# Swimming of larval zebrafish: fin–axis coordination and implications for function and neural control

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## Summary

Adult actinopterygian fishes typically perform steady forward swimming using either their pectoral fins or their body axis as the primary propulsor. In most species, when axial undulation is employed for swimming, the pectoral fins are tucked (i.e. adducted) against the body; conversely, when pectoral fins are beating, the body axis is held straight. In contrast to adults, larval fishes can combine their pectoral fin and body-axis movements during locomotion; however, little is known about how these locomotor modes are coordinated. With this study we provide a detailed analysis of the coordinated fin and axial movements during slow and fast swimming by examining forward locomotion in larval zebrafish (*Danio rerio* L.). In addition, we describe the musculature that powers pectoral fin movement in larval zebrafish and discuss its functional implications. As larvae, zebrafish alternate their pectoral fins during slow swimming ($0.011\pm0.001$ mm ms$^{-1}$) in conjunction with axial undulations of the same frequency (18–28 Hz). During fast swimming ($0.109\pm0.030$ mm ms$^{-1}$; 36–67 Hz), the fins are tucked against the body and propulsion occurs by axial undulation alone. We show that during swimming, larval fishes can use a similar limb–axis coordination pattern to that of walking and running salamanders. We suggest that the fin–axis coordination observed in larval zebrafish may be attributed to a primitive neural circuit and that early terrestrial vertebrates may have gained the ability to coordinate limbs and lateral bending by retaining a larval central pattern generator for limb–axis coordination in the adult life history stage.

Key words: kinematics, biomechanics, gait, *Danio rerio*, larva, musculature, locomotion, mechanical design, central pattern generator, pectoral fin.

## Introduction

Locomotion by fishes has traditionally been subdivided into swimming with paired or median fins, or with the body axis and caudal fin (e.g. Webb, 1994). Often, slow swimming is powered by the pectoral fins actuated in phase (synchronously) with one another (Blake, 1983). At higher relative speeds, pectoral fin locomotion may be supplemented by intermittent caudal fin movement (Drucker and Jensen, 1996a). Frequently, at high swimming speeds, the pectoral fins are tucked along the sides of the body and axial movement alone generates propulsive thrust (Webb, 1994).

Many species, including adult zebrafish (Thorsen et al., 2004), use axial body bending at all speeds, and the fins to maneuver and stabilize (Webb, 1994). When used for maneuvering, pectoral fins have been shown to alternate out of phase temporally, such that abduction of one fin coincides with adduction of the contralateral fin (Drucker and Lauder, 2003). Other species employ their pectoral fins in synchrony as their primary mode of locomotion during steady swimming across a wide range of speeds (Webb, 1973, 1993; Gibb et al., 1994; Drucker and Jensen, 1996a,b; Walker and Westneat, 1997).

The morphologically unusual burrfish (*Chilomycterus schoepfi*) alternates the pectoral fins during swimming, which are active simultaneously with the caudal fin (Arreola and Westneat, 1996).

Research in locomotion of larval fishes has focused on axial movements during steady swimming, turning, prey capture (Budick and O'Malley, 2000; Borla et al., 2002), startle behaviors (Batty, 1981; Hale, 1996, 1999; Budick and O'Malley, 2000; Müller and van Leeuwen, 2004) and swimming performance (Fisher et al., 2000; Bellwood and Fisher, 2001; Fisher and Bellwood, 2003). Work by Batty (1981), and Müller and van Leeuwen (2004), demonstrated that plaice larvae (*Pleuronectes platessa*) and zebrafish larvae, respectively, can swim with simultaneous axial and pectoral fin movements. However, the detailed kinematics and role of coordinated pectoral fin and body movements have gone unstudied primarily due to the technical difficulty of visualizing pectoral fins of larvae (Budick and O'Malley, 2000).

The combined movement of the limbs and axis during

locomotion has been studied in depth in tetrapods. Axial bending is often coordinated with limb rhythms so that a flexion–extension limb cycle corresponds to one cycle of axial bending (Ritter, 1992; Ashley-Ross, 1994). The limbs within a fore limb or hind limb pair alternate with each other so that, for most of the stride cycle, one side is in its swing phase while the other is in its support phase (Biewener, 2003). There is a short period of overlap when both limbs are on the ground with one limb at the beginning of the support phase and the other at the end during walking. The production of axial movements *via* standing or traveling waves of bending (Williams et al., 1989; Frolich and Biewener, 1992; Ritter, 1992; Reilly and Delancey, 1997) varies among species, developmental stage and gait. The basic temporal pattern of this locomotor activity involves the integrated activity of central pattern generators (CPGs) in the spinal cord (for reviews, see: Stein, 1978; Grillner, 1981; McClellan, 1996).

Pectoral fin muscles of adult fishes have been studied in many species and include an array of muscles that control fin adduction and abduction during different locomotor modes (e.g. Winterbottom, 1974; Geerlink, 1979, 1983, 1989; Westneat, 1996). Several muscles, including their subdivisions and individual bundles, perform various roles in actuating the fin during locomotion (Thorsen and Westneat, in press). Despite widespread interest in limb development (e.g. Sordino et al., 1995; Ahn et al., 2002), the muscle morphology and function of early developing fish fins remains to be explored.

To investigate the use of pectoral fins in larval zebrafish locomotion, we examined axial bending and fin movement during routine swimming, and compared it with swimming following the startle response, a behavior thought to be produced at near-peak velocity that does not involve fin movement. Both our preliminary observations and reports in the literature (Batty, 1981; Borla et al., 2002; Müller and van Leeuwen, 2004) found that the fins and axis were active simultaneously during routine larval fish swimming. Previous work on tetrapod locomotion demonstrating that alternation of the limbs and lateral bending of the axis are coordinated tightly during locomotion (Ashley-Ross, 1994; Bennett et al., 2001) drove our hypothesis that relative movements of limbs and axis of larval fish would be similarly patterned. Through the comparison of slow and fast swimming we suggest that the use of fins may be associated with the hydrodynamics experienced by the fish at different swimming speeds. In addition, we describe the pectoral fin musculature and discuss its functions in fin movement.

This work complements the previous work of Budick and O'Malley (2000), and Müller and van Leeuwen (2004), and focuses on the coordination of fin movements during slow swimming and the neural implications of kinematic patterns. Based on our data in larval zebrafish, and similar data in plaice larvae (Batty, 1981), we suggest that fishes and tetrapods may use similar neural coordination of axial and appendicular structures, and that the mechanisms for that coordination may have been conserved from an ancestral condition.

