JOURNAL OF EXPERIMENTAL ZOOLOGY 307A (2007)

Heterochrony and the Development of the Escape Response: Prehatching Movements in the Rainbow Trout *Oncorhynchus mykiss*

ALICE C. GIBB*, CORINA LIU, AND BROOK O. SWANSON
Department of Biology, Northern Arizona University, Flagstaff, Arizona

ABSTRACT Teleost fishes produce coordinated escape responses (C-starts) at hatching. This implies that essential swimming morphologies and motor behaviors develop during the incubation interval while the embryo is in the chorion. We examined prehatching motor behaviors in rainbow trout *Oncorhycus mykiss* (considered morphologically mature at hatching) and compared this species with zebrafish *Danio rerio* (considered morphologically immature) and assessed two hypotheses concerning the development of escape behavior. (1) Escape behavior is associated with the formation of key elements of the musculoskeletal and nervous systems; thus, the escape response appears early in ontogeny, when these elements form. (2) Escape behavior is not directly associated with the formation of underlying morphological elements; instead, it appears at hatching (i.e. when needed). We find that rainbow trout, like zebrafish, respond to touch early in the incubation interval, but do not demonstrate a complete C-start (including the second, propulsive stage) until shortly before hatching. At hatching, rainbow trout and zebrafish are similar in the degree of development of the chondocranium, paired fins and visceral arches (which comprise the larval jaw and gill support); however, rainbow trout have incipient rays in their unpaired fins (dorsal, anal and caudal), whereas zebrafish retain the embryonic fin fold. Although rainbow trout are more mature in axial swimming morphology at hatching, the essential neural and musculoskeletal systems that produce a coordinated escape response are functional at hatching in both species. This finding supports the evolutionary hypothesis that an effective escape response is critical for the survival of newly hatched teleost fishes. *J. Exp. Zool. 307A, 2007.* © 2007 Wiley-Liss, Inc.

How to cite this article: Gibb AC. Liu C, Swanson BO. 2007. Heterochrony and the development of the escape response: prehatching movements in the rainbow trout *Oncorhynchus mykiss*. J. Exp. Zool. 307A:[page range].

The ability to escape a predator is important for individual survival and is likely under directional selection (O'Steen et al., 2002; Walker et al., 2005). In teleost fishes, a coordinated escape response, the C-start, is present in newly hatched individuals of many different species (e.g. Kimmel et al., '74; Fuiman, '93; Gibson and Johnston, '95; Shepherd et al., 2000; Sugisaki et al., 2001). Because 94–99% of mortality occurs before fish become juveniles (Houde, '94) and much of this mortality may occur due to predation (Cushing, '74; Leggett and Deblois, '94), it is important for teleosts to perform the escape behavior effectively during early life history stages.

The C-start behavior of larval, juvenile and adult fishes consists of two phases, an initial C-shaped bend about the center of mass (COM), followed by an S-shaped bend to the opposite side (Weihs, '73). These movements are generated by lateral flexion of the axial skeleton via contraction of the myomeres (Eaton et al., '77a; Jayne and Lauder, '93; Goldbogen et al., 2005). This behavior requires the ability to sense a negative stimulus and then stimulate the axial musculature to generate the appropriate response. If a larval fish needs to evade predators as soon as it leaves the

---

Grant sponsor: National Institutes of Health; Grant number: T34GM065101; Grant sponsor: MARC U*STAR

*Correspondence to: Alice C. Gibb, Department of Biology, Northern Arizona University, Flagstaff, AZ, USA 86011-5640.
E-mail: Alice.Gibb@nau.edu

The current address of Brook O. Swanson is Department of Biology, Gonzaga University, Spokane, WA, USA 99258-0008.

Received 22 March 2007; Revised 25 June 2007; Accepted 26 June 2007

Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jez.409

chorion, then it must produce effective escape movements immediately upon hatching.

Extensive research on limb development in embryonic birds and mammals demonstrates that the neural circuitry for generating locomotor movements appears early in development, and that cyclic stepping behaviors can be observed weeks before hatching or birth (e.g. Bradley and Bekoff, '90; Muir, 2000). These movements are important for motor development and apparently allow the pathways that produce coordinated movements to be established before they are needed for locomotion. Indeed, the early establishment of these movement patterns likely contributes to the ability of animals with precocial (i.e. progeny are morphologically mature and mobile from birth or hatching) life history strategies to move effectively immediately after hatching or birth.

Like birds and mammals, teleost fish demonstrate a range of life history strategies, where some species produce altricial progeny (relatively immature and immobile at hatching) while others produce precocial progeny (as above). In teleosts, these strategies hinge on the presence or absence of a larval stage, and are often termed indirect (altricial; with a larval stage) and direct (precocial; without a larval stage) development (Balon, '81). Teleosts with indirect development typically provision their eggs with small yolks, and the progeny hatch and subsequently make the transition from endogenous (yolk) feeding to exogenous feeding with immature (larval) morphology. Teleosts with direct development provision their eggs with large yolks, and may even be ovoviviparous (i.e. eggs develop inside the female); progeny complete their development in the chorion and enter the external environment with adult morphology (but are considered juveniles, as they are reproductively immature). Some teleosts employ an ''intermediate'' strategy, where eggs are provisioned with large yolks and progeny demonstrate a truncated larval stage. In this strategy, progeny may hatch at an advanced stage of morphological development and do not begin exogenous feeding until they approach adult morphology (Balon, '81; Pavlov and Osinov, 2004).

Most studies of escape behavior in newly hatched teleosts have focused on species with indirect developmental strategies. C-starts are present at (or immediately after) hatching in cod, drum, pollock, herring, various species of flatfish, carp, razorback sucker and zebrafish (Kimmel et al., '74; Bailey and Batty, '84; Yin and Blaxter, '87; Gibson and Johnston, '95; Wakeling et al., '99; Wesp and Gibb, 2003; Smith and Fuiman, 2004; Gibb et al., 2006). Thus, for both marine and freshwater teleosts with indirect development, coordination of the elements that produce the escape response is achieved before hatching, even though adult morphology has not been attained. Relatively few studies, have examined the escape response in species with intermediate development. However, C-start behavior is present at hatching in the few species with intermediate development examined to date (Hale, '99; Wesp and Gibb, 2003). Thus, it seems that coordinated C-starts are present at hatching in teleosts with indirect and intermediate development. Two hypotheses concerning the relationship between morphological and behavioral development could explain this pattern (Fig. 1):

(A) In species with intermediate development, key structures that enable the escape behavior may form early in development; when these structures reach a certain state of maturity the behavior could appear as a functional ''byproduct.'' Consequently, escape behavior would appear early in development, but only become apparent upon hatching. According to this hypothesis, we would expect the appearance of the escape behavior to be consistently associated with the formation of both elements of the musculoskeletal (i.e. myomeres and notochord) and nervous (i.e. sensory, inter- and motor neurons) systems.

