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Prey Tracking by Larval Zebrafish: Axial Kinematics and Visual Control

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Key Words

Zebrafish · Prey capture · Tracking · Vision · Behavior · Teleost · Kinematic · Locomotion · Spinal cord · Brainstem

Abstract

High-speed imaging was used to record the prey-tracking behavior of larval zebrafish as they fed upon paramecium. Prey tracking is comprised of a variable set of discrete locomotor movements that together align the larva with the paramecium and bring it into close proximity, usually within one body length. These tracking behaviors are followed by a brief capture swim bout that was previously described [Borla et al., 2002]. Tracking movements were classified as either swimming or turning bouts. The swimming bouts were similar to a previously characterized larval slow swim [Budick and O'Malley, 2000], but the turning movements consisted of unique J-shaped bends which appear to minimize forward hydrodynamic disturbance when approaching the paramecium. Such J-turn tracking bouts consisted of multiple unilateral contractions to one side of the body. J-turns slowly and moderately alter the orientation of the larva – this is in contrast to previously described escape and routine turns. Tracking behaviors appear to be entirely visually guided. Infra-red (IR) imaging of locomotor behaviors in a dark environment revealed a complete absence of tracking behaviors, even though the normal repertoire of other locomotive behaviors was recorded. Concomitantly, such larvae were greatly impaired in consuming paramecia. The tracking behavior is of interest because it indicates the presence of sophisticated locomotor control circuitry in this relatively simple model organism. Such locomotor strategies may be conserved and elaborated upon by other larval and adult fishes.

Introduction

The neural control of locomotion has been examined in non-mammalian vertebrates with the hope of revealing organizational features and principles that might be difficult to discern in mammals [see e.g., Stein et al., 1997; Bass and Baker, 1997; Kiehn et al., 1998]. Studies of the escape behavior of the goldfish [Faber et al., 1989], and the swimming behaviors of *Xenopus* tadpoles and lamprey [Roberts et al., 1998; Grillner, 2003], offer prominent examples of the value of such model organisms. However, even these 'simpler' vertebrate animals confront researchers with daunting challenges in terms of both neural coding and neuroanatomical complexity [see e.g., Buchanan, 1999, 2001; Zelenin et al., 2000, 2001]. For these reasons, a model vertebrate organism of yet greater 'simplicity' is desirable. The larval zebrafish has been established as such an organism, having many individually identifiable neurons in both brainstem and spinal cord [Kimmel et al., 1982, 1985; Westerfield et al., 1986; Bernhardt et al., 1990; Hale et al., 2001; and see Lee and Eaton, 1991; Lee et al., 1993]. Zebrafish are popular for studies of neural development [Driever et al., 1996; Granato et al., 1996; Eisen, 1999; Drapeau et al.,

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2001] and its optically accessible CNS offers many opportunities for the in vivo study of neural control systems [Fetcho and O'Malley, 1995; O'Malley et al., 1996; Fetcho et al., 1998; Liu and Fetcho, 1999; Ritter et al., 2001; Gahtan and O'Malley, 2001, 2003; Gahtan et al., 2002; Roeser and Baier, 2003; Gahtan and Baier, 2004; Masino and Fetcho, 2005].

In larval zebrafish, locomotor behaviors are controlled by the approximately 300 neurons that descend from the brainstem into spinal cord [O'Malley et al., 2003]. In an effort to better understand the neural control systems underlying axial locomotor movements, the larva's locomotive repertoire had previously been surveyed [Budick and O'Malley, 2000]. This initial study categorized larval turning and swimming behaviors into four distinct types. Subsequently we described a capture swim bout, a brief swim bout that concludes a series of maneuvers used in capturing paramecium. Kinematic analysis of the capture swim showed that zebrafish larvae are able to dynamically modulate bend location, amplitude and frequency, a capability referred to as fine axial motor control [Borla et al., 2002]. However, the series of locomotor movements leading up to the capture swim had not been described in any detail.

The locomotor aspects of prey capture are variable amongst fish species. Herring larvae *(Clupea harengus)* exhibit slower swim patterns in the presence of a prey patch [Munk and Kiorboe, 1985], whereas larval clownfish *(Amphiprion perideraion)* increase the speed of swimming when entering a concentrated prey area [Coughlin et al., 1992]. The larval clownfish were observed to increase both the number of turns and the turn angles which allowed them to stay longer within a small patch of prey. The cottid fish *(Clinocottus analis)* approaches its prey to within 0.24 body lengths and then pauses before initiating the final attack [Cook, 1996]. Although these studies have provided important information about the evolutionary diversity of larval prey capture, there are few details available concerning the precise axial kinematics that underlie prey tracking in these species. Most high-speed recordings of both larval and adult prey-capture behaviors have focused on jaw movements [Lauder, 1980; Gibb, 1997; Wainwright and Shaw, 1999; Hernandez, 2000; Ferry-Graham and Lauder, 2001], although sometimes in conjunction with axial locomotive maneuvers [Rand and Lauder, 1981; Drost and van den Boogaart, 1986; Drost, 1987]. Studies of the axial kinematics used in adult prey-capture show use of a held S-bend from which the fish darts forward to capture prey [Harper and Blake, 1991], but there are no reports of precise sequences of distinct locomotive maneuvers, such as those that will be described here.

The means by which the prey capture sequence is initiated and guided are also quite variable. In visually poor environments, electroreception can be used, perhaps exclusively in the case of paddlefish, to detect and capture zooplankton [Wilkens et al., 2002]; this sensory information is sufficient to guide complex and agile kinematic maneuvers. The lateral line can also play a central role in prey capture as when rainbow trout integrate information from both superficial neuromasts and subdermal lateral line canals to capture prey in the dark [Montgomery et al., 2003]. Distinct strike kinematics can arise out of morphological necessity even if the same sensory modality is used, as has been demonstrated for sphyrnid and carcharinid sharks that use electroreception to detect and approach prey [Kajiura and Holland, 2002]. The integration of multiple sensory modalities has also been reported, as in the case of the black ghost knifefish that utilizes electrosensory and mechanosensory information in a highly dynamic fashion during the capture of insect larvae [Nelson et al., 2002; Coombs et al., 2002]. In addition to direct detection of prey, these systems can be used to follow or track prey, utilizing, for example, hydrodynamic fish trails possibly in conjunction with chemical cues [Montgomery et al., 2002], which are also important in prey recognition [Finger, 1997, 2000; Kanwal and Finger, 1997].

Vision can, of course, be a particularly powerful and effective tool as highlighted by the sandlance, which possesses novel visual capabilities and was observed to strike successfully at live prey on two-thousand successive attempts recorded with a high-speed camera [Pettigrew et al., 2000].

In many teleost species, vision appears to be the most important sensory modality for prey capture, allowing tracking and capture of small and large prey alike [Hairston et al., 1982]. Herring larvae alter their prey capture behavior depending on the light intensity: they filter-feed in total darkness but rely on biting at higher light intensities [Batty et al., 1990]. There is evidence that many teleost species require light to feed [Blaxter, 1968; Job and Bellwood, 1996; Pettigrew et al., 2000; Downing and Litvak, 2001; Job and Shand, 2001; Neuhauss, 2003]. Visual fixation prior to prey capture has been suggested in Atlantic salmon alevins [Coughlin, 1991] as well as larval carp [Drost and van den Boogaart, 1986] and visual information might be integrated with other sensory modalities during different phases of a prey capture sequence [New et al., 2001].

Because the role of vision in larval zebrafish feeding had not been systematically described, we evaluated the role of light in several aspects of feeding. We report that vision is necessary for robust consumption of paramecium and for the performance of locomotive maneuvers unique to prey tracking and prey capture. In this context we describe a novel turning behavior, called a J-turn, which is integral to the prey tracking behavior and helps larvae approach and align themselves with their prey. A preliminary version of some of this work had been reported previously [Borla and O'Malley, 2002].

Materials and Methods

Animals

Fertilized eggs were collected from a laboratory stock of zebrafish *(Danio rerio)* and maintained in 10% Hanks solution at a temperature of approximately 25°C, in a 14–10 h light/dark cycle [Westerfield, 1995; O'Malley and Fetcho, 2000]. Unfed larvae at ages 6–8 days post-fertilization (dpf) were used throughout this study. All protocols were carried out in accordance with the guidelines of the National Institutes of Health and were approved by the Northeastern IACUC Committee.

Experimental Protocol

The high-speed imaging protocol was described previously [Borla et al., 2002]. Briefly, larvae were transferred to a small dish (approximately 1.3 cm in diameter) containing 10% Hanks solution. To observe feeding, a solution of paramecia (either *Paramecium caudatum*, or *Paramecium multimicronucleatum*) was pipetted into the recording dish. An MD4256 high-speed digital camera (EG&G Reticon, Sunnyvale, Calif., USA) mounted on a Zeiss dissecting microscope was used to record prey capture behaviors as well as spontaneous swimming and turning behaviors (which were recorded in the absence of paramecia). Recordings were made at frame rates between 500 and 800 frames/s and all behavioral observations were saved to disk. Frame-by-frame playback of the sequences was used to classify each behavior and to select frames for quantitative analyses.

Image Analysis: Quantification of Tracking, Swimming and Turning Behaviors

Quantification of most kinematic variables analyzed was previously described in detail in Borla et al. [2002] and Budick and O'Malley [2000]. They are summarized here along with additional analyses specific to tracking behavior elements, such as the turning maneuver that we refer to as a 'J-turn'. J-turns were recognized and so classified based on the following criteria: repetitive (at least 2), unilateral J-shaped bends with caudal bend locations, and an initial bend amplitude that exceeds 90 degrees, thus forming the trunk and tail into a 'J' shape. In addition to analyzing the prey tracking behaviors, we compared those movements with routine swimming and turning behaviors made in the absence of prey. The kinematic variables analyzed included:

Number of tracking behaviors preceding prey capture (n = 17). The numbers of slow swim and turning movements that took place during tracking of the paramecium were manually counted. The high-speed camera maintains in its frame buffer a rolling set of the 2000 most recently acquired frames, which equates to about 3 to 4 s worth of data at the image acquisition rates being used. When an apparent capture event is observed, the experimenter halts further image acquisition and then examines the previous 2000 frames in the frame buffer. Because of the 2000 frame limit, the earliest locomotor movements in a particular capture episode might not have been recorded in some very rare instances. But in the great majority of cases there was a long quiescent period preceding the initiation of movement towards a paramecium and so we are confident that the entire tracking behavior was recorded.

Orientation and Angular Deviation. The orientation or heading of the larva (n = 19) was obtained by drawing a line from the anterior end of the swim bladder through the midpoint of the rostral end of the larva. The angle between the larva's orientation and the direction to the paramecium (also drawn from the anterior end of the swim bladder) was then measured using Image J, a PC-compatible version of NIH Image.