## Materials and methods

### Animals

Eggs of wild-type zebrafish *Danio rerio* Hamilton 1822 and α-actin GFP transgenic zebrafish (Higashijima et al., 1997) were obtained from a breeding laboratory population maintained at 28.2°C. Embryos and larvae were raised at 28.2°C on a 14 h:10 h light:dark cycle until 5 days post-fertilization (dpf). Ten wild-type individuals were used at 5 dpf for behavioral experiments [3.94±0.17 mm total length (TL), mean ± S.D.]. Muscle morphology was examined at 5 dpf in five α-actin transgenic individuals (3.97±0.07 mm TL). We found no difference (Student's *t*-test, $P=0.6948$) in size between wild type and α-actin populations.

### Digital video recording of locomotion

For behavioral imaging, larvae were transferred to 10% Hanks solution and placed into Petri dishes (3.5 cm in diameter). Behavioral observations were made after acclimation to room temperature (25°C) for 15 min. Fish were placed under a Leica MZ 6 microscope (Wetzlar, Germany) with an attached high-speed Redlake MotionScope PCI 2000S video camera (San Diego, CA, USA). Black and white video at 1000 frames s⁻¹ and 240×210 pixel resolution was saved directly to a PC utilizing the Redlake Imaging MotionScope 2.21.1 software. Only spontaneous swimming events were collected for slow swimming trials. A glass micropipette was directed at the caudal region of the fish to elicit fast swimming responses.

### Behavior analysis

Behavioral trials for slow swimming (30 total; three trials per individual for ten fish) and post-startle swimming [15 total; three trials per individual for five fish (a subset of the individuals used in slow swimming trials)] were analyzed with a customized program for digitizing the axial midline using LabView 5.0.1 software (National Instruments, Austin, Texas, USA; with virtual instruments designed by J. R. Fetcho, Cornell University, NY, USA). In addition, the timing and coordination of fin movements and parameters used to calculate Reynolds number ($Re=\mathbf{V}L\rho/\mu$, where **V** and $L$ are the velocity and length of the fish, and $\rho$ and $\mu$ are the density and viscosity of water) were determined by viewing trials frame-by-frame in NIH Image 1.62 (NIH, Bethesda, MD, USA). For each trial, we quantified kinematic data during the middle of a straight swimming bout for one tail-beat cycle and the three fin strokes that overlapped it (two on one side of the body, one on the other).

We defined each fin cycle (locomotor cycle) using three events: the frame just prior to start of fin abduction, the frame of maximum lateral abduction, and the first frame post adduction. The refractory period between fin cycles was defined by indeterminate fin activity, which results from a fin positioned adjacent to the body. Points of maximal medial axial curvature correspond to when the tip of the fish's tail changes direction (Budick and O'Malley, 2000). Only swimming after the first two tail strokes was examined, both

for fast and for slow locomotion, to avoid the asymmetric initial bends and acceleration associated with the initiation of movement. Asymmetrical bends begin when maximal convexity is achieved for the first time in the same direction as the initial turn (which is at the end of the second beat), and ends when the axis cycles back to this configuration. All statistical tests were performed with JMP 3.1.6 (SAS Institute, Cary, NC, USA).

### Morphological imaging and analysis

A subset of the α-actin transgenic zebrafish were stained with Calcein green (Molecular Probes, Eugene, OR, USA) to visualize the cleithrum and endoskeletal components of the pectoral fin. Fish were immersed in a 0.2% Calcein green solution following Du et al. (2001) for 15 min and allowed to swim freely. Fish were then rinsed in 10% Hanks and anesthetized with MS222 and embedded in agar for confocal imaging. α-actin GFP fish and Calcein-stained fish (Calcein green + α-actin GFP) were positioned with their left side down in 1.2% agar on a glass coverslip floor of a small Petri dish. The agar was covered with a 50% mixture of 10% Hanks solution and MS222 to prevent desiccation and fish movement while imaging. The pectoral girdle musculature was imaged under a Zeiss LSM 510 laser-scanning confocal imaging system (Thornwood, NY, USA).

Single optical sections and image stacks (40× objective, 1028×1028 pixel resolution, 100 slices, 0.8 µm interval for three-dimensional reconstruction) of the pectoral girdle musculature and fin membrane were saved to a PC. Three-dimensional reconstructions were produced using Zeiss LSM 510 software. Fin surface area was calculated in ImageJ 1.30 (NIH, USA) using three-dimensional lateral view projections of the fin (musculature and membrane). The number of muscle fibers constituting the pectoral musculature were counted using three-dimensional projections and Z stacks to aid in the visualization of the fin.

## Results

### Behavior

We found that slow and fast swimming in larval zebrafish represent distinct swimming gaits. Larval zebrafish synchronize their pectoral fin movements with the body axis (18–28 Hz) during slow swimming and tuck their fins against their body during fast swimming. During slow swimming, the axial muscle bends the body with the same frequency as the fins such that one left–right axial cycle corresponds to one abduction–adduction cycle of the fins (Fig. 1). When the right fin is initially at maximum abduction and ready to initiate adduction toward the body, the left fin is in its adducted position against the body. As the right fin is adducted, the tail flips toward it while the left fin is abducted. The right fin becomes fully adducted while the left fin is fully abducted. This cycle is then repeated with the other fin and subsequent tail-flip towards it.

Slow swimming with the fins and axis was significantly



0 ms
Right fin extension, left fin flexion

10 ms
Midstroke

20 ms
Left fin extension, right fin flexion

30 ms
Midstroke

40 ms
Right fin extension, left fin flexion

50 ms
Midstroke

60 ms
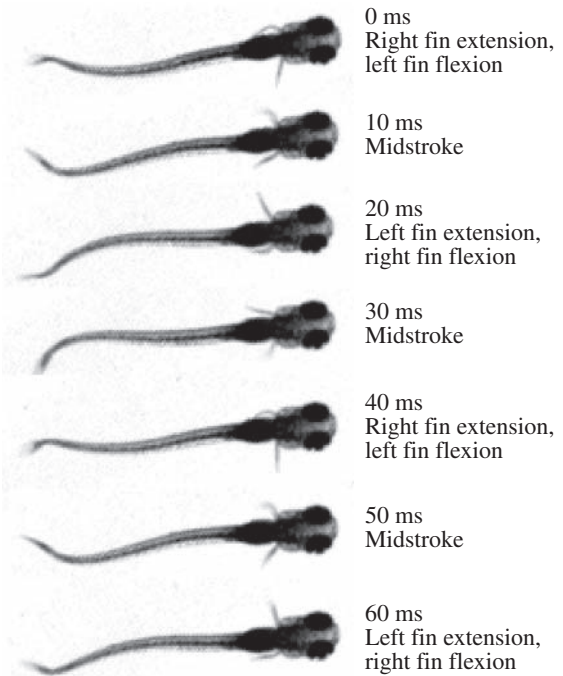Left fin extension, right fin flexion