(B) Alternately, the escape behavior may demonstrate heterochrony, or a change in the timing or rate of development of a particular trait in a given species, relative to the ancestral condition (Gould, '77). According to this hypothesis, we might expect the appearance of the escape response in species with intermediate development to be disassociated from development of specific features of the musculoskeletal and nervous systems. Here, the necessary morphology may form early in development, but the behavior may manifest only when it is useful to the individual—at or near hatching, when the behavior can be employed to evade predators.

Salmonids (trout, grayling, salmon, etc.) are the quintessential example of intermediate development (Balon, '81): embryos hatch from eggs at an advanced stage of morphological development

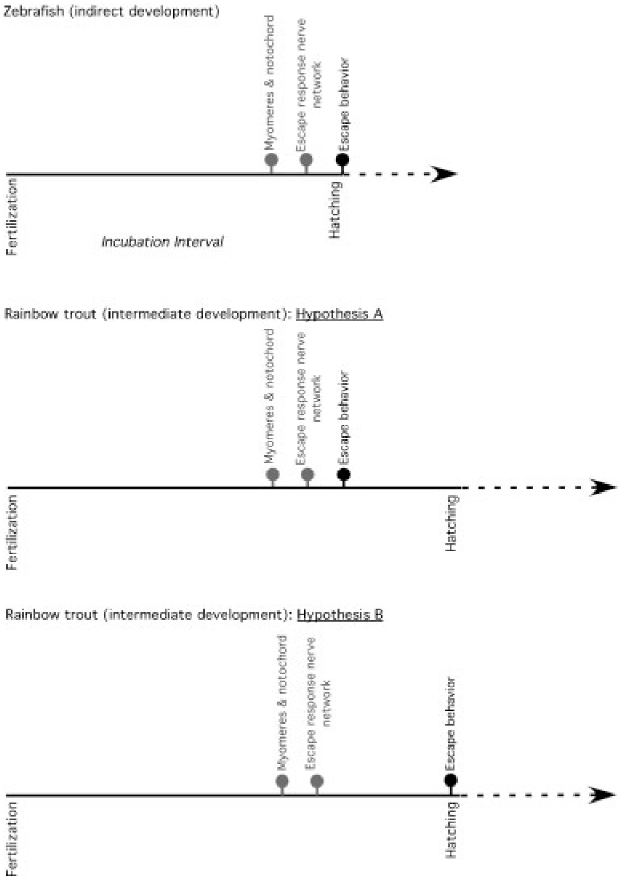

Fig. 1. A priori models of the relationship between development of key morphological elements and appearance of the C-start, or escape response behavior, in zebrafish *Danio rerio* (top panel) and rainbow trout *Oncorhynchus mykiss* (middle and lower panels). See text for details.

with a large yolk, and fish do not begin exogenous feeding until they approach juvenile morphology (Pavlov and Osinov, 2004). Previous studies have documented the presence of the C-start at hatching in several salmonid species (Hale, '99; Wesp and Gibb, 2003). However, although certain early motor behaviors have been described for this group (Whiting, '55; Wright et al., 2003), it is not known if the ability to perform a coordinated C-start appears before hatching, or if it appears at hatching. If it appears well before hatching, this would support hypothesis A (Fig. 1)—development of the escape response is associated with the formation of specific morphological features. If coordinated C-starts appear only at or near the time of hatching, this would suggest that escape behavior demonstrates developmental heterochrony (i.e. the timing of the appearance of the behavior has been changed relative to the ancestral condition) and support hypothesis B (Fig. 1).

Here, we artificially remove rainbow trout *Oncorhynchus mykiss* from their chorions (i.e. decapsulation) to examine motor behaviors in developing embryos; this allows us to determine when during development the escape response first appears and to assess the two hypotheses outlined above. In addition, we determine if the development of particular morphological structures occurs concomitantly with the appearance of escape behavior in rainbow trout, and we compare this pattern to that demonstrated by a teleost with indirect development, the zebrafish *Danio rerio*, by synthesizing information from the literature (e.g. Kimmel et al., '95; Saint-Amant and Drapeau, '98). Finally, we compare prehatching motor behaviors in trout to previous studies of prehatching motor behaviors in *Danio rerio* (Kimmel et al., '74; Saint-Amant and Drapeau, '98) to determine if embryonic motor behaviors are similar across divergent teleost species.

METHODS

Rainbow trout *Oncorhynchus mykiss* "eyed" eggs were obtained from Troutlodge, Sumner, Washington (April, 2004); eyed eggs represent approximately stage 23 of development (Ballard, '73). The eggs were divided among eight rearing chambers (43 cm long × 26 cm wide × 15 cm deep) and provided with filtered, recirculated fresh water at 12±1°C. The room containing the chambers was maintained on a light: dark photoperiod of 14 hr:10 hr. Eggs arrived approximately 5 days before their natural hatching date, were given 24 hr to acclimate to laboratory conditions, and were sampled every day after that until the day that natural hatching occurred, and then on one subsequent day. Thus, rainbow trout were sampled on days −4, −3, −2, −1, 0 and +1, relative to the day of natural hatching (day 0); these days represented approximately 85, 88, 92, 96, 100 and 105% of the total incubation interval (the time from fertilization to hatching) of 22 days at 12°C for this species (additional details below).

For the motor behavior experiments, eggs were removed from the rearing chambers and placed in large glass beakers maintained in a chilled water bath at 12±1°C. For an individual trial, an egg was removed from the beaker, immersed briefly in a ~0.1% pronase solution (to make the chorion rigid, Westerfield, '95), and then placed in a glass bowl with 12±1°C water. The bowl was placed under a dissecting scope, and two pairs of number 5 forceps were used to pinch the chorion and gently tear it open. After the embryo was freed from the chorion, it was transferred to another small glass bowl (6 cm diameter × 2 cm deep) containing freshwater for the imaging trials.

A high-speed, digital-imaging system was used to record both spontaneous and experimenter-initiated motor behaviors at 250 frames per second (Redlake PCI 1000s, Redlake Inc., Tucson, AZ 85706, USA). During the trials, the camera was mounted above the testing chamber with the lens parallel with the water surface, a 0.5-cm grid was placed below the testing chamber for scale, and the chamber was illuminated with a fiber optic light. A surrounding water bath connected to a recirculating water chiller maintained the water temperature in the experimental chamber at $12\pm1^{\circ}$C. Experimenter-initiated motor behaviors were elicited by using a small blunt probe to apply a noxious stimulus to the fish's yolk sac; spontaneous motor behaviors that occurred during the trial interval were also recorded, and two to seven responses (10–30 min trial time) were obtained for each individual. Over the course of the study, 41 individuals were examined, with a minimum of five individuals examined on any given day. After multiple motor behaviors were recorded, embryos were euthanized using a solution of 1% MS222 (3-aminobenzoic acid ethyl ester) and measured (length and mass).