Distance to prey (n = 19). The approach of the larva towards the paramecium was determined by measuring, at successive time points, the distance from the paramecium to a point on the larva located in the midline of the head, just between the eyes. This is a relative distance because the paramecium is also moving, but it mainly reflects the distance traveled by the zebrafish because its movement during these time epochs is much greater than that of the paramecium.

Bend Amplitude. A line was drawn through the midline of the larva and tangent lines were drawn at the most rostral and most caudal portions of this curve. The angle between these lines, at the timepoint of maximal bending (see next section) is called the maximal bend angle. Subtracting this angle from 180 degrees yields the bend amplitude.

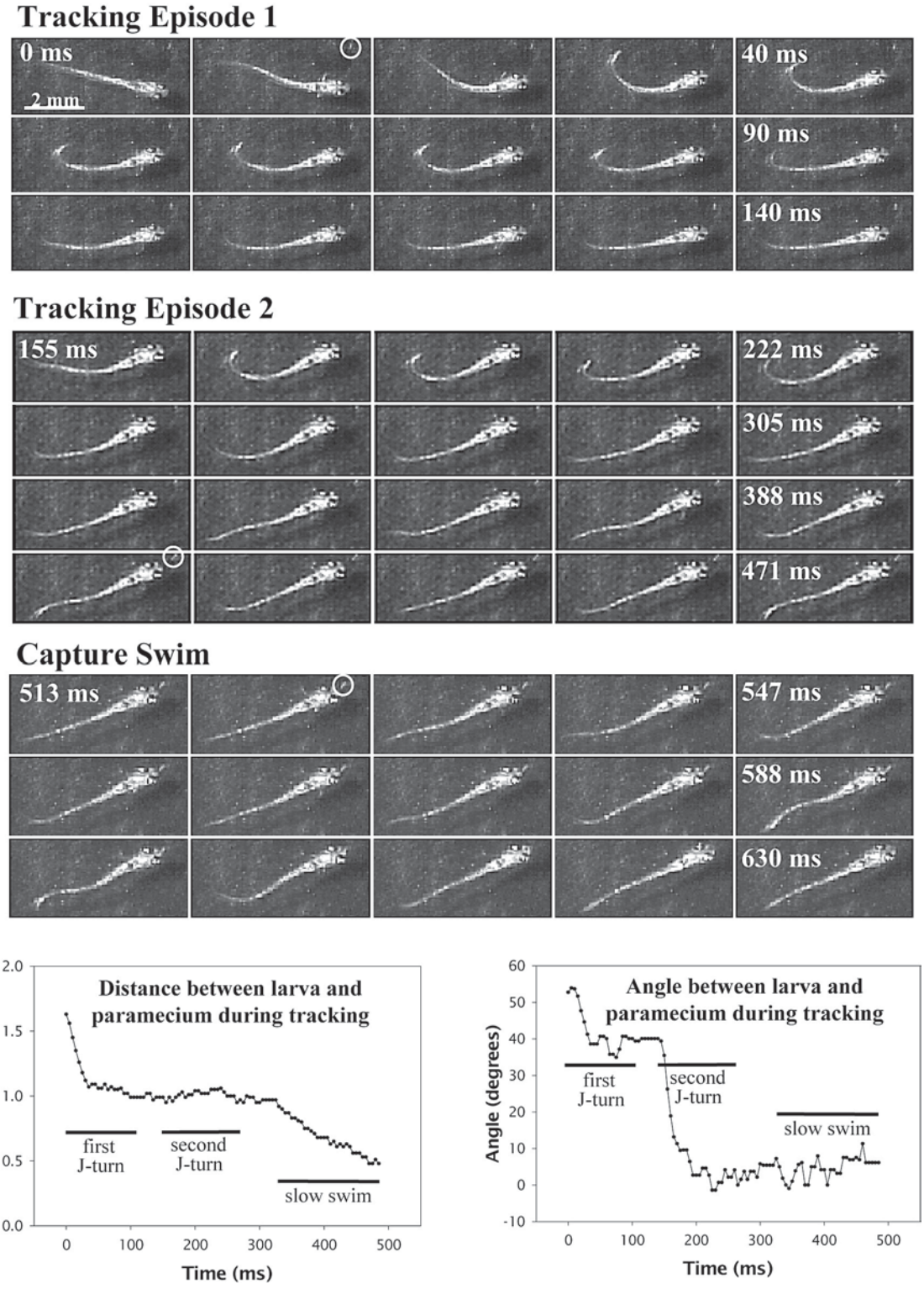
Bend Location. The bend location was recorded for each of the maximal bends in a locomotor sequence (n = 41 for J-bends). The time point of maximal bending in each half-cycle is approximated by the time at which the lateral movement of the tip of the tail reverses direction – see Budick and O'Malley [2000] and Borla et al. [2002]. The maximal bend location at such time points was determined by drawing the shortest possible line between the vertex of the bend angle and the midline of the larva. The intersection of these two lines is the midpoint of the bend. The distance from the rostral point of the fish to this point was measured to determine bend location as a percentage of total body length (TL).

Bend Duration. The elapsed time between the initiation of the bend and the time point of maximal bending.

Angular Velocity. The bend amplitude of the initial bend of each analyzed turn was divided by the duration of that bend, from initiation to the time point of maximum bend angle. The angular velocity of the initial bend for each routine turn was recorded from 6 behavioral episodes. Angular velocities for the J-turns were calculated from 14 J-turn episodes.

Visual Control of Prey Tracking

The role of vision in prey tracking was investigated in two separate experiments. To evaluate feeding in light vs. darkness, larvae were placed individually into small plastic Petri dishes (35 mm diameter, solution depth = 1 mm) with 10% Hanks solution. A known number of paramecia (15–20) were then individually pipetted into the dish, as well as into control dishes which did not contain a


Tracking Episode 1
0 ms
2 mm
40 ms
90 ms
140 ms
Tracking Episode 2
155 ms
222 ms
305 ms
388 ms
471 ms
Capture Swim
513 ms
547 ms
588 ms
630 ms
Distance between larva and paramecium during tracking
Distance (mm)
2.0
1.5
1.0
0.5
0.0
first J-turn
second J-turn
slow swim
0
100
200
300
400
500
Time (ms)
Angle between larva and paramecium during tracking
Angle (degrees)
60
50
40
30
20
10
0
-10
first J-turn
second J-turn
slow swim
0
100
200
300
400
500
Time (ms)

larva. All dishes were covered with a transparent lid to prevent evaporation of the fluid. The dishes were then placed into either a light-tight box (dimensions = 75 × 57 × 43 cm, temp = 24°C) or under a lamp approximately 22 cm above the dish. Both the larva-containing and control dishes were left in these conditions for 24 h. At the end of this time the number of paramecia left in each dish was manually counted under a dissecting microscope.

Infrared (IR) Imaging Experiments. Larvae (6–8 days post-fertilization) were placed into a 35 mm Petri dish containing a 10% Hanks solution and many paramecia. The experimental conditions were identical to those used during the prey-capture recordings except for the lighting conditions. Larvae were imaged and recorded under normal lighting conditions until a prey capture event was observed. This was confirmed by frame-by-frame playback of the recording. The dish containing the larva was then placed on a 50 × 50 mm infrared light source emitting at 880 nm ± 20 nm (LED Backlight, 880 nm, Edmund Industrial Optics, Barrington, N.J., USA). The behaviors performed under IR light were imaged with a Zeiss dissecting microscope attached to a high-speed camera (Redlake, San Diego, Calif., USA). Because the light intensity during the IR recordings is lower than during the visible-light recordings, IR behaviors had to be recorded at a slower frame rate of 150 frames/s. However, this did not affect our ability to classify the recorded behaviors. To record behaviors occurring in the dark (i.e., under IR illumination) a light-impenetrable cloth was placed over the entire imaging apparatus and each larva was imaged for 2 h.

Statistical Analyses

T tests and linear regression analyses were performed using Microsoft Excel statistical routines, with the least-squares method being used for the linear regressions. SPSS 11.0 (Chicago, Ill., USA) was used to determine p values for the linear regressions. Wilcoxon, Mann-Whitney, and ANOVA statistics were calculated using GraphPad Prism Software, version 4.0 (GraphPad Software, Inc., San Diego, Calif., USA).

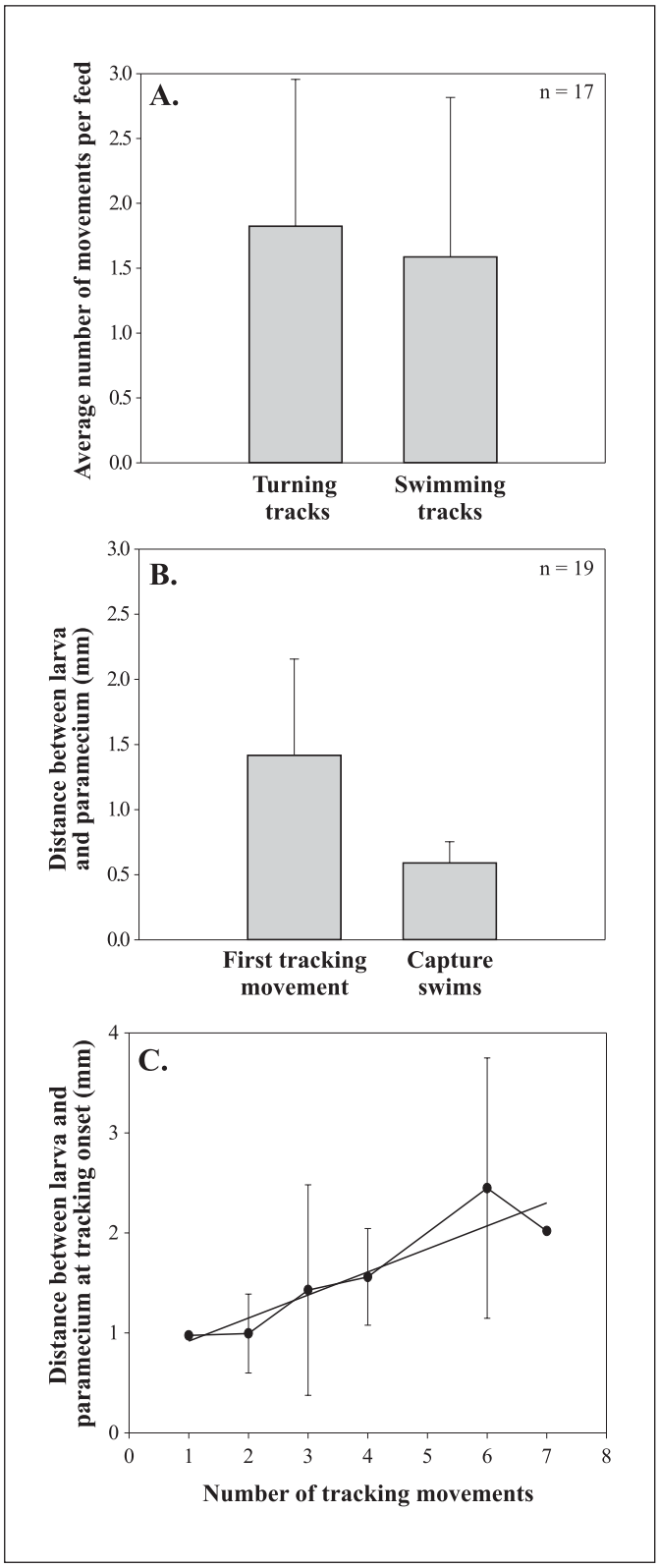
Fig. 1. Prey tracking and capture sequence. This larva (7 dpf) performs two tracking movements (turns) separated by a brief pause (episodes 1 and 2), along with a forward swimming movement (end of episode 2). The turning movements consist of unilateral J-bends that orient the larva towards the paramecium. In the second tracking sequence, after the J-bends, there is a mild undulatory swimming pattern (bottom two rows). Paramecia can be difficult to see in still images and are highlighted by white circles in select frames; in movies the targeted paramecium is easier to see because of its motion. In the capture swim bout, the paramecium is seen immediately in front of the larva's mouth in the first frame. The paramecium enters the larva's mouth during the first two frames in the bottom row. The total elapsed time from the onset of tracking is shown at the end of each row. Images were collected at 600 frames/s. Every 6th frame is shown in Track 1, every 10th frame in Track 2 and every 5th frame in the Capture Swim. Lower panels: The distance (lower left panel) and angle (lower right panel) between the larva and the paramecium decreases throughout this tracking sequence.

