

Development of the Escape Response in Teleost Fishes: Do Ontogenetic Changes Enable Improved Performance?*

Alice C. Gibb[†]

Brook O. Swanson[‡]

Heather Wesp[§]

Cydney Landels

Corina Liu

Department of Biological Sciences, Box 5640, Northern
Arizona University, Flagstaff, Arizona 86011-5640

Accepted 5/1/2005; Electronically Published 11/30/2005

ABSTRACT

Teleost fishes typically first encounter the environment as free-swimming embryos or larvae. Larvae are morphologically distinct from adults, and major anatomical structures are unformed. Thus, larvae undergo a series of dramatic morphological changes until they reach adult morphology (but are reproductively immature) and are considered juveniles. Free-swimming embryos and larvae are able to perform a C-start, an effective escape response that is used to evade predators. However, escape response performance improves during early development: as young fish grow, they swim faster (length-specific maximum velocity increases) and perform the escape more rapidly (time to complete the behavior decreases). These improvements cease when fish become juveniles, although absolute swimming velocity (m s^{-1}) continues to increase. We use studies of escape behavior and ontogeny in California halibut (*Paralichthys californicus*), rainbow trout (*Oncorhynchus mykiss*), and razorback suckers (*Xyrauchen texanus*) to test the hypothesis that specific morphological changes improve escape performance. We suggest that formation of the caudal fin improves energy transfer to the water and therefore increases thrust production and swimming velocity. In addition, changes to the axial skeleton during the larval period produce increased

axial stiffness, which in turn allows the production of a more rapid and effective escape response. Because escape performance improves as adult morphology develops, fish that enter the environment in an advanced stage of development (i.e., those with direct development) should have a greater ability to evade predators than do fish that enter the environment at an early stage of development (i.e., those with indirect development).

Introduction

Teleost fishes typically emerge from their chorions and encounter the environment as free-swimming embryos, which rely on yolk for nutrition, or larvae, which obtain nutrition from the environment (see Balon 1999). These early stages are morphologically distinct from juveniles and adults, and many structures are incompletely formed. For example, fins and jaws exist only as rudimentary structures in young larvae, if at all. These structures form and mature across larval development until they reach adult morphology, at which point the fish are considered juveniles. Interestingly, early life-history stages are characterized by extreme mortality, and mortality decreases exponentially as fish grow older (for a review, see Houde 1997).

Development is composed of two interrelated processes: growth, typically an increase in both length and mass, and ontogenetic changes, the formation of new structures or the modification of existing ones (Fuiman and Higgs 1997). Both of these processes likely contribute to a decreased vulnerability to predation across development and an increased ability to procure food from the environment (Werner and Gilliam 1984; Houde 1997). An increase in absolute size may be beneficial because it allows fish to reach a "size refuge," where they simply become too big to be handled by potential predators (Miller et al. 1988). Ontogenetic changes, such as the formation of the jaws and eyes, improve a fish's ability to capture prey and to detect and evade predators (Fuiman and Higgs 1997). However, size and shape transformations occur concomitantly. Consequently, it is difficult to determine precisely which developmental changes improve the performance of ecologically relevant tasks and thus contribute to decreased mortality in older fishes.

The ability to evade predators may be particularly important during early life-history stages (Houde 1997). The most common escape response behavior exhibited by teleost fishes across

* This article was presented at the symposium "The Ontogeny of Performance in Vertebrates," Seventh International Congress of Vertebrate Morphology, Boca Raton, Florida, 2004.

[†]Corresponding author; e-mail: alice.gibb@nau.edu.

[‡]Present address: Department of Biology, Pacific University, 2043 College Way, Forest Grove, Oregon 97116.

[§]Present address: Montcalm Community College, 2800 College Drive, Sidney, Michigan 48885.

all life-history stages is a rapid acceleration involving bending about the center of mass followed by a single propulsive tail stroke and burst swimming (Fig. 1). These escape responses (or C-starts) are employed by fishes to escape attacks by invertebrate (e.g., Seale and Binkowski 1988; Horn et al. 1994) and vertebrate predators (e.g., Webb 1976; Katzir and Camhi 1993). Correlations between escape performance and survival have been demonstrated by several studies (Swain 1992a, 1992b; O'Steen et al. 2002), making studies of escape performance relevant for evaluating the potential effects of predation on early life-history stages of fish.

Studies of scaling in the escape responses of adult fish suggest that individuals become slower as they grow larger because of body allometry. Typically, larger fish take longer to complete a behavior, and they swim more slowly in body lengths per second (e.g., Webb 1976; Domenici and Blake 1993; James and Johnston 1998). This pattern is apparently a result of the allometric relationship between the mass of fish that must be moved during an escape response and the cross-sectional area of the axial musculature used to generate the force necessary to move. Assuming isometric growth, as a fish grows larger, cross-sectional area increases with the square of length ($A \approx L^2$), whereas mass increases with the cube of body length ($M \approx L^3$). Thus, our a priori expectation is that maximum length-specific swimming speed during an escape behavior will decrease as fish grow larger. Additionally, it may take longer to produce the behavior, because the absolute distance covered by body elements will increase as fish grow larger.

However, several species of salmonids (i.e., trout, salmon, and their relatives) are known to improve their escape performance as they mature from larvae into juveniles (Hale 1999). This improvement is likely a function of ontogenetic changes; during this time period, fish undergo a change in overall body shape and development of structures (e.g., true fins) that should improve swimming performance. However, precisely which on-

togenetic changes account for improvement is not known. Here we consider five developmental changes that have potential ramifications for escape performance.

1. Absorption of the yolk and associated changes in body shape. Many young teleosts possess a large yolk sac that contributes to body inertia but does not contribute to thrust production (Hale 1999). Thus, as yolk is converted to body tissue, it may improve the ability of a fish to perform the escape response.

2. Changes in hydrodynamic regime. The hydrodynamic environment experienced by a fish depends on the physical properties of water (i.e., viscosity and density), the size of the fish, and the velocity at which the fish moves through the water (Webb and Weihs 1986). During ontogeny, the hydrodynamic regime experienced by a fish must change because it will become both larger and faster. In particular, the ratio of inertial to viscous forces acting on the fish will change, and this can be estimated by calculating the Reynolds number (Weihs 1980). This shift in hydrodynamic regime contributes to changes in routine swimming behavior as fish develop (Weihs 1980; Webb and Weihs 1986; Fuiman and Webb 1988; Osse and van den Boogaart 2000) and could also affect escape performance (Hale 1996).