Motor behaviors produced during the trials were replayed as digital movies and resulting movements described and categorized. Two previous studies on development of motor behaviors in zebrafish (*Danio rerio)* outlined several categories of motor behaviors that appeared during development in this species (Kimmel et al., '74; Saint-Amant and Drapeau, '98). Behaviors exhibited by rainbow trout appeared similar to those described for zebrafish, so in most instances behaviors observed for rainbow trout were placed into categories proposed for zebrafish (see Results). Every recorded behavioral response ($n = 2$–7) was categorized for each of the 41 individuals, for a total of 188 responses across 6 days encompassing the day of natural hatching.

Previous studies of embryonic and larval morphogenesis were used to assess the timing of morphological development in rainbow trout and zebrafish (Leghissa, '42; Ballard, '73; Verraes, '73; Eaton et al., '77b; Kimmel et al., '95; Cubbage and Mabee, '96; Ali et al., 2000; Bird and Mabee, 2003; Ristovska et al., 2006). Rainbow trout and zebrafish are reared at different temperatures, and might have different developmental time courses even if rearing temperature were the same. Thus, the time between fertilization and hatching was standardized by dividing the time to a given developmental milestone or major event (e.g. the appearance of retinal pigment) by the total number of incubation days (time from fertilization to hatching). In this manner, the developmental events for rainbow trout and zebrafish can be compared to one another across a 0 to 100% incubation interval. Information about the development of motor behaviors for zebrafish (Kimmel et al., '74; Saint-Amant and Drapeau, '98) and early developmental stages of rainbow trout (Whiting, '55; Wright et al., 2003) were similarly collected from the literature and standardized. Although the morphological data and certain behavioral data used in this study were published previously, we note that this comparison represents a novel transformation and metaanalysis of this information.

RESULTS

Prehatching and posthatching movements in rainbow trout

Rainbow trout exhibited four prehatching motor behaviors similar to those described for zebrafish, and one motor behavior that was undescribed previously (Fig. 2). The five prehatching motor behaviors of rainbow trout were:

A Spontaneous, coiling contractions (''alternating, coiling contractions'' in Saint-Amant and Drapeau, 2000): without stimulation, embryos coiled the body and tail around the yolk sac repeatedly, alternating sides for each sequential coil.

B Touch-initiated, coiling contractions [type IV response (Kimmel et al., '74), ''touch-evoked rapid coils'' (Saint-Amant and Drapeau, 2000)]: after receiving a negative stimulus, embryos coiled the body and tail around the yolk sac repeatedly, alternating sides for each sequential coil; these were not obviously distinct from the spontaneous coiling contractions.

C Touch-initiated, single coil contraction (undescribed previously): embryos coiled the body and tail around the yolk sac a single time with no subsequent contralateral response.

D C-start only (type II response in Kimmel et al., '74): a coordinated escape response involving an initial C-bend, followed by a subsequent S-bend

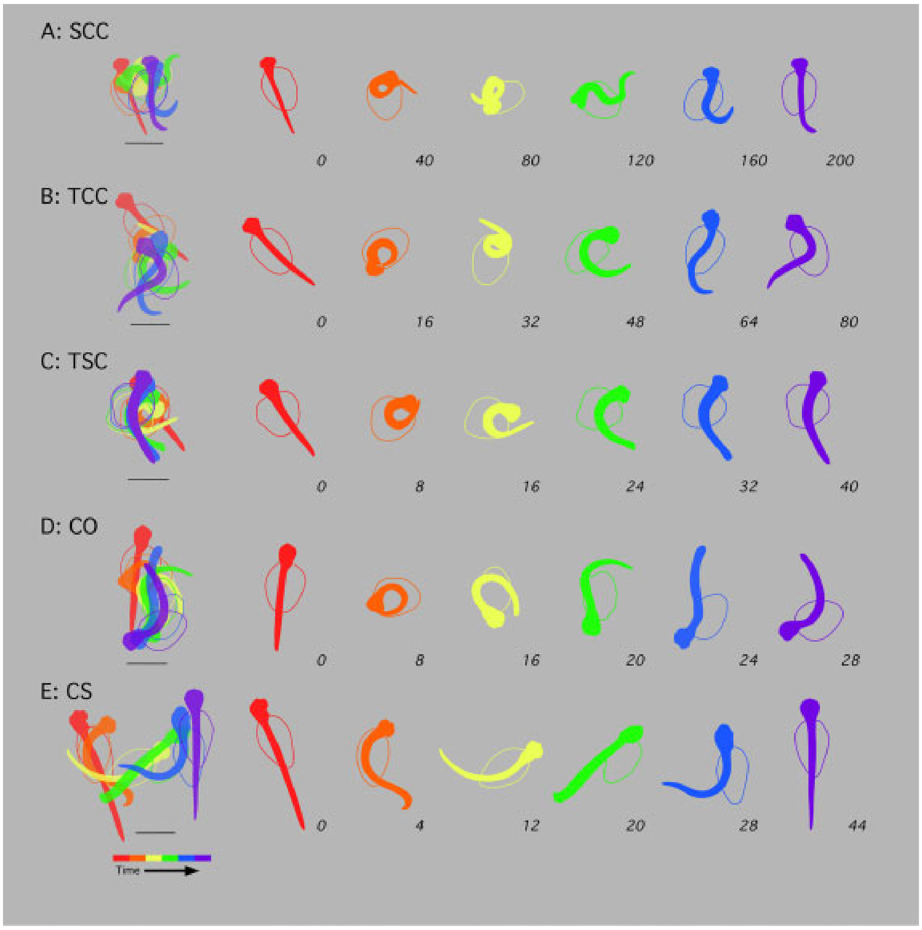

Fig. 2. Images depicting five motor behaviors repeatedly observed in embryonic and newly hatched rainbow trout: (**A**) Spontaneous, coiling contractions (“SCC,” Individual 1, 85% incubation interval). (**B**) Touch-initiated, coiling contractions (“TCC,” 15, 92%). (**C**) Touch-initiated, single coil contraction (“TSC,” 17, 92%). (**D**) C-start only (“CO,” 26, 100%). (**E**) C-start with swimming (“CS,” 30, 100%). Images were produced by tracing outlines of embryos directly from digital images; scale bar below each image sequence indicates 1 cm. Left column: sequence of six images overlaid to demonstrate displacement during the behavior; image color indicates relative order of each image, where red indicates the first image and violet the last (see legend in figure). Right columns: six images from each behavior shown individually to reveal postures adopted during the behavior. Time (in milliseconds) is given at lower right of each image, with the frame captured immediately before the fish began to move considered as time 0. The time increment between sequential illustrated images varies both among and within behaviors.

to the contralateral side, with no subsequent swimming.