Fig. 1. Fin and axial locomotion during slow swimming of the larval zebrafish. Fins are actuated in alternating abduction–adduction cycles. The axial muscle bends the body with the same frequency as the fins so that one axial cycle corresponds to one abduction–adduction cycle of the fins. The timing of fin abduction and adduction coincides with maximum axial bending. At 0 ms the right fin is maximally abducted, ready to initiate adduction toward the body. The left fin is in its adducted position against the body. At 10 ms (mid-stroke), the right fin is adducting while the left is abducting. By 20 ms the right fin is fully adducted while the left fin is at fully abducted. This cycle is repeated with the right fin abducting forward and the left adducting back.

slower, approximately an order of magnitude lower, than fast swimming (Fig. 2A). Fast swimming was more effective at propelling the larval fish forward, covering more than four times the distance traveled during slow swimming (Fig. 2B). The average duration of locomotor cycles was significantly shorter during fast swimming events (Fig. 2C). *Re* calculated for the slow swimming condition averaged 43±3 (Fig. 2D). During fast swimming, in which the fins are tucked and the axis alone propels the fish, *Re* numbers were significantly higher (427±31) than those of slow swimming, corresponding to a change in velocity (*P*<0.0001, Fig. 2A). Axial movement of zebrafish swimming possesses attributes of traveling and standing waves with a loose node present slightly posterior to the pectoral girdle (in agreement with Müller and van Leeuwen, 2004).

We further investigated the fin movements and coordination of the fins and axis in the slow swimming gait (Table 1). The duration of a complete fin abduction–adduction cycle, including the refractory period (Drucker and Jensen, 1996a) when a fin is positioned against the body, averaged 41.23±0.94 ms and is not significantly different from the

duration of a tail-beat cycle (40.23±0.94 ms, *P*=0.4602, Fig. 3A). The mean duration of the refractory period was 5.27±0.62 ms. The mean durations of the abduction and adduction phases across three fin cycles were not significantly different (17.66±0.44 ms *vs* 18.30±0.54 ms, *P*=0.3680;
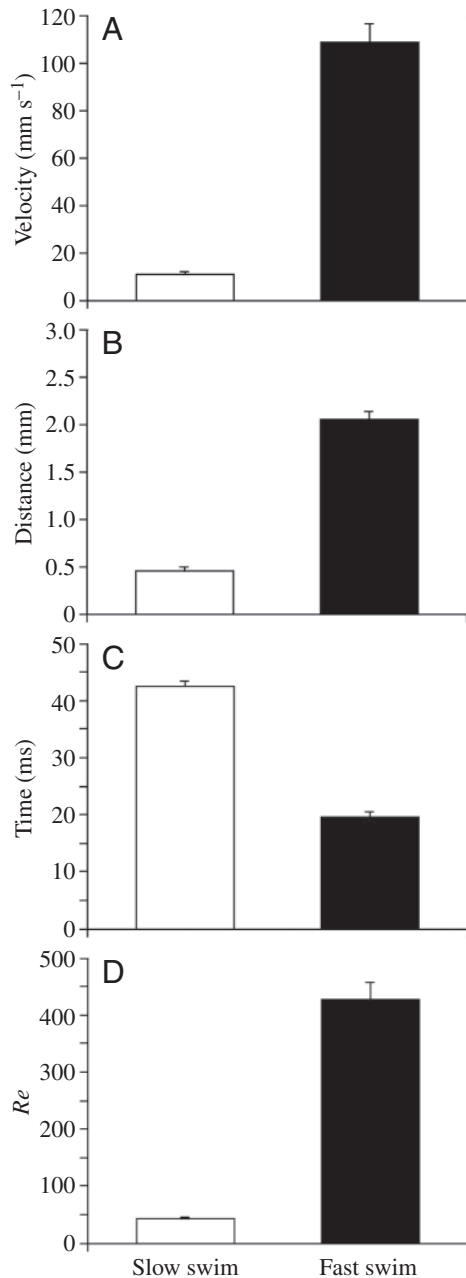


Fig. 2. Slow and fast straight swimming of zebrafish larvae demonstrating the distinct swimming gaits during straight swimming. (A) Average velocity across one locomotor cycle, (B) distance traveled in a locomotor cycle, (C) duration of a locomotor cycle, (D) Reynolds (*Re*) number. All comparisons are significantly different (*P*<0.0001). All values are given as mean of three trials for each of 10 individuals for slow (*N*=30) and five individuals for fast swimming (*N*=15). All data consist of one analyzed tail beat from a longer swimming event. Fish effects were present in two individuals and were not correlated to length.

Fig. 3B). The mean time points of maximum fin abduction during slow swimming events (–0.10, 19.80 and 40.07 ms) coincided with, and were not significantly different (*P*>0.05) than, maximum axial bending (1.00, 21.17 and 40.30 ms), indicating that the fins and axis are highly coordinated (Table 1).

### *Morphology*

Pectoral fins in the larval stage are composed of a flexible endoskeletal disk (Grandel and Schulte-Merker, 1998), fin membrane with actinotrichia, and muscles that actuate the fin membrane. The fin musculature, composed of two relatively simple muscles, is separated along the sagittal plane by an endoskeletal disk. Confocal microscopy sections (Fig. 4A–C) through these muscles in a transgenic fish that expresses green fluorescent protein in muscle fibers (Higashijima et al., 1997) indicate the position of the abductor/adductor musculature along the fin. Planar views of the abductor and adductor muscles (Fig. 4A,B,E,F), illustrate that muscle fibers run in a sheet on the fin extending upwards from its base. The abductor muscle is located on the rostral side of the fin and pulls the fin forward when it contracts. The adductor muscle is located on the caudal side of the fin and pulls the fin back against the body when it contracts. The abductor and adductor originate along the anterolateral and anteromedial surface of the cleithrum, respectively, and insert onto the fin membrane (Fig. 4F).