3. Formation of true fins. Free-swimming embryos and young larvae possess shallow fin folds that are not stiffened by cartilaginous or bony elements. It has been suggested that escape performance improves as true fins form (Hale 1999) presumably because true fins stiffened by bony rays create a deeper, thinner, low-mass fin that will improve the transfer of momentum between the body and the surrounding fluid (Weihs 1973).

4. Formation and coordination of the nervous system. Studies on escape responses of young fishes have shown that free-swimming embryos of some species produce uncoordinated escape responses shortly after hatching (Kimmel et al. 1974;

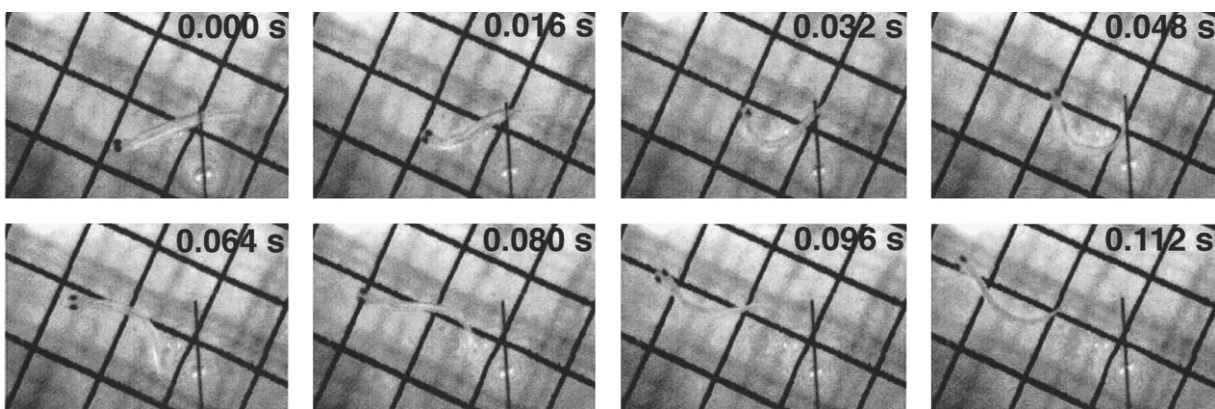


Figure 1. These images depict a razorback sucker performing an escape response. A 0.5-cm grid is shown in the background; time from the beginning of the response is shown in seconds. The first four panels depict the preparatory phase (the "C"), the next two depict the propulsive phase, and the final two depict free swimming. This figure is modified from Wesp and Gibb (2003).

Wesp and Gibb 2003). Escape performance may improve across early life-history stages because the ability to integrate sensory input and motor output improves as organisms develop (Carrier 1996).

5. Formation of the vertebral column. In free-swimming embryos and young larvae, the notochord stiffens the body. Formation of the vertebral column has been hypothesized to improve escape response performance (Hale 1999) presumably by increasing the ability of the axial skeleton to transmit propulsive forces from the axial musculature to the caudal fin (Long et al. 2002; Long and Nipper 1996).

In this study, we use the comparative method to evaluate these five predictions about the ramifications of ontogenetic change on escape performance. To this end, we examine escape response performance data from three species of teleost fish: California halibut (*Paralichthys californicus*), rainbow trout (*Oncorhynchus mykiss*), and razorback suckers (*Xyrauchen texanus*). Data were originally collected for these three species for other purposes (Gibb and Dickson 2002; Wesp and Gibb 2003). However, these three species represent distinct phylogenetic lineages with varied life-history strategies; this allows us to address the hypotheses outlined above and describe patterns in the ontogeny of escape performance across teleost fishes.

Material and Methods

Study Species

California halibut (Pleuronectiformes) are marine fish that produce many very small eggs. In this species, embryos hatch when they are approximately 2.1 mm in standard length and possess a small, round yolk mass. The free-swimming embryos quickly absorb the yolk (it is depleted by 3 d after hatching in embryos held at 18°–20°), and acquisition of adult morphology occurs during the larval period (Gisbert et al. 2002). This pattern of development is considered indirect development by Balon (1999).

Razorback suckers (Cypriniformes) are freshwater fish that produce small eggs. These eggs hatch when embryos are approximately 7.3 mm in standard length, and the free-swimming embryos have a small but elongate yolk sac. This yolk sac is rapidly absorbed (it is depleted by 7 d after hatching in embryos held at 13°–17°), and acquisition of adult morphology occurs during the larval period (Minckley and Gustafson 1982). This pattern of development is also considered indirect development by Balon (1999).

Rainbow trout (Salmoniformes) are freshwater fish that have relatively large eggs. Rainbow trout eggs hatch when trout are approximately 10.2 mm in standard length with a very large, oval yolk sac. This yolk sac is absorbed over a fairly long period of time (it is depleted by 20 d after hatching in embryos held at 18°), and many adult structures are formed during this extended free-swimming embryo, or eleutheroembryo, period (Hale 1999). Thus, rainbow trout become juveniles very soon

after absorbing the yolk and consequently have a very short larval period. This pattern of development is considered intermediate development by Balon (1999). These three species represent divergent teleost lineages that share a common ancestor near the origin of the teleosts (Nelson 1994).

Performance Data

All of the data used in this study come from two previously published and/or ongoing studies by A. C. Gibb and coworkers. We refer the reader to the published studies for additional details of the data collection methods (Gibb and Dickson 2002; Wesp and Gibb 2003); only a brief summary of the methods is provided here. Because the studies were originally conducted to address two different questions, slightly different methods were used for each study. However, several comparable kinematic variables were collected in the two studies.

