

Prey Capture by Larval Zebrafish: Evidence for Fine Axial Motor Control

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Key Words

Fish · Teleost · Motor control · Brainstem · Spinal cord · Kinematics · Swimming · Locomotion · Reticulospinal

Abstract

Swimming and turning behaviors of larval zebrafish have been described kinematically, but prey capture behaviors are less well characterized. High-speed digital imaging was used to record the axial kinematics of larval zebrafish as they preyed upon paramecia and also during other types of swimming. In all types of swim bouts, a series of traveling waves of bending is observed and these bends propagate along the trunk in the rostral to caudal direction. The prey capture swim bouts appeared to be more complex than other swim patterns examined. In the capture swim bouts, the initial bends were of low amplitude and were most prominent at far-caudal locations during each individual traveling wave of bending. Later bends in the bout (occurring just prior to prey capture) appeared to originate more rostrally and were of larger amplitude. These changes in bending pattern during capture swims were accompanied by a marked increase in tail-beat frequency. Associated with these axial kinematics were changes in heading and an abrupt increase in velocity close to the moment of prey capture. These changing patterns of bending suggest precise, bend-to-bend, neural control over both the timing and

the rostral-caudal locus of bending. This degree of 'fine axial motor control' has not previously been described in the teleost behavioral literature and is notable because it occurs in larval zebrafish, where descending control signals are funneled through the roughly three-hundred neurons that project from brain into spinal cord. These findings will necessitate a significant increase in the complexity of current models of descending motor control in fishes.

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Introduction

The prey capture behaviors of fishes may offer insight into the descending motor systems that control locomotive behaviors. Larval fishes are of interest because of their reduced complexity (relative to adult fishes and especially to mammals). Different species of larval fishes have evolved different prey capture strategies and behaviors. Herring larvae (*Clupea harengus*), for example, exhibit brief bouts of 'beat and glide' swimming [Batty et al., 1991] and tend to slow down when they encounter patches of prey [Munk and Kiorboe, 1985]. In contrast, larval clownfish (*Amphiprion perideraion*) swim continuously and, upon encountering a patch of prey, exhibit an increased speed of swimming, a greater frequency of turning and higher turn angles which collectively result in a

more localized search path [Coughlin et al., 1992]. The mechanism of capture may occur via either ram or suction prey-capture strategies [see e.g., Lauder, 1980; Coughlin, 1991; Cook, 1996; Von Herbing et al., 1996; Ferry-Graham, 1998; Budick and O'Malley, 2000a]. A video-rate study of larval clownfish shows that they develop a suction component to feeding within 5 days post-hatching [Coughlin, 1994], whereas a hydrodynamic analysis of feeding by carp larvae (*Cyprinus carpio*) suggests that a combined ram-suction strategy may increase the efficiency of prey capture [Drost et al., 1988a, b]. Larval fishes also gradually improve their prey capture strike accuracy, especially when fed live prey [Drost, 1987; Coughlin, 1991; and see Webb and Skadsen, 1980]. What has not been examined in detail is the kinematics of the axial musculature during prey capture, yet these axial bending patterns may offer clues to the organization of the underlying neural architectures.

Several key aspects of the spinal and descending neural controls for locomotor behaviors appear to be conserved amongst vertebrate animals. Central pattern generators (CPGs), for example, are present in all vertebrate spinal cords examined to date [Kiehn et al., 1998]. Spinal circuitry appears to be fairly well-conserved in anamniotes, based on similarity of spinal interneuron cell types [Fetcho, 1992]. The arrangement of hindbrain rhombomeres and cranial nerves is also well conserved amongst many anamniotes, birds and mammals [Trevarrow et al., 1990; Keynes and Lumsden, 1990; Szekely and Matesz, 1993; Bass and Baker, 1997; Glover, 2001]. However, the full extent to which locomotor control systems might be conserved across vertebrate animals is difficult to assess. Mammals exhibit an extremely complex CNS with elaborate motor control systems that may involve billions of neurons. Technical problems exacerbate the difficulties of comparative studies. For example, descending neural controls from brainstem to spinal cord exhibit great complexity and intermingling [see e.g., Peterson, 1979; Siegel and Tomaszewski, 1983] and individual 'classes' of descending neurons in mammals, such as vestibulospinal and reticulospinal neurons, exhibit considerable diversity [Newman, 1985a,b; Matsuyama et al., 1997, 1999; Perlmutter et al., 1998]. Even in adult fishes the situation is complex, as in the lamprey which has an estimated 1800 descending neurons, a sampling of which revealed many distinct functional types [Zelenin et al., 2001]. This complexity limits the comparative analyses of these diverse systems. A simpler model system, where the neural architectures could be more comprehensively examined, and where neural controls could be more directly related to

behaviors, might be useful in revealing organizational principles common to vertebrate descending locomotor control.

The larval zebrafish (*Danio rerio*) is an attractive model organism for such an undertaking foremost because of the many neurons in brain and spinal cord that can be individually identified [Kimmel et al., 1982, 1985; Metcalfe et al., 1986; Westerfield et al., 1986; Bernhardt et al., 1990; Hale et al., 2001]. The species is experimentally tractable, having a transparent CNS that is accessible to a variety of anatomical and physiological techniques [Fetcho et al., 1998]. Physiological studies have now linked a number of individually identified neurons, in both brain and spinal cord, to specific behaviors such as escaping and swimming [Fetcho and O'Malley, 1995; O'Malley et al., 1996; Liu and Fetcho, 1999; Ritter et al., 2001]. The entire descending projection in the larval zebrafish is estimated to be comprised of about 300 neurons, and until recently there was little information about what (if any) physiological role the great majority of these neurons might play. A recent optical recording survey has suggested that a majority of the descending neurons are active during escape behaviors [Gahtan et al., 2002; also see Bosch et al., 2001], but laser ablation studies have indicated that many of these same neurons are not necessary for the escape behavior [Budick and O'Malley, 2000b; Gahtan and O'Malley, 2000]. It is thus possible that many of these neurons might be supernumerary, vestigial, or otherwise limited in their contribution to locomotive behaviors. The previously known locomotive behaviors of the larvae did not seem to require a control system of this magnitude and so a more complete description of the locomotive repertoire seemed worthwhile as it could reveal behaviors that might require more sophisticated descending controls, and thus might concomitantly reveal further capabilities of this anatomically well-defined set of descending neurons.

We had previously examined the locomotive repertoire of the zebrafish using a high-speed camera and had classified movements into two distinct types of turning behavior (routine and escape turns) and two distinct types of swim bouts (slow and burst swims) [Budick and O'Malley, 2000a; also see Kimmel et al., 1974; Fuiman and Webb, 1988]. These larvae are also able to capture prey within a few days after hatching, and one possibility was that prey capture would consist of piecing together a series of simpler locomotive elements (e.g., routine turns and slow swims). Our initial observations of larvae preying upon paramecia revealed that prey capture is comprised of two distinct phases: an initial series of small 'tracking'

turns followed by a single discrete 'capture swim' bout where the larva strikes at the paramecium [Budick and O'Malley, 2000a]. Neither behavior has been described in detail and we focus here on the capture swim. Using high-speed digital imaging, we observed bend-to-bend changes in several axial kinematic parameters including tail-beat frequency, bend location and bend amplitude. These findings indicate that capture swims involve more than the simple combination of elemental locomotive behaviors, such as routine turns and slow swims. The sophistication of this control system has direct implications for the underlying neural circuitry and will require substantial elaboration of existing models of descending motor control.

Materials and Methods

Animals

Fertilized eggs were collected from a breeding laboratory population of zebrafish (*Danio rerio*), transferred to 10% Hanks' solution, and maintained at approximately 25 °C [Westerfield, 1995; O'Malley and Fetcho, 2000]. After hatching, the larvae were kept under the same conditions for the duration of the study. Larvae were not fed prior to the imaging of feeding behaviors. Behavioral observations were performed on larvae aged 4–6 days post-hatching. Their sizes ranged from 3.7 to 4.1 mm in total length. All protocols were carried out under the guidelines of the National Institutes of Health and were approved by the Northeastern University IACUC committee.

Experimental Protocol

To observe feeding and locomotor behaviors, larvae were individually transferred to small wells, 1.3 cm in diameter, containing 10% Hanks solution. During feeding observations, a suspension containing approximately 1000 *Paramecium caudatum* (or *P. aurelia*) per ml was added to the dish. Larval behaviors were recorded using a high-speed, MD4256 digital camera (EG&G Reticon, Sunnyvale, CA) mounted on a Zeiss dissecting microscope. Feeding data were collected at 600–700 frames/s. Slow swim and burst swim data were collected at 800 frames/s. All experiments were conducted at room temperature, usually 25–26 °C. However, due to evaporative cooling, the temperature of the solution in the dish was approximately 22 °C. The fiber-optic lights used (Fiber-Lite, Dolan-Jenner, Lawrence, MA) during the recording sessions raised the solution temperature slightly, to between 22–23 °C. The fluid layer is about 1 mm deep (vs. a larval depth of about 0.5 mm) and so the larval behaviors occur in what is essentially a 2-dimensional arena (chamber volume = 0.13 ml). The proximity of the larvae to the wall of the chamber might, in some instances, have resulted in wall-effect influences on the axial kinematics [Webb, 1993], but recording several capture swim bouts in a larger arena (2.2 cm, 550% of the length of the larvae; 1 mm deep) revealed capture swim patterns that were indistinguishable from those presented here. In a shallow chamber, kinematics may also be influenced by interactions with the water surface or the dish bottom. Although the behavioral output of the fish is likely influenced by wall and surface interactions (and more substantially by the viscosity of the surrounding medium) our kinematic analyses

specifically compare slow swims with capture swims (see below), which are being recorded in an identical environment. Thus, these experiments are designed so that any observed differences in the two comparison behaviors will be due to actions of the larvae and not due to the environment.

Recording of Feeding and Swimming Behaviors

Capture swims and spontaneous slow swims were recorded for each larva. For some fish, we first recorded slow swim bouts, and subsequently, in the same imaging session, capture swim bouts. In other fish the order was reversed. In both cases, the capture-swim episodes analyzed were, we believe, among the first prey-capture events executed by these larvae (although we observed relatively few 'misses' in these early capture attempts, we did not observe enough events to address capture efficiency). A swim was defined operationally as three or more consecutive cycles of bending that did not involve large changes in direction. One example of a burst swim was also recorded and included for comparison. A detailed kinematic description of both slow and burst swims of zebrafish larvae has been reported [Budick and O'Malley, 2000a]. For capture swims, the lighting was adjusted to permit simultaneous visualization of both the larva and the paramecium; use of heat absorbing glass (Edmund Scientific, Barrington, NJ) prevented excessive heating of the chamber. After allowing 10 min for the larva to acclimate to the adjusted lighting, a glass micropipette was used to add paramecia to the dish. The larva was then recorded feeding on one or more paramecia. It was necessary to periodically add 10% Hanks solution to the dish in order to maintain a sufficient solution level. Once a successful feeding sequence was captured, the images in the camera's frame buffer were saved to disk. Moderately fast playback of the saved sequence was used to accurately determine the location of the paramecium. In figure 1, the location of the paramecium was digitally enhanced because it is difficult to see the paramecium in still images at the low magnification needed to image tail movements during feeding bouts. When viewing movies of capture swim episodes, however, the location of the paramecium is quite certain due to its motion (www.omalleylab.neu.edu); the paramecia are also directly visible in higher magnification images (e.g. fig. 10a, 11).

Image Analysis

After visually observing and recording behaviors, variable speed playback was performed using the Reticon software to confirm that we had collected complete recordings of both slow swims, and successful capture swims, where the paramecium could be seen entering the larva's mouth. Observation of both swim types within the same 4-hour recording session occurred infrequently and so we report here paired recordings for 5 larvae in which both swim types were successfully collected. Detailed manual measurements of axial kinematics were performed on individual frames taken from the behavioral episodes. These measurements were made on printed copies whose brightness and contrast had been digitally enhanced to improve visualization of the larva (Adobe PhotoShop, San Jose, CA). In addition to the paired recordings, we recorded multiple feeding episodes for individual larvae ($n = 4$ larvae) to examine the extent of intra-fish variability in capture swim bouts.