The fin musculature represents a functional fin blade surface area of 25590±993 $\mu m^2$ (82055±2535 $\mu m^2$ total fin blade area) in the lateral plane of the abductor (Table 2). The majority of the fin musculature is only one muscle fiber thick. The abductor and adductor muscles are composed of essentially the same number of muscle fibers (54.4±1.4 and 51.8±1.9, respectively; *P*=0.1902; Table 2). A number of fibers converge at the origination of the fin musculature, along the midline of the fin, which is about two muscle fibers thick. Muscle fibers along the midline run parallel from origination to insertion. The musculature servicing the leading edge and trailing-edge of the fin travel at opposite angles of curvature with respect to the midline fibers, with trailing-edge fibers having the largest relative curvature (Fig. 4A,B,E).

### Discussion

Many fishes can use axial and fin movements during swimming. However, in adult fishes, these two modes of locomotion tend to function independently of one another (e.g. Webb, 1994). By contrast, slow swimming of larval zebrafish is characterized by the coordinated movement of the fins and body axis. The pattern of coordination identified for slow swimming is similar to that observed in basal tetrapod groups, and may be controlled by an evolutionarily conserved neural circuit. Here we use locomotor behavior and morphology to develop hypotheses of locomotor function.

### *Locomotion in larval zebrafish*

Axial locomotion in larval zebrafish has been well described

Table 1. *Fin and axial coordination of slow swimming*

| Variable | Time (ms) | S.E.M. |
| --- | --- | --- |
| Tail beat duration | 39.30 | 0.60 |
| Fin abduction duration | 18.30 | 0.54 |
| Fin adduction duration | 17.67 | 0.44 |
| Refractory period | 5.27 | 0.62 |
| Abduction/adduction cycle | 35.97 | 0.81 |
| Abduction/adduction cycle, including refractory period | 41.23 | 0.78 |
| Fin 1, maximum lateral abduction | −0.10 | 0.14 |
| Tail position 1, at maximal axial curvature | 1* | 0.00 |
| Fin 2, maximum lateral abduction | 19.80 | 0.65 |
| Tail position 2, at maximal axial curvature | 21.17 | 0.47 |
| Fin 3, maximum lateral abduction | 40.07 | 0.78 |
| Tail position 3 of maximal axial curvature | 40.30 | 0.60 |

All values are given as mean of three trials for each of 10 individuals for slow swimming ($N$=30 for each entry) during three complete fin beat cycles. Maximal axial curvature as defined by Budick and O'Malley (2000). *Tail position one used as a reference for coordination calculations. A repeated measures analysis of variance found no significant differences ($P$>0.05) between tail position and fin extension position indicating coordination between the two.

in several recent studies (Budick and O'Malley, 2000; Müller and van Leeuwen, 2004), the first at 6 and 9 dpf, the second at 2–5, 7 and 14 dpf. We chose to focus on 5 dpf larvae because previous work on the neural control of swimming (e.g. Liu and Fetcho, 1999; Hale et al., 2001) and previous kinematic studies

(e.g. Budick and O'Malley, 2000; Borla et al., 2002) have been done at that age. In the present study we further examine the pectoral fins, focusing on the steady swimming component of the slow start and compare it with straight forward swimming following the, previously described, fast start (Budick and O'Malley, 2000; Müller and van Leeuwen, 2004).

To examine the relationship between fin and axial movement during steady swimming, we examined only the component of slow swimming in which the fish is swimming straight with equivalent left and right angular head movement, and with little change in angle between tail strokes (limiting initiation and end movement bias). We restricted trials because of our primary interest in the coordination of steady forward locomotion. For these components of the swimming trials, we found that pectoral fin beats matched both the frequency and phase of tail beats. We conclude that the fins and axis are highly coordinated by showing that the number of fin movements matched the number of points of maximal axial curvature (Fig. 1) and that there are no significant differences between the timing of fin maximum lateral abduction and maximum axial curvature (Table 1). This differs slightly from the findings of Müller and van Leeuwen (2004) that the pectoral fins are active during slow starts at the same frequency (typically below 30 Hz) but not necessarily the same phase as axial movements during what they call 'slow-start swimming'. The pectoral fins and tail were found to be in phase in most sequences of slow start swimming in Müller and van Leeuwen (2004), although during burst swimming the pectoral fins were occasionally found to be out of phase with the tail (U. Müller, personal communication). We attribute our findings of tight fin–axial coordination to the extent of the slow swimming events examined (i.e. steady swimming – no burst of acceleration or deceleration).
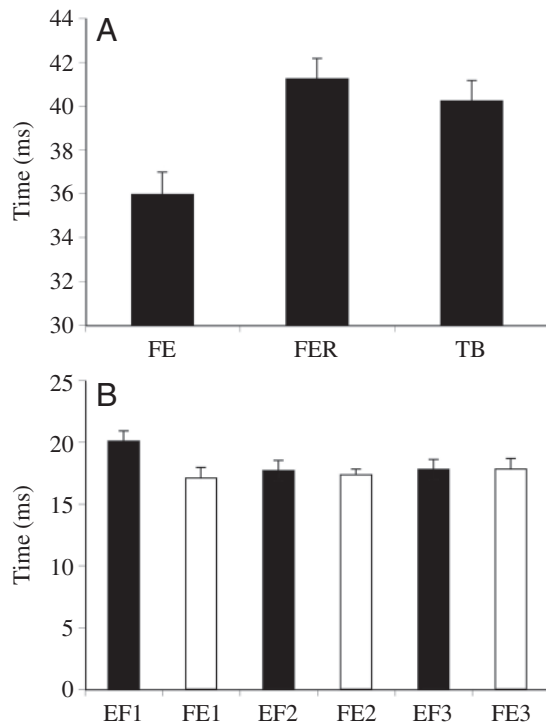


Fig. 3. Coordination of the fin–axis during slow swimming. (A) Duration of an abduction–adduction cycle. FE, fin cycle excluding refractory period; FER, fin cycle including refractory period; TB, tail beat. *Significantly different ($P$<0.05). (B) Duration of fin abduction *vs* adduction (ms). Black bars, adduction; white bars, abduction. EF1 is significantly different ($P$<0.05) from FE1 and FE2. Values are plotted as mean ± S.E.M.