California halibut *Paralichthys californicus* were obtained as larvae or juveniles from a local hatchery (Redondo Beach California Halibut Hatchery Program), held at 18°C in 1-L containers of filtered seawater, and fed daily on *Atermia* nauplii or squid pieces. Individual larvae were transferred to a small glass petri dish and videotaped with a Peak Performance Technologies high-speed video camera mounted over the dish, with the lens perpendicular to the base of the dish. Individual juveniles were transferred to small rectangular aquaria and videotaped from a lateral view (with the lens perpendicular to the side of the tank). This view is anatomically a dorsal view of the fish, since by the juvenile stage, California halibut have settled onto their right side (now the “blind” side), with their left side (or “eyed” side) oriented upward and toward the water column. Both videotaping arenas contained a calibrated grid in the field of view.

Escape responses were triggered by gently placing a blunt probe against the side of the fish. Escape movements for all three fish species always outpaced the movement of ripples produced in the water’s surface by the probe; thus, there was no distortion present in images used to calculate kinematic parameters (e.g., see Fig. 1). California halibut escape responses were recorded to VHS tape at 120 Hz, the maximum frame rate for the Peak Performance Technologies system. Although this recording rate is low relative to the digital imaging system used for the other two species reported here, it represents an adequate sampling rate for determining maximum velocity during an escape response (Walker 1998a). This video imaging process was repeated for 38 California halibut larvae and juveniles, and multiple escapes were recorded for each individual.

Sequential video images of each escape response were uploaded field by field to a Macintosh personal computer and saved as image files. Image files were transferred to a personal computer and analyzed using Didge custom image analysis software (Cullum 1999). This program uses the total length of the fish (obtained from postexperiment morphological mea-

surements) and 11 points along the midline of the fish to divide the fish into 10 equal-length segments mathematically. The 11 points anchoring these segments were tracked over time, and movements of the point closest to the center of mass were used to calculate displacement and velocity for each of three escape responses per individual. The mean value of these three responses was used for further analyses.

Razorback sucker *Xyrauchen texanus* eggs were obtained from the Willow Beach Fish Hatchery, Arizona. Rainbow trout *Oncorhynchus mykiss* eggs were obtained from the Lost River Trout Hatchery, Idaho. For each species, eggs were divided among eight tanks (43 cm long × 26 cm wide × 15 cm deep) with a 14L : 10D photoperiod. Each tank contained recirculated and filtered fresh water. Four tanks were maintained at 12°C and four at 18°C, and fish were fed daily on *Artemia* nauplii or a protein-rich dried food (razorback suckers were given a custom-formulated dried food containing plankton flakes, brine shrimp flakes, krill flakes, *Spirulina* flakes, and microencapsulated protein that was provided by the fish hatchery; rainbow trout were given a commercially available aquaculture feed). Fish were reared for the duration of embryonic and larval development and into early juvenile stages (approximately 2 mo). Individual fish were placed in a circular glass testing chamber (6 cm in diameter × 2 cm deep or 10 cm in diameter × 6 cm deep) and allowed to acclimate for at least 5 min. A 0.5-cm grid was placed below the testing chamber for scale, the chamber was illuminated with a fiber optic light, and a camera was mounted above the testing chamber with the lens perpendicular to the base of the dish. Escape sequences were recorded at 500 frames per second using a high-speed digital imaging system (Redlake Motionscope 1000). Escape responses were triggered by gently placing a blunt probe against the side of the fish. This process was repeated for 24 razorback suckers at 12°C and 39 at 18°C (e.g., see Fig. 1). Similarly, 28 rainbow trout were tested at 12°C and 30 at 18°C. Multiple escapes were recorded for each individual. Individuals included in these samples were selected from the rearing tanks at 2–3-d intervals over a 70-d rearing period to represent an ontogenetic series.

For razorback suckers and rainbow trout, the single best escape response for each individual, determined by the fastest maximum velocity and/or shortest response duration, was used for quantitative analysis. As above, Didge custom image analysis software (Cullum 1999) was used to determine the coordinates of 11 points along the midline of the fish. These points were tracked over time, and the point closest to the center of mass was used to determine the movement of the fish during the escape response. In this analysis, data from the consecutive frames were uploaded into QuickSAND software (Walker 1997), and a cubic-spline algorithm with an estimated error variance (based on the pixel to centimeter ratio) was used to reduce mathematically the effects of digitizing error, effectively smoothing the data. The program was also used to determine

velocity and acceleration by taking the first and second derivatives of the smoothed displacement data (Walker 1998a).

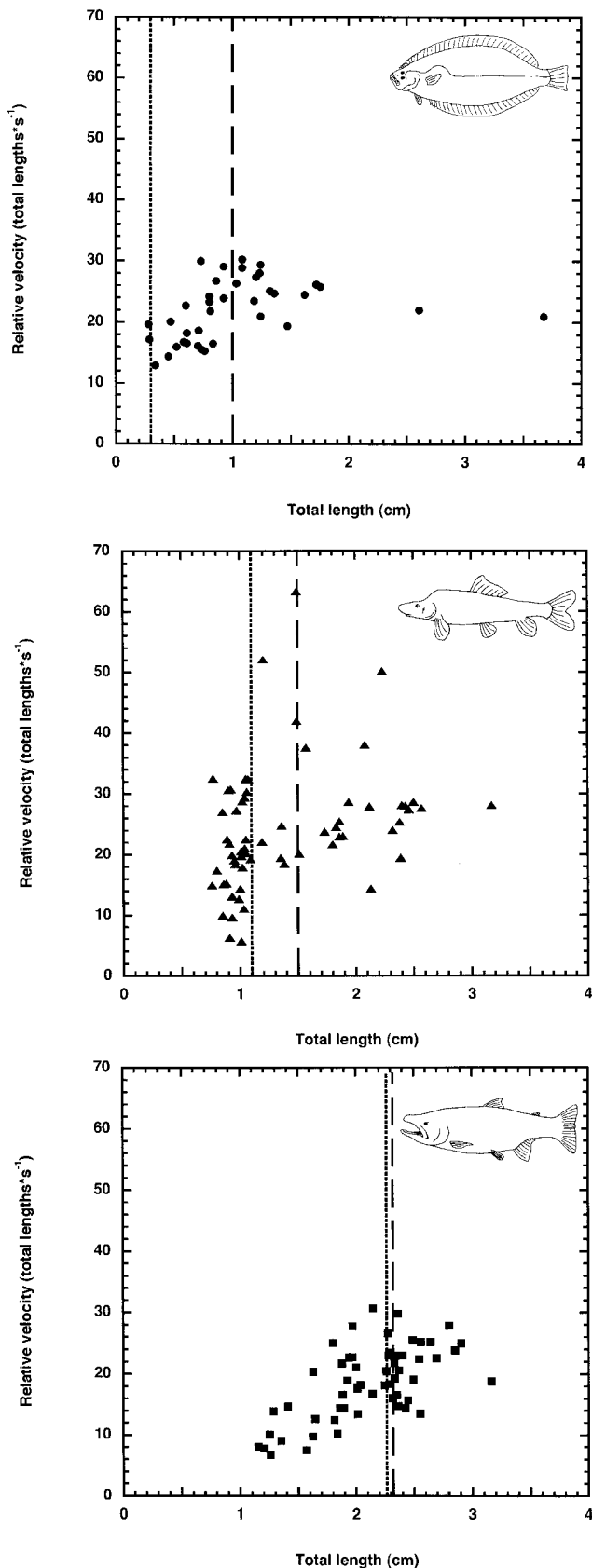
We also quantified bending produced during the preparatory phase for razorback suckers and rainbow trout by determining body curvature at nine positions along the body (using the 11 points to create 10% intervals along the entire body for the nine positions, with the tip of the snout representing 0% total length and the tip of the tail representing 100% total length, respectively). Length-specific curvature was calculated using the program QuicKurve (Walker 1998b). This program determines the radius of curvature at preassigned increments along the body and divides them by total fish length to produce length-specific curvature (or K) for each location on the body.