Variables Manually Analyzed in Characterizing the Swim Bouts

The following variables were manually measured:

(i) *Orientation*: the heading of the larva at each point during the swim bout. The heading at the time of prey capture was defined to be

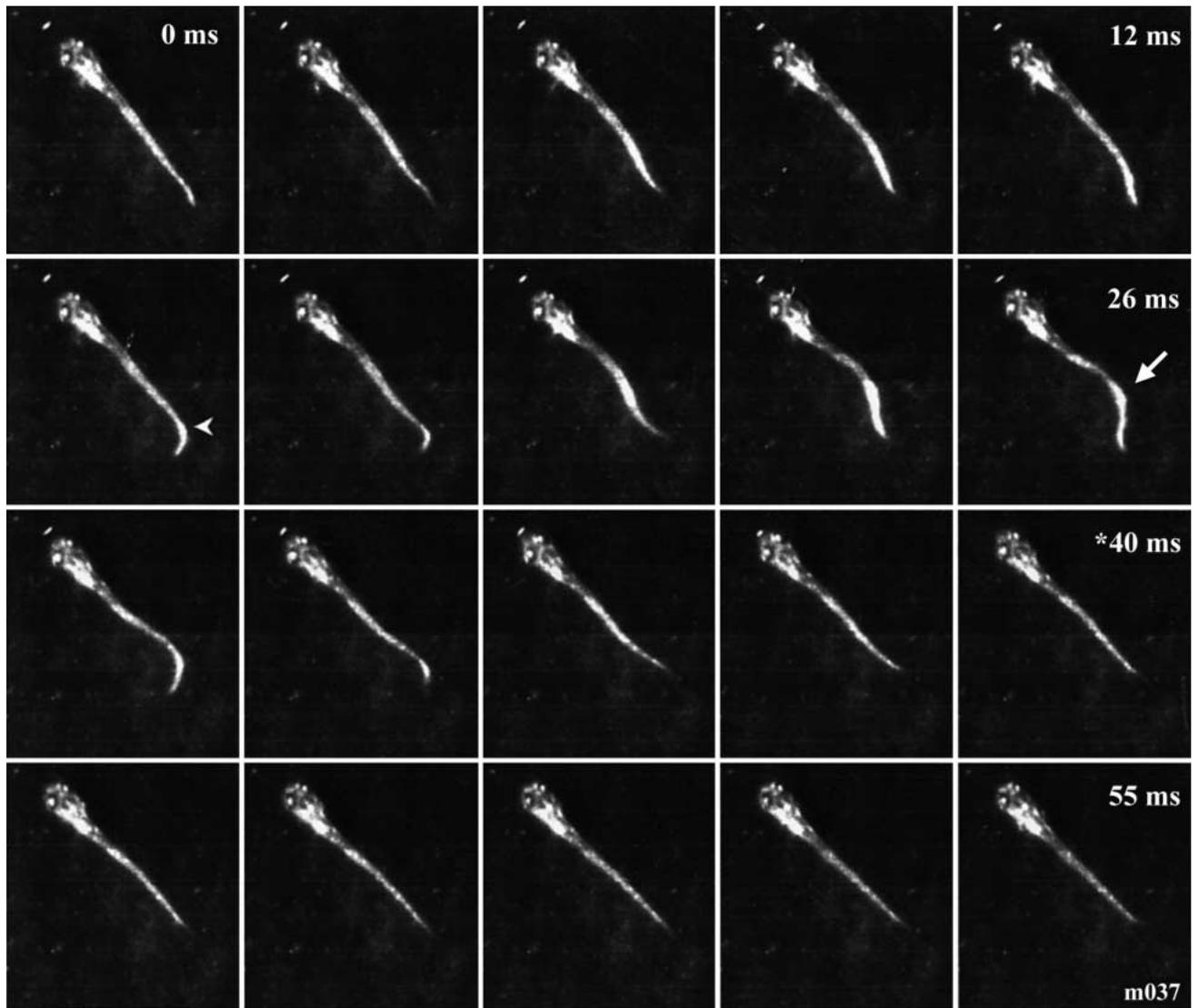


Fig. 1. Example of a capture swim. Images of a 4-day post-hatching larva, feeding on a paramecium, were collected at 700 frames/s; every other frame is shown. Note the small, far-caudal bend in the beginning of the sequence (arrow-head). The bends become larger and more rostral (arrow) prior to the prey-capture event which occurs about 40 ms into the sequence (asterisk). After capture, the swim bout abruptly ends. Use of a dark background and oblique illumina-

tion facilitated simultaneous visualization of the paramecium and the larva. At this age, the caudal fin extends about 0.1 mm beyond the trunk, so the bending patterns observed are those of the trunk. The paramecium was digitally highlighted in these still images, but its location is quite certain when viewing movies of the event. This larva is 4.0 mm long (total length).

zero degrees and the orientation at other time points were normalized to this value. The heading was determined by drawing a line from the midline of the anterior end of the swim bladder to the midline of the snout. It was calculated at 3-frame intervals.

(ii) *Time-point of maximal bending*: for each traveling wave of bending that occurs during a swim bout, a time-point that captures the 'time-point of maximal bending' is the time at which the rostral-caudal traveling wave causes the caudal tip of the larva's tail to flip direction [see Budick and O'Malley, 2000a]. Our objective was to

select a time point when the greatest fraction of the fish's trunk was maximally bent. This definition provides an easily discerned time point during each traveling wave when the bending of the trunk is quite pronounced. Specification of this time point is useful because associated variables, such as the bend angle and the rostral-caudal midpoint of this 'maximum' bend, can be determined. Many details of the muscular contractions and trunk biomechanics are unknown, more rostrally located bends have the appearance of being initiated in more rostral myotomes.

(iii) *Bend angle*: the angle formed between the rostral and caudal portions of the bend of the trunk at the time point of maximal bending. This angle was determined by drawing a line along the midline of the fish, generating a curve that defines the bending of the larva. A line is then drawn tangent to the most rostral portion of the caudal curve, and a second line is drawn tangent to the most caudal portion of this curve. The angle at the intersection of these two lines is the caudal bend angle [see figure 1 in Budick and O'Malley, 2000a].

(iv) *Maximal bend location*: the point along the rostral-caudal axis at the midpoint of the bend at the time point of maximal bending. This point was determined by drawing the shortest possible line between the vertex of the bend angle and the midline of the fish. The intersection of these two lines is defined as the midpoint of the bend. To allow between-fish comparisons, the distance from the rostral end of the larva to this point, expressed as a percentage of total body length, is defined as the 'maximal bend location'.

(v) *Half-cycle duration*: the time required for the larva to complete half of a swimming cycle. A complete cycle of swimming can be defined as the time from the bend maximum on one side to the next bend maximum on that same side. A half-cycle is the interval from the time of the bend maximum on one side until the immediately following bend maximum on the *opposite* side.

(vi) *Tail-beat frequency (TBF)*: the reciprocal of the duration of a complete cycle of bending (or 'tail-beat') is the tail-beat frequency for that cycle. Because the capture swim's rhythm changes rapidly over time, we measured the time for each individual bend or 'half-cycle' and calculated an 'instantaneous' TBF or iTBF as: $iTBF = (1/(\text{half-cycle duration (seconds)} \times 2))$.

(vii) *Velocity*: velocity was calculated by dividing the distance traveled during each time interval by that time interval. Velocity was measured by tracking a point defined by the intersection of the larva's midline with a line drawn between the center of the two eyes. Velocity was calculated over successive 3-frame intervals because the distances traveled between individual frames was often too small to measure accurately.

Statistical Analyses

Statistical comparisons of the differences in peak iTBF and peak velocity between the capture and slow swims were performed using a two-tailed t test. For analysis of the changes in bend amplitude and bend location, paired two-tailed t tests were used. In these analyses, we compared for the capture swims the bend amplitude of the first bend in the capture swim with the bend amplitude at the time of prey capture. Bend location was compared at the same two time points. For slow swims, we made the same comparisons at the same relative time points in the aligned slow swim bouts.

Laser Ablation

Retrograde labeling and laser ablation of reticulospinal neurons were performed according to previously published methods [O'Malley et al., 1996; Liu and Fetcho, 1999; O'Malley and Fetcho, 2000; Gahtan and O'Malley, 2001]. Briefly, anesthetized larvae were injected in the caudal spinal cord with Oregon-green dextran (50% wt/vol solution, Molecular Probes, Eugene, OR) and allowed to recover overnight. Larvae were then embedded in agar and inspected for labeled Mauthner neurons using a BioRad MRC 600 laser scanning confocal microscope. In larvae that had bilateral labeling of the Mauthner cells, the laser beam was focused, at maximal intensity, on a Mauthner cell for about 10 min. Liu and Fetcho [1999] have documented the efficacy of this technique for killing the Mauthner cell.

The contralateral Mauthner neuron was then ablated in the same way, after which the larvae were released from the agar and allowed to recover overnight. Only larvae in which both Mauthner cells had disappeared ($n = 5$) were used to evaluate the effects of laser-ablation on prey capture; blunt stumps of the Mauthner axons were usually evident. Other larvae within the same batch that also showed bilateral labeling of the Mauthner cells, but were not laser-ablated, were used as 'injected-controls' ($n = 4$). Previous studies have shown that mere labeling of the Mauthner cells does not affect their high-performance escape behaviors [Liu and Fetcho, 1999; Gahtan and O'Malley, 2001]. The prey capture behaviors of the laser-ablated and control larvae were analyzed in the same manner as described above (see *Image Analysis* and *Variables Manually Analyzed in Characterizing the Swim Bouts*); only one feeding trial per larvae was recorded so as to avoid practice effects.

Results

Swim Patterns Exhibited by Larval Zebrafish

Examination of a swim bout culminating in prey capture, i.e. a 'capture swim', reveals a series of small bends that propel the larva a short distance forward to capture the paramecium (fig. 1). The capture swim bout is distinct from other types of swim bouts exhibited by larval zebrafish and typically incorporates several prominent features including: (1) a number of small, far-caudal bends, that produce very slight adjustments in position (arrowhead), (2) one or more larger, more rostral bends (arrow) that precede the moment of capture (asterisk) and appear to provide substantial propulsion, and (3) an abrupt cessation of swimming immediately after the capture. None of the bends occurring during the capture-swim approached the magnitude of the C-bends that are produced during escape behaviors of larval zebrafish [see e.g., Liu and Fetcho, 1999; Budick and O'Malley, 2000a]. Capture swims are also markedly different from burst swims. Burst swims are high-speed swims with large bend angles that often follow escape behaviors (fig. 2). Burst swims involve vigorous yaw and sufficiently high velocities such that the larva would swim completely across the field of view shown in figure 1 within about 70 ms. Capture swims are most similar to another type of swim bout termed a 'slow swim', a low-speed swim that involves a more caudal locus of bending.

A slow swim, recorded from the same fish as in figure 1, is shown in figure 3. Although slow swims might appear to consist of a simple back and forth waving of the tail, closer inspection reveals rostral-to-caudal propagation of individual traveling waves of bending. Slow swims appear similar to capture swims in that both involve low swim speeds and small bend angles. Slow swims typically involve a fairly caudal bend location and a small bend

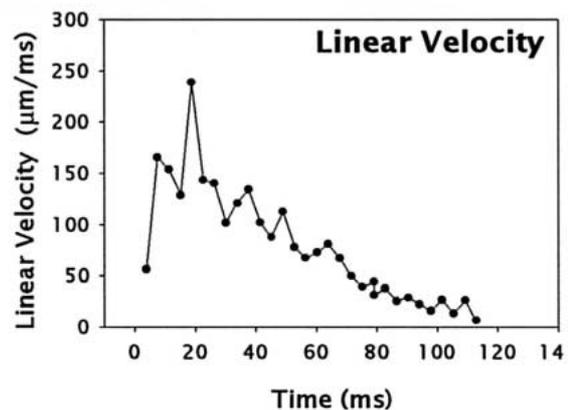
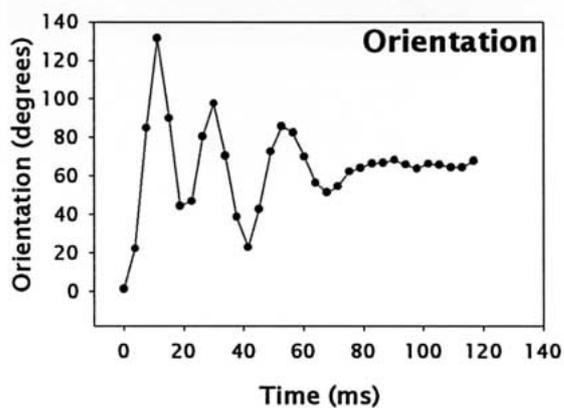
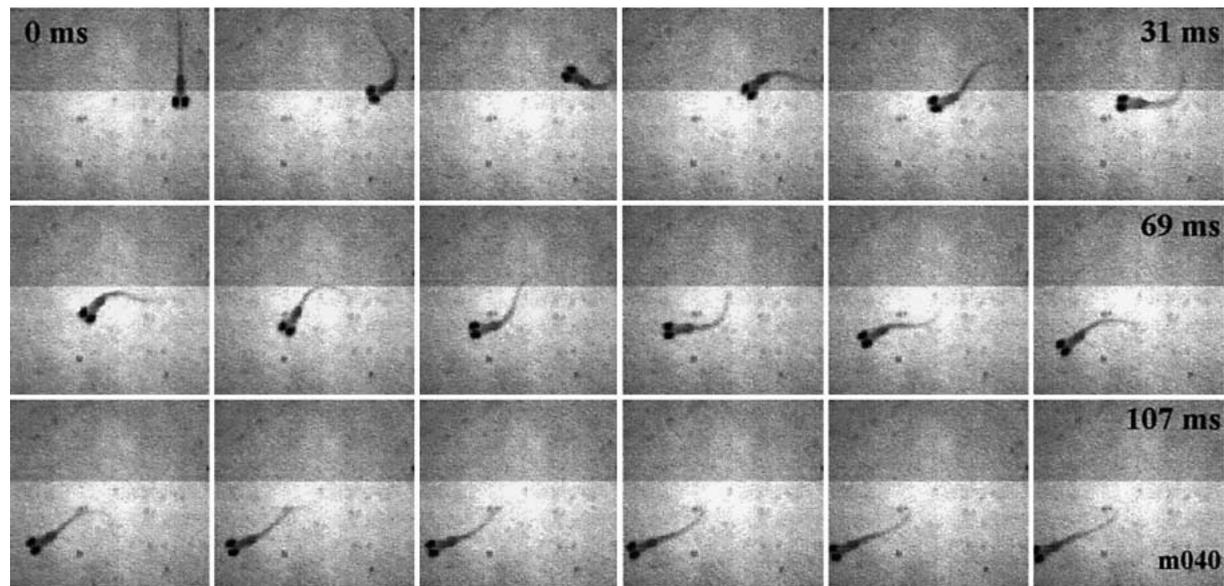