E C-start with swimming (type III response in Kimmel et al., ’74): a coordinated escape response involving an initial C-bend, then a subsequent S-bend to the contralateral side, followed by free swimming; in this behavior, the embryo often moves a significant distance (typically more than one body length).

All of these behaviors were observed in rainbow trout before hatching, although some behaviors appeared later in the incubation interval than others (Fig. 3).

The youngest rainbow trout examined in this study demonstrated the ability to respond to negative stimuli. Indeed, at 85% of the incubation interval (4 days before hatching), rainbow trout displayed three of the five behaviors described above: (A) spontaneous, coiling contractions, (B) touch-initiated, coiling contractions, and (C) touch-initiated, single coil contraction (Fig. 3). These three behaviors were also the only ones observed at 88% of the incubation interval, or three days before hatching (Fig. 3). Although two of these three behaviors (touch-initiated, coiling contractions and touch-initiated, single coil contraction) represent the ability to respond to a

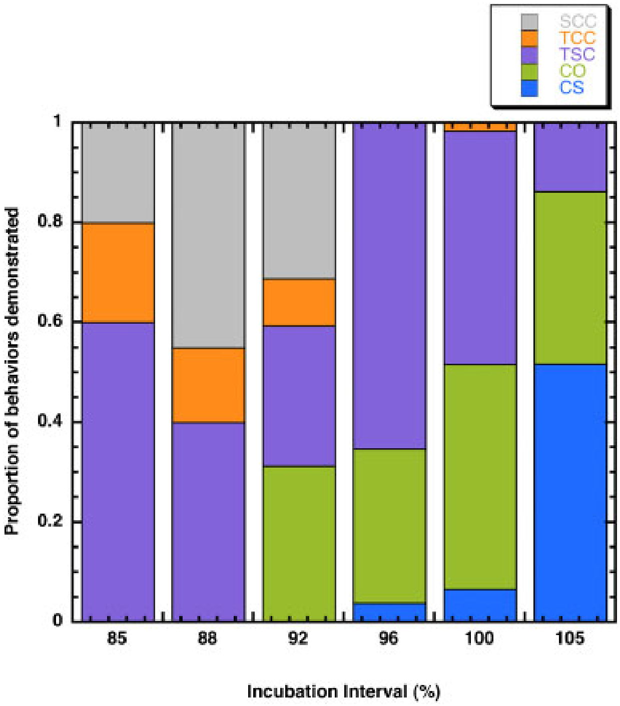

Fig. 3. Bar graph depicting motor behaviors demonstrated by embryonic and newly hatched rainbow trout during the later stages of the incubation interval, where 100% is the day of natural hatching. Behaviors are color-coded as follows: gray, spontaneous, coiling contractions (“SCC”); orange, touch-initiated, coiling contractions (“TCC”); purple, touch-initiated, single coil contraction (“TSC”); green, C-start only (“CO”); blue, C-start with swimming (“CS”).

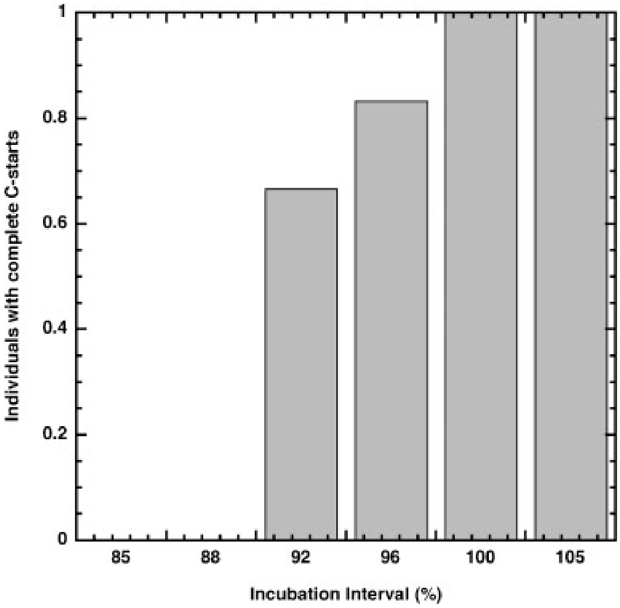

Fig. 4. Bar graph depicting appearance of a complete C-start (with both preparatory and propulsive phases) in individual rainbow trout during the incubation interval, where 100% incubation interval is the day of natural hatching. By the day of natural hatching, all individuals produced at least one complete C-start behavior.

negative stimulus, none of the three behaviors results in a rapid, efficient or reliable movement of the COM of the fish away from the negative stimulus. Rather, any movement of the COM that occurred during these behaviors appeared to be a byproduct of a series of uncoordinated contractions that occurred over a long period of time (e.g. Fig. 2B). In fact, the touch-initiated coiling contractions observed here may represent the ‘‘struggle’’ behavior that has been well characterized in embryos of *Xenopus laevis* (Kahn and Roberts, ’82) and also reported in zebrafish (Hale et al., 2001).

Effective escape behaviors, defined here as behaviors that could rapidly and reliably move the COM away from a negative stimulus, appeared in close proximity to the day of natural hatching. At 92% of the incubation interval (2 days before hatching), some individuals produced a coordinated and complete (i.e. with a preparatory and propulsive phase) escape response, or C-start (Fig. 3). In fact, four out of six individuals produced at least one coordinated C-start on this day (Fig. 4), although the other motor behaviors recorded for these individuals typically were not C-starts (Fig. 3). At 96% of the incubation interval (1 day before natural hatching), five out of six individuals produced at least one C-start or C-start with swimming, and by the day of natural hatching (100% of the incubation interval) all ten individuals produced at least one C-start or C-start with swimming (Fig. 4). Thus, the C-start behavior appeared at 92% of the incubation interval, and all fish were capable of producing complete C-starts by the day of hatching. Conversely, less effective behaviors (i.e. behaviors A–C above) appeared to decline in frequency across development (Fig. 3).

Morphological development

Zebrafish hatch after only 3 days of incubation at 28.5°C (Kimmel et al., ’95), whereas rainbow trout hatch after approximately 22 days at 13°C (Ballard, ’73). In addition, because of their developmental strategies (zebrafish, indirect; trout, intermediate), our a priori expectation was that trout would hatch at an advanced state of morphological development. However, a comparison of major developmental events taken from the literature (Leghissa, ’42; Ballard, ’73; Verraes, ’73; Eaton et al., ’77b; Kimmel et al., ’95; Cubbage and

Mabee, '96; Ali et al., 2000; Bird and Mabee, 2003; Ristovska et al., 2006), and compared across the incubation interval (time from fertilization to hatching) for rainbow trout and zebrafish, revealed several similar developmental patterns (Table 1).