Data for razorback suckers and rainbow trout were originally collected as part of a temperature acclimation study. However, a two-way MANCOVA on performance variables (using species and temperature as potential effects and size as the covariate) indicated that there was no significant temperature effect after acclimation ($F = 1.1$, $P > 0.05$). Because there was no significant temperature effect, data for both temperatures have been combined for a given species (rainbow trout or razorback sucker) in this analysis (for details of the temperature acclimation study, see Wesp and Gibb 2003).

Developmental Changes and Performance Variables

Morphological observations were made using previously published descriptions of posthatching development (Minckley and Gustafson 1982; Gisbert et al. 2002) and using razorback sucker and rainbow trout individuals from our studies that we cleared and stained (Taylor 1967) to reveal the presence of cartilaginous and bony skeletal elements. Images of these specimens (with a calibrated grid in the background) were taken using a high-resolution digital camera mounted to a dissecting microscope and uploaded to a personal computer. Observations made from these images included both presence and degree of development of adult structures (e.g., formation of the caudal fin) and size of anatomical structures (e.g., notochord radius). All measurements of anatomical structures were taken from the digital images using Image-J (version 1.32) for Macintosh.

Plots were constructed of relevant performance data (e.g., maximum velocity, maximum length-specific velocity, time to maximum velocity) versus a metric of individual size (total length or mass), which served as a proxy for ontogenetic stage (Hale 1999). Morphological “milestones” (e.g., absorption of the yolk) were overlaid on performance plots to test hypotheses about correlations between performance and ontogenetic changes. To evaluate the relationships between performance and size statistically, data were log transformed and simple linear regressions were fitted to the data using Microsoft Excel (version 10 for Macintosh). For the analysis of maximum length-specific velocity, statistics were performed on data that were first arcsine transformed (to ensure the data were normally



distributed), and then log transformed to allow the use of a linear model to describe the data. The correlation coefficients (Pearson r values) from these regressions were then evaluated to determine whether the correlations were significant using a P value of 0.05.

Additional calculations for variables assessing fluid dynamics and biomechanical properties of the skeleton were made using morphological and performance variables measured for these fishes. Reynolds number (Re) was used to estimate the ratio of inertial to viscous forces experienced by free-swimming embryos, larvae, and juveniles at their maximum escape velocity and was calculated using the formula

$$Re = \rho V L \mu^{-1},$$

where ρ is the fluid density at a given temperature, V is the maximum swimming velocity of the fish, L is the total length of the fish, and μ is the dynamic viscosity of the fluid at a given temperature.

Similarly, estimates of flexural stiffness (EI) of the axial skeleton of free-swimming embryos or larval fish were made using Young's modulus (E) values determined for the notochord of sturgeon and the intervertebral discs of marlin (both are approximately 1 MPa) made by Long (1992, 1995) to estimate E for a larval fish notochord. We multiplied E by the second moment of area (I), as calculated from a subset of our rainbow trout specimens. (We did not have enough preserved razorback sucker specimens to determine I for this species.) To determine I for the rainbow trout, we used the radius (r) of the notochord and the formula for the second moment of area for a cylinder:

$$I = \frac{\pi}{4} \times r^4.$$

Both Re and EI were plotted versus fish size (total length) to test hypotheses about the effects of hydrodynamic regime and changes to the axial skeleton on escape response performance.

Results

All three species of teleost fish increased their relative escape response performance across embryonic and larval development (Fig. 2; Table 1). For all species, newly hatched individuals were able to swim at no more than 10 total lengths per second. However, escape ability rapidly improved as fish grew older and

Figure 2. Plots depicting length-specific velocity (total lengths per second) for (top) California halibut (circles), (middle) razorback suckers (triangles), and (bottom) rainbow trout (squares). Stippled line represents the size at which the yolk sac has been completely absorbed; dashed line represents the size at which the caudal fin has reached adult morphology.

Table 1: Linear regressions describing the relationship between total length (cm) and performance variables in log transformed data

Variable	California Halibut ($n = 38$)		Razorback Sucker ($n = 63$)		Rainbow Trout ($n = 57$)	
	Regression	r^2	Regression	r^2	Regression	r^2
Relative velocity (total lengths s^{-1})	$y = .23x + 1.34$.29*	$y = .08x + 1.23$.06*	$y = 1.12x + .89$.52*
Maximum acceleration (cm s^{-2})	NA	NA	$y = 1.21x + 2.43$.11*	$y = 4.47x + 1.33$.45*
Time to complete stage 1 (s)	NA	NA	$y = -.47x - 1.51$.15*	$y = -.90x - 1.30$.33*
Time to maximum velocity (s)	NA	NA	$y = -.77x - 1.31$.17*	$y = -.51x - 1.28$.04

Note. Data for California halibut (*Paralichthys californicus*), rainbow trout (*Oncorhynchus mykiss*), and razorback suckers (*Xyrauchen texanus*). Statistics were performed on data that were first arcsine transformed to ensure the data were normally distributed, and then log transformed to allow the use of a linear model to describe the data. However, for ease of interpretation of scaling relationships, the equations given here describe data that have not been arcsine transformed. NA = not applicable.