Fig. 2. Example of a burst swim. In this burst swim bout, the larva (3.9 mm in total length) swims nearly two body lengths in 75 ms, which is a much greater distance than that traveled during the capture swim in figure 1. The burst swim involves substantial bending of the body and robust side-to-side movements of the head (yaw). Yaw

is represented below by plotting the cyclical changes in head orientation during this swim bout. The plot of linear velocity shows an initial rapid acceleration followed by a gradual slowing. For further details on larval burst swims see Budick and O'Malley [2000a].

angle that tends to decline over the course of the swim bout [see Budick and O'Malley, 2000a, for more details]. They also exhibit, over several cycles of swimming, a moderate and relatively constant tail-beat frequency (TBF). From a neural control perspective, slow swims might be viewed as a relatively 'simple' behavior involving transient activation and subsequent run down of a central pattern generator. To quantify potential differences between slow and capture swims, we recorded both

types of swim bouts from 5 larvae that were 4–6 days old (post-hatching) and had never been fed paramecium.

The Axial Kinematics of Capture Swims Are Distinct from Slow Swims

A comparison of paired slow and capture swim bouts revealed a number of potentially important differences. In slow swims, the maximal bend location, measured once for each successive traveling wave of bending, occurred at

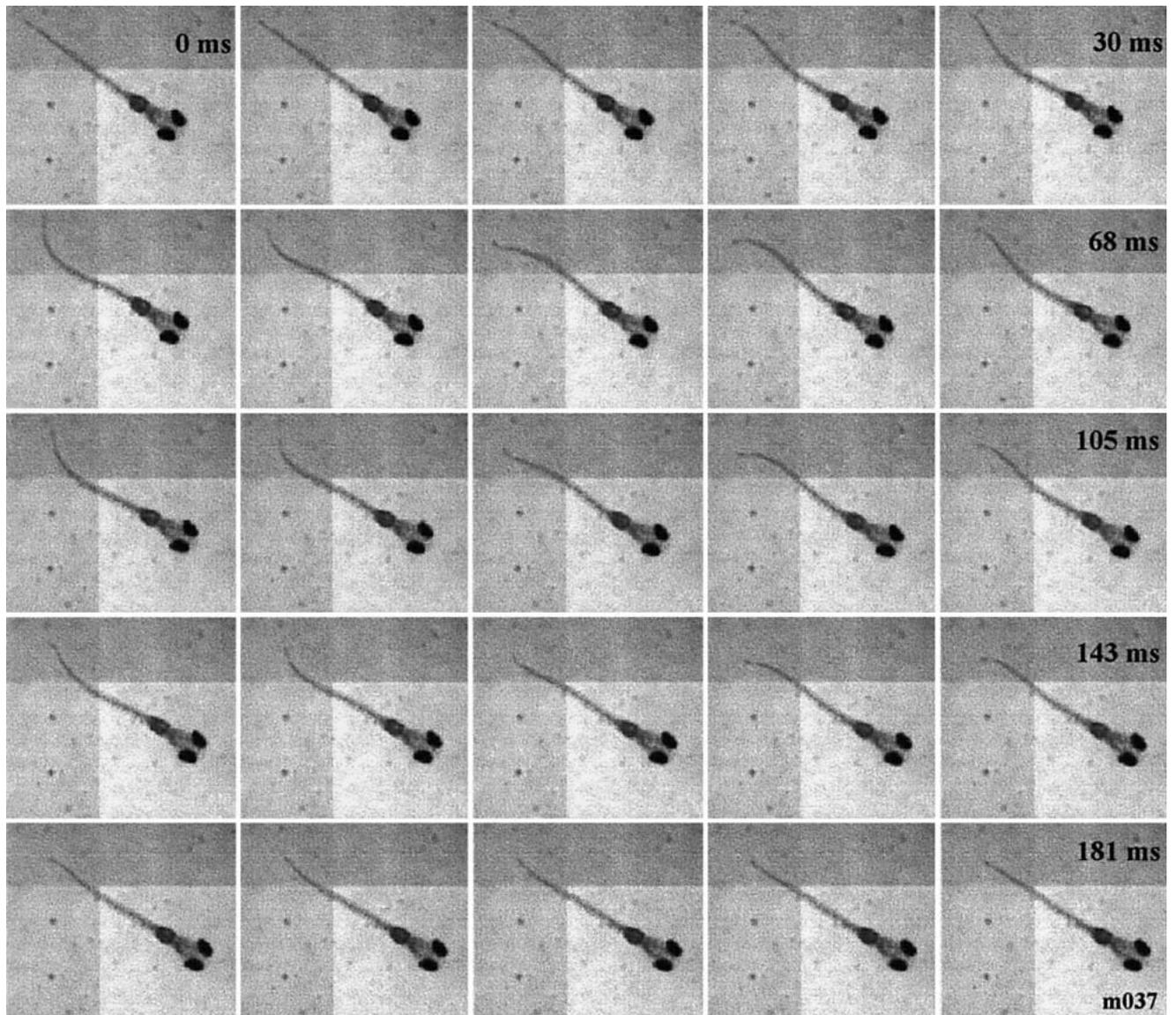


Fig. 3. Example of a slow swim from the same larva shown in figure 1. Images were collected at 800 frames/s and every 6th frame is shown. The traveling waves of bending that make up all larval swim bouts (that we have observed) are most evident in this series, where the swim speed is low and the bending to alternate sides relatively symmetrical. Note, for example, the bending on the left side of the larva's trunk which begins about frame #3 of the first row and then shifts caudally and becomes more pronounced in the next two

frames. Successive, alternating traveling waves of bending can be observed over the next 12 frames. Transmitted illumination was used in recording slow swims because it provided better contrast and therefore better visualization of the tail. The small variations in background in different sectors of the image are an artifact of the EG&G Reticon camera and did not materially impair visualization or analysis of the behavior.

about 80–90% of the total body length towards the tail (fig. 4A, B). Capture swims also showed far-caudal maximal bend locations during the initial cycles of bending (fig. 4C), but these were followed by a shift of bend-location in the rostral direction (fig. 4D). Also note the relatively slight bending of the caudal half of the body early in

the capture swim bout (bend angle = 42° in fig. 4C) vs. the more pronounced, 'scythe-like' bending later in the capture swim (bend angle = 74° in fig. 4D). It seems likely that the more vigorous bending late in the capture swim bout would provide greater propulsion. Figures 5–8 show analyses of several swim parameters from the set of 5 larvae

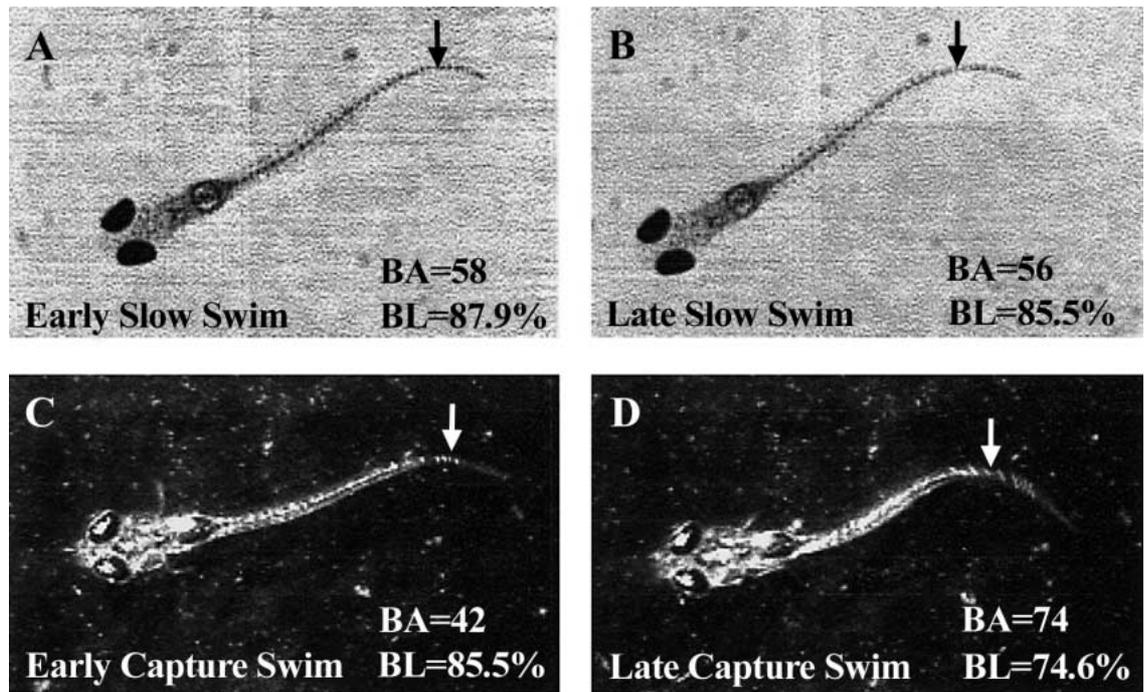
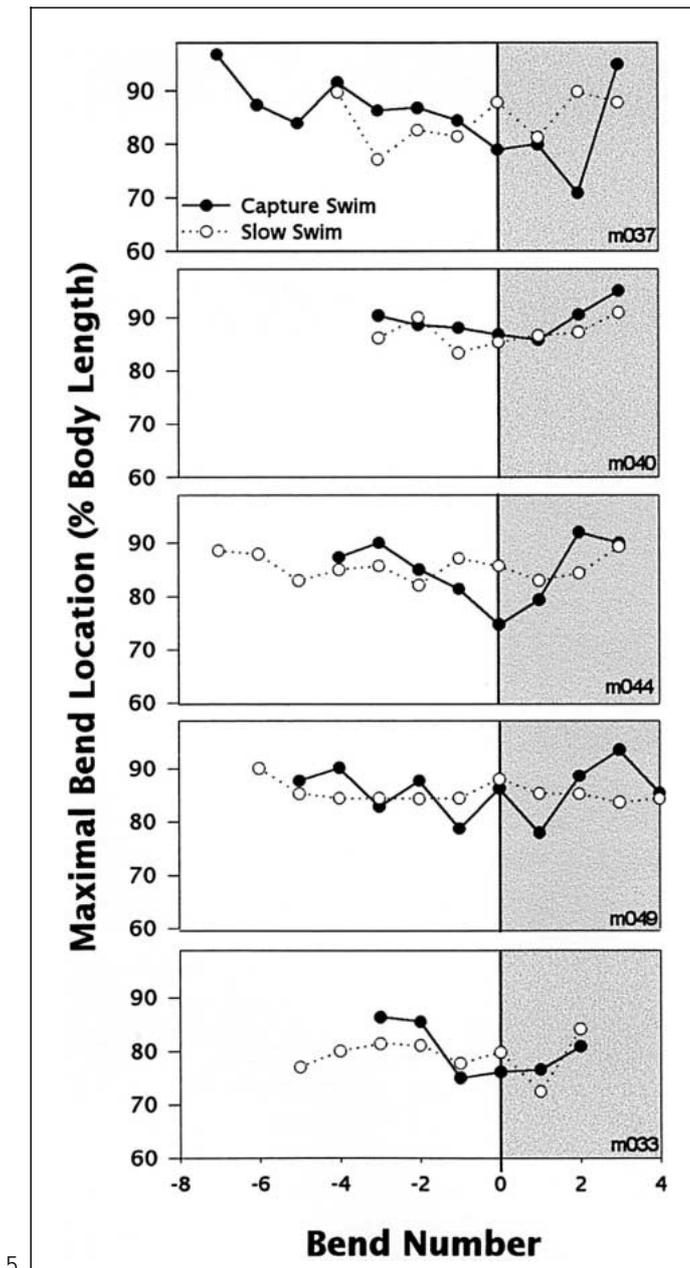


Fig. 4. Comparison of tail bends during slow and capture swims. Both behaviors were recorded from the same 6-day post-hatching larva. In the slow swim (A and B), there is a slight rostral shift in maximal bend location (arrows) and the bend angle is similar at both times. During the capture swim (C and D), there is a larger rostral shift in maximal bend location. Such rostral shifts precede capture events. The more rostral bends are often of larger amplitude and so would seem likely to provide more propulsive force for the strike. Early in the capture swim, the rostral portion of the body is relatively straight, but the larva attains a more 'scythe-like' shape later in the bout. This larva was 4.0 mm in length.

for which paired slow and capture swim recordings were obtained. In figure 5, the maximal bend location is plotted vs. bend number and each panel shows data for a slow swim and a capture swim recorded from an individual fish. The observed rostral shift in maximal bend location, during successive traveling waves of bending, was more marked in some capture swim episodes than others, but in all cases bend location shifts rostrally in the bending cycles occurring prior to the capture event (unshaded portion of the plots). The data have been plotted so that for capture swims, bend #0 is the bend occurring immediately prior to capture of the paramecium. For illustration purposes, the slow swim bouts have been plotted so that the last bend in the slow-swim bout is aligned with the last bend of the capture swim bout recorded from the same fish.