*Comparison to limb–axis coordination in other taxa*

The pattern, and relative timing, of fin and axial movement

observed during slow swimming (Fig. 5A) bears a striking resemblance to the fore limb and axial coordination of some amphibians and reptiles, and the walking and running gait of many tetrapods (Fig. 5C,D; Daan and Belterman, 1968; Ritter, 1992). In most cases, the limbs are coordinated so that one cycle of axial bending corresponds to one limb cycle. Periods of maximal axial curvature generally coincide with maximal extension of limbs. In tetrapods, coordination of these behaviors involves the integrated activity of central pattern generators controlling the abduction–adduction rhythms of the limbs and lateral bending of the body (Devolvé et al., 1997; Bem et al., 2003).
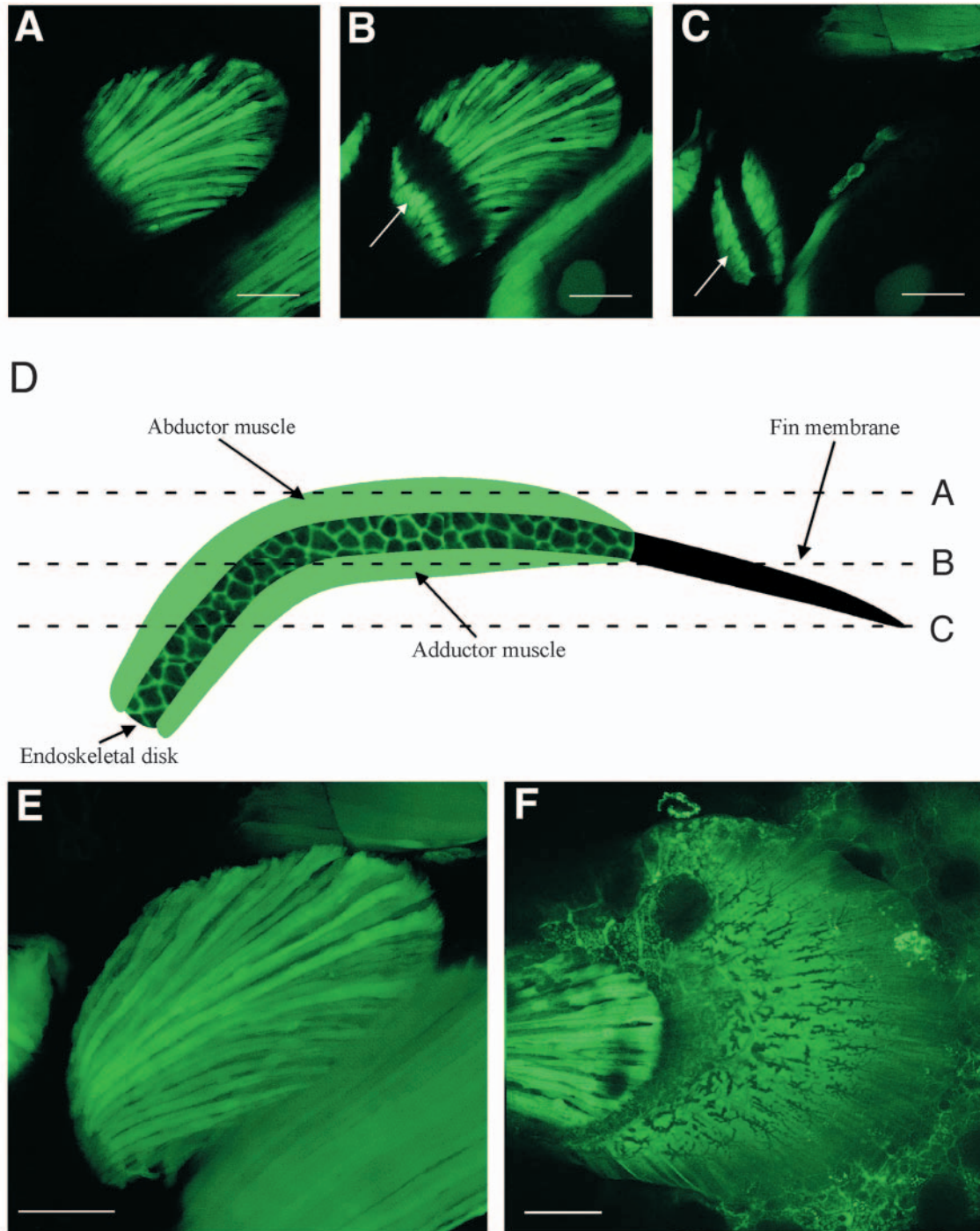


Fig. 4. Optical sections through pectoral fin muscle of an α-actin transgenic zebrafish expressing GFP. Rostral is to the left in all images. Pectoral fin muscle is in the middle of the images. (A) Planar section through the abductor muscle. (B) Adductor muscle (right) and cross section although abductor muscle (left, with arrow), (C) Cross sections through the abductor muscle (left with arrow) and adductor muscle (right), (D) Orientation of the muscle sections A through C of the entire pectoral fin, (E) abductor muscle, (F) abductor muscle and fin membrane. Scale bars, 50 μm.

Table 2. *Muscle morphology of the pectoral fin at five days post fertilization*

| Variable | Mean | S.E.M. |
|---|---|---|
| Fin area ($\mu m^2$) | 56465 | 2021 |
| Muscle area ($\mu m^2$) | 25590 | 993 |
| Total fin area ($\mu m^2$) | 82055 | 2535 |
| Ratio fin area/muscle area | 2.2 | 0.1 |
| Fin area (%) | 68.8 | 1 |
| Muscle area (%) | 31.2 | 1 |
| Number of extensor fibers | 54.4 | 0.9 |
| Number of flexor fibers | 51.8 | 1.6 |

All values are given as mean of the pectoral fin musculature ($N=5$) of the left pectoral fin of 5 dpf fish. Fin and muscle areas calculated from lateral view projections. There is no significant difference between the number of extensor and flexor fibers ($t$-test, $t=1.431$, $P=0.1902$).