Results

Prey Tracking Behavior

Each prey tracking episode is comprised of a variable number of discrete turning and swimming movements (from one to seven, n = 17) that occur prior to a final discrete swim bout, termed a capture swim. Because the capture swim bout is distinct from the tracking movements, and had been analyzed in detail previously [Borla et al., 2002], it is not included in the present analysis (the capture swim was defined operationally as the discrete bout in which the paramecium is captured, but its distinct kinematic nature makes it easily distinguishable from the tracking maneuvers described here). The individual components of the tracking behavior are typically separated by brief pauses, allowing individual tracking movements to be identified and analyzed. Figure 1 illustrates a representative feeding sequence. The first panel (tracking episode 1) shows a series of asymmetric bends (turns) that orient the larva towards the paramecium (the paramecium is highlighted by a white circle in select frames). The second panel (tracking episode 2) shows a similar sequence of asymmetric bends (top two rows), followed by a pause and then a slow, symmetrical forward swimming pattern that is quite similar to a previously described slow swim pattern [Budick and O'Malley, 2000]. For completeness, the final capture swim bout is shown (bottom panel of images). Each discrete tracking movement consists of multiple bends that primarily decrease either the distance or the angle between the larva and paramecium, thus allowing the larva to approach and align with the paramecium. The bottom two plots show the declining distance and angle between the larva and the paramecium that was being tracked; the corresponding turning and swimming phases from the above images are indicated on the plots. Such behavioral sequences were not observed in the absence of paramecia.

The tracking movements could be categorized based upon whether they consisted of serial, asymmetric bends (shown in tracking episode 1 and the first half of tracking episode 2) or a symmetrical slow-swim bout (bottom two rows of tracking episode 2). These two distinct tracking maneuvers are employed in varying combinations to approach a paramecium, but when summed over all analyzed feeding episodes they were used in about equal numbers (fig. 2a). In an analysis of 17 separate feeding episodes, there was no significant difference between the average number of turns 1.82 (± 1.13 SD) and slow swims 1.59 (± 1.23 SD) used during prey tracking. Turning behaviors were observed during tracking when the



paramecium was at an angle of between 8 and 130 degrees away from the larva's heading. Turns were not observed when tracking was initiated at angular deviations of less than 8 degrees (n = 8). The tracking behavior decreases the distance and angle separating the larva from its prey as shown for an individual larva (fig. 1) and for the aggregate data from 19 larvae (fig. 2b). At the onset of tracking, the average distance between the larvae and the paramecium was 1.42 mm (± 0.74 mm SD), which was significantly further than the average distance at the onset of the capture swim bout (0.59 mm; ± 1.63 mm SD; fig. 2b; $p < 0.001$, $t = -5.09$, d.f. = 18). The tracking behavior was also analyzed to determine if the number of tracking movements per feeding episode was correlated with the initial separation distance between larva and paramecium. Larvae were binned according to the total number of tracking movements performed, and the distance between larva and prey at the onset of tracking was then averaged for each group (fig. 2c). We observed a positive correlation ($r^2 = 0.849$, $p < 0.001$) between the number of tracking movements, and the initial distance, showing that more tracking movements were used when larvae began tracking from a greater distance.

J-Turns and Slow Swims

Tracking behaviors that involve substantial changes in orientation are generally accomplished by repetitive, unilateral, far-caudal bends that gradually orient the larva toward the side on which the bending is occurring. Due to the 'J' shape of the tail, the individual bends are referred to as J-bends (fig. 1 and 3). Because each discrete turning bout typically consisted of multiple unilateral J-bends, used in smooth succession, we refer to the overall turning bout as a J-turn. Approximately 95% of J-turns involve two to four J-bends, i.e., two to four unilateral

Fig. 2. Tracking decreases the distance and angle between larva and paramecium. **A** Larvae use on average about the same number of turning tracks (1.82 ± 1.13 SD) and swimming tracks (1.59 ± 1.23 SD) in each feeding episode. **B** Tracking decreases the distance between the larva and the paramecium prior to the onset of the capture swim. **C** With an increasing starting distance between larva and prey, the average number of tracking movements used per feeding episode is also greater. Larvae were binned according to the number of tracking movements used during a feeding episode. The distance separating the larva and the prey at the onset of the first tracking movement was then averaged within each group.

contractions of the tail. Figure 3 illustrates two J-turns used in succession in a single tracking behavior. Each consisted of three successive unilateral contractions, i.e., J-bends, whose peaks are indicated by asterisks. Note that the tail does not return back to its original (unbent) position in between successive J-bends. Generally, the tail will remain slightly bent to the side on which the J-bends are occurring. These repetitive unilateral bends make J-turns unique and distinguish them from routine turns, the most similar larval behavior that had been previously analyzed [Budick and O'Malley, 2000]. To quantify these differences we compared J-turn kinematics with spontaneously occurring routine turns performed by the same larvae; routine turns were recorded in the absence of paramecia. J-turns were almost exclusively associated with the prey capture behavior. Of the 20 recorded sequences that included J-turns, 19 (95%) occurred in the context of feeding, i.e., in the presence of paramecia. Furthermore, 79% of feeding sequences contained one or more J-turns. In contrast, no routine turns were observed during these same feeding sequences.

J-turns were compared to routine turns in regards to several kinematic variables of relevance to the underlying neural controls. Specifically, we compared maximal bend location, angular velocity, linear velocity and orientation between routine turns and J-turns that were recorded from the same larva (fig. 4 and 5). In figure 4a paired examples from the same larva illustrate the maximum bend during a routine turn and a J-bend. Figure 4b shows image sequences of turns from two different larvae. Routine turns appear to involve the near simultaneous bending of a major fraction of the trunk and appear to be initiated in rostral axial musculature. In contrast, J-bends show a more caudal locus of bending that involves fewer body

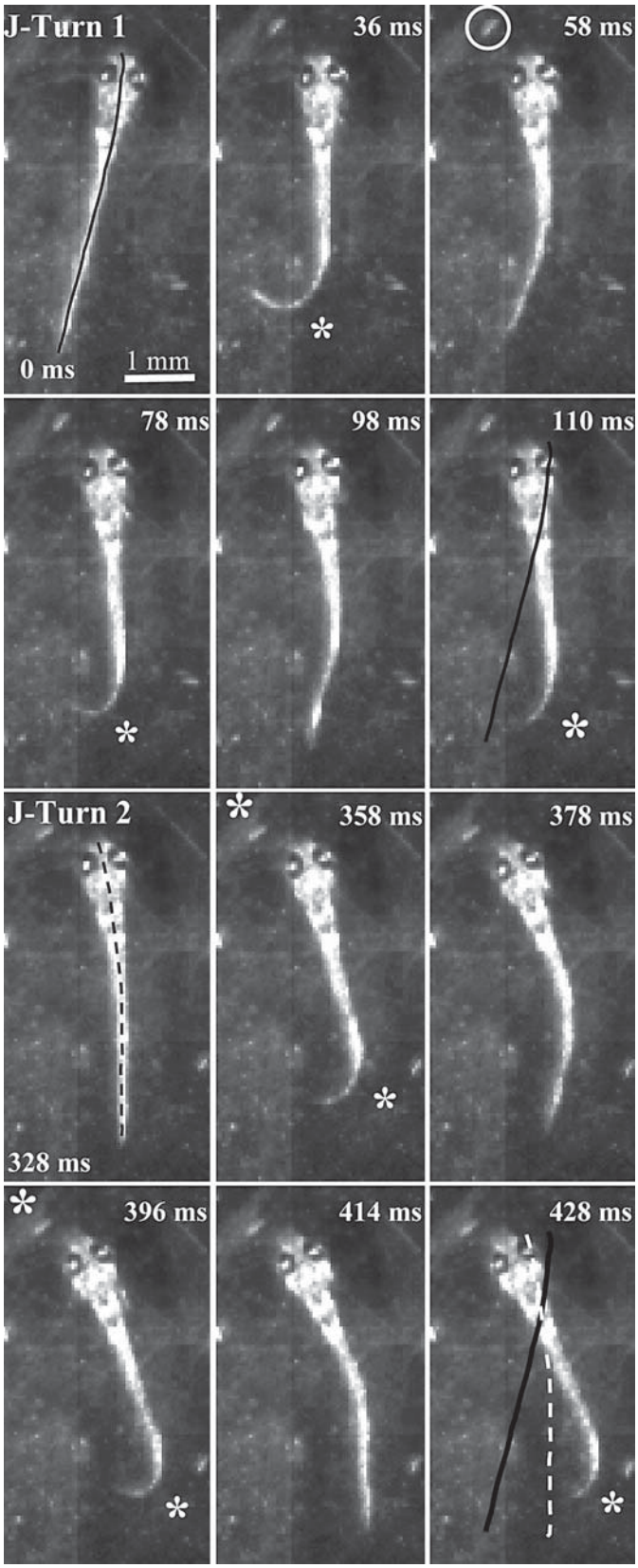


Fig. 3. Example J-turns. This larva (7 dpf; 4 mm total length, TL) performed two successive J-turns, each composed of rhythmic unilateral J-bends. The J-shaped bending of the trunk indicates a far-caudal locus of contraction. Successive maximal bends are indicated by asterisks and three are present in each of the two J-turns shown. Within a J-turn, the tail relaxes between consecutive contractions, but does not return to its original position. Between the first and second J-turn, there is a pause of about 200 ms. The midline of the larvae at the onset of the first and second J-turn is indicated by a solid and dashed line respectively; comparison with the final position of the larva (last frame) indicates the total change in orientation. The number of J-bends per J-turn, and the number of J-turns per tracking behavior are variable between feeding episodes.