* Correlations that are significant at $P < 0.05$.

larger and typically peaked at about 25 total lengths per second (although suckers showed variability in maximum performance; Fig. 2).

Razorback suckers and rainbow trout also improved their escape performance in terms of the time it took to complete the preparatory phase of the escape response (stage 1), the time it took to reach maximum swimming velocity, and their maximum acceleration during the response (Fig. 3; Table 1). The improvement in timing variables was particularly extreme; for example, razorback suckers decreased the time it took to complete the preparatory phase by almost an order of magnitude (Fig. 3, *top*). Similar trends were observed for California halibut, but the relatively low sampling rate made it difficult to determine accurate values for timing variables.

Both razorback suckers and rainbow trout produced decreased axial bending across ontogeny, although there were differences between the two species in the degree of bending produced for some body regions. In general, the two species differed in length-specific curvature for the anterior 40% of the body but not for the posterior 60% of the body. Differences in anterior bending across species are likely caused by differences in the shape of the yolk sac and the way it attaches to the abdomen (A. C. Gibb, personal observation); these results will be explored in greater detail elsewhere. Length-specific curvature in the posterior 60% of the body declined as the fish grew, but the two species were indistinguishable from one another at any given size (e.g., Fig. 4). As above, a qualitative assessment of developmental changes in California halibut suggests that this species also produced decreased axial bending across development, but a low sampling rate made quantitative analysis impractical.

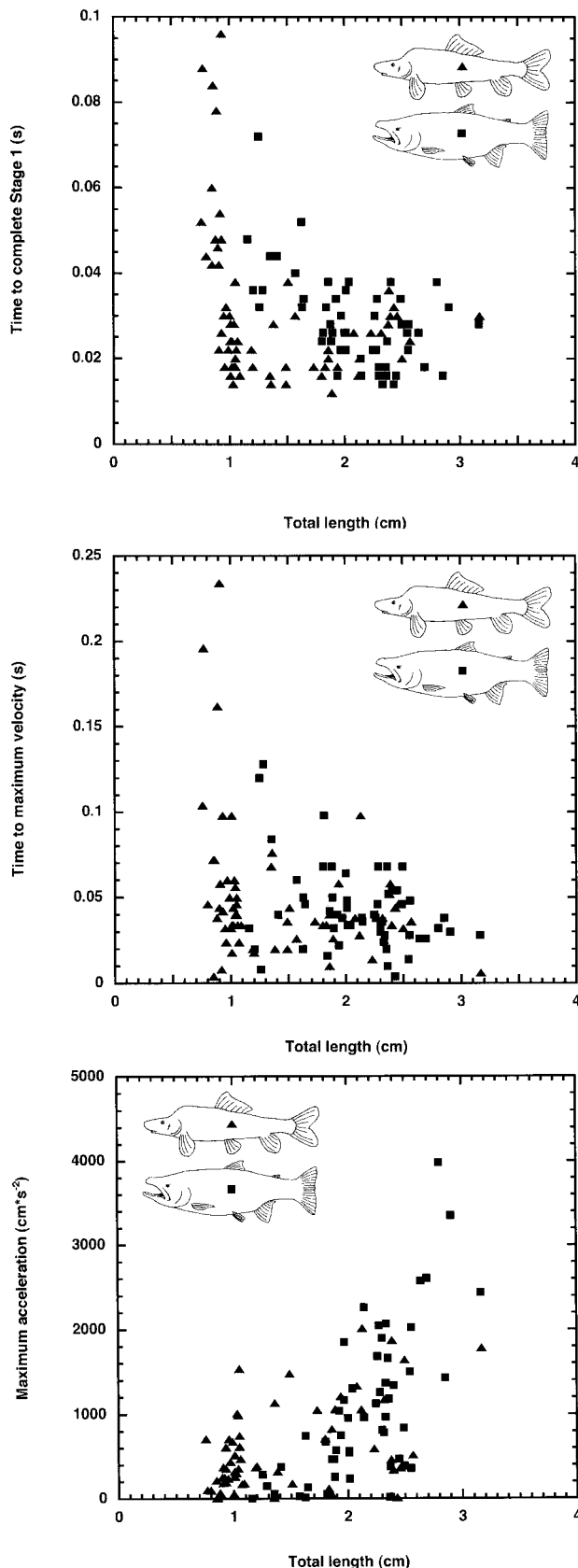
Major ontogenetic changes for structures proposed to have consequences for escape response performance are given in Table 2 and indicated in Figures 2 and 4. As would be expected on the basis of previous developmental studies, California halibut and razorback sucker undergo dramatic morphological changes after the yolk has been depleted. In contrast, rainbow

trout demonstrate a near-adult morphology by the time the yolk is depleted. We also noted, on the basis of published studies of larval allometry, that the caudal fin undergoes a dramatic change in shape and depth in the California halibut (Fig. 5; Gisbert et al. 2002).

Calculations of Reynolds number for all three species across ontogeny suggest that only California halibut experience a wide range of hydrodynamic regimes at maximum swimming velocity. The smallest halibut in the study appear to experience a hydrodynamic regime dominated by the effects of viscosity, even when they are swimming at peak velocity; as they grow larger, halibut move through an “intermediate” flow regime, and as large larvae and juveniles they experience a hydrodynamic regime dominated by inertial effects (Fig. 6). Razorback suckers and rainbow trout are substantially larger than California halibut at hatching. Although a few of the smallest razorback suckers experience intermediate flow regimes at maximum swimming velocity, the majority of the razorback suckers and all of the rainbow trout experience a hydrodynamic regime dominated by inertial effects (Fig. 6).