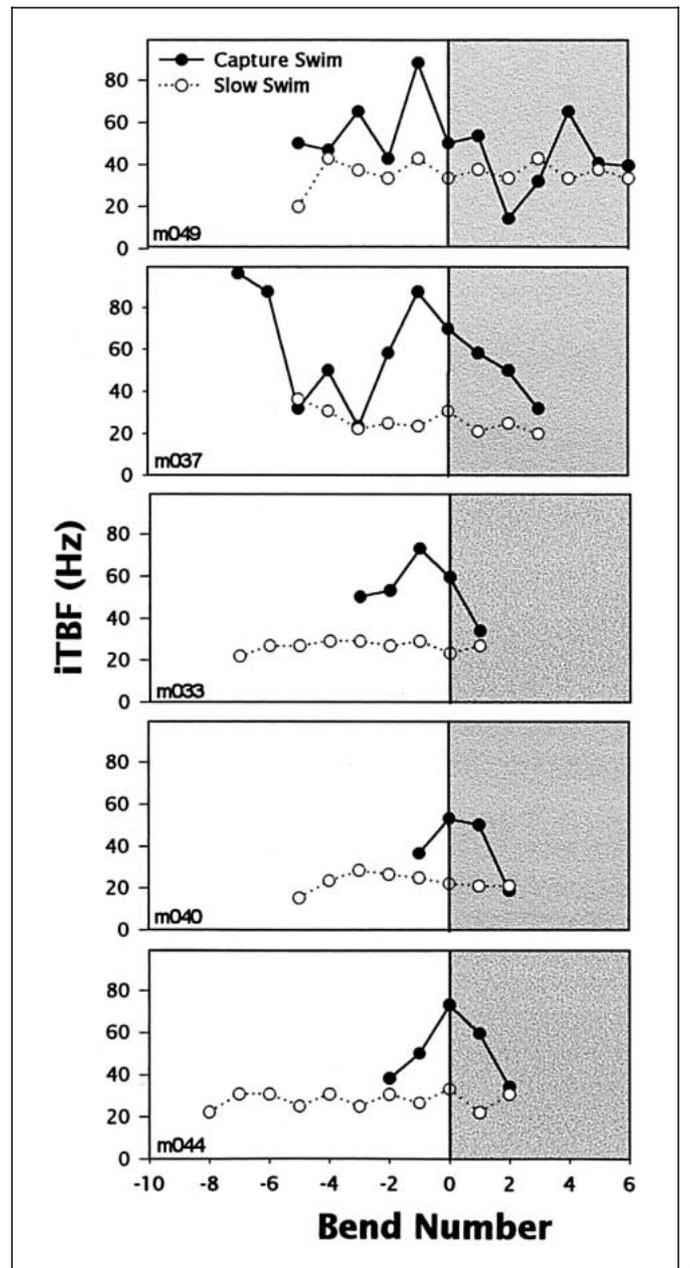
A quantitative comparison of bend parameters was made for the slow and the capture swims to determine if the observed changes in bend amplitude and location were significant. For the capture swim bouts, the maximal

bend location of the first bend in each bout, expressed in terms of % of body length (mean \pm SD = $89.72 \pm 4.23\%$), was compared with the bend immediately preceding prey capture (mean \pm SD = $80.53 \pm 5.64\%$), and the latter bend was found to be shifted significantly rostrally ($t = 3.06$, d.f. = 4, $p < 0.05$). Slow swim bouts did not show this shift; the location of the first bend location (mean \pm SD = $86.28 \pm 5.41\%$) was similar to that of a later bend (mean \pm SD = $85.33 \pm 3.34\%$) that occurred at the same relative time point in the slow swim bout, i.e., bend #0 ($t = 0.097$, d.f. = 4, $p = 0.37$). The bend amplitude also increased prior to prey capture. The initial bend amplitude (mean \pm SD = $26.4 \pm 17.2^\circ$) was substantially smaller than the bend preceding prey capture (mean \pm SD = $68.9 \pm 17.2^\circ$; $t = 2.99$, d.f. = 4, $p < 0.05$). Comparison of the bend amplitude at corresponding times during the slow swims revealed that the first bend (mean \pm SD = $42.0 \pm 23.0^\circ$) was similar in size to the later bend (mean \pm SD = $45.9 \pm 15.7^\circ$); there was no significant difference ($t = 0.27$, d.f. = 4, $p = 0.80$).



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Fig. 5. Comparison of maximal bend location during slow and capture swims. Maximal bend location is plotted vs. bend number for 5 different larvae. In each graph, a capture swim is plotted along with a slow swim recorded from the same fish. During slow swims, the maximum bend location occurs at a relatively constant location, whereas in the capture swim, the maximal bend location tends to shift rostrally over successive traveling waves of bending leading up to the capture of the paramecia (unshaded portion of plot). In the final stages of both types of swim bout, maximal bend location tends to shift caudally as the amplitude of bending wanes. For capture swims, bend #0 is defined as the bend immediately prior to or at capture. Other bends are numbered relative to this bend. The portion of the swim bout after capture has been shaded grey to highlight differences in the kinematics that occur before and after prey capture. To illustrate



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slow swims, the last bend in each slow swim is aligned with the last bend in the capture swim in figure 5, 6. Note that because the tail beat frequency changes from bend to bend (especially for capture swims, see fig. 6), the x-axis is a non-linear representation of time.

Fig. 6. Tail-beat frequency increases prior to capture. In all cases, there was a marked increase in iTBF in the bends just prior to or at capture. These capture-swim iTBFs far exceeded those present in slow swims. The iTBF during prey capture ranged from 14 to 88 Hz. During slow swims it ranged from 15 to 42 Hz. The relative constancy of the slow swim iTBFs (within individual larvae) indicates the precision with which these measurements can be made. The more variable iTBFs during capture swims are thus of biological origin and presumably relate to variable outputs of spinal CPGs or circuitry.

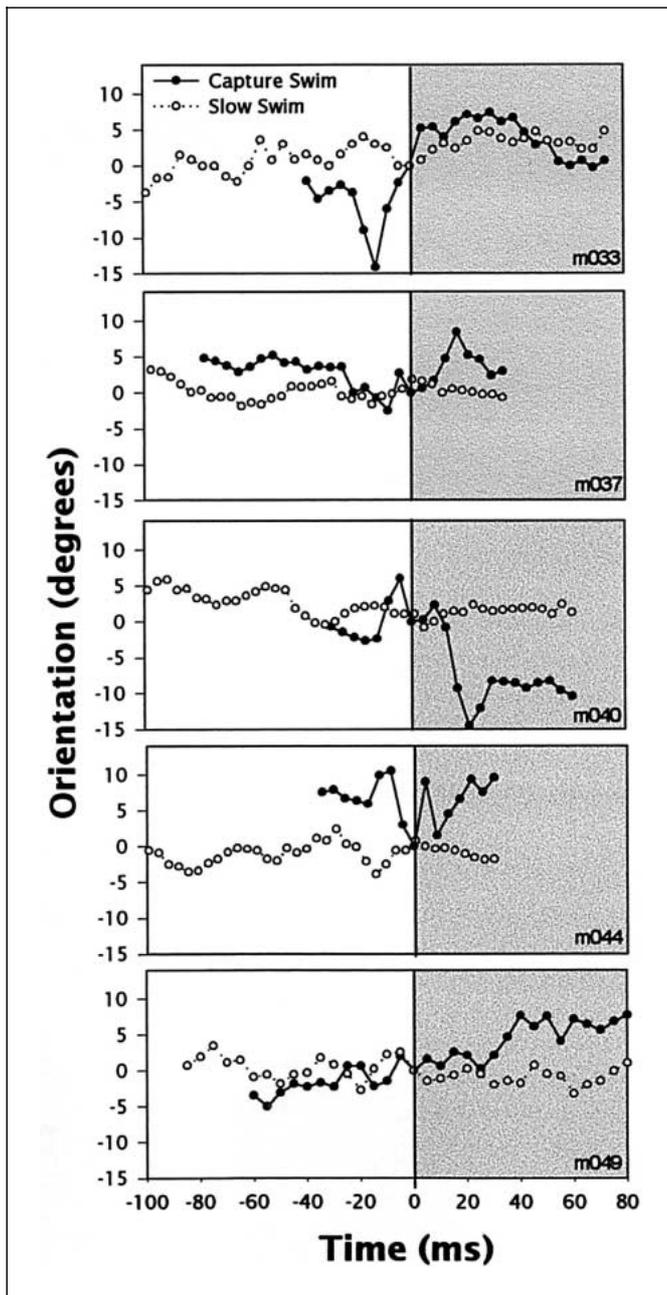


Fig. 7. Abrupt changes in orientation may occur during capture swims. The heading or orientation of the larva at the time of capture is set to 0 degrees, and all other orientations during the swim bout are normalized to this value. Orientation was measured and plotted for every third frame throughout each swim bout. Slow swim bouts exhibited cyclical changes in orientation that corresponded to the TBF of those swim bouts (the last data point from each slow swim was aligned with the last data point from the corresponding capture swim). These cyclical changes in orientation constitute the ‘yaw’ of the swim bouts, i.e., the side-to-side bending of the head during the swim. In the capture swim bouts there are periods of quite minimal yaw preceding more abrupt changes in orientation that occur close to the time of capture.