The timing of limb activity, specifically the duration of abduction–adduction phases, varies among larval and adult fishes as well as tetrapods. Unlike in larvae, the abduction–adduction phases of pectoral swimming of adult fishes are not equal in duration. Studies by Gibb et al. (1994) on bluegill sunfish, Walker and Westneat (1997) on a wrasse, and Drucker and Lauder (1997) on a surfperch, demonstrate that fin adduction is shorter in duration than abduction. A comparison of timing of larval fin cycles to the swing/stance cycle in tetrapods reveals that cycle duration varies depending on locomotor speed (Biewener, 2003). Work on the salamander, *Dicamptodon* (Ashley-Ross, 1994), has shown that the stance and swing phase durations during the step cycle are nearly equal (Fig. 5C). However, kinematics of lizard limbs

have shown that stance is longer in duration than the swing phase during running (Reilly and Delancey, 1997). Differences in timing of limb movements in vertebrates may reflect specializations based on morphological, behavioral and function requirements.

Fricke and Hissman (1992) have shown that the coelacanth (*Latimeria chalumnae*) can coordinate its pectoral fins with the caudal fin in a similar fashion to tetrapods and larval fishes. Fins were coordinated with a phase difference of 180° (abduction of one fin and adduction of the other). Pectoral fins were employed in an alternating fashion during accelerated forward movement, and have the ability to synchronize after a sudden start and during the following behaviors: curve swimming, accelerated movement to gliding, and upside down swimming (Fricke and Hissman, 1992). The pattern observed in the coelacanth provides additional behavioral evidence that a fin–axis motor pattern may be primitive among Osteichthyes (Sarcopterygii and Actinopterygii).

We suggest that the neural control of fin–axis coordination observed in tetrapods and larval fishes evolved prior to the split of sarcopterygian (lobe-finned) and actinopterygian (ray-finned) fishes and, although not common in the swimming modes of adult fishes, may have been conserved in the larvae of some species. Work by Grillner and Wallen (1985) suggests that the neural circuits controlling rhythmic axial oscillation in lamprey, one of the most basal vertebrate lineages, could be employed with limb CPGs to generate the pattern of axial muscle activity observed in tetrapods. Our data and other larval data (Batty, 1981) support this hypothesis by demonstrating that an axial traveling wave of bending, typical of fishes, can occur with rhythmic limb movements and may represent an intermediate
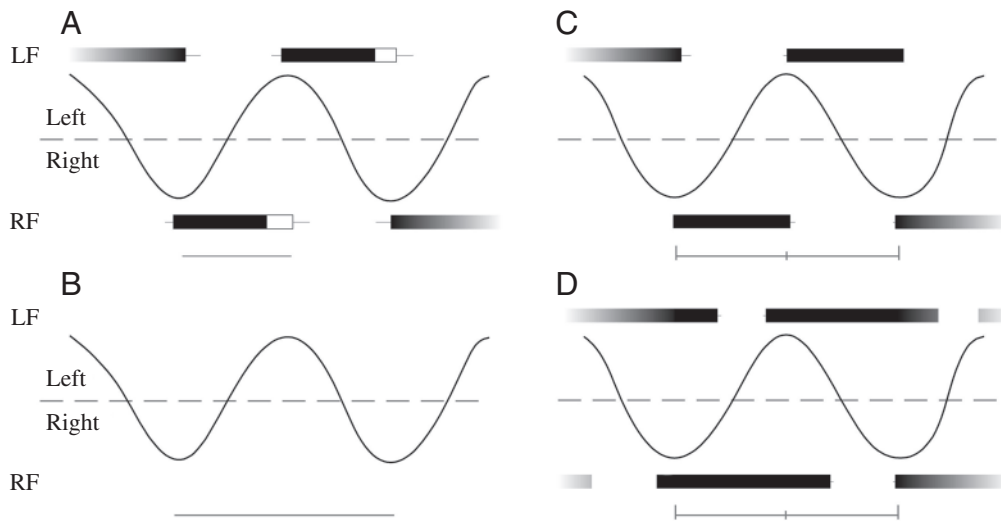


Fig. 5. Limb–body axis coordination illustrating the similarity in limb–axis locomotor patterns among tetrapods and fishes. (A) Slow swimming in 5 dpf larval zebrafish ($N=10$) and (B) fast swimming ($N=5$), (C) running ($N=16$) and (D) walking ($N=20$) for the salamander *Dicamptodon tenebrosus* (modified from Ashley-Ross, 1994). Scale bar in A,B, 20 ms; C,D, 100% of step cycle; LF, left fore foot/fin; RF, right fore foot/fin. Standard errors are indicated. Black bars indicate fin/limb extension. Fin adduction is followed by a short refractory period (open bars) characterized by limb position indeterminably adjacent to body. Body bending, represented as a wave form, and limb extension continues until maximum axial curvature.

condition of circuit coordination in which the axial bending and fore limb CPGs are integrated temporally but without substantial modification to the axial movement pattern. Furthermore, axial kinematics of adult eels (Gillis, 1996), in which axial movement alone generates propulsion, demonstrate a similar pattern to slow swimming in zebrafish, suggesting that use of pectoral fins does not necessarily alter axial patterns.

The diversity of vertebrates provides many opportunities to examine the diversity and evolution of coordination of limbs and axis. For example, Azizi and Horton (2004) recently found that the elongate salamander (*Siren lacertina*), which lacks hind limbs, is able to decouple appendicular movements and tail movements during aquatic walking, which the authors suggest may be related to elongation. This example highlights one of several possible evolutionary modifications of a primitive limb–axial circuit.

### Fin function during slow swimming

The presence of coordinated fin activity during slow swimming does not necessarily mean that the fins are participating in generating propulsive force. Fin movement may contribute to respiration (Osse and van den Boogaart, 1999) or may be used to stabilize the body during swimming. Equal abduction and adduction phases of pectoral fin movements are highly coordinated with axial movement. As suggested by Batty (1981) for pectoral and axial movements in plaice larvae, the synchronization of these pectoral fin movements with axial movements may serve to offset head yaw by counteracting the recoil effect produced by the tail movement. Larval zebrafish pectoral fin strokes are timed precisely to do this, improving efficiency by reducing drag induced by axial swimming movements. The functions of the fins in respiration, stability and propulsion remain to be tested. Clarifying the roles of fin and axial coordinated movement patterns may provide important insight into the evolution and diversification of vertebrate locomotion.