Three events relating to the circulatory system occurred approximately the same time in the incubation period for both species. The heart begins beating very early in zebrafish and trout (33% incubation interval for both species) and blood flow is evident in the second through fourth aortic arches (which serve the gills) at approximately two-thirds of the way through the incubation interval (67% zebrafish, 64% trout). Gill filaments begin to form at around the time of hatching in both species.

The development of the chondocranium also showed a generally similar pattern across species. Both species hatched with substantial chondrification (cartilage formation) in the base of the neurocranium (the basal plate, trabecular bar, ethmoid plate, etc.) and in the anterior visceral arches (e.g. Meckel's cartilage and the palatoquadrate). However, trout were more morphologically mature in a few aspects of development of the chondocranium; for example, the otic capsule shows some chondrification in newly hatched trout, but not in newly hatched zebrafish.

TABLE 1. Developmental milestones (major events) observed during the incubation interval (time between fertilization and hatching) for zebrafish Danio rerio and rainbow trout Oncochynchus mykiss as summarized from the literature[1–13] and the present study

	Percent incubation interval (%)	
Developmental event	Zebrafish	Rainbow trout
Morphological and physiological		
V-shaped trunk somites	22[1]	32[2]
Heart begins to beat	33[1]	33[10]
First trunk pigment	33[1]	68[2]
First retina pigment	42[1]	82[2]
Tail free of yolk sac; "straight tail"	42[1]	45[2]
Pectoral fin apical ridge prominent	58[1]	41[2]
Circulation present in 2–4 aortic arches	67[1]	64[2]
Paddle-shaped pectoral fins	83[1]	82[2]
Gill filaments form	100[1]	100[2]
Head-trunk angle ∼180°	100[1]	100[2]
Mauthner neuron with measurable activity (patch clamp)	36[9,13]	Unknown
Base of chondocraium (e.g., ethmoid and basal plates, trabecular bar) present	100[5]	100[6,7]
Anterior visceral arches (inc. palatoquadrate and Meckel's cartilage) present	100[5]	100[6,7]
Chondrofication of the otic capsule begins	Posthatching[5]	100[6,7]
Caudal fin flexion	Posthatching[8]	64[2]
Fin rays form in caudal fin	Posthatching[8]	82[2]
Fin rays form in medial fins	Posthatching[8]	100[2]
Fin rays form in pelvic and pectoral fins	Posthatching[8]	Posthatching[2]
Behavioral		
Spontaneous coiling contractions appear	24[9]	36[10]
"Touch response" appears	29[9]	52–57[10,11]
Spontaneous coiling contractions disappear	36[9]	91[14]
Effective C-start (with stage 2) appears	100[12]	91[14]

[1] Kimmel et al. ['95].
[2] Ballard ['73].
[3] Eaton et al. ['77b].
[4] Leghissa ['42].
[5] Cubbage and Mabee ['96].
[6] Ristovska and others [2006].
[7] Verraes ['73].
[8] Bird and Mabee [2003].
[9] Saint-Amant and Drapeau ['98].
[10] Whiting ['55].
[11] Wright et al. [2003].
[12] Kimmel et al. ['74].
[13] Ali et al. [2000].
[14] Present study.

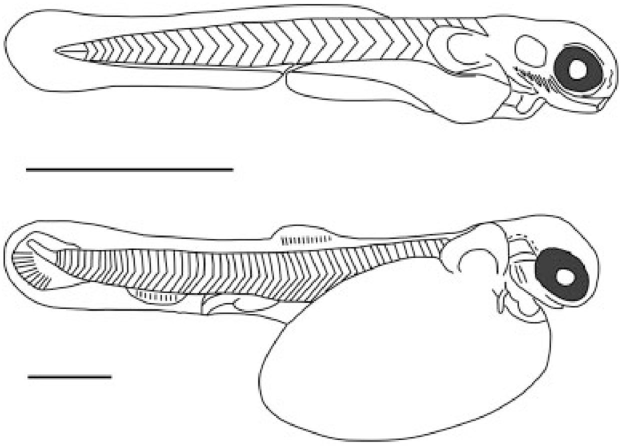

Fig. 5. Line drawings of newly hatched zebrafish and rainbow trout, after Kimmel et al. (1995) and Ballard (1973), respectively. Scale bar below each fish represents 1 mm. Upper panel: a zebrafish *Danio rerio* after 72 hr (3 days) development at 28.5°C. Lower panel: a rainbow trout *Oncorhynchus mykiss* after approximately 22 days of development at 13°C. Both species hatch with a well-developed eye, both possess cartilage precursors to the visceral arches and the lower jaw (Meckel's cartilage) and paddle-shaped pectoral fins. However, in the rainbow trout, the caudal fin of has undergone flexion and the cartilage precursors of fin rays have appeared; in the zebrafish, the embryonic fin fold is retained.

Several events related to paired fin formation also occurred at similar points in the incubation interval for both species. The apical ridge of the pectoral fin formed approximately mid-way through the incubation interval in both zebrafish (58%) and trout (41%), and the fin became paddle-shaped shortly before hatching (zebrafish, 82%; trout, 83%). However, pelvic fins are not formed before hatching in either species, and pelvic and pectoral fin rays ossify after hatching in both trout and zebrafish.

Several events potentially relevant to the development of the escape response occur at similar times during the incubation interval in both species: the tail straightens away from yolk sac approximately midway through the incubation interval (zebrafish, 42%; trout, 45%); the angle between head and trunk approaches 180° and the Mauthner neurons (key interneurons of the central nervous system that coordinate the escape response; Weiss et al., 2006) achieve adult morphology at hatching. In addition, trunk somites develop the V-shaped morphology necessary for axial bending early in the incubation interval in both species (zebrafish, 22%; trout, 32%).

However, the comparison of trout and zebrafish embryonic development also yielded evidence of differences in the timing of particular developmental events (Table 1). Development of both trunk and retina pigment occurred much earlier in zebrafish than trout, although the relative order of pigment appearance (first trunk, then retina) remained unchanged. In contrast, formation of the unpaired (dorsal, anal and caudal) fins occurred much earlier in trout than in zebrafish. In trout, caudal fin flexion (dorsal flexion of the notochord in the developing tail) and formation of the fin rays in the dorsal, anal and caudal fins all began before hatching, whereas these events occur during the larval period (posthatching) in zebrafish (Table 1; Fig. 5).