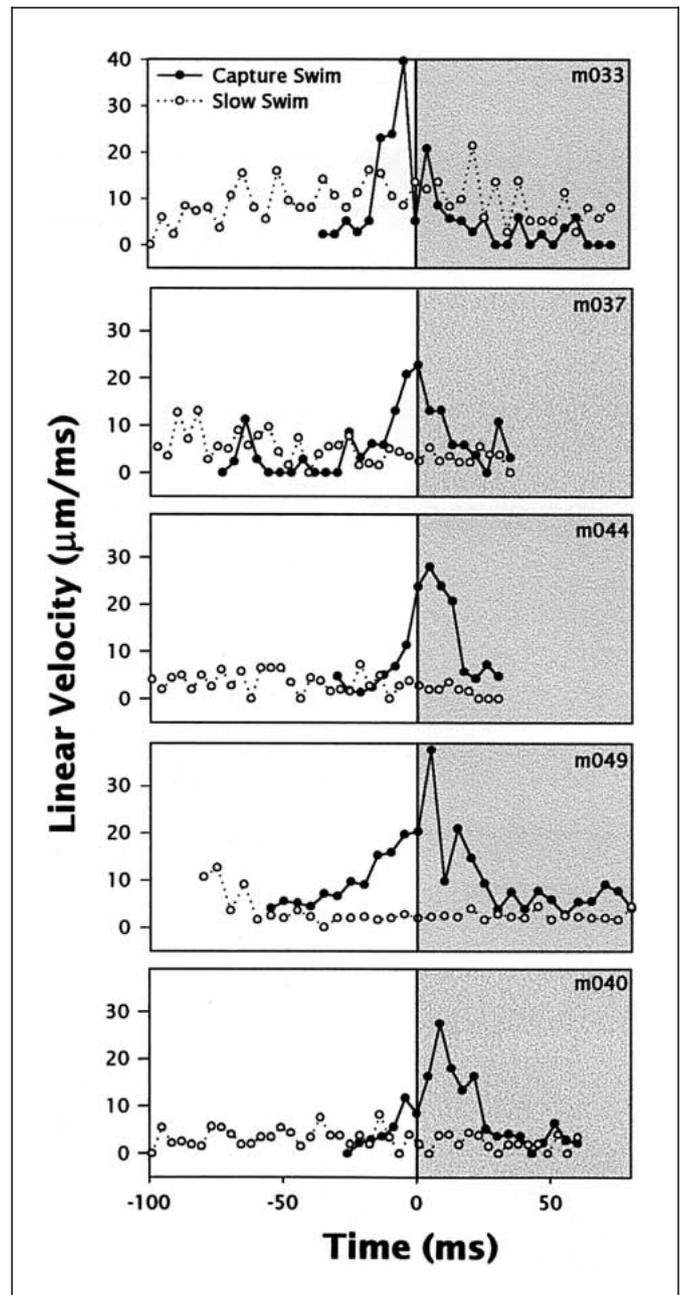


Fig. 8. Propulsive bursts accompany capture swims. Velocity was plotted at 3-frame intervals for both slow and capture swim bouts, with time 0 corresponding to the frame at or just prior to prey capture. A marked spike in velocity accompanied each capture event. This peak can occur immediately prior to or at prey capture (top 2 panels), or just following it (bottom three panels). Velocity increases are likely related to both bend location and iTBF (fig. 5 and 6, respectively). The abrupt increase in velocity propels the larva through the location of the paramecium. In contrast, slow swims are characterized by relatively constant velocities that are much slower than the peak velocities of capture swim bouts.

Timing of Slow vs. Capture Swims

To compare the timing of capture and slow swims, the frequency of bending, or instantaneous Tail Beat Frequency (iTBF), is plotted as a function of bend number (fig. 6), for the same 5 larvae analyzed in figure 5. Bend #0 is again the bend immediately prior to capture (for the capture swims). The iTBF is calculated based on the elapsed time from the preceding (contralateral) bend maximum to the time of the current bend maximum (see Methods). Although 'iTBF' is not a frequency in the sense of a stable ongoing rhythm, it does represent the bend-to-bend frequency of contraction in terms that can be compared to bending rates reported for other patterns of swimming. Slow swims exhibit iTBFs that are fairly constant throughout the swim bout, with most values falling between 20 and 40 Hz. The capture swim does not have a stable rhythm, but instead exhibits an iTBF that varies considerably, as in fish m037 where it ranges from below 25 Hz to over 80 Hz within a single bout. In all larvae there was an increase in iTBF in the bends shortly before the capture event, with most capture swim bouts exhibiting peak iTBFs of at least 60 Hz and some exceeding 80 Hz, well above the range for slow swims and into the range of burst swims [see Budick and O'Malley, 2000a]. The differences in peak iTBF for slow swims (mean \pm SD = 34.0 ± 5.9 Hz) vs. capture swims (mean \pm SD = 75.1 ± 14.2 Hz) was statistically significant ($t = 5.963$, d.f. = 8, $p < 0.001$). The large changes in iTBF over the course of the capture swim bouts suggest the existence of neural circuitry that controls iTBF on a bend-to-bend basis. Overall, the observed changes in bend location, amplitude and timing appear to play a role in orienting and propelling larvae towards the paramecia. Analyses of the movements of the larval zebrafish in the moments leading up to the capture event are consistent with this idea.

Changes in Heading and Velocity Associated with Prey Capture Events

The orientation or heading of the larva (defined by a line from the anterior end of the swim bladder to the mid-point of the snout), was measured over the course of both slow and capture swim bouts (fig. 7). The larva's heading at the time of capture is normalized to 0° ; values at other time points thus illustrate how far away from the paramecium the larva is oriented. Slow swims have also been normalized to 0° at the same time-point in the paired traces. Slow swims showed small, rhythmic changes in heading (i.e., yaw) that corresponded to the larva's undulatory motion during swimming. In contrast, capture swims showed initially stable headings, but often included

one or more larger, abrupt changes in heading both prior to and, in some cases, after the capture event. Somewhat unexpectedly, these abrupt changes in heading, in some instances, transiently oriented the larvae away from its final heading at the time of capture. The paramecia move only a short distance during the final stages of the capture swims (less than $100 \mu\text{m}$, typically), so these abrupt heading changes do not seem to reflect an attempt to track or chase the paramecium. Rather, the heading changes away from the paramecium appear to be a mechanical consequence of the larger amplitude bends that occur just prior to the capture event. Another notable feature of the capture swims is that the initial heading of the larva (i.e., at the beginning of the capture swim) is fairly close to its final orientation at the moment of capture – within about $3\text{--}7^\circ$ for the capture swims shown. This indicates that the capture swim produces only small adjustments in heading, perhaps to fine-tune its orientation relative to the paramecium. We should note that prior to the capture-swim bouts, there are usually several brief bouts of swimming/turning that produce larger changes in heading and might be involved in tracking and coarse alignment of the larvae with their prey [Budick and O'Malley, 2000a]. Such 'tracking' swims are beyond the scope of this report.

Perhaps the most striking aspect of the capture swim is an abrupt acceleration that precedes or accompanies the actual capture of the paramecium (fig. 8). Plots of velocity of the larvae show, in all cases, an increase in velocity shortly before the time of the capture event. In some cases the velocity peaks just prior to or at capture (top 2 panels), whereas in other cases the peak velocity occurs just after the capture event (bottom 3 panels). This variability might reflect differences in the distance between larva and paramecium at the time of the strike. In contrast, slow swims had a relatively constant velocity and none of the slow swims reached the peak velocities of the capture swims. Indeed, most capture swims reached peak velocities that were nearly three times higher than the corresponding slow swim velocity. The difference in peak velocities (mean \pm SD = $31.4 \pm 7.3 \mu\text{m}/\text{ms}$ for capture swims and $12.8 \pm 5.1 \mu\text{m}/\text{ms}$ for slow swims) was statistically significant ($t = 4.622$, d.f. = 8, $p < 0.002$). As great as this difference was, the peak capture-swim velocities were far slower than those of burst swims (less than $40 \mu\text{m}/\text{ms}$ for capture swims vs. $240 \mu\text{m}/\text{ms}$ for the burst swim shown in fig. 2).

Intra-Fish Variability

In the above experiments, where paired slow and capture swims were recorded from a set of 5 larvae, we ana-

lyzed only the first capture swim episode observed. This was done to avoid the possible confound of learning or practice effects that might occur as the larvae fed on additional paramecia. A limitation with this approach is that one cannot determine if the observed variability in the capture swim bouts is due to differences among individual larvae or is an inherent feature of the capture swim sequences themselves (perhaps due to differences in distance and direction to the paramecium). To address this issue, we recorded multiple capture swim bouts from several larvae ($n = 4$) and analyzed them in the same manner as described above for the paired capture swim episodes. A summary of 4 capture swim bouts performed by 1 larva over a 5-hour recording session (fig. 9) reveals that these bouts exhibit a degree of variability similar to that shown in figure 5–8. Specifically, the number of bends constituting the swim, the exact timing of the peak velocity in relationship to the moment of capture and the exact magnitude of the changes in bend location, and iTBF all showed a spread of values that appeared similar to the results from the paired slow/capture swim data set. This variability notwithstanding, these capture swims exhibited the same overall trends as observed in the earlier trials: increases in bend amplitude and iTBF; a rostral shift in bend location prior to capture; increased direction changes about the time of capture, and a marked peak in velocity also occurring about the time of capture. Thus, although the prey capture behavior has several variable elements, key characteristics that seem important for the propulsive burst are conserved. The plots in figure 9 are arranged chronologically and do not show any obvious trend that we might associate with learning or refinement of prey capture.

Other Aspects of Prey Capture

In some instances, purely suction-mediated prey capture may occur when the larva is stationary (fig. 10A), so swimming is not a necessary component of the larval prey capture behavior. A suction component to prey capture is often indicated by an abrupt acceleration of the paramecium into the mouth of the larva. In the five prey capture episodes analyzed in figure 5–8, however, locomotion through the location of the paramecium did occur, so there was a significant ‘ram’ or engulfment component to those capture events. Figure 10B illustrates an example of ram feeding where the paramecium is actually pushed away from the larva before it is captured – note the movement of the paramecium, highlighted by the vertical bar, relative to the fixed horizontal reference mark in frames #1 and #5. Analysis of this capture event results in a ram-

suction index of greater than one, which indicates a ram-dominated strategy, according to the formula of Norton and Brainerd [1993]. In figure 10B the paramecium was barely visible in the still images and so was digitally enhanced, but in figure 10A the paramecium can be directly observed. Instances of both ram and suction feeding were observed during the first recorded feeding episode of individual larvae, although ram feeding was observed more frequently under the conditions of our experiments. A recent report describes fine details of the jaw movements used during prey capture by larval zebrafish [Hernandez, 2000].

There is ongoing movement of the pectoral fins throughout capture swim bouts. Pectoral fins are often used in an alternating pattern early in the swim bout (fig. 11, arrowheads), whereas later in the swim bout, the pectoral fins may be bilaterally extended (arrows) in what appears to be a ‘braking’ maneuver that coincides with a sharp drop in the velocity of the fish (fig. 8). Indeed, some larvae move noticeably backwards in this terminal phase of the behavior, apparently due to forward movement of the extended pectoral fins. A very slight backwards movement is present in the example in figure 11 (compare the larva’s position in the last frame in row 2 vs. the first frame in row 4). Such pectoral fin movements were not evident in the slow swim bouts. It is not obvious why such a braking maneuver occurs during prey capture events, but we expect that the local fluid dynamics accompanying the abrupt acceleration, deceleration and jaw movements will be quite complex [Drost et al., 1988a, b; also see Drucker and Lauder, 2000; Muller et al., 2000]. Other potential complexities of capture swim bouts have not been analyzed, e.g., changes in pitch or roll of the larva that may be occurring. Because the capture events occur in a chamber with a relatively shallow depth of solution, about twice the vertical height of the fish, the behavior occurs largely within a two-dimensional plane. Any changes in pitch or roll that may have occurred in this arena were neither obvious nor dramatic. Extension of the arena to a deeper, more three-dimensional situation would add another degree of freedom and might reveal further complexities of this larval behavior.

In regards to the neural control of prey capture, there has been considerable interest regarding the possible role of the Mauthner cell. The C-bend that occurs during escape behaviors of zebrafish, and many other teleost fishes, is believed to be triggered by firing of the Mauthner cell [Zottoli, 1977; Nissanov et al., 1990]. Such a C-bend is obviously lacking in the capture swims (fig. 1, 4), but because several reports have indicated possible involve-

