) in larval zebrafish exposed to low DO water (Jonz and Nurse, 2005). In contrast, some marine species of larval fish have been reported to increase the number of body movements in response to low oxygen conditions (e.g. Hunter, 1972; Weihs, 1980). Beyond the sustained responses examined here, a first, rapid response to low oxygen, or an avoidance behavior (rapid movement, struggling, etc.) has been reported in other species (Jones, 1952; Weltzien et al., 1999). Other stressors evoke similar immediate responses in many fish including larval zebrafish (Eaton and Farley, 1975; Saint-Amant and Drapeau, 1998). We attempted to minimize the effects of any initial avoidance response to low oxygen in our data set by waiting 1 min after placing the fish in low or high DO conditions to begin video recording.

Species-specific differences in the response to low oxygen may be due to the differences in the habitats of the animals. Zebrafish are very adaptable to variability in oxygen levels (Rees et al., 2001; Roesner et al., 2006) and, in their natural habitats of warm, still water (Engeszer et al., 2007), may encounter substantial variation in oxygen levels [see Kramer (Kramer, 1987) for a discussion of environmental factors, such as temperature and water currents, affecting oxygen availability]. Examining larvae from different habitats, their fin movements and related flow patterns, may elucidate variation in larval fin function among fishes.

While in more mature fish oxygen absorption occurs through the gills over which water is actively moved, in larvae oxygen uptake through the skin relies on diffusion through the water that surrounds the body. Weihs used a mathematical model to predict that when a larval fish arrives at a new location, the flux of oxygen at the skin will rapidly decrease from an initial value, as long as the fish remains still (Weihs, 1980). There are two possible ways to increase the local DO levels under this condition: by moving to an area of higher DO to increase available oxygen for diffusion or by moving high DO fluid into the area around the body, replacing the oxygen-depleted water. The ability to increase available oxygen by moving to a new environment is limited by the size of the boundary layer around the fish. In larvae this layer is relatively thick. As discussed by Müller and colleagues, it extends laterally a distance of approximately one body width (Müller et al., 2008). The boundary layer greatly reduces mixing of fluid adjacent to the body with more distant (more oxygenated) water. Fin movements may provide an advection-based mechanism to augment the oxygen in the boundary layer and to facilitate oxygen uptake across the skin.

Conclusions

Although swimming in adult fishes has been studied broadly, much less is known about swimming in larvae. The combined pectoral fin-body movement pattern of larval zebrafish is not typical of adult fishes and showed unexpected trends in kinematics across a range of speeds, including the constancy of fin and tail beat frequency across speeds, as well as the decline of fin beat amplitude and increase of tail beat amplitude with increasing speed. Our results also indicate that the functions of the fins differ markedly between larvae and adults.

Although many adult fish use the pectoral fins as major or sole propulsors (e.g. Webb, 1973; Blake, 1979; Drucker and Jensen, 1996a; Drucker and Jensen, 1996b; Walker and Westneat, 1997) during steady forward locomotion, the data presented in this study suggest that larval fins are not major propulsors in forward swimming but do serve a respiratory function. Across fishes, larvae demonstrate remarkable morphological and ecological diversity and greater exploration of this diversity will likely provide new perspectives on movement systems and behaviors.

APPENDIX

Frames of an input video were first background subtracted using ImageJ. In the first frame of an input video, when the full extent of the body and caudal fin is visible, a point on the rostral tip of the snout and a point on the caudal tip of the tail were manually digitized to provide an accurate estimate of TL. The program then automatically tracked the position of the midline of the body in each video frame *via* the following iterative algorithm.

Each video frame was thresholded, which set pixels on the body of the fish to have a value of 1 and pixels in the background to have a value of 0. Any small particles in the water that are not eliminated by thresholding were removed using MATLAB Image Processing Toolbox function *bwareaopen*. The 2D spatial coordinates of the pixels with value 1 were computed and stored in a matrix, from which the mean spatial position (centroid) was subtracted. Singular value decomposition (MATLAB function *svd*) was applied to this matrix to obtain the principle components. The longest axis of the body is the rostrocaudal axis during slow swimming, so the first principle component was oriented approximately along this axis. Next, the coordinates of the body pixels were rotated so that the first principle component was horizontal, with the direction of motion positive. Following rotation, a spline curve, restricted to be linear on the head, was fitted to points on the tail using least-squares (MATLAB Spline Toolbox function *spap2*), resulting in 51 points along the midline of the body. Because of the symmetry of the body about its midline, least-squares fitting results in a curve along the midline.

To correct for any errors in the total length of the midline fit due to poor visibility of the caudal fin in some frames, the position of the most caudal midline point was re-estimated using linear extrapolation so that the total length of the fitted midline matched the value determined from the manual digitization in the first frame. In all trials, this length correction was never more than 2% of the body length. After length correction, midline points were adjusted to be spaced uniformly, and rotated and translated back to the original coordinate system. The presence of pectoral fins did not alter the midline estimation because the profile of the pectoral fins in the dorsal view was small and did not significantly alter the symmetry of the body across the midline. This algorithm is specific to linear swimming paths, and cannot accommodate behaviors with large body bends, such as the escape response. Other more advanced tracking algorithms have been developed for this purpose (e.g. Fontaine et al., 2008). The benefit of our algorithm is that it is fast and easy to use.

LIST OF SYMBOLS

A	amplitude
d.p.f.	days post fertilization
f	frequency
Re	Reynolds number
TL	total body length
$\bar{U}$	mean swimming speed
U	instantaneous swimming speed
κ	curvature

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