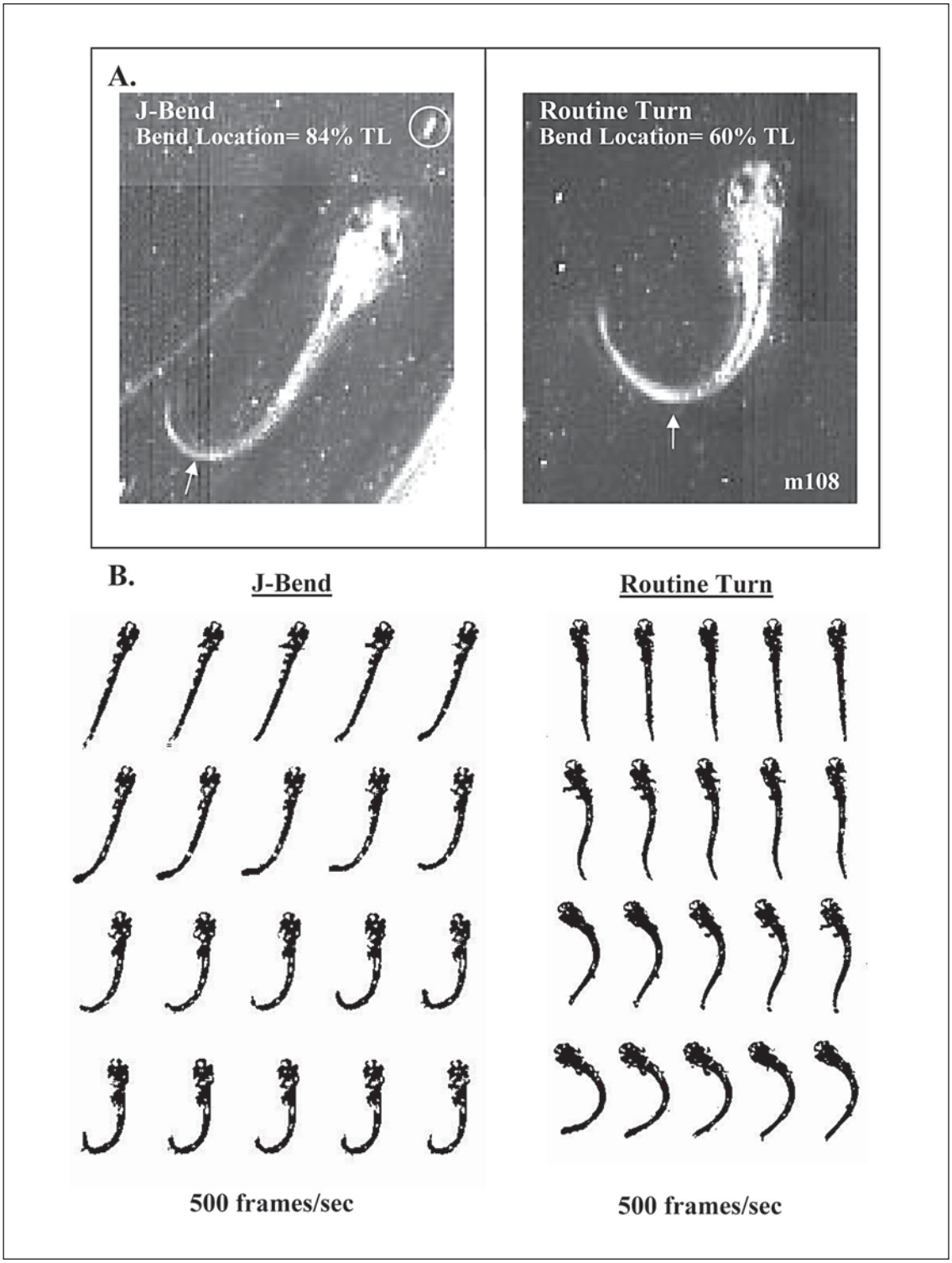


Fig. 4. J-Turns are distinct from Routine Turns. **A** The bend maximum in the J-bend is located at 84% TL compared to ~60% TL for the routine turns (indicated by arrows; larva TL = 4 mm; 6 dpf). **B** Silhouettes of the bending sequences from a representative J-turn and a routine turn (two different larvae). J-bends appear to be initiated in a more caudal region of the trunk, in comparison to routine turns. Note the greater initial head movement in the routine turn. Also note that routine turns involve more substantial bending of the rostral trunk. Although the J-bend is far caudal, it is a high-amplitude bend in comparison to the bending that occurs during slow swims (see fig. 7).

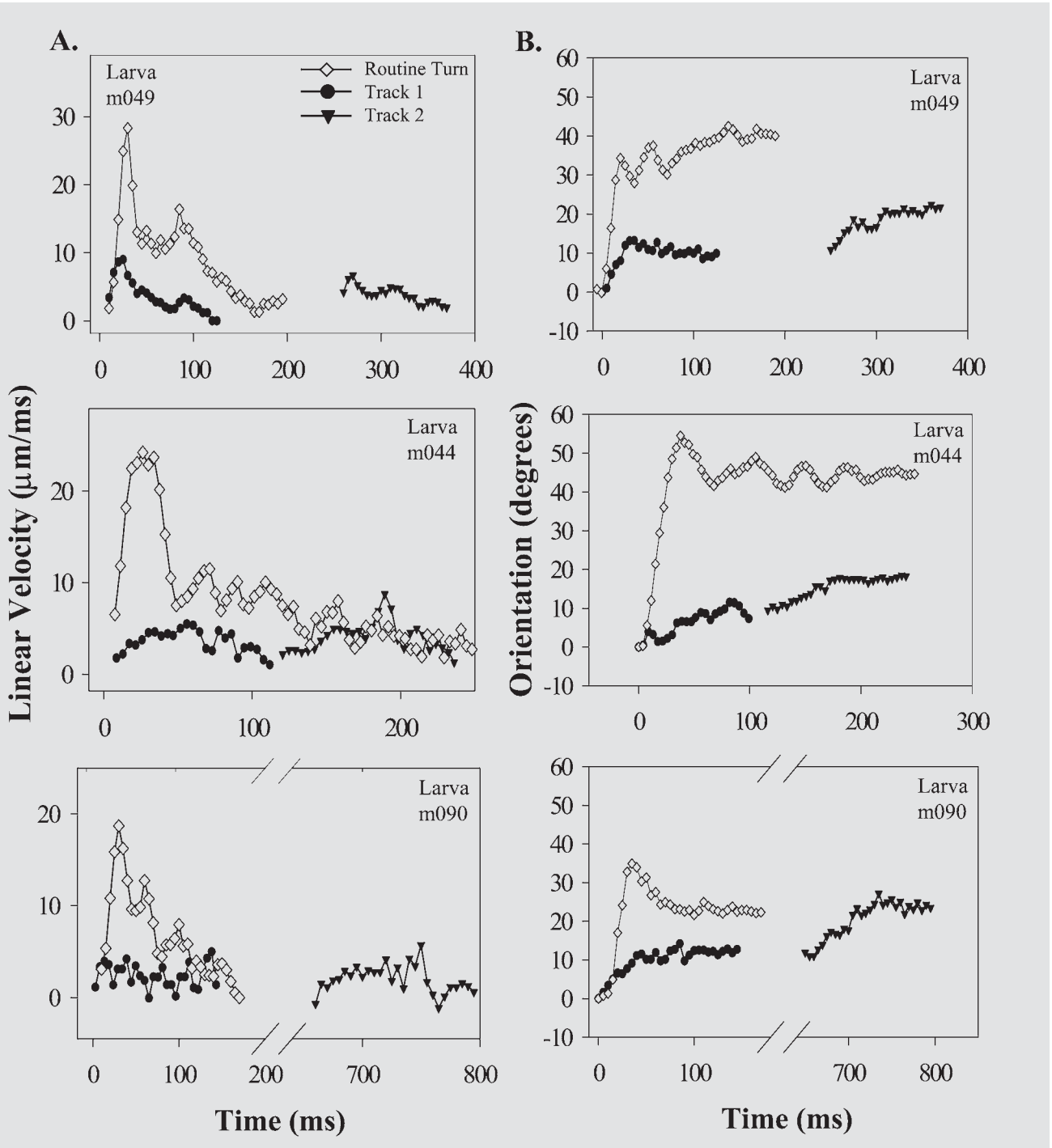


Fig. 5. J-Turns have low linear velocities and small orientation changes. **A** The peak linear velocities of routine turns, which can approach 30 μm/ms, are markedly faster than those of J-turns which ranged between 5 and 10 μm/ms. Each panel illustrates a paired tracking sequence and routine turn from an individual larva. All larvae are between 6–9 dpf, and are approximately 4 mm in total length. The peak linear velocity for all routine turns analyzed was 27.5 μm/ms (± 6.3 μm/ms SD) and for J-turns it was 11.5 μm/ms (± 4.3 μm/ms SD, n = 4). **B** Routine turns result in greater orientation changes compared to J-turns. Routine turns can alter the orientation of the larva by >90 degrees [Budick and O'Malley, 2000]. J-turns, in contrast, tend to be considerably smaller, with most turns shown here being less than 15 degrees (but see fig. 6). Complete orientation profiles were determined for 4 different larvae. A running average of n = 3 was used to smooth the data.

segments. Quantification of maximal bend location confirms that the bends are more caudal in J-turns (85.5% TL ± 3.6% SD) vs. routine turns (66.9% TL ± 5.2% SD; $p < 0.001$, $t = 11.82$, d.f. = 46).

Both angular and linear velocities tended to be smaller for J-turns as compared to routine turns (in similarly sized larvae). The average angular velocity of the routine turns was 5.37 degrees/ms as compared to 3.57 degrees/ms for tracking J-turns. A comparison of linear velocities between J-turns and routine turns in paired recordings from the same fish is shown for 3 different larvae in figure 5a. For each larva, the linear velocity of the routine-turn, measured at its center of mass (19–29 μm/ms), was much higher than that of the J-turns (5–10 μm/ms; $p < 0.001$, $t = 5.31$, d.f. = 11). Also, routine turns produce orientation changes that often exceed 30 degrees [fig. 5b and see Budick and O'Malley, 2000] in as little as 20 ms. The middle panel illustrates a routine turn in which the larva changed direction by 45 degrees in about 20 ms. Such turns are not considered escape turns (C-starts) because they do not achieve the angular velocities apparent in C-starts and also do not exhibit the pronounced counter-turn and high-yaw burst swim that accompanies typical larval C-starts [Budick and O'Malley, 2000]. In this instance, after the initial large turn the larva follows a relatively constant heading with moderate yaw about this heading: 4 cycles of bending are evident in the trace. In contrast, in the J-turn examples rhythmic yaw is lacking. Individual J-turns result in more modest orientation changes than routine turns, in the range of 10–25 degrees. However, when J-turns occur in succession, the cumulative orientation changes can equal or surpass that observed during routine turns (fig. 5b, bottom panel). In each of these 3 paired examples, the larva has the ability to produce routine turns, but in the context of prey capture consistently uses the smaller J-turns to orient, perhaps to avoid hydrodynamic disturbance of the targeted prey item (see Discussion).