Behavioral development

Rainbow trout demonstrate several prehatching behaviors that are similar to those described previously for zebrafish. These behaviors appear in the same order as in zebrafish, but later during the incubation interval (Table 1). The first distinct motor behaviors demonstrated by zebrafish are spontaneous, coiling contractions (24% of incubation interval); this is also the first motor behavior to appear in rainbow trout (36% of incubation interval). The touch response next appears in the zebrafish (29%), followed by the disappearance of spontaneous, coiling contractions (36%), and finally by the appearance of the coordinated and complete C-start (~100%). The same sequence is found in developing rainbow trout, although it occurs entirely in the second half of the incubation interval: the touch response appears at 52–57%; the spontaneous, coiling contractions disappear and the C-start appears at 92% of the incubation interval. Interestingly, the C-start appears at approximately the same time the Mauthner neuron becomes electrically active in the zebrafish (Table 1).

DISCUSSION

We initially assumed, based on the work of others (Adriaens and Vandewalle, 2003; Pavlov and Osinov, 2004; Ristovska et al., 2006), that the large-yolked rainbow trout embryo would hatch at an advanced stage of morphological maturation, relative to the small-yolked zebrafish embryo. On the basis of this assumption, we considered two alternate hypotheses: (A) C-start development is associated with the development of key morphological features and appears early in trout ontogeny, or (B) C-start development is not directly associated with development of key morphological features and appears at or near hatching. We found that a coordinated C-start appears in rainbow trout immediately before hatching. However, we also determined that the developmental trajectories of rainbow trout and zebrafish were more similar than we had expected, and that our a priori assumption that rainbow trout are more morphologically mature at hatching was only partially correct. In fact, trout and zebrafish are very similar in many aspects of their morphological development at hatching. Many structures (heart, aortic arches, visceral arches, gill filaments, pectoral fins) appear or become functional at similar increments of the incubation interval for both species.

Although some cranial elements, aspects of the circulatory system and the paired fins had achieved a similar level of development in the two species by hatching, the rainbow trout were more mature in their axial swimming morphology, specifically formation of the unpaired fins. Thus, our initial expectation that the skeletal elements of the rainbow trout would be more mature at hatching was true for the dorsal, anal and caudal fins (Fig. 5). We have shown previously (Gibb et al., 2006) that the development of the caudal fin enables improved escape response performance because fins stiffened by bony rays provide more effective thrust-producing surfaces (Weihs, '73) than the larval fin fold. Thus, because of the biomechanical ramifications of fin formation, a newly hatched rainbow trout should produce a more effective posthatching escape response than a zebrafish (Gibb et al., 2006). We also posit that it is difficult for rainbow trout to displace the large mass of the body plus the yolk sac during the escape response. However, it is difficult to ascertain cause and effect: do rainbow trout have better developed unpaired fins to overcome body and yolk sac inertia during escape responses, or do rainbow trout develop well-formed unpaired fins because they have a large supply of energy in their yolk sac available for skeletogenesis?

Development of the unpaired fins shows a highly conserved pattern across teleost groups, and *Hox* genes likely regulate this process (Mabee et al., 2002). A comparison of unpaired fin development in zebrafish and rainbow trout supports this hypothesis. Although the role of the anal and dorsal fins in the escape response is not yet well understood, it is clear that the caudal fin is key in producing acceleration during the escape response (Webb, '77). In fact, all three unpaired fins show a change in developmental timing in rainbow trout relative to zebrafish: all three fins form *early* relative to the development of the neurocranium, visceral arches, paired fins, the circulatory system and gill elements (Fig. 6). A change in the timing of the expression of a suite of *Hox* genes may allow these fins to be produced during embryonic development in rainbow trout, whereas the same suite of genes is likely expressed later, during larval development, in zebrafish.

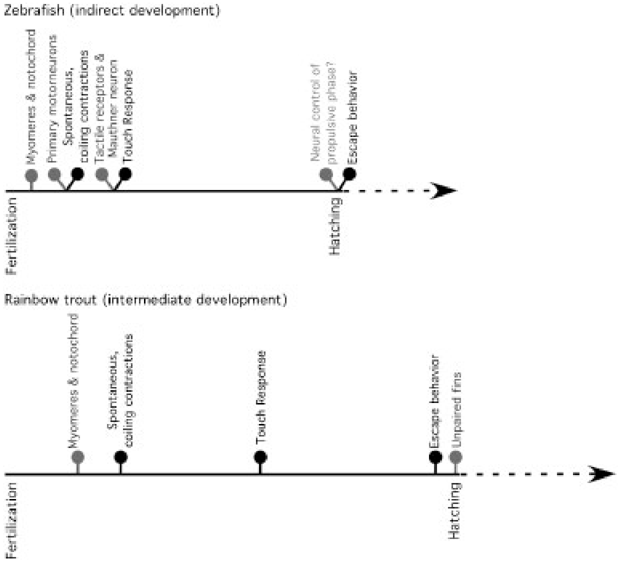

Fig. 6. Modified model of relationship between morphological, neurophysiological and behavioral development for zebrafish (top panel) and rainbow trout (lower panel).

To complete an effective escape response, a fish must be able to sense a noxious stimulus using the mechanosensory (i.e. lateral line neuromasts or tactile receptors) or other sensory system (visual, olfactory, etc.), transmit this information through a series of interneurons in the hindbrain, activate the axial muscles of the contralateral side of the body (preparatory phase), and then activate a portion of the ipsilateral axial muscles (propulsive stroke). Thus, this behavior requires adequate development of sensory, neuronal processing and motor systems (including motor neurons and axial muscles). On the basis of behavioral data for rainbow trout and zebrafish, it is clear that certain elements of this system are functional well before an effective C-start is produced.

(1) Spontaneous coiling contractions appear early in embryonic development, indicating that the nervous system is capable of stimulating the muscles to produce axial bending. In zebrafish, these spontaneous motor behaviors appear at the same time the primary motor neurons innervate the axial musculature appear and

become electrically active (Saint-Amant and Drapeau, '98, 2000).

(2) Embryos are able to respond to touch before an effective C-start appears, which reveals that afferent sensory systems have developed. In larval zebrafish, mechanosensory (tactile) neurons on the head and tail begin to transmit information to the central nervous system at the same time the touch response appears (Kimmel et al., '81; Drapeau et al., 2002).

(3) Incoming sensory information is coordinated with motor output to produce the touch response. In zebrafish embryos, the Mauthner neuron is active during the touch response (Saint-Amant and Drapeau, '98; Drapeau et al., 2002). This hindbrain interneuron is a component of a ''brainstem escape network'' (Eaton et al., 2001) and initiates the preparatory phase (the ''C'') of a C-start in larval, juvenile and adult fishes. However, it does not initiate the subsequent propulsive (the ''S'') phase required to complete the escape response.