We observed that the vertebral column forms near the end of the larval stage for razorback suckers and rainbow trout (Table 2; Fig. 4). Thus, the formation of this structure apparently could not account for changes in escape performance. However, radius of the notochord increased across early development. In fact, notochord radius increased faster than would be expected because of simple scaling effects ($r \approx L$) and scaled with length to the second power ($r \approx L^2$). Any increase in radius, even one due to simple isometry, will have clear consequences for flexural stiffness of the axial skeleton. On the basis of our estimates of EI , rainbow trout flexural stiffness due to the notochord increased by four orders of magnitude during the free-swimming embryo and larval developmental periods (Fig. 7).

Finally, some razorback sucker individuals performed an O-start behavior (Westneat et al. 1998; Wesp and Gibb 2003). In these instances, fish would perform the initial C-bend and then



continue bending to form an O or Q shape (Fig. 8). The O-start appears to occur as a consequence of an extreme C-bend in fish with an elongate body shape and was more likely to occur in young razorback suckers than in older ones (Wesp and Gibb 2003). In contrast, the O-start was never observed in rainbow trout (Wesp and Gibb 2003). California halibut sometimes produced uncoordinated responses early in development, but they never appeared to produce an O-start (A. C. Gibb, personal observation). However, it is possible halibut are too short to form an O-shape, even when bending is extreme.

Discussion

Previous work on development of the escape response in salmonid species emphasized the effect of the yolk sac on escape performance. Hale (1999) demonstrated that escape performance in three salmonids peaked around the time the yolk was absorbed; we found a similar pattern with a fourth species of salmonid, the rainbow trout. Razorback suckers also demonstrate a performance peak near the time of absorption of the yolk sac. However, California halibut do not follow this pattern. In California halibut, the yolk is completely absorbed when the larvae are still quite small, and length-specific maximum velocity is still low. In fact, maximum length-specific velocity increases in California halibut throughout the larval period and peaks near the larval-juvenile transition. Thus, although transformation of the “dead weight” of the yolk mass into morphological structures may contribute to improved performance in rainbow trout and razorback suckers, it certainly does not contribute to an identical pattern of improvement observed in California halibut.

Previous research analyzing the effect of the hydrodynamic regime on the escape response during ontogeny has been equivocal. Some researchers have proposed that even small larvae experience high Reynolds number environments, and thus hydrodynamic regimes dominated by inertial forces, when they are swimming at maximum velocity during the escape response (Webb and Weihs 1986). Others have suggested that even large larvae may experience hydrodynamic regimes dominated by viscous forces during the early stages of the escape response, when larvae must accelerate their bodies from a standstill (Hale 1996). We note that there are at least two nonexclusive mechanisms by which changes in fluid regime could potentially result in improved escape response performance. First, it is possible that as larvae experience higher Reynolds numbers during ontogeny, they move into a hydrodynamic regime that allows them to attain greater swimming velocities while employing the same

Figure 3. Plots depicting (*top*) time to complete the preparatory phase, (*middle*) time to maximum velocity, and (*bottom*) maximum acceleration during the escape response for razorback suckers (*triangles*) and rainbow trout (*squares*).

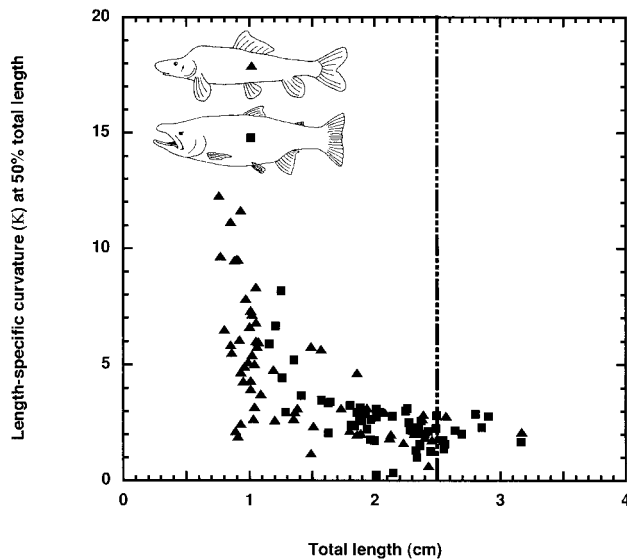


Figure 4. Plot depicting length specific curvature (K) at 50% of the total length of the body during the preparatory phase of the escape response for razorback suckers (*triangles*) and rainbow trout (*squares*). Power functions describing the relationship between total length and length-specific curvature are as follows: for razorback sucker, $y = 5.2x^{-1.15}$; for rainbow trout, $y = 5.6x^{-1.21}$. A single dashed line indicates the size at which the vertebral column forms for both razorback suckers and rainbow trout.

behavior, thereby improving length-specific performance. For example, as inertial forces become dominant at higher Reynolds numbers, the acceleration reaction (Daniel 1984) may play a greater role in producing thrust during the escape response, and this could contribute to increased maximum swimming velocity. Second, it is also possible that a change in the relative contribution of viscous forces allows fish to improve their ability to accelerate from a standstill during ontogeny. This hydrodynamic change might enable larvae to complete the escape behavior more rapidly as they grow larger.

Although we acknowledge a change in hydrodynamic regime likely contributes to improved escape performance during ontogeny (see McHenry 2004), we suspect this change is not the primary factor underlying improved performance for several reasons. First, when all three species are compared, there is no

apparent correlation between the observed changes in length-specific swimming performance and Reynolds number. For example, California halibut reach their maximum length-specific performance ($\sim 30 L s^{-1}$) at a total length of approximately 1 cm (Fig. 2). For halibut, this length corresponds with when young fish experience an inertia-dominated hydrodynamic regime. However, although rainbow trout also experience an inertia-dominated hydrodynamic regime when they are 1 cm in total length, these individuals still demonstrate poor length-specific swimming performance ($< 10 L s^{-1}$) at this size (Fig. 2). Changes in length-specific performance are more closely associated with morphological changes (i.e., caudal fin formation; see below) than they are with hydrodynamic transitions.