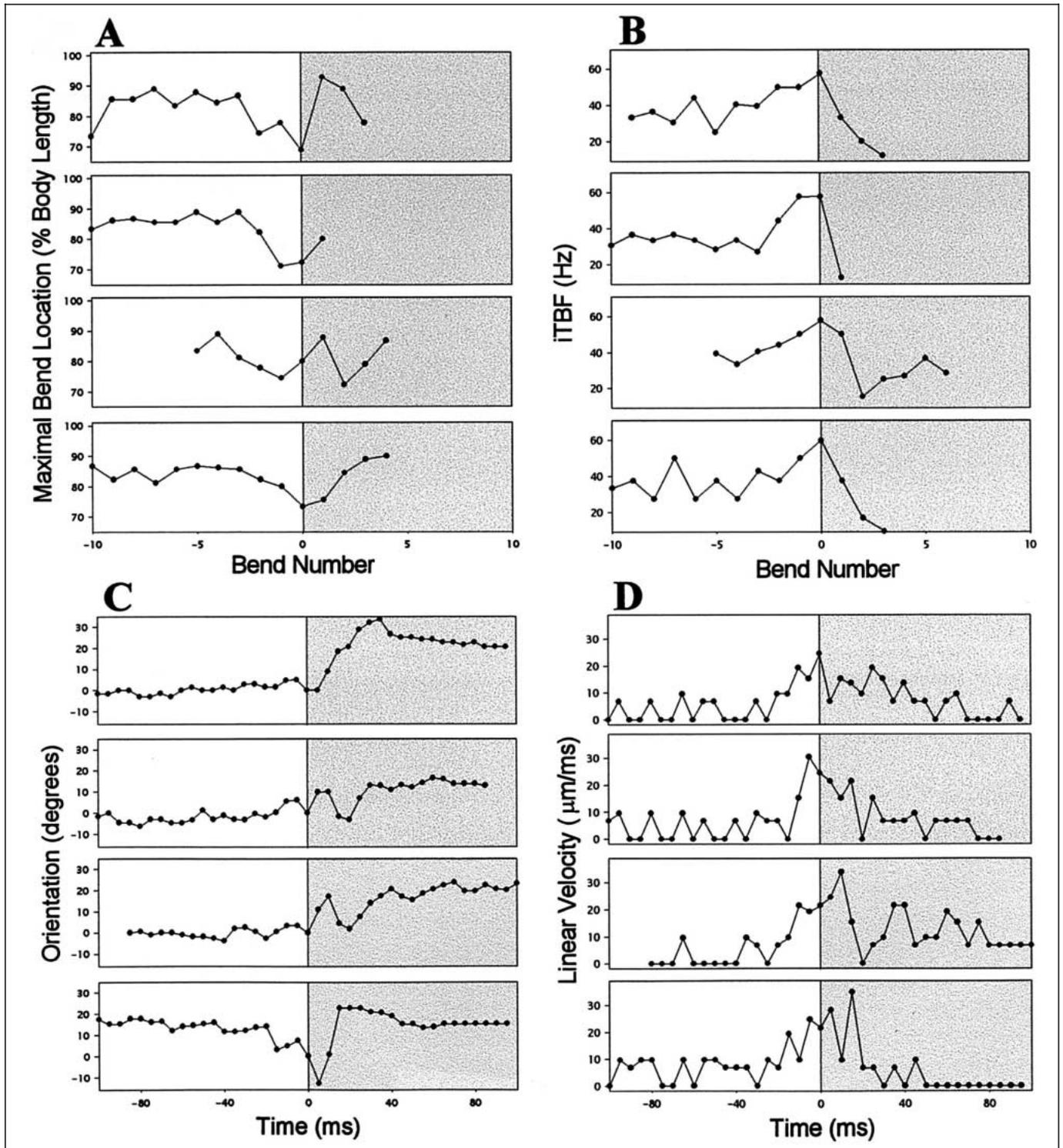


Fig. 9. Intra-fish variability in prey capture. Four consecutive capture swim episodes, exhibited by a single larva and acquired during a 5 hour recording session were analyzed in terms of A bend location, B iTBF, C orientation and D velocity. Each of the capture swim bouts has features similar to the capture swims analyzed in figures 5–8, the most obvious being the increase in iTBF and the spike

in velocity about the time of capture. These core features of the capture swim were conserved, but the bouts differed in terms of the number of bends prior to capture, the absolute magnitudes of the variables measured and the degree to which they changed from bout to bout. This variability appears similar to that observed in figures 5–8.

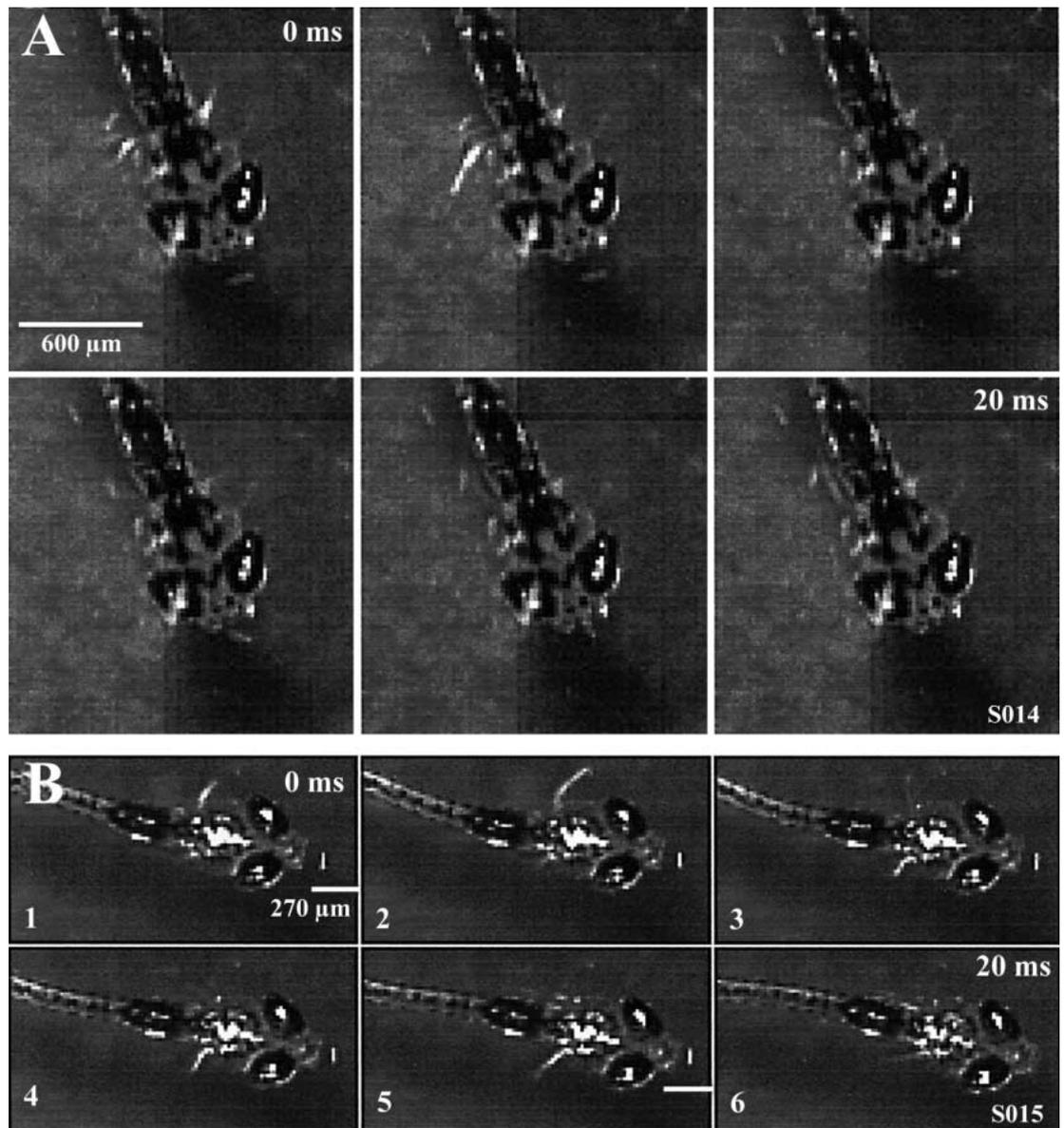


Fig. 10. Examples of suction and ram feeding. A In this example of suction feeding (recorded from a 5-day post-hatching larva, 3.8 mm long), the paramecium abruptly changes direction and accelerates into the mouth of the larva, while the larva is essentially stationary. The paramecium was not digitally highlighted in A. In B, a ram-dominated feeding event is shown where the paramecium (marked by vertical bar) appears to be displaced away from the fish (3.9 mm long) by the entrained water mass just prior to capture. Note its movement relative to the horizontal marker bar in frames #1 and #5 (bar is in a fixed position relative to the dish). A suction component of feeding was occasionally observed during capture swim bouts, so those instances would constitute a combined ram/suction strategy.

ment of the Mauthner cell, we examined prey capture in larvae lacking Mauthner cells. Mauthner cells were retrogradely labeled by injection of fluorescent dextran into far caudal spinal cord (fig. 12A) and killed by sustained exposure to a confocal imaging laser, which results in the disappearance of the cells and the appearance of axonal

stumps (fig. 12B, recorded 6 h after the image in fig. 12A). The axonal stumps are a clear indication of a successful laser-ablation [see Liu and Fetcho, 1999, for further details]. In larvae where there was clear anatomical evidence that both Mauthner cells had been killed ($n = 5$), the prey-capture behaviors (fig. 12C) were similar to

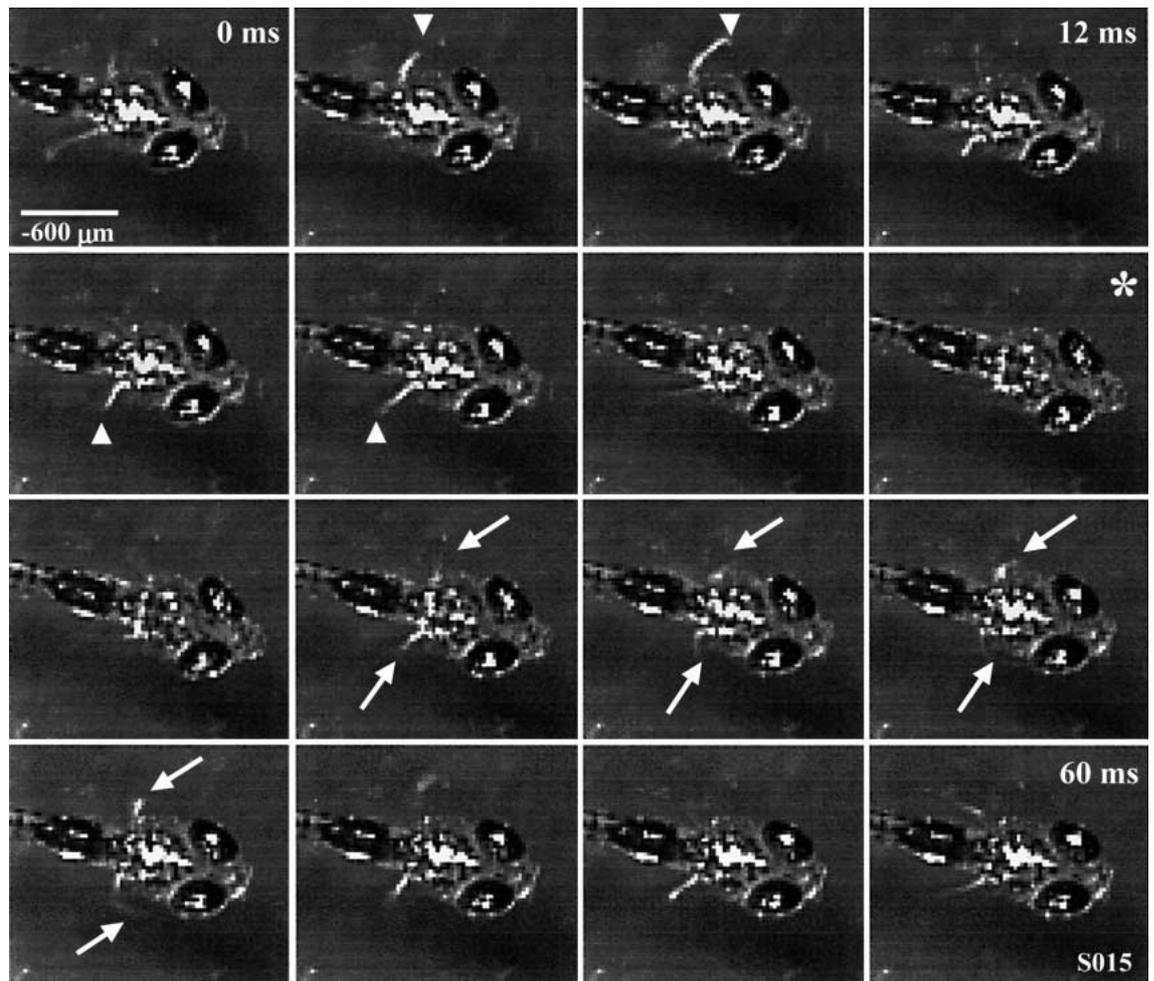
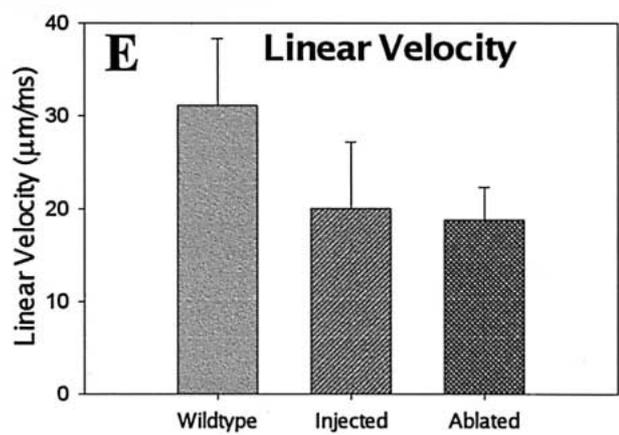
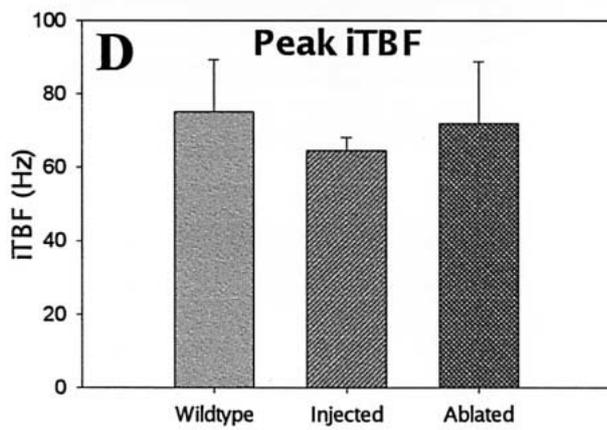
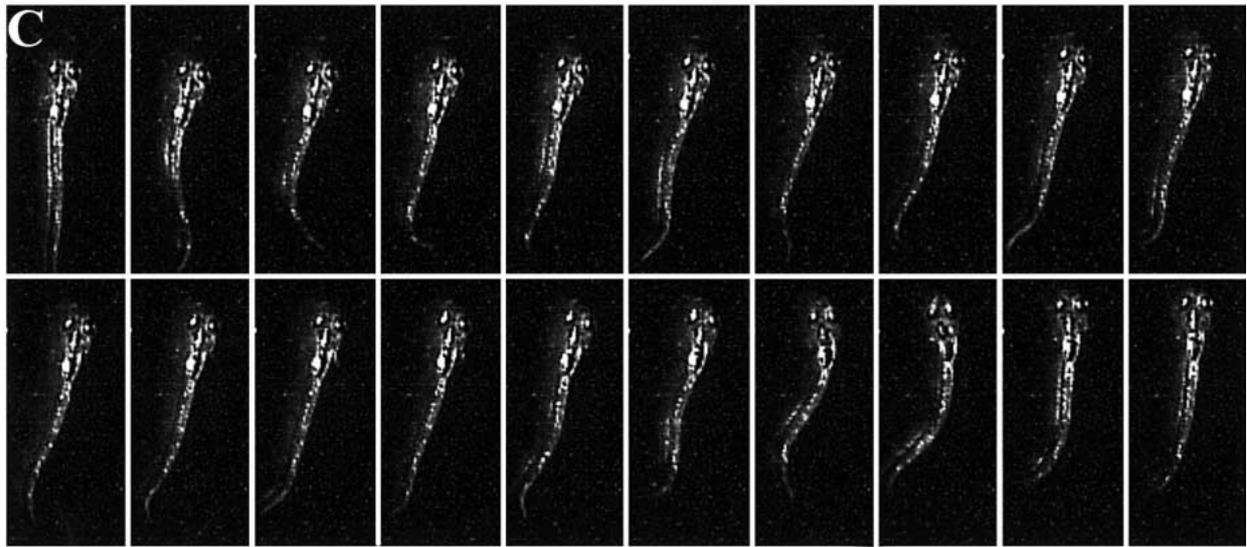
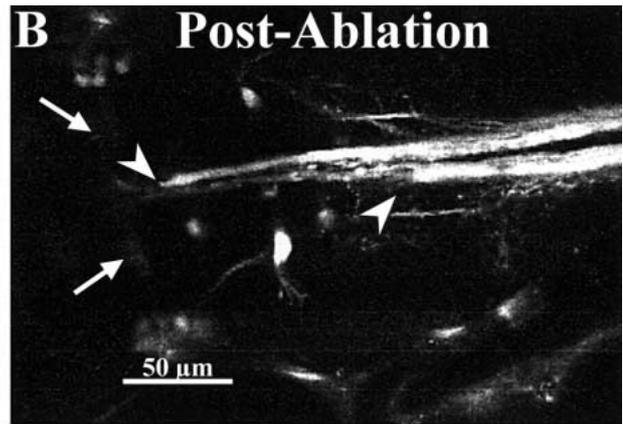
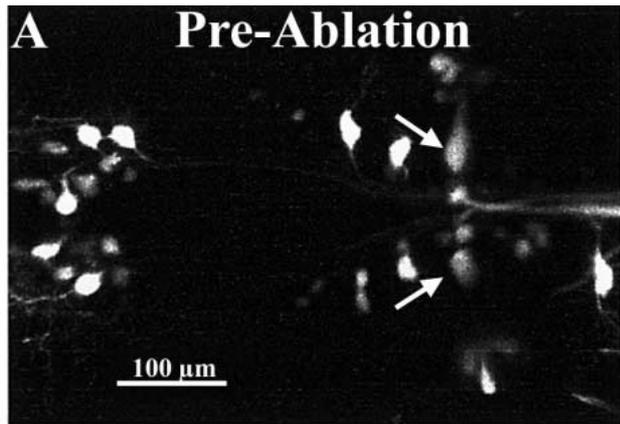