The number and magnitude of J-turns executed per feeding episode was correlated with the initial angular difference between the larva and paramecium. Figure 6a shows that when larvae initiate tracking at greater angular deviations, they tend to use larger numbers of J-turns (each vertical bar represents the number of turns used in a complete feeding episode for a single larva). Likewise, at greater angular deviations, individual J-turns tend to result in greater orientation changes (fig. 6b). In regards to further distinguishing J-turns from routine turns, we determined their temporal association with other locomotive behaviors (fig. 6C). J-turns occurred on average within 113 ms (± 121 ms SD, n = 47) of other axial locomotor behaviors (including capture swims, slow swims and other J-turns). This contrasts with the routine turns that occurred within an 'average' of 615 ms (± 399 ms SD, n = 14) of other locomotor behaviors. But the routine turn average is only a lower limit because of the finite duration of our high-speed recording procedures. Many recorded routine turns occurred in isolation of any other recorded locomotive behaviors. To be conservative we excluded from our analysis those routine turns that occurred in isolation (see fig. 6 legend). In spite of this restriction, the difference in the temporal association of these two turn types with other behaviors was statistically significant based on a one-tailed t test ($p < 0.001$, $t = -4.63$, d.f. = 14). J-turns are thus used in temporal proximity to other locomotive behaviors and specifically in conjunction with slow and capture swim maneuvers that lead to successful prey capture.

Slow swims are one of two previously-described classes of spontaneous forward-swimming patterns, that is they occur in the absence of overt stimulation of the larvae. Spontaneous slow swims (fig. 7a) are characterized by minimal yaw, low forward velocities, short distances of travel, small bend amplitudes and a more caudal locus of bending than the far more vigorous burst swims that occur spontaneously, but more typically accompany escape behaviors [Budick and O'Malley, 2000; Thorsen et al., 2004]. The slow swim bouts observed during prey tracking (fig. 7b) appear similar to the previously described slow swims in all these respects. In our observation of 28 slow swim bouts that were integral to prey tracking episodes, we did not detect any kinematic features that were notably different from spontaneous slow swims. The slow swim bout thus appears to be a common locomotive maneuver that occurs both spontaneously and as part of prey tracking [it also constitutes an early part of the capture swim bout; Borla et al., 2002]. In both tracking and prey capture swims, the slow swim component has clear ethological significance, but the function of spontaneous slow swim bouts is less certain. Spontaneous bouts could serve a developmental purpose, e.g., helping to establish appropriate neural connections for future performance of the same locomotor pattern, but they might also, within their natural environment, serve a localized exploratory or navigational purpose.

Vision and Prey Capture

The role of vision in prey capture was examined by evaluating the extent of feeding in total darkness and by imaging locomotive behaviors under infrared light (880-

nm band-pass filter). Larvae kept in total darkness fed markedly less than larvae kept in lit conditions for 24 h (fig. 8a). Larvae in total darkness left an average of about 67% of the paramecia in the dish after 24 h, whereas larvae kept in the lit condition consumed almost every paramecium. A non-parametric, paired one-tailed Wilcoxon test indicated that larvae kept in darkness fed significantly less than larvae in visible light ($p < 0.001$). The number of paramecia remaining in darkened dishes that contained a single larva was not statistically different from control dishes that lacked larvae, as determined by a one way ANOVA analysis: 66.8% (± 38.3% SD) of the paramecia remained in the darkened dishes containing larvae, whereas the percentages were 88.8% (± 28.6% SD) for the dark control dishes and 99.4% (± 29.8% SD) for the lit control dishes ($p > 0.05$). These data cannot rule out the possibility that a small of number of paramecium were being consumed in the dark, perhaps by a suction feeding mechanism after either random encounters or as a result of prey detection by other sensory modalities.

Larvae were also imaged under infra-red (IR) illumination to determine if prey tracking and capture maneuvers would be performed under dark conditions. Because it can take a variable and substantial amount of time for

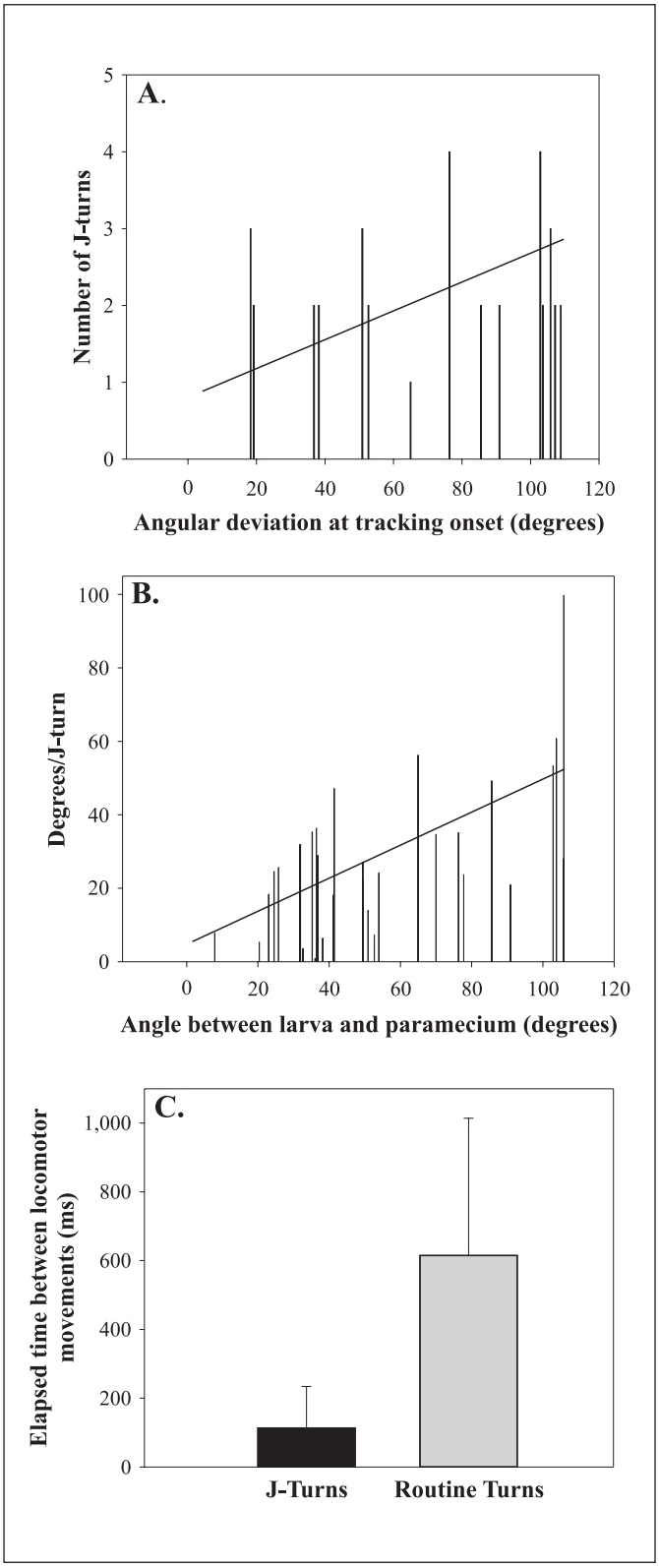


Fig. 6. Size and numbers of J-turns. Both the number of J-turns and their angular size increase with increasing angular deviation between larva and paramecium. **A** The number of J-turns executed during single feeding episodes (vertical bars, 1 per larva) is correlated with the initial angular deviation between the larva and paramecium at the onset of prey tracking: larvae tend to execute more J-turns at increased angular deviations ($r^2 = 0.334$, $p < 0.01$). **B** The orientation change produced by each J-turn is also increased at greater angles between the larva and paramecium ($r^2 = 0.392$, $p < 0.001$). **C.** J-turns are temporally associated with other axial behaviors whereas routine turns occur in a more isolated context. J-turns occurred on average within 113.3 ms (± 120.5 ms SD) of other behaviors including other J-turns, slow swims and the capture swim. Routine turns were more isolated occurring within 615 ms (± 400 ms SD) of other behaviors, for those recording sequences in which other locomotive behaviors were observed. Many routine turns occurred in complete isolation from other locomotive behaviors and we took the conservative approach of not including such routine turns in our analysis. Had we included those routine turns, and assigned them a temporal proximity value equal to the full duration of the recording period, the calculated difference in temporal proximity would have been much greater. In contrast, all of the observed J-turns occurred in close temporal proximity to other locomotive maneuvers.