Second, the change in timing variables also appears to be correlated with ontogenetic transitions and not with hydrodynamic transitions. Hale (1999) compared escape performance in several species of salmonid across a range of sizes and developmental stages. Improvement in the duration of the escape response showed a clear correlation with ontogenetic state and not with absolute size (Figs. 5, 6 in Hale 1999). If timing improvements were due to hydrodynamic transitions, fish size should play a large role in determining performance. Additionally, our calculations of instantaneous Reynolds number from moment to moment during an individual escape response suggest that even relatively slow 1-d-old rainbow trout larvae experience high Reynolds numbers ($> 1,000$) within 4 ms of beginning the escape response. Thus, we concur with Webb and Weihs (1986), who suggested that rapid body movements allow even small fish to function in an inertia-dominated hydrodynamic regime during the escape response.

We do expect that hydrodynamic regime would have a clear effect on one variable not measured here: distance traveled within a given time period after initiation of the escape response. Fish often produce an escape response that consists of the preparatory phase followed by a single propulsive stroke. Thus, a measure of distance traveled at a particular time after the initiation of the escape response would likely reflect the ability of the fish to “coast” through the fluid after the propulsive stroke. We anticipate that this parameter would be heavily influenced by the relative dominance of inertial forces

Table 2: Approximate total length (cm) at which morphological changes occur during the development of California halibut, razorback sucker, and rainbow trout

Species	Yolk Assimilated	Caudal Fin Ossified	Vertebrae Formed
California halibut	.3	1.0	Unknown
Razorback sucker	1.1	1.6	2.5
Rainbow trout	2.3	2.3	2.5

Note. Data given here were obtained from previous studies of development (Minckley and Gustafson 1982; Gisbert et al. 2002) and observations made from preserved specimens.

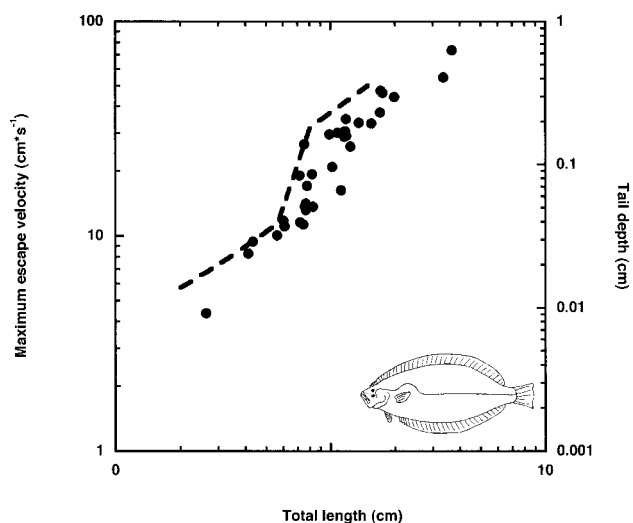


Figure 5. Plot depicting absolute swimming speed (circles) and tail depth (dashed line) versus total length for California halibut. Tail depth is given here from power functions in a published study of larval allometry (Gisbert et al. 2002).

and thus would show a correlation with changes in the hydrodynamic regime.

It has previously been proposed that formation of true fins affects escape performance (Hale 1999). Our data provide support for this hypothesis: all three species show an improvement in length-specific swimming velocity that corresponds with formation of the caudal fin. In all three species, peak performance occurs at, or shortly before, the caudal fin reaches adult morphology. Additionally, California halibut, which have the most dramatic change in caudal fin morphology, show a pattern of change in absolute swimming speed (cm s^{-1}) that closely follows ontogenetic changes in caudal fin depth (Gisbert et al. 2002). We suggest that this performance improvement is due to the increased stiffness caused by the formation of the bony rays within the caudal fin and to the increased surface area of the adult-morphology caudal fin, which together create a deep, thin, low-mass fin that increases the efficiency of transfer of momentum from the body to the surrounding water (Weihs 1973). Such correlations between tail fin area and escape response performance are expected on the basis of hydrodynamic theory (Weihs 1973; Webb 1978), and reductions in swimming performance when the tail fin area is surgically reduced have been demonstrated for adult rainbow trout (Webb 1977) and frog tadpoles (Hoff and Wassersug 2000; Van Buskirk and McCollum 2000).

We also note that axial depth increases during larval development and that the dorsal and anal fins form at approximately the same time as the caudal fin (Wakeling et al. 1999; Gisbert et al. 2002); both of these factors will serve to increase the lateral body profile during ontogeny. This increase in lateral

profile will create a large virtual mass when the posterior region of the body is rapidly moved through the water during the escape response and should maximize thrust production (Weihs 1973). In fact, teleost fish are known to erect the bony rays of their median fins during the escape response to maximize their lateral profile (Webb 1977). Thus, during teleost ontogeny, the median fins (dorsal, anal, and caudal) form and axial depth increases; all of these changes should combine to maximize lateral profile and enhance thrust during the escape response.

The mechanism underlying improvements in timing of the escape response across development has been particularly opaque. Previous research has emphasized the role of neuromuscular coordination in improving the time it takes to produce the preparatory phase of the escape response (Hale 1996, 1999). We find some evidence to support this hypothesis: razorback suckers and California halibut often produce uncoordinated responses shortly after hatching. In some cases, these behaviors clearly contribute to poor escape performance (Wesp and Gibb 2003). However, these timing variables also improved in rainbow trout, which demonstrated no apparent change in coordination across development.

Formation of the vertebral column is thought to contribute to improved escape performance across development, but it has generally been implied that the underlying mechanism is improved force transmission (from the axial musculature to the caudal fin), and an improvement of this nature would likely manifest as increased maximum velocity. We note that formation of the vertebral column occurs late in development for

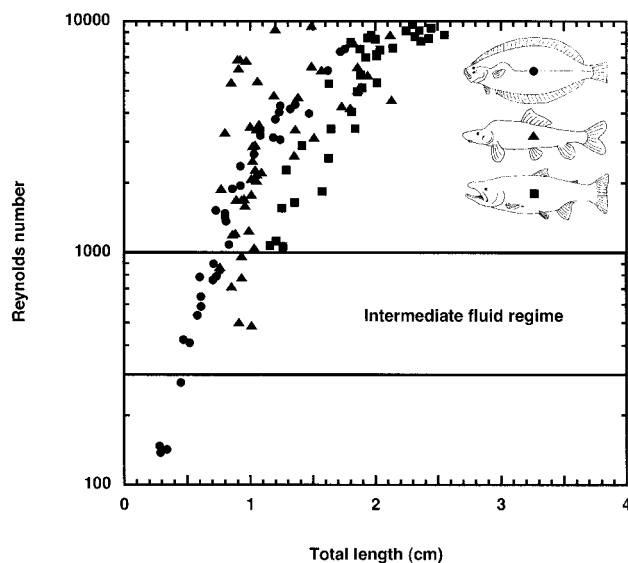


Figure 6. Plot depicting Reynolds number (Re) as calculated for three species swimming at maximum escape velocity: California halibut (circles), razorback suckers (triangles), and rainbow trout (squares). The transitional, or “intermediate,” fluid regime is enclosed within the boxed area.