The difference in $Re$ number between slow and fast swimming suggests that hydrodynamic forces may be related to fin use during steady swimming. For larval fishes, the pattern of fin–axis locomotor coordination (Batty, 1981; Müller and van Leeuwen, 2004; this paper) seems to be associated with swimming in low $Re$ conditions. There was a tenfold difference in $Re$ values between slow swimming with pectoral fins and fast swimming with axial movement alone ($43\pm3$ and $427\pm31$, respectively). $Re$ values and movement pattern reported here are similar to those described by Batty (1981), and Müller and van Leeuwen (2004), in larval plaice and zebrafish, respectively. Zebrafish maintain pectoral fin and axial coordination in a significantly decreased $Re$ environment ($Re$ ranging from 3–11), which was achieved by increasing the viscosity of water using polyvinyl pyrrolidone (Sigma-Aldrich, Saint Louis, MO, USA; D.H.T. and M.E.H., unpublished). This finding suggests that coordinated alternating fin movements with the axis can occur through a wide range of low $Re$ numbers.

### Fin muscle structure and implications for function

The 5 day time period of the zebrafish studied here represents the first phase of pectoral fin development (Grandel and Schulte-Merker, 1998). Despite their early development, larval zebrafish pectoral fins are fully functional and perform normal locomotor behaviors. Based on kinematic and morphological data (Grandel and Schulte-Merker, 1998; Thorsen et al., 2004; this paper), we believe that larval zebrafish musculature moves along with the fin membrane and is a functional component of the fin blade. We predict the abductor/adductor muscles are able to bend with the fin through its full range of motion. The only stationary structure of the pectoral girdle appears to be the cleithrum, which anchors both abductor and adductor muscles.

Muscle fibers are relatively evenly distributed along the fin membrane, although the muscle fibers inserting at the midline of the fin are longer than those of the leading or trailing-edge fibers. A distributed network of muscle fibers along the abductor and adductor muscles suggests an even force distribution along the fin. Neural innervation patterns (Thorsen et al., 2004) suggest independent control of the leading, middle and trailing-edge components of the fin musculature. We predict then, that in the larval condition, the pectoral musculature has variable control of the fin due to innervation patterns and muscle curvature enabling asymmetries in fin movement. High-resolution, high-speed video technology could be used to test these predictions.

### Conclusion

The patterns of movement described here suggest a similarity in the neural control of limbs and the body axis. We suggest that the same basic limb–axis motor control circuit has been conserved evolutionarily and is present in fishes and salamanders; however, in fishes it is only used during early development when animals experience low $Re$ conditions, whereas tetrapods have retained and modified it for function in adults. We believe that a number of factors, including $Re$, stability, fin musculature and a primitive neural circuit, contribute to produce the behavior of the zebrafish during slow swimming. Many questions remain regarding the function of fins throughout development, how fins are controlled through sensory–motor mechanisms, neural circuitry for generating fin abduction–adduction rhythms and fine control of motion. The simplicity of the pectoral fin musculature composed of one muscle at one limb joint makes the larval zebrafish an excellent model to address many of these questions.

## References

**Ahn, D., Kourakis, M. J., Rohde, L. A., Silver, L. M. and Ho, R. K.** (2002). T-box gene *tbx5* is essential for formation of the pectoral limb bud. *Nature* **417**, 754-758.

**Arreola, V. I. and Westneat, M. W.** (1996). Mechanics of propulsion by multiple fins: kinematics of aquatic locomotion in the burrfish (*Chilomycterus schoepfi*). *Proc. R. Soc. Lond. B.* **1377**, 1689-1696.

**Ashley-Ross, M. A.** (1994). Hind limb kinematics during terrestrial locomotion in a salamander (*Dicamptodon tenebrosus*). *J. Exp. Biol.* **193**, 255-283.

**Azizi, E. and Horton, J. M.** (2004). Patterns of axial and appendicular movements during aquatic walking in the salamander, *Siren lacertina*. *Zool.* **107**, 111-120.

**Batty, R. S.** (1981). Locomotion of plaice larvae. *Symp. Zool. Soc. Zool.* **48**, 53-69.

**Bellwood, D. R. and Fisher, R.** (2001). Relative swimming speeds in reef fish larvae. *Mar. Ecol. Prog.* **211**, 299-303.

**Bem, T., Cabelguen, J. M., Ekeberg, O. and Grillner, S.** (2003). From swimming to walking: a single basic network for two different behaviors. *Biol. Cybern.* **88**, 79-90.

**Bennett, W. O., Simons, R. S. and Brainerd, E. L.** (2001). Twisting and bending: the functional role of salamander lateral hypaxial musculature during locomotion. *J. Exp. Biol.* **204**, 1979-1989.

**Biewener, A. A.** (2003). *Animal Locomotion*. Oxford, UK: Oxford University Press.

**Blake, R. W.** (1983). Medial and paired fin propulsion. In *Fish Biomechanics* (ed. P. W. Webb and D. Weihs), pp. 214-247. New York, USA: Praeger.

**Borla, M. A., Palecek, B., Budick, S. and O'Malley, D. M.** (2002). Prey capture by larval zebrafish: evidence for fine axial motor control. *Brain Behav. Evol.* **60**, 207-229.

**Budick, S. A. and O'Malley, D. M.** (2000). Locomotor repertoire of the larval zebrafish: swimming, turning and prey capture. *J. Exp. Biol.* **203**, 2565-2579.

**Daan, S. and Belterman, T.** (1968). Lateral bending during the locomotion of some lower tetrapods, I and II. *Proc. Konk. Akad. Wetensch. Ser. C.* **71**, 245-266.

**Devolvé, I., Bem, T. and Cabelguen, J. M.** (1997). Epaxial and limb muscle activity during swimming and terrestrial stepping in the adult newt. *J. Neurophysiol.* **78**, 638-650.

**Drucker, E. G. and Jensen, J. S.** (1996a). Pectoral fin locomotion in the striped surfperch. I. Kinematics effects of swimming speed and body size. *J. Exp. Biol.* **199**, 2235-2242.

**Drucker, E. G. and Jensen, J. S.** (1996b). Pectoral fin locomotion in the striped surfperch. II. Scaling swimming kinematics and performance at a gait transition. *J. Exp. Biol.* **199**, 2243-2252.

**Drucker, E. G. and Lauder, G. V.** (1997). Kinematic and electromyographic analysis of stedy pectoral fin swimming in the surfperches. *J. Exp. Biol.* **200**, 1709-1723.

**Drucker, E. G. and Lauder, G. V.** (2003). Function of pectoral fins in rainbow rout: behavioral repertoire and hydodynamic forces. *J. Exp. Biol.* **206,** 813-826.

**Du, S. J., Frenkel, V., Kindschi, G. and Zohar, Y.** (2001). Visualizing normal and defective bone development in zebrafish embryos using the fluorescent chromophore Calcein. *Dev. Biol.* **238**, 239-246.