Fig. 11. Pectoral fins are active during prey capture. Early in the capture sequence, the pectoral fins are used in an alternating manner (arrow heads), which might contribute to stabilization or adjustment of orientation. Following prey capture (asterisk), both pectoral fins are extended in parallel (arrows) in what may be a 'braking' maneuver; velocity often declines sharply after prey capture (fig. 8). Because pectoral fins are visible only at higher magnification, they could not be examined simultaneously with the tail movements (larva was 3.9 mm long). These changing patterns of fin usage, however, are frequently observed during capture swims. The paramecium is faint but visible in these still images. Frames were collected at 500 frames/s and every other frame is shown.

those of wild-type larvae and indistinguishable from injected control larvae ($n = 4$) that had labeling of both Mauthner cells, but had not undergone laser-ablation (fig. 12D, E). There was a difference between non-injected and injected larvae, in that peak velocity was decreased in the injected animals (fig. 12E), possibly due to mechanical damage affecting the efficacy of the propulsive mechanism. Killing of the Mauthner cells, however, had no discernible effect on the prey capture behavior, confirming that the Mauthner cells are not necessary for successful prey capture by larval zebrafish.

Discussion

We describe here a *capture swim* used by larval zebrafish to prey upon paramecium. Capture swims were easily distinguished from the most closely related *slow swim* pattern exhibited by these larvae. In comparison to the slow swims, capture swim bouts exhibited significantly greater complexity in terms of bend-to-bend changes in tail-beat frequency, bend amplitude and bend location. These parameters varied in a logical way leading up to the moment of capture, but other aspects of the capture swim



bout were more variable. For example, the total number of bends in each capture swim bout, the exact pattern of the changes in bend location and iTBF, and the pattern of orientation changes were not uniform across capture swim bouts. In our experiments, we made no attempt to control the relative location of predator and prey, but instead allowed the zebrafish larvae to feed in as natural a manner as possible. These variations were not due to between-fish differences, because the recording of multiple capture events from single larva revealed similar variability (fig. 9). One possibility is that variations in the capture swim bouts were due to differences in proximity of the paramecium to the zebrafish, but an alternative idea is that these bouts are just early efforts in a behavior that appears to improve (and therefore change) with practice [Drost, 1987; Coughlin, 1991; Batty and Domenici, 2000]. A rigorous assessment of these issues would require many more trials than is currently practical. Our more immediate interests concern those aspects of the prey capture behavior that were consistent among capture swim bouts and that already seemed effective and important for successful capture of the paramecia. As discussed below, these typical features of the capture swim bout suggest that the descending signals used to control capture swims are considerably more complex than those that control the previously described larval locomotive behaviors.

Fig. 12. Effects of Mauthner cell ablation on prey capture. A Both Mauthner cells (arrows), along with other neurons, were retrogradely labeled with Oregon-green dextran and visualized in vivo using confocal microscopy. The larva was embedded in agar before imaging. B The success of the laser-ablation is shown at higher magnification by the presence of axon stumps (arrowheads) observed 6 h after the laser ablation. The Mauthner cell bodies are now missing (arrows). After laser ablating both Mauthner cells, larvae were released from the agar, allowed to recover overnight, and fed paramecia the following day. C Larvae lacking Mauthner cells exhibited normal locomotive patterns during prey capture. Frames were collected at 600 frames/s and every 5th frame is shown. D, E Quantification showed that the Mauthner-less fish were indistinguishable from injected-embedded controls, in terms of tail-beat frequency and peak linear velocity. The only difference noted was that strike velocities of the injected larvae did not reach the peak values of non-injected controls, but this effect was independent of whether or not the Mauthner cells were laser ablated. Both the injected controls and the Mauthner-ablated fish showed a clear, albeit smaller, velocity peaks and were able to successfully capture prey. Larva shown in C was 3.7 mm in total length.

Existing Data on the Neural Control of Prey-Capture Locomotion

The neural control of prey capture locomotion is largely unknown. A possible source of gustatory input to the reticulospinal system has been described in the channel catfish, *Ictalurus punctatus* [Kanwal and Finger, 1997]. Most other available data concerns possible overlap between escape and prey capture circuitry [reviewed in Batty and Domenici, 2000], although the evidence for escape circuitry playing a major role in prey capture locomotion is not compelling. In the case of S-starts, the similarities between escape S-starts and predation S-starts might suggest shared underlying neural controls for escapes and prey capture locomotion [Harper and Blake, 1991; Domenici and Blake, 1997]. The Mauthner cell, a hindbrain neuron known to initiate C-start type escape behaviors [Zottoli, 1977; Kimmel et al., 1980; Eaton et al., 1981; reviewed in Faber et al., 1989; Zottoli and Faber, 2000; Eaton et al., 2001], appears in the case of the goldfish (*Carassius auratus*) to be involved with prey-capture events [Canfield and Rose, 1993, 1996]. In this instance, the Mauthner cell does not contribute to the capture-event proper, but instead appears to drive a post-strike maneuver that likely reduces risks associated with prey capture. Casual observation of adult zebrafish also suggests the possible occurrence of a Mauthner cell initiated C-bend after a feeding event. A more direct role of a C-bend (possibly Mauthner-cell initiated) in prey capture has been reported in larval clownfish, where a C-bend appears to precede capture of the prey and may serve to propel the larva through the prey location [Coughlin, 1994]. A role for the Mauthner cell in prey capture behaviors has also been suggested by other authors [Zottoli et al., 1992; discussed in Domenici and Blake, 1997]. This issue had not previously been evaluated for the feeding behavior of larval zebrafish.

In the case of the larval zebrafish, our observations show that the Mauthner cell is not necessary for successful prey capture (at least in a laboratory setting), because bilateral laser ablation of the Mauthner cell had no apparent effect on their ability to capture prey (fig. 12). The presence of axonal stumps makes it fairly certain that the Mauthner cells have been killed. Liu and Fetcho [1999] reported a marked increase in escape latency after using this kind of laser ablation to kill just the Mauthner cell, demonstrating that such ablations are able to reveal instances where small numbers of cells make a substantial contribution to a behavior. If the Mauthner cell were making a similarly large contribution to the capture swim, which one might expect given its substantial output to primary motoneurons [Celio et al., 1979; Fetcho and Faber,

1988; Fetcho and O'Malley, 1995], then it should have been detected in our laser ablation experiments. We should also note that even the largest capture swim bends that we observed are much smaller (in terms of bend angle) than the escape C-bends that are produced by adult goldfish after firing of just a single action potential in the Mauthner cell [Nissanov et al., 1990]. Such data are not available for larval zebrafish, but given the neuroanatomical conservation of descending control systems in larval and adult zebrafish and goldfish [Metcalf et al., 1986; Fetcho and Faber, 1988; Fetcho, 1992; Lee and Eaton, 1991; Lee et al., 1993; Eaton et al., 2001], the simplest explanation for these collective data is that the Mauthner cell does not fire during larval prey capture. This said, our results do not eliminate the possibility of the Mauthner cell playing a significant role in larval prey capture; such a contribution might be masked by either redundant neural circuits or by functional reorganization after the laser-ablation. Of greater import to the neural controls underlying prey capture are the details of the kinematic measurements.

Observed Kinematics and Their Implications for Underlying Neural Controls

To what extent can behavioral observations provide insight into underlying neural controls? Comparing multiple behaviors helps to answer this question. In the larval sea lamprey (*Petromyzon marinus*), kinematic analysis of swimming and burrowing suggests reconfiguration of spinal networks during behavioral switching [Paggett et al., 1998]. Our previous study of different turning and swimming behaviors reported significant differences in tail-beat frequencies, bend amplitudes and bend locations [Budick and O'Malley, 2000a]. It seemed likely from those data that there must be significant differences in the output of spinal circuitry (e.g., rostral-caudal locus of excitation, CPG oscillation frequency) in order to account for the different behaviors. In regards to capture swims, simple visual inspection reveals that they are far different from escape C-bends [see e.g., Liu and Fetcho, 1999; Budick and O'Malley, 2000a]. Capture swims are also easily distinguished from burst swims in that they exhibit little of the yaw (side-to-side bending of the head) that is so pronounced during burst swimming (fig. 1, 2, 7), and their peak velocity is more than 5-fold slower (fig. 8). In addition, capture swims show more caudal bend locations, which are distinct from the mid-body bend location of burst swims.