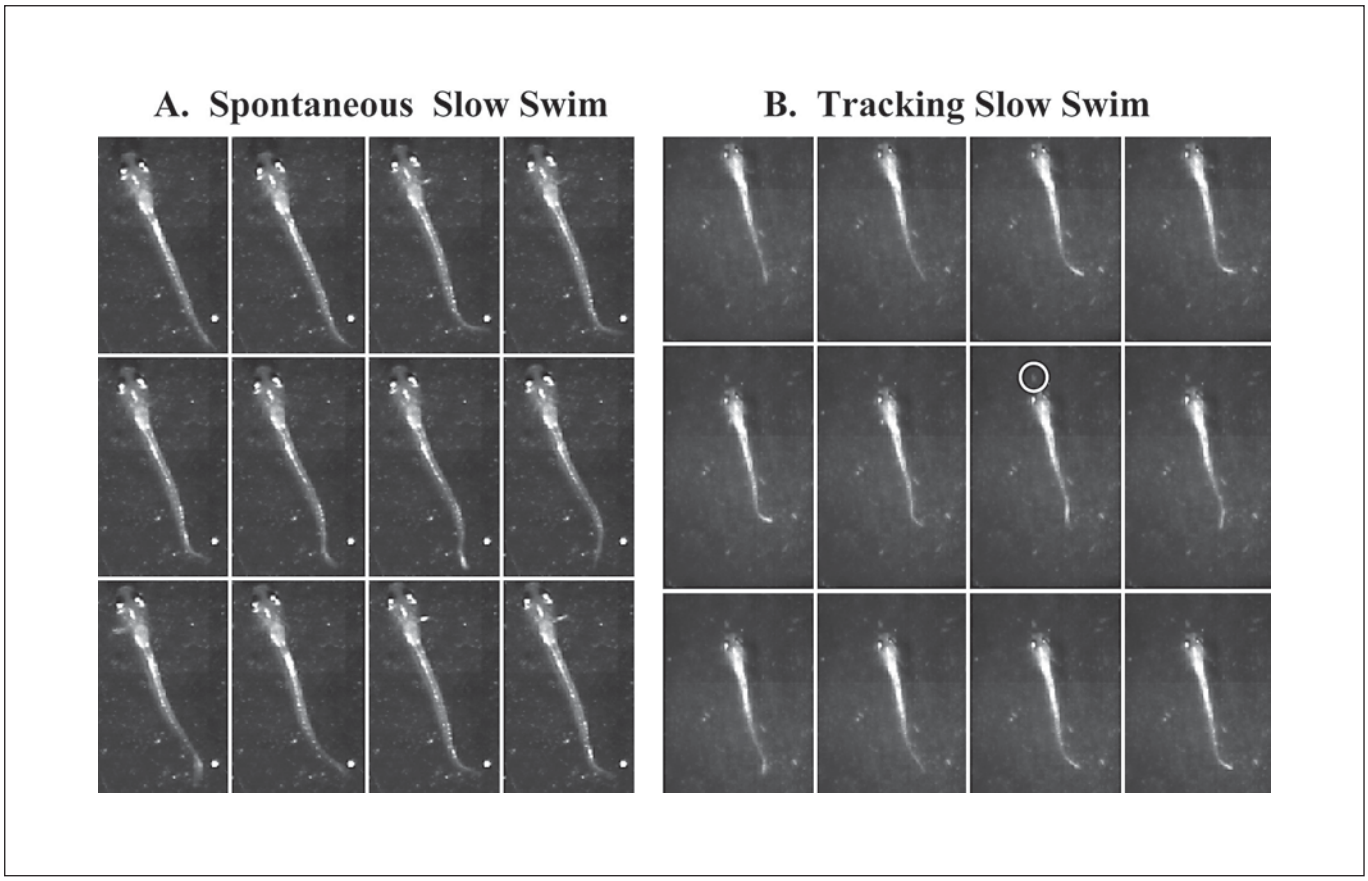


Fig. 7. Tracking slow swims and spontaneous slow swims exhibit similar axial patterns. Although a detailed analysis comparing spontaneous slow swims (**A**) to tracking slow swims (**B**) has not been performed, the basic axial patterns seem similar: there were no obvious differences in the rostral-to-caudal pattern of wave propagation, bend amplitude or bend location. Tail-beat frequency and linear velocity also appeared similar for the two behaviors. Tracking slow swims (**B**) can be used alone, or in conjunction with J-turns to approach the paramecium.

a larva's first-feeding event to take place, and to demonstrate that each larva was capable of feeding, larvae were first observed with paramecia under normal lighting conditions until they captured one paramecium, which took on average 80 min (fig. 8b). Following the initial prey capture, the dish containing the larvae and the remaining paramecia was either kept under normal lighting or placed under IR light; subsequent behaviors were recorded with the high-speed camera. Larvae kept in the light consumed a second paramecium, on average, within about 25 min. In contrast, none of the 5 larvae observed under IR light fed within a 2-hour recording period and so if any of them could have fed, it would have taken in excess of the 120 min indicated by the black bar in figure 8b. These larvae that failed to feed in the dark were observed during the same recording periods performing normal non-feeding behaviors including spontaneous slow swims (fig. 9a), spontaneous routine turns (fig. 9b), and spontaneous escapes (fig. 9c). The total amount of spontaneous activity recorded with IR light appeared similar to that recorded under visible light (these experiments were recorded about mid-day in the larva's circadian time). Thus, the failure to exhibit feeding-specific locomotive maneuvers (J-turns and capture swims) in the dark cannot be explained by some non-specific disturbance of the larvae, but more likely reflects an inability of the larvae to see and track paramecia in the dark.

Other Locomotive Maneuvers Associated with Feeding

Although each tracking episode generally resulted in a successful capture event, in rare instances larvae ap-

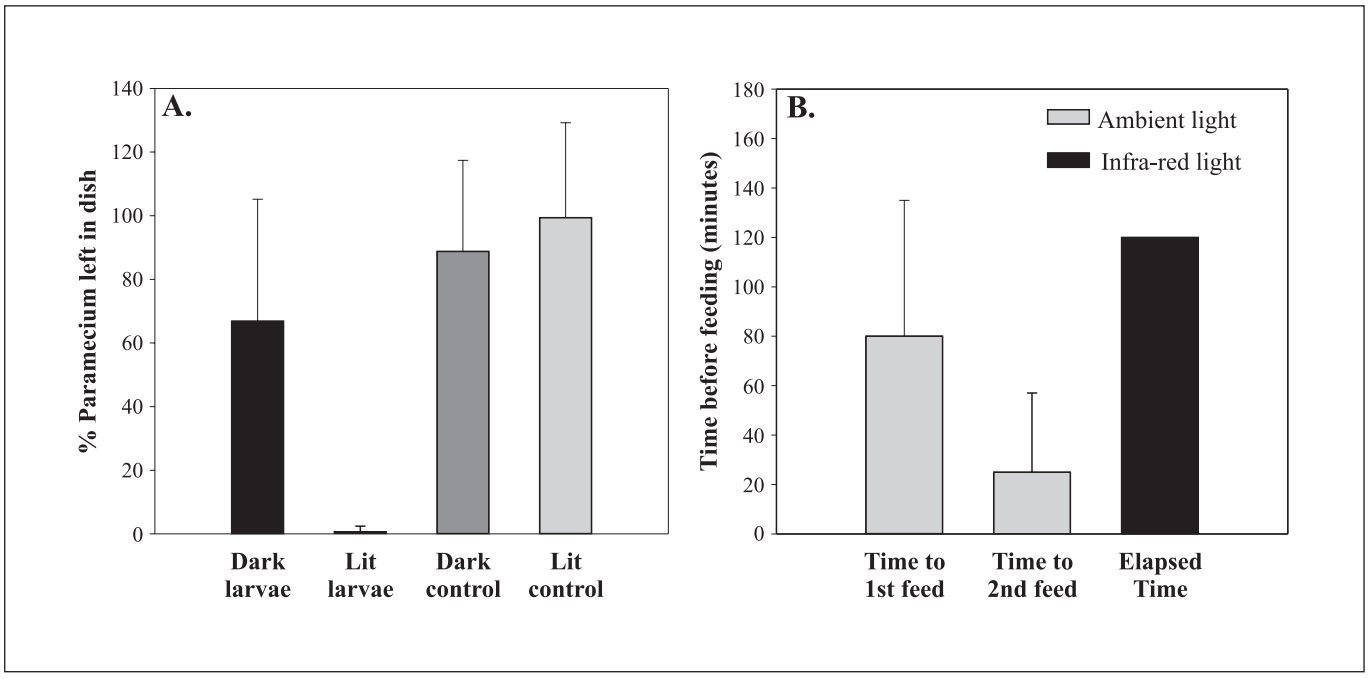
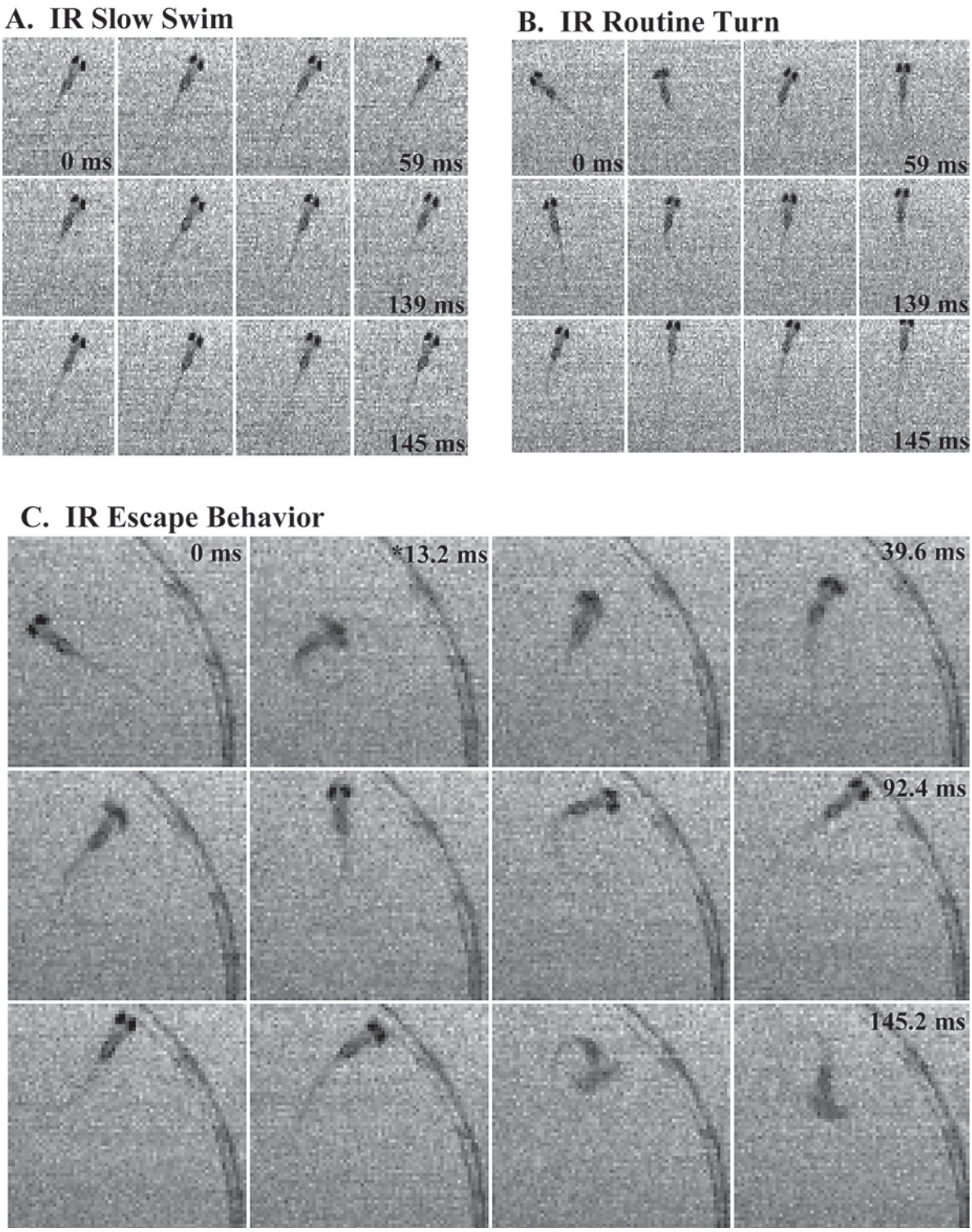