**Fisher, R., Bellwood, D. R. and Job, S. D.** (2000). Development of swimming abilities in reef fish larvae. *Mar. Ecol. Prog.* **202**, 163-173.

**Fisher, R. and Bellwood, D. R.** (2003). Undisturbed swimming behaviour and nocturnal activity of coral reef fish larvae. *Mar. Ecol. Prog.* **263**, 177-188.

**Fricke, H. and Hissmann, K.** (1992). Locomotion, fin coordination and body form of the living coelacanth *Latimeria chalumnae*. *Environ. Biol. Fish.* **34**, 329-356.

**Frolich, L. M. and Biewener, A. A.** (1992). Kinematic and electromyographic locomotion in the salamander *Ambystoma tigrinum*. *J. Exp. Biol.* **162**, 107-130.

**Geerlink, P. J.** (1979). The anatomy of the pectoral fin in *Sarotherodon niloticus* trewavas (Cichlidae). *Neth. J. Zool.* **29**, 9-32.

**Geerlink, P. J.** (1983). Pectoral fin kinematics of *Coris formosa* (Teleostei, Labridae). *Neth. J. Zool.* **33**, 515-531.

**Geerlink, P. J.** (1989). Pectoral fin morphology: a simple relation with movement patterns? *Neth. J. Zool.* **39**, 166-193.

**Gibb, A. C., Jayne, B. C. and Lauder, G. V.** (1994). Kinematics of pectoral fin locomotion in the bluegill Sunfish *Lepomis macrochirus*. *J. Exp. Biol.* **189**, 133-161.

**Gillis, G. B.** (1996). Undulatory locomotion in elongate aquatic vertebrates: anguilliform swimming since Sir James Gray. *Am. Zool.* **36**, 656-665.

**Grandel, H. and Schulte-Merker, S.** (1998). The development of the paired fins in the zebrafish (*Danio rerio*). *Mech. Dev.* **79**, 99-120.

**Grillner, S.** (1981). Control of locomotion in bipeds, tetrapods and fish. In *Handbook of Physiology, Motor Control* (ed. V. Brooks), pp. 1179-1236. Bethesda, USA: American Physiological Society.

**Grillner, S. and Wallen, P.** (1985). Central pattern generators for locomotion, with special reference to vertebrates. *Annu. Rev. Neurosci.* **8**, 233-261.

**Hale, M. E.** (1996). Ontogeny of fast-start ability in fishes. *Am. Zool.* **36**, 695-709.

**Hale, M. E.** (1999). Effects of size and ontogeny on the fast-start performance of several salmonid species. *J. Exp. Biol.* **202**, 1465-1479.

**Hale, M. E., Ritter, D. A. and Fetcho, J. R.** (2001). A confocal study of spinal interneurons in living larval zebrafish. *J. Comp. Neurol.* **437**, 1-16.

**Higashijima, S., Okamoto, H., Ueno, N., Hotta, Y. and Eguchi, E.** (1997). High-frequency generation of transgenic zebrafish which reliably express GFP in whole muscles or the whole body using promoters of zebrafish origin. *Dev. Biol.* **192**, 189-199.

**Liu, K. S. and Fetcho, J. R.** (1999). Laser relations reveal functional relationships of segmental hindbrain neurons in zebrafish. *Neuron* **23**, 325-335.

**McClellan, A. D.** (1996). Organization of spinal locomotor networks: contributions from model systems. *Commun. Theor. Biol.* **4**, 63-91.

**Müller, U. K. and van Leeuwen, J. L.** (2004). Swimming of larval zebrafish: ontogeny of body waves and implications for locomotory development. *J. Exp. Biol.* **207**, 853-868.

**Osse, J. W. M. and van den Boogaart, J. G. M.** (1999). Dynamic morphology of fish larvae, structural implications of friction forces in swimming, feeding and ventilation. *J. Fish Biol.* **55**, 156-174.

**Reilly, S. M. and Delancey, M. J.** (1997). Sprawling locomotion in the lizard *Sceloporus clarkii*: quantitative kinematics of a walking trot. *J. Exp. Biol.* **200**, 753-765.

**Ritter, D.** (1992). Lateral bending during lizard locomotion. *J. Exp. Biol.* **173**, 1-10.

**Sordino, P., van der Hoeve, F. and Duboule, D.** (1995). *Hox* gene expression in teleost fins and the origin of vertebrate digits. *Nature* **375**, 678-681.

**Stein, P. S. G.** (1978). Motor systems, with special reference to locomotion. *Annu. Rev. Neurosci.* **1**, 61-81.

**Thorsen, D. H. and Westneat, M. W.** (in press). Diversity of pectoral fin structure and function in fishes with labriform propulsion. *J. Morphol.*, in press.

**Thorsen, D. H., Cassidy, J. J. and Hale, M. E.** (2004). Development of pectoral fin morphology and locomotion in the zebrafish *Danio rerio*. *Am. Zool.* **43**, 903.

**Walker, J. A. and Westneat, M. W.** (1997). Labriform propulsion in fishes: kinematics of flapping aquatic flight in the bird wrasse *Gomphosus varius* (Labridae). *J. Exp. Biol.* **200**, 1549-1569.

**Webb, P. W.** (1973). Kinematics of pectoral fin propulsion in *Cymatogaster aggregata*. *J. Exp. Biol.* **59**, 697-710.

**Webb, P. W.** (1993). Swimming. In *The Physiology of Fishes* (ed. D. H. Evans), pp. 47-73. Boca Raton, FL, USA: CRC Press.

**Webb, P. W.** (1994). The biology of fish swimming. In *Mechanics and Physiology of Animal Swimming* (ed. L. Maddock, Q. Bone and J. V. M. Rayner), pp. 45-62. Cambridge, UK: Cambridge University Press.

**Westneat, M. W.** (1996). Functional morphology of aquatic flight in fishes: kinematics, electromyography, and mechanical modeling of labriform locomotion. *Am. Zool.* **36**, 582-598.

**Williams, T. L., Grillner, S., Smoljaninov, V. V., Wallen, P., Kashin, S. and Rossignol, S.** (1989). Locomotion in lamprey and trout: the relative timing of activation and movement. *J. Exp. Biol.* **143**, 559-566.

**Winterbottom, R.** (1974). A descriptive synonymy of the striated muscles of the teleostei. *Proc. Acad. Nat. Sci. PA* **125**, 225-317.