Thus, although the majority of the components needed to trigger the escape response appear to be present by midway through the incubation interval in both species, a complete C-start (with both preparatory and propulsive phases) does not appear until at or near hatching. Before the approximate time of hatching, embryos of both species apparently lack the ability to produce the propulsive tail beat that completes the escape response and allows the COM to be moved rapidly away from a negative stimulus. Work in zebrafish and goldfish suggests that ''A2'' reticulospinal neurons (interneurons that comprise one element of the complex ''brainstem escape network'') coordinate the axial movements produced during the propulsive tail beat (Eaton et al., 2001). These interneurons may be inactive or immature until near hatching in both rainbow trout and zebrafish; however, neurophysiological data are necessary to test this hypothesis.

On the basis of the patterns we observed, we reject our a priori models of development as too simplistic. In both rainbow trout and zebrafish, the musculoskeletal elements that create the movements that enable the C-start form early in development; however, the behavior itself does not appear until near hatching. In zebrafish, the morphological and physiological maturation of elements of the nervous system that control and coordinate the escape behavior come ''on-line'' one by one, and each of these neurophysiological events is associated with the appearance of a specific motor behavior (Fig. 6). We assume that when these same motor behaviors appear in rainbow trout that they are triggered by similar neurophysiological events, although further work is necessary to test this assumption. In many ways rainbow trout morphological and behavioral development was quite similar to zebrafish development, but stretched out over a longer incubation interval, and this change may be driven, at least in part, by temperature. The only clear change in developmental timing, and possible incidence of heterochrony, was in the formation of the unpaired fins, which formed much earlier in the rainbow trout than in the zebrafish (Fig. 6).

CONCLUSIONS

Because many teleost embryos hatch and become free swimming while retaining the yolk sac, hatching has sometimes been considered an arbitrary developmental milestone. Complete absorption of the yolk has been proposed to represent a more critical functional milestone because fish must transition from endogenous to exogenous feeding (Balon, '81). We posit that hatching is also an important milestone, as before hatching a young fish has no way to evade its potential predators and must rely on parental care and/or crypsis to avoid being consumed. In contrast, after hatching, a young fish potentially has the ability to swim, or at least to displace its COM, when attacked by a potential predator. Thus, the incubation interval (time between fertilization and hatching) allows the embryo to develop the morphological features and behaviors that are important for predator evasion.

We found that escape responses appear late in embryonic development in rainbow trout, and we predict that this is also true for other species that provision their embryos with large yolks. Rainbow trout, like zebrafish, develop the escape response very shortly before hatching (i.e. when it is needed), and the appearance of this behavior in both species is associated with the appearance of the propulsive phase of the escape response. We also found that rainbow trout hatch from the chorion with unpaired fins that are more mature than those of the zebrafish, and our previous biomechanical studies suggest that these morphological differences will allow rainbow trout to produce a more effective escape response (Gibb et al., 2006).

These results reveal the complexity of developmental strategies possible for fishes. Teleosts are not simply ''indirect,'' ''intermediate'' or ''direct'' developers, but rather allocate resources differentially to particular structures (i.e. those critical for producing feeding or swimming behaviors) to facilitate success in specific aspects of their life history. Embryonic rainbow trout may allocate energy to fin formation because their large yolk sac delays the demand for exogenous feeding. In contrast, larval zebrafish may use energy acquired during early larval development to fuel the formation of the osteocranium, which should improve their ability to catch prey (Hernandez, 2000; Hunt von Herbing, 2001). However, although trout and zebrafish demonstrate differences in aspects of morphological development, the essential neural and musculoskeletal systems required for the escape response become functional near hatching in both species, which points to the importance of escape behavior for the survival of newly hatched teleost fishes.

ACKNOWLEDGMENTS

We thank Matt O'Neill, Cinnamon Pace, Teresa Hunt and Mike Manning for technical assistance on the project and helpful feedback on early drafts of the manuscript. James Liao and an anonymous reviewer provided us with thoughtful comments on the final draft of this paper. Financial support for this research was provided by National Institutes of Health T34GM065101 MARC U*STAR grant to Fernando Monroy, Northern Arizona University.

LITERATURE CITED

Adriaens D, Vandewalle P. 2003. Embryonic and larval development in catfishes. In: Arratia G, Kapoor AS, Chardon M, Diogo R, editors. Catfishes. Enfield, NH: Science Publishers, Inc. p 639–666.

Ali DW, Drapeau P, Legendre P. 2000. Development of spontaneous glycinergic currents in the Mauthner neuron of the zebrafish embryo. J Neurophysiol 84:1726–1736.

Bailey KM, Batty RS. 1984. Laboratory study of predation by *Aurelia aurita* on larvae of cod, flounder, plaice and herring: development and vulnerability to capture. Marine Biol 83: 287–291.

Ballard W. 1973. Normal embryonic stages for salmonid fishes based on *Salmo gairdneri* and *Salvelinus fontinalis*. J Exp Zool 184:7–26.

Balon EK. 1981. Saltatory processes and altricial to precocial forms in the ontogeny of fishes. Am Zool 21:573–596.

Bird NC, Mabee PM. 2003. Developmental morphology of the axial skeleton of the zebrafish, *Danio rerio* (Ostariophysi: Cyprinidae). Dev Dyn 228:337–357.

Bradley NS, Bekoff A. 1990. Development of coordinated movement in chicks: I Temporal analysis of hindlimb muscle synergies at embryonic days 9 and 10. Dev psychobiol 23:763–782.

Cubbage CC, Mabee PM. 1996. Development of the cranium and paired fins in the zebrafish *Danio rerio* (Ostariophysi, Cyprinidae). J Morphol 229:121–160.

Cushing DH. 1974. The possible density-dependence of larval mortality and adult mortality in fishes. In: Blaxter JHS, editor. The early life history of fish. Berlin: Springer-Verlag. p103–111.

Drapeau P, Saint-Amant L, Buss RR, Chong M, McDearmid JR, Brustein E. 2002. Development of the locomotor network in zebrafish. Prog Neurobiol 68:85–111.

Eaton RC, Bombardieri RA, Meyer DL. 1977a. The Mauthner-initiated startle response in teleost fish. J Exp Biol 66:65–81.

Eaton RC, Farley RD, Kimmel CB, Schabtach E. 1977b. Functional development in the Mauthner cell system of embryos and larvae of the zebrafish, *Brachydanio rerio*. J neurobiol 8:151–172.

Eaton RC, Lee RKK, Foreman MB. 2001. The Mauthner cell and other identified neurons of the brainstem escape network of fish. Prog Neurobiol 63:467–485.

Fuiman LA. 1993. Development of predator evasion in Atlantic herring, *Clupea harengus L*. Anim Behav 45: 1101–1116.

Gibb AC, Swanson BO, Wesp H, Landels C, Liu C. 2006. Development of the escape response in teleost fishes: do ontogenetic changes enable improved performance? Physiol Biochem Zool 79:7–19.