Fig. 8. Darkness impairs prey capture. **A** In constant light (lit larvae), zebrafish larvae consume almost every paramecium in the dish over 24 h, with only 0.7% remaining (± 1.76% SD). In darkness, a majority of the paramecia survive a 24-hour incubation with a larva (dark larvae, 66.8% remaining ± 38.3% SD). Fewer paramecia remained in the dish of the 'dark' larvae compared to the dark control (24 h, no larva) 88.8% (± 28.6% SD) or the lit control 99.4% (± 29.8% SD) but this difference was not statistically significant. **B** Following their first feeding episode, larvae begin to feed more frequently. It took an average of 80 min (± 55 min SD) for a larva to begin feeding after paramecia were first pipetted into the recording dish. This time is variable and can extend to 150 min, or beyond (at which point the recording was terminated). In contrast, after the first feeding episode, the time to the next feeding, in the presence of light, occurred on average in 25 min (± 32 min SD). Following a successful first feed under visible light, five larvae were transferred into a darkened environment (by gently moving the Petri dish to a nearby microscope stage) and observed under IR illumination. None of the 5 larvae were observed feeding during a 2-hour observation period, although they did exhibit a variety of non-feeding locomotive behaviors.

peared to miss the paramecium. Following such failed feeding attempts, three separate individuals were observed 'backing up'. In the instance shown in figure 10, the larva moved backwards and appeared to reorient towards the paramecium (highlighted by white circle). Several J-turns were utilized, resulting in backwards motion relative to a marker shown on the image (vertical white bar shown in the first frame of sequence A and the last frame of sequence B). Each J-turn was directed towards the side on which the missed paramecium was located. This behavior was observed too rarely for statistical analysis, but the larvae appeared to use synchronized pectoral fin extensions (arrows in B) as well as J-bends to move backwards and re-orient towards the prey. After reorienting towards the prey, larvae were again observed tracking the paramecium, and were sometimes successful, as was observed later in this tracking episode (capture not shown). One reason why this noticeable degree of backing-up was rarely observed is that from the first recorded feeding episodes, zebrafish larvae are highly successful in capturing paramecium, showing an overall success rate of 82% (71 successes out of 87 recorded feeding attempts). In the more typical J-turns used during tracking, a very slight backwards motion was sometimes observed. Such backwards movements appear to be a side effect of the caudal trunk moving forward and pushing against the water; they were almost imperceptible in size and did not result in consequential repositioning with respect to the paramecium's location.

In regards to other usage of the pectoral fins, there was no apparent coordination between J-turn trunk movements and fin movements. Although the pectoral fins abducted bilaterally at the outset of some J-turns, the subsequent alternating fin movements were not performed

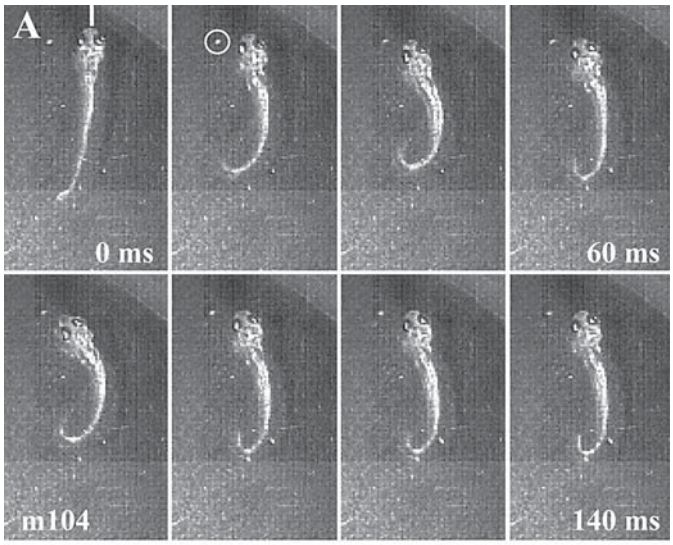

A. IR Slow Swim
0 ms
59 ms
139 ms
145 ms
B. IR Routine Turn
0 ms
59 ms
139 ms
145 ms
C. IR Escape Behavior
0 ms
*13.2 ms
39.6 ms
92.4 ms
145.2 ms

9

in synchrony with ongoing axial trunk movements. There was inter-fish as well as inter-trial variability with regard to the pattern of pectoral fin usage and a consistent pattern was not detected. This is in contrast to the usage of pectoral fins during the capture swim where these fins are used in alternation during the propulsive phase and then extended bilaterally in a braking maneuver at the completion of prey capture [Borla et al., 2002]. Another behavior-specific usage of pectoral fins by zebrafish larvae was recently reported for different speeds of forward swimming [Thorsen et al., 2004]. Our observations are limited by the tradeoffs inherent in recording axial movements vs. fin movements: to record the entire larva and its tracking of the paramecium requires a low magnification that is not well suited for the high-resolution analysis of fin movements.

Discussion

High-speed recording of predation by larval zebrafish revealed a prey-tracking phase that consists of a variable number of swimming and turning maneuvers. These maneuvers enable the larva to orient towards and approach a slow moving prey item, such as a paramecium, in preparation for a final capture swim. Our previous study of the capture swim [Borla et al., 2002] revealed the presence of descending motor signals that dynamically modulate spinal neural circuits during prey capture locomotion. The present study reveals further capabilities of the descending motor control system and extends the known locomotive repertoire of this model organism. Notably, the far-caudal bend location observed during J-turns confirms that larvae can exert fine rostral-caudal control over axial musculature. Also, the directed nature of the overall



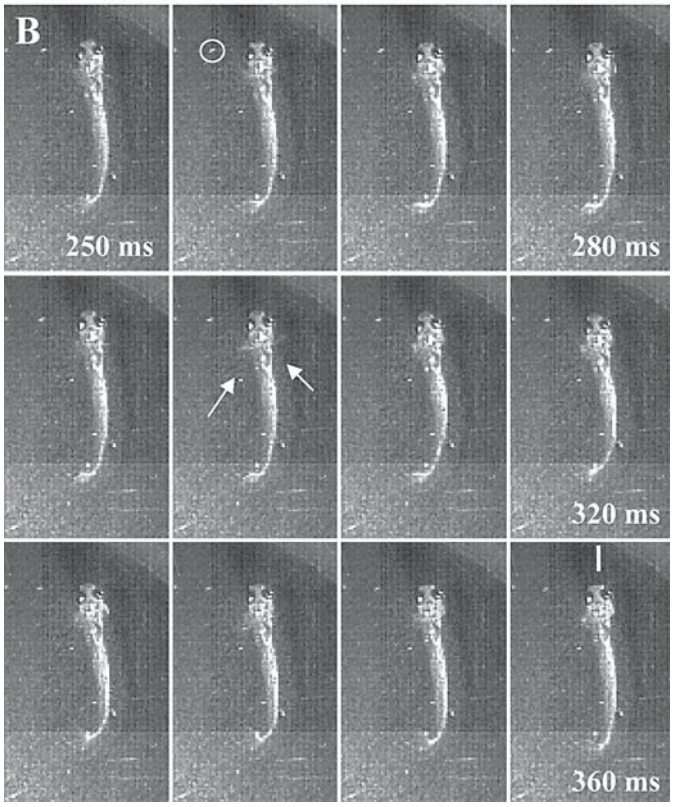


Fig. 9. Larvae exhibit a normal locomotor repertoire under IR light. Larval swimming and turning behaviors recorded in total 'darkness' using IR illumination (880 nm) appear the same as the behaviors observed under visible light. **A** A slow swimming behavior of a larva recorded under IR illumination appears similar to normal slow swims (see fig. 7). The tail rhythmically alternates from side-to-side with a rostral-to-caudal propagating wave of bending. **B** A routine turn from the same larva as in **A** (8 dpf) appears quite similar to those recorded under visible light. **C** Another larva (also 8 dpf) performs a spontaneous escape behavior. The C-bend has reached its maximum angle in frame #2. It subsequently performs a counter-bend and burst swimming bout with kinematics similar to those observed under visible light [see e.g., Budick and O'Malley, 2000].

Fig. 10. Use of J-bends to back up and reorient towards a paramecium. This larva (approximately 4 mm in length and 7 dpf) used a series of J-bends, along with a synchronized extension of the pectoral fins (arrows in B), to back up and reorient towards a paramecium (high-lighted by the white circle). The bar shown in the first frame of **A** is in the same location as that in the last frame of **B** and is provided to indicate the changing position of the larva. This episode consisted of two J-turn maneuvers, the first in **A** lasting 140 ms, and the second in **B** lasting 110 ms.

prey capture sequence, from initiation of tracking through to the moment of prey capture, demonstrates that at this early larval stage, zebrafish are able to execute a complex, well-coordinated and adaptive locomotor sequence. Their ability to do this from varying angles and distances, and using variable combinations of turns and swims, suggests the persistent activation of a goal-oriented motor 'program' that is regularly updated by current sensory information.

The Tracking Behavior

Because capture swims are executed only when larvae are closely aligned with and in close proximity to the paramecium (fig. 1, 2), the apparent function of tracking is to guide the larva to a location from which the capture swim can be triggered. The number and types of tracking movements used by larval zebrafish to approach the paramecium is variable from fish to fish (fig. 2, 6), as well as within individuals (data not shown). Variability in motor output during feeding has been reported previously [Sanderson, 1988; Wainwright et al., 1989, 2001; Wainwright and Friel, 2000], but in the case of the larval zebrafish, the variable combinations of swimming and turning maneuvers used can be explained, at least in part, by the variety of distances and angles from which the larvae begin tracking. Indeed, the number of tracking movements performed per feeding episode increases with increasing initiation distance (fig. 2c), and the number of J-turns increases with increased angular deviation between the larva's heading and the direction to the paramecium (fig. 6a). Likewise, the size of the individual J-turns is correlated with increasing angular deviation (fig. 6b).

The scenario that emerges is that a specific stimulus – e.g., a moving object of a certain size (spanning a certain visual angle at a certain distance) and within a certain angle from the larva's heading – will trigger the overall prey capture behavior. Slow swims and J-turns reduce the angle and distance to the paramecium until the sensory criteria for triggering the capture swim are met. The capture swim trigger might consist of a bilateral and fairly symmetrical stimulation of the two retinas by an object that appears sufficiently close and small that it has a good probability of being captured. In making the earlier decision to track, the larval brain has to determine if the stimulus is predator, prey or neither [Hart, 1993; Miklósi and Andrew, 1999]. It might do this by integrating multiple sensory modalities, as the Mauthner cell does when triggering the escape behavior [Faber et al., 1991; Canfield and Rose, 1996; Eaton et al., 2001; Canfield, 2003], but it is presently unknown if non-visual stimuli (olfactory, mechanical or electrical) contribute to either the decision to track or the release of the capture swim. Once a decision to track has been made, the larva seems to perform a further series of binary decisions: at each step of the tracking behavior it must perform either a slow swim or a J-turn. The brief pauses between tracking movements might be used to visually update the tracking program, with greater angular deviation biasing the decision towards a J-turn and greater straight-line distance triggering slow swim bouts. This serial decision-making process is terminated when the larva attains an orientation and proximity sufficient to release the capture swim behavior. The capture swim bout takes less than 50 ms, so there is insufficient time for further visual updating of the larva's position relative to the prey during this bout. Although this scenario appears to be the simplest explanation for the observed behavior, it is presently unknown (at the cellular level) how the initial predator-prey-neither decision is made, nor how the motor programs for J-turns, slow swims and capture swims are stored and selected.