Capture swims are most similar to slow swims and a first impression might be that their neural controls were

similar. Detailed inspection revealed, however, significant differences. Bend location, for example, tends to be far caudal at the outset of a capture swim bout and shift rostrally prior to prey capture (fig. 5). This is not seen in other types of swims. One interpretation is that localized bending reflects relatively localized firing of spinal motoneurons. If so, the rostral shift in bend location indicates that the locus of excitation can be shifted along the rostral-caudal axis. Tail-beat frequency also changes before the prey capture event (fig. 6). Initially, these frequencies fall within the slow-swim TBF range, but they accelerate into the highest range of TBFs observed in larval zebrafish, which were previously observed only during burst swims [Budick and O'Malley, 2000a]. Because these changes in bend parameters accompany successful prey capture events, a natural explanation is that they are controlled by descending signals from the brainstem. The behavioral relevance of these changes is indicated by the abrupt increase in velocity. Velocities are initially quite low, overlapping with slow swim velocities recorded from the same larva, but they show a marked peak about the time of the capture event (fig. 8). This coincidence with successful prey capture indicates that the bend-to-bend changes in timing and locus of bending that precede the strike are purposeful in nature.

The pattern of bends exhibited during prey capture suggests the presence of a complex set of descending control signals. It is useful to first consider what neural controls might be required to produce slow swims. The slow swim may be the result of transient activation of rostral spinal CPGs by a trigger signal from brainstem. Such a trigger signal could, in principle, initiate a series of rostral-to-caudal traveling waves of bending, much as a single touch does in the case of the *Xenopus* tadpole [Roberts et al., 1998]. Such a series of bends would be maintained by the rhythmic activity of spinal CPGs, with such activity (in the case of the zebrafish larvae) winding down over successive cycles of CPG oscillation/swimming, possibly due to inherent properties of the spinal circuitry. That a temporally and spatially 'simple' signal might produce swimming is indicated by the observation that bath application of NMDA, to the isolated spinal cord, is able to evoke rhythmic fictive locomotion. In the case of capture swims, we suggest that dynamic descending control signals are required to account for the ongoing changes in bend amplitude, location and iTBF. An alternative view is that a global (i.e., whole-cord) increase in excitation might change bend location, amplitude and frequency simultaneously. For example, a global increase in contraction strength might bend more of the body, thereby shift-

ing the bend rostrally. During capture swims, however, the very caudal tip of the tail (fig. 1) can be bent at a more extreme angle than occurs during the slow swim (fig. 3); and during tracking turns these larvae can achieve a 'J' shape where the tip of the tail is bent greater than 90° [Borla and O'Malley, unpublished observations]. These data indicate that bend location can be varied independently of other parameters. This is also true for bend amplitude because there can be large increases in bend amplitude, with only a slight shift in bend location, as seen by comparing figure 4C with figure 4D. This relatively local increase in bend amplitude is in contrast to the whole-body bending seen during burst swims (fig. 2). A parsimonious view is that the neural controls for slow swims are a small subset of the descending controls that orchestrate capture swims.

Another notable feature is the yaw during capture swims, or more precisely, the lack of yaw. During high-speed burst swimming episodes, large yaw movements occur on each cycle of bending, ranging from 14 to 28° (fig. 2). During slow swims, yaw is much reduced, but can still be seen to oscillate in time with the tail-beat frequency (fig. 7). The yaw during capture swims is distinct from both in that the yaw is usually quite minimal even during abrupt accelerations that substantially exceed that occurring during slow swim bouts (e.g., larvae m037 and m049; fig. 7, 8). From a neural and motor control perspective, the 'simpler' pattern of a constant heading during an abrupt acceleration may actually be quite complex because compensatory adjustments may be required to stabilize head orientation. In other capture swim bouts yaw increased transiently about the time of prey capture. These orientation changes might be a byproduct of the bends used to generate thrust because these changes often orient the larvae away from the paramecium (m033, m040 in fig. 7). In the instances where there is similar acceleration but less direction change, it may be that yaw is being actively minimized by such muscular activities as stiffening and/or pectoral fin movements. Stabilization of orientation while producing maximal thrust was suggested as an important aspect of the feeding S-starts of adult Northern Pike [Harper and Blake, 1991]. More direct evidence for minimization of yaw has been reported in a study of the probing behavior of the electric fish *Eigenmannia virescens*. When *Eigenmannia* is electrically 'examining' an object, the rostral half of the fish is held straight while the tail rhythmically bends towards an object so as to electrically probe the location and other features of the object. Lesions of the cerebellum abolish their ability to perform this 'simple' kinematic behavior,

and the entire fish bends back and forth when it attempts to probe [Behrend, 1984]. This increase from essentially zero yaw to a large and maladaptive yaw suggests that, in *Eigenmannia*, the cerebellum is able to orchestrate active suppression of yaw.

Implications for Models of Undulatory Swimming

A partial basis for the observed locomotive complexity occurs at the level of the spinal cord, where distinct neural controls exist for red and white muscle which are used for slow and fast swimming, respectively [Westerfield et al., 1986; Liu and Westerfield, 1988; De Graaf et al., 1990; Roberts and Mos, 1992; Jayne and Lauder, 1994; Gillis, 1998]. In zebrafish, there are distinct classes of spinal interneurons [Bernhardt et al., 1990; Hale et al., 2001] and some appear dedicated to specific locomotive behaviors [Ritter et al., 2001]. Descending neurons from brainstem might tap into these different spinal elements to produce different speeds and patterns of swimming. Our observations suggest another level of complexity by indicating that these descending neurons drive spinal circuitry with spatial and temporal precision along the rostral-caudal axis of the spinal cord. The anatomical substrate for this control is likely in the brainstem where a total of about 300 neurons project from brainstem into the spinal cord [Kimmel et al., 1985; Metcalfe et al., 1986; Gahtan and O'Malley, 2001]. Many of these descending neurons traverse nearly the full length of the spinal cord, giving off frequent axon collaterals along the length of their trajectory [Gahtan and O'Malley, unpublished observations]. Determining the synaptic targets of these descending neurons and further characterizing the spinal interneurons would be useful steps toward the development of a realistic model of how the pattern of bends exhibited during prey capture is generated.

Among the most detailed neural models of aquatic locomotion are cellular-level models of *Xenopus* tadpole spinal cord that describe the generation of the alternating left-right contractions used during undulatory swimming [Roberts and Tunstall, 1990; Dale, 1995; Roberts et al., 1998]. Undulatory swimming also involves a rostral-caudal propagation of neural activity and muscular contraction; this has been modeled in lamprey using chains of coupled segmental oscillators [see e.g., Skinner and Molloney, 1998; McClellan and Hagevik, 1999; Miller and Sigvardt, 2000]. Fewer models have addressed turning behaviors. Foreman and Eaton [1993] proposed an organization of the Mauthner array that could account for directional control of the escape behavior [also see Eaton et al., 1982, 1991, 2001; Meyers et al., 1998]. This model

subsequently received substantial experimental support [O'Malley et al., 1996; Liu and Fetcho, 1999; Zottoli et al., 1999]. 'Turning while swimming' has been examined in the lamprey, where McClellan and Hagevik [1997] propose three different mechanisms by which the brainstem might control spinal oscillators [also see Svoboda and Fetcho, 1996]. Any model to explain the axial kinematics during prey capture would need to add considerably to these existing models because it would need to provide temporally precise inputs to specific locations along the rostral-caudal axis and would need to change these inputs on a bend-to-bend basis to produce the observed bend patterns and strike trajectories.

Our current state of knowledge of brainstem control systems indicates that any realistic model of descending motor control will be quite complex, possibly involving reorganizing or distributed architectures [discussed in Morton and Chiel, 1994] and probably involving cooperative actions of large numbers of neurons. Studies in lamprey have revealed widespread processing of vestibular signals in reticulospinal neurons [Deliagina et al., 1992; Orlovsky et al., 1992], and considerable heterogeneity to these neurons in terms of their pattern of spinal outputs [Zelenin et al., 2001]. Recent studies of trout and zebrafish brainstem indicate a widespread distribution of neurons involved in escape behaviors [Bosch et al., 2001; Gahtan et al., 2002]. Other evidence suggests that these descending inputs are highly redundant or distributed and therefore not easily disrupted [Budick and O'Malley, 2000b; Gahtan and O'Malley, 2001], escape latency being a notable exception to this idea [Liu and Fetcho, 1999]. Our behavioral analyses might be viewed as complementary to these prior anatomical and physiological studies. Acquiring a detailed description of the locomotive behaviors that the CNS generates and controls seems an essential step in the development of a comprehensive description of descending locomotor control systems.

Evolutionary Aspects of Fine Axial Motor Control

To what extent is fine axial motor control present amongst the fishes? High-speed imaging of the bending parameters analyzed here has not been reported for other fishes, but the available data suggest that this capability may be widespread. The most detailed axial kinematic studies have focused on adult fishes that use S-starts to strike at prey [Webb and Skadsen, 1980; Rand and Lauder, 1981]. Although there are several variations of S-starts [Harper and Blake, 1991], the essence of the behavior is the bending of the trunk into an S-shape, which is held until the fish abruptly strikes at the prey. Maintain-

ing and abruptly releasing the S-position would require descending control capabilities distinct from those exhibited by zebrafish larvae during capture swims, but in terms of overall complexity, the S-start controls might approach or exceed those used during prey capture. Another behavior, the C-start escape, might also involve fine axial motor control in that precise control of the relative timing of C-bend and counter-turn, and perhaps the rostral-caudal extent of muscular activation, may be occurring. This is suggested by the ability of the goldfish to escape to any compass direction within its plane of swimming, exhibiting varying amplitudes of initial C-bend and counter-turn [Foreman and Eaton, 1993; Eaton et al., 2001]. C-start and S-start escape and prey capture behaviors have been documented in a number of other fishes [see e.g., Domenici and Blake, 1997; Meyers et al., 1998; Hale, 2002], so although the degree of fine descending control is not well characterized, it is likely present amongst many species of fish.

Many details of the neural basis of these diverse, complex behaviors are unknown, but a common thread is that they all rely on spinal circuitry. At the level of the spinal cord there may be significant conservation of form and function, based on similarities amongst spinal interneuron anatomy, and similarities in CPG functioning, as reported for a variety of fishes and larval amphibians [Fetcho, 1992; Grillner et al., 1998; Roberts et al., 1998]. A spinal system that is highly adaptable to different situations and environments, i.e., one that can be effectively and purposefully modulated by descending signals, would likely be beneficial to many fishes. Such a system is present in the larval zebrafish. This system requires only a limited set of spinal interneuron and motoneuron types [Westerfield et al., 1986; Bernhardt et al., 1990; Hale et al., 2001] and is modest in terms of total numbers of neurons per segment, yet it is capable of transducing a variety of descending signals and producing a complex repertoire of adaptive locomotive behaviors. It is thus tempting to speculate that the larva's spinal system constitutes a basic plan that is highly conserved amongst fishes. If spinal circuitry is highly conserved, the question then arises as to where and how the diversity of piscine locomotive behaviors arises.

The prey capture strategies and behaviors of larval fishes are also diverse and include the beat-and-glide swimming pattern used by herring larvae [Batty et al., 1990, 1991] and the apparent use of a C-bend during strikes by clownfish [Coughlin et al., 1992]. A key question is to what extent these behaviors (and the fine motor control behaviors discussed above) reflect distinct evolu-

tionary adaptations of the nervous system, as opposed to inherent flexibility in the locomotor control circuits in the brain and spinal cord. Another way to pose this question is to ask what would happen if we took the descending and spinal motor-control systems of the larval zebrafish (for example) and placed it in the body of the lamprey. One would predict that this 'zebrafish-lamprey' hybrid would initially have difficulties, but to the extent that the CNS is capable of functionally reorganizing, we might see that the larval zebrafish CNS is able to produce respectable lamprey behaviors (i.e., it is able to generate patterns of muscular activation that are suitable for the lamprey's body and ecological needs). This *gedanken* experiment is, of course, not technically feasible, but to the extent that the CNS of the zebrafish could generate lamprey-like behaviors it would suggest that (1) the descending and spinal control systems are highly conserved and (2) the behavior-

al variations observed are not dependent on hard-wired evolutionary changes in these systems. There is evidence for neuroanatomical conservation of the descending control systems in closely related fishes such as zebrafish and goldfish [Lee and Eaton, 1991; Lee et al., 1993; Suwa et al., 1996], and, to varying degrees, in less closely related fishes [Rovainen, 1967; Timerick et al., 1992; New et al., 1998; Bosch and Roberts, 2001], but a great deal more behavioral, anatomical and physiological data would be needed to evaluate the conservation of these control systems amongst the many species of extant fishes.

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