Gibson S, Johnston IA. 1995. Temperature and development in larvae of the turbot *Scophthalmus maximus*. Marine Biol 124:17–25.

Goldbogen JA, Shadwick RE, Fudge DS, Gosline JM. 2005. Fast-start muscle dynamics in the rainbow trout *Oncorhynchus mykiss*: phase relationship of white muscle shortening and body curvature. J Exp Biol 208:929–938.

Gould, SJ. 1977. Ontogeny and phylogeny. Cambridge, MA: Harvard University Press.

Hale ME. 1999. Locomotor mechanics during early life history: effects of size and ontogeny on fast-start performance of salmonid fishes. J Exp Biol 202:1465–1479.

Hale ME, Ritter DA, Fetcho JR. 2001. A confocal study of spinal interneurons in living larval zebrafish. J Comp Neurobiol 437:1–16.

Hernandez LP. 2000. Intraspecific scaling of feeding mechanics in an ontogenetic series of zebrafish, *Danio rerio*. J Exp Biol 203:3033–3043.

Houde ED. 1994. Differences between marine and freshwater fish larvae: implications for recruitment. ICES (International Council for the Exploration of the Sea). J Marine Sci 51: 91–97.

Hunt von Herbing IH. 2001. Development of feeding structures in larval fish with different life histories: winter flounder and Atlantic cod. J Fish Biol 59:767–782.

Jayne BC, Lauder GV. 1993. Red and white muscle activity and kinematics of the escape response of the bluegill sunfish during swimming. J Comp Physiol A Sens Neural Behav Physiol 173:495–508.

Kahn JA, Roberts R. 1982. The neuromuscular basis of rhythmic struggling movements in embryos of *Xenopus laevis*. J Exp Biol 99:197–205.

Kimmel CB, Patterson J, Kimmel RO. 1974. The development and behavioral characteristics of the startle response in the zebra fish. Dev psychobiol 7:47–60.

Kimmel CB, Sessions SK, Kimmel RJ. 1981. Morphogenesis and synaptogenesis of the zebrafish Mauthner neuron. J Comp Neurol 198:101–120.

Kimmel CB, Ballard WW, Kimmel SR, Ullmann B, Schilling TF. 1995. Stages of embryonic development of the zebrafish. Dev Dyn 203:253–310.

Leggett WC, Deblois E. 1994. Recruitment in marine fishes: is it regulated by starvation and predation in the egg and larval stages? Neth J Sea Res 32:119–134.

Leghissa S. 1942. Le basi anatomiche nella evoluzione del ''comportamento'' durante lo sviluppo embrionale e post-embrionale di Trota (Salmo fario, irideus e lacustris). Anat Embryol 111:601–675.

Mabee PM, Crotwell PL, Bird NC, Burke AC. 2002. Evolution of median fin modules in the axial skeleton of fishes. J Exp Zool 294:77–90.

Muir GD. 2000. Early ontogeny of locomotor behaviour: a comparison between altricial and precocial animals. Brain Res Bull 53:719–726.

O'Steen S, Cullum AJ, Bennett AF. 2002. Rapid evolution of escape ability in Trinidadian guppies (*Poecilia reticulata*). Evolution 56:776–784.

Pavlov DA, Osinov AG. 2004. Main features of early ontogeny in salmoniforms (Salmoniformes) and other representatives of the protacanthopterygii in relation to the phylogeny. Voprosy Ikhtiologii 44:293–312.

Ristovska M, Karaman B, Verraes W, Adriaens D. 2006. Early development of the chondrocranium in *Salmo letnica* (Karaman, 1924) (Teleostei: Salmonidae). J Fish Biol 68: 458–480.

Saint-Amant L, Drapeau P. 1998. Time course of the development of motor behaviors in the zebrafish embryo. J Neurobiol 37:622–632.

Saint-Amant L, Drapeau P. 2000. Motoneuron activity patterns related to the earliest behavior of the zebrafish embryo. J Neurosci 20:3964–3972.

Shepherd TD, Costain KE, Litvak MK. 2000. Effect of development rate on the swimming, escape responses, and morphology of yolk-sac stage larval American plaice, *Hippoglossoides platessoides*. Mar Biol (Berl) 137:737–745.

Smith ME, Fuiman LA. 2004. Behavioral performance of wild-caught and laboratory-reared red drum *Sciaenops ocellatus* (Linnaeus) larvae. J Exp Mar Biol Ecol 302:17–33.

Sugisaki H, Bailey KM, Brodeur RD. 2001. Development of the escape response in larval walleye pollock (*Theragra chalcogramma*). Mar Biol 139:19–24.

Verraes W. 1973. Contribution to the functional morphology of the constituting elements of the head of *Salmo gairdeneri*, Richardson, 1836 (Pisces, Teleostei) during postembrionic ontogeny with special attention to the cranium and muscles of the head. Belgium: University of Ghent.

Wakeling JM, Kemp KM, Johnston IA. 1999. The biomechanics of fast-starts during ontogeny in the common carp *Cyprinus carpio*. J Exp Biol 202:3057–3067.

Walker JA, Ghalambor CK, Griset OL, McKenney DM, Reznick DN. 2005. Do faster starts increase the probability of evading predators? Funct Ecol 19:808–815.

Webb PW. 1977. Effects of median-fin amputation on fast-start performance of rainbow trout (*Salmo gairdneri*). J Exp Biol 68:123–135.

Weihs D. 1973. The mechanism of rapid starting of slender fish. Biorheology 10:343–350.

Weiss SA, Zottoli SJ, Do SC, Faber DS, Preuss T. 2006. Correlation of C-start behaviors with neural activity recorded from the hindbrain in free-swimming goldfish (*Carassius auratus*). J Exp Biol 209:4788–4801.

Wesp HM, Gibb AC. 2003. Do endangered razorback suckers have poor larval escape performance relative to introduced rainbow trout? Trans Am Fisheries Soc 132:1166–1178.

Westerfield M. 1995. The zebrafish book: a guide for the laboratory use of zebrafish *Danio (Brachydanio) rerio*. Eugene: University of Oregon Press.

Whiting HP. 1955. Functional development in the nervous system. In: Waelsch H, editor. Biochemistry of the developing nervous system: proceedings of the first international neurochemical symposium. Magdalen College, Oxford, 13-17 July 1954: New York: Academic Press. p 85–103.

Wright PJ, Noltie DB, Tillitt DE. 2003. Comparison of prehatch C-start responses in rainbow trout and lake trout embryos by means of a tactile stimulus test. Trans Am Fisheries Soc 132:988–996.

Yin MC, Blaxter JHS. 1987. Escape speeds of marine fish larvae during early development and starvation. Mar Biol (Berl) 96:459–468.