Function of J-Turns

The J-turn is a distinctive locomotive maneuver that had not previously been described, to our knowledge, for any aquatic vertebrate. These repetitive, J-shaped bends are unlike any turning or swimming behavior previously reported for zebrafish larvae [Kimmel et al., 1974; Fuiman and Webb, 1988; Budick and O'Malley, 2000; Borla et al., 2002, Schneider et al., 2003; Müller and van Leeuwen, 2004; Watkins et al., 2004; Larson et al., 2004; Thorsen et al. 2004; O'Malley et al., 2004] or other larval fishes [see e.g., Blaxter, 1968; Munk and Kiorboe, 1985; Drost, 1987; Batty et al., 1990; Coughlin et al., 1992]. Because J-turns consist of multiple, unilateral J-bends (fig. 3), they must be driven by a highly asymmetric control signal that is distinct from the more symmetrical neural activity underlying forward swimming [Stein et al., 1997; Kiehn et al., 1998; Grillner and Wallen, 2002; Grillner, 2003]. Compared to routine turns, J-turns have a more caudal bend location (fig. 4), are of lower linear velocity (fig. 5a), and result in smaller orientation changes (fig. 5b). They are associated almost exclusively with prey tracking (fig. 6c). In contrast, routine turns are almost never observed when paramecia are present, and so are unlikely to play any substantive role in prey tracking or capture, at least for slow moving prey items.

Why, during tracking, do larvae use a J-turn rather than a routine turn? Routine turns tend to be larger and faster and so would allow, in principle, a more rapid ap-

proach towards a prey item. The exclusive use of J-turns suggests a significant adaptive advantage over routine turns. One possibility is that J-turns (and capture swims as well) are designed to minimize hydrodynamic disturbance at the paramecium's location. As seen in figures 1 and 2, zebrafish larvae are often within one body length's distance from the paramecium when turning. If the higher velocity routine turn were used, that might generate a forward hydrodynamic disturbance that could push the paramecium away. There is no direct evidence for this because routine turns are not used during prey tracking or capture, but interruption of small sets of descending nerve fibers can perturb feeding maneuvers and result, in some instances, in abnormal yaw that sends a targeted paramecium tumbling away [Borla et al., 2004]. In the case of the normal J-turn, its lower angular velocity and more caudal bend location seem to minimize forward disturbance of the water surrounding the paramecium – this seems true independently of the size of the J-turn or the larva's proximity to the targeted paramecium.

Neural Control Schemes for J-Turns

The J-bend, with a maximal bend location of about 86% of total body length, is further caudal than any other larval bending pattern. Several possible neural mechanisms might explain this pattern of bending. First, if a specific class of descending neurons was found to give off axonal terminals only in far caudal spinal cord, such neurons could (in principle) selectively activate far-caudal musculature. Intracellular labeling experiments, however, have thus far failed to reveal descending neurons with such an arborization pattern in zebrafish or any other vertebrate species [see Discussion in Gahtan and O'Malley, 2003]. An alternative means of generating J-bends might be accomplished, in part, by bilaterally activating the rostral trunk to stiffen it. Descending neurons that project just to rostral cord in larval zebrafish have been reported. In this scenario, the rostral trunk would be stiffened bilaterally, while the caudal trunk would be unilaterally and rhythmically activated by other neurons that arborized along the entire rostral-caudal extent of cord. Neurons with this latter termination pattern were described in Gahtan and O'Malley [2003] and should be able to selectively and unilaterally bend the caudal trunk, as long as the rostral trunk remains rigid [modeled in Hill et al., 2005]. Such a mechanism could, in principle, operate independently of the spinal segmental oscillators, but it is also possible that spinal CPGs participate in the behavior, albeit with gating of their output so as to not excite motoneurons contralateral to the J-bend. The same spinal CPGs might be used during slow swims, but the rostral-to-caudal strength of excitation must vary between the two behaviors given the more extreme bending of the caudal trunk during J-turns.

However J-turns are generated, there must be some mechanism whereby spinal neurons are rhythmically and unilaterally activated, perhaps as a consequence of a lateralized, visually-modulated hindbrain signal, which might have originated in the optic tectum [Bass, 1977; Meek, 1981; Bosch and Paul, 1993]. In lamprey, lateral turning movements are produced by asymmetric activation of either left or right reticulospinal neurons which in turn provide asymmetric excitation to spinal cord thereby increasing bend amplitude on one side [Kozlov et al., 2002; Grillner and Wallen, 2002]. Several variations on this theme have been advanced in more detailed models of lamprey turning [McClellan and Hagevik, 1997]. Although the lamprey's body form and swimming style differ greatly from that of the zebrafish, the architecture of the underlying CPG might be similar. CPGs are found in all vertebrate spinal cords [Kiehn et al., 1998] and spinal interneuron types appear similar in anamniotes [Fetcho, 1992]. If the larval zebrafish possesses a spinal architecture similar to that of other vertebrates, such as lamprey, *Xenopus* and cat [Stein et al., 1997; Roberts et al., 1998; Butt et al., 2002], then the descending control strategies used to dynamically modulate the spinal circuitry might also be conserved.

Visual Control of Prey Tracking

In comparison to larvae kept in the light, larvae in the dark fed dramatically less, and perhaps not at all, over a 24-hour time period (fig. 8a). This indicated that prey tracking was impaired during dark conditions. Zebrafish larvae are unable to see at wavelengths above 800 nm [Saszik et al., 1999; McDowell et al., 2004; and R. Baker, personal communication] and fishes in general have poor or no ability to image small objects at wavelengths above 800 nm [Govardovskii et al., 2002; Neumeyer, 2003]. Thus, IR imaging with 880-nm light should not allow the larvae to visually detect or track a paramecium. In dark conditions, during subjective day, there was no apparent decrease in the overall locomotor activity as compared to that observed under normal light conditions, in agreement with studies of larval circadian activity [Hurd and Cahill, 2002]. Larvae spontaneously performed slow swims, routine turns and escape behaviors in the dark (fig. 9). But over 2 h of continuous IR observation for each examined larva, we never observed an apparent feeding behavior, such as a J-turn or capture swim. These larvae

had all fed at least once previously under visible light – which is important because once larvae feed on their first paramecium they are more likely to continue feeding within a short time frame (fig. 8b); such larvae are apparently far from satiated. Collectively, these data indicate that the visual system is the primary sensory system used in prey tracking and is necessary for successful larval feeding. Although the use of suction feeding to capture a small number of paramecium in the dark has not been ruled out (fig. 8a), we did not observe, during the periods of direct IR observation, any instances of suction feeding.

Our results correspond well with other reports in the teleost literature. Batty et al. [1990] used IR video imaging to study the effects of light intensities on the feeding of herring *(Clupea harengus)*. At light intensities exceeding 0.001 lux, fish fed using a biting technique. In darkness, herring were unable to bite their prey, and could only filter-feed. Unpublished observations by George Streisinger's group had suggested that zebrafish larvae feed as a function of both paramecium concentration and light intensity; blind larvae did not consume a detectable amount of paramecium [reviewed in Neuhauss, 2003]. The possibility of suction feeding in the dark is suggested by observations of suction feeding in zebrafish larvae [Hernandez, 2000; Budick and O'Malley, 2000; Borla et al., 2002] and other teleost larvae [Lauder, 1980; Drost and Van den Boogaart, 1986; Drost et al., 1988; Coughlin, 1991; Ferry-Graham, 1998]. Although vision appears critical for survival of zebrafish larvae, such visually-guided feeding might be augmented by the lateral line [New et al., 2001; Montgomery et al., 2002] or olfactory system [Friedrich et al., 2004], especially in the case of the capture swim which is initiated in very close proximity to the paramecium.

Evolution of the Prey Capture Behavior

How has the neural control of prey capture evolved in the vertebrate lineage? As noted in our introduction, fishes use a variety of sensory systems to detect and track prey including electroreception, lateral line, chemical cues and vision. The extent to which common locomotor controls might be used in diverse strategies of prey capture is of interest in that such controls might represent the ancestral condition. One thing these capture strategies have in common is the need for the predator to align itself with the prey item – this requirement is independent of the sensory modality used. Some of these studies have described axial kinematics using high-speed imaging, but there has not been a concerted effort to record and analyze such behaviors with the specific intent of inferring details of the underlying neural controls. Nonetheless, these studies collectively demonstrate a powerful computational skill set that enables fast sensorimotor transformations and the execution of fast and precisely controlled sequences of axial and jaw movements.

How do the neural controls used by the larval zebrafish relate to these widespread capture capabilities? One possibility is that the neural circuits used by larval zebrafish to track and capture prey represent conserved computing elements that are present in many fish clades, as opposed to being a derived characteristic of, for example, the otophysan grouping. Orienting towards and tracking a prey item appears to require precise modulation of spinal circuitry. In regards to swim-rhythm generation, the spinal circuitry in anamniotes has conserved features dating to the earliest vertebrates studied [Fetcho, 1992]. To the extent that spinal cord is conserved, the descending control of spinal circuits underlying tracking and prey capture might also be conserved and should rely upon the oldest pathways projecting from brainstem to spinal cord, including nMLF and the reticulospinal and vestibulospinal pathways.

In larval zebrafish the entirety of the descending projection appears to consist of about 300 neurons [O'Malley et al., 2003]. Larval tracking maneuvers must therefore be controlled by some subset of this discrete neuronal population. This 'minimal' teleost locomotor control system appears capable of producing almost any desired pattern of bending, being able to modulate laterality of bending, bend amplitude, bend location and tail-beat frequency in a dynamic and goal-oriented fashion [present findings and Borla et al., 2002]. We suggest that this capability of fine axial motor control is a primitive trait exhibited by all clades of vertebrates, at least beginning with lamprey [Zelenin et al., 2000, 2001]. It is presumably elaborated upon in adult fishes, given that adult zebrafish, for example, have a far more elaborate forebrain and cerebellum than their larvae [Wullimann et al., 1996]. Adult systems should therefore enable greater locomotive dexterity and perhaps more powerful tracking strategies.

Acknowledgments

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