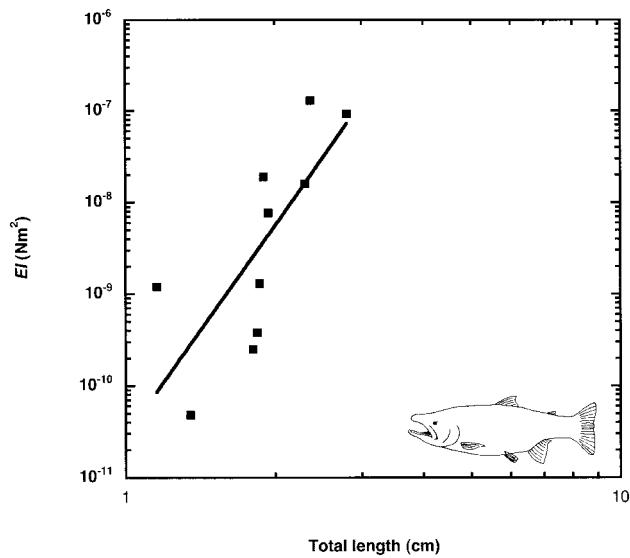


Figure 7. Plot depicting estimated axial skeleton flexural stiffness (EI) for individual free-swimming embryos and larval rainbow trout versus size. Flexural stiffness increases with total length according to the following formula: $y = 2.8 \times 10^{11} \times x^{2.6}$, $r = 0.68$.

two of the species examined here (and we suspect it also occurs late in development for California halibut). Thus, formation of the vertebral column cannot account for improvements in length-specific velocity that occur early in development. However, improvements in timing do occur concomitantly with developmental changes in length-specific curvature. Therefore, we propose an alternate model of the ramifications of axial development on escape performance.

Very young fish have small notochords (i.e., small in radius) that grow larger as the fish grow longer. Any increase in notochord radius should result in increased flexural stiffness of

the fish because bending moment (muscle force [a result of muscle cross-sectional area $\approx L^2$] \times distance $[L] =$ moment [$\approx L^3$]) cannot increase as rapidly as flexural stiffness ($\approx L^4$), even if the notochord scales isometrically. In fact, our measurements of the radius of the notochord across early development in rainbow trout suggest that it increases twice as rapidly as would be expected because of isometry. Thus, ontogenetic changes to axial morphology in young fish will generate greatly increased flexural stiffness across development. We suggest that the low flexural stiffness of very young fish is disadvantageous for two reasons: (1) fish tend to “overbend” during the preparatory phase, which increases the time it takes to complete this step; (2) an axial skeleton with low flexural stiffness cannot transfer energy effectively or store significant amounts of elastic energy to be recovered during the propulsive stroke (this idea will be expanded on elsewhere; Swanson and Gibb 2004).

There is one additional suite of ontogenetic changes we have not considered here that has obvious potential ramifications for escape performance: changes to the fiber type and morphology of the axial musculature. Young fishes undergo a change in fiber types of the axial musculature; typically, slow oxidative fibers represent a greater proportion of the axial musculature in larvae than in juveniles (e.g., Nag and Nursall 1972). Thus, increased speed of the escape behavior across ontogeny may be due, in part, to the increased representation of fast fibers in the axial musculature. Additionally, myomeres change in overall shape during free embryo and larval development. Myomeres in young fish are more vertical in orientation, and any one myomere spans a very small proportion of the axial skeleton. Myomeres in older fish, on the other hand, have a more pronounced “W” morphology consisting of pronounced anterior and posterior pointing “cones” of muscle and span a greater proportion of the axial skeleton (e.g., Gisbert et al. 2002). A thorough analysis of the ramifications of these changes

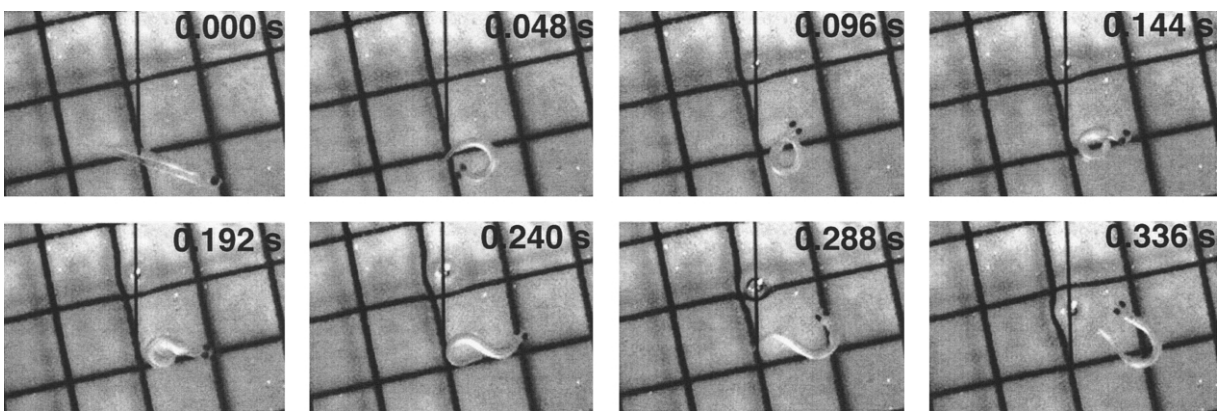


Figure 8. These images depict a razorback sucker performing an O-start in response to stimulation. A 0.5-cm grid is shown in the background; time from the beginning of the response is shown in seconds. Note that the center of the mass of the fish moves very little from the first panel to the last panel. This figure is modified from Wesp and Gibb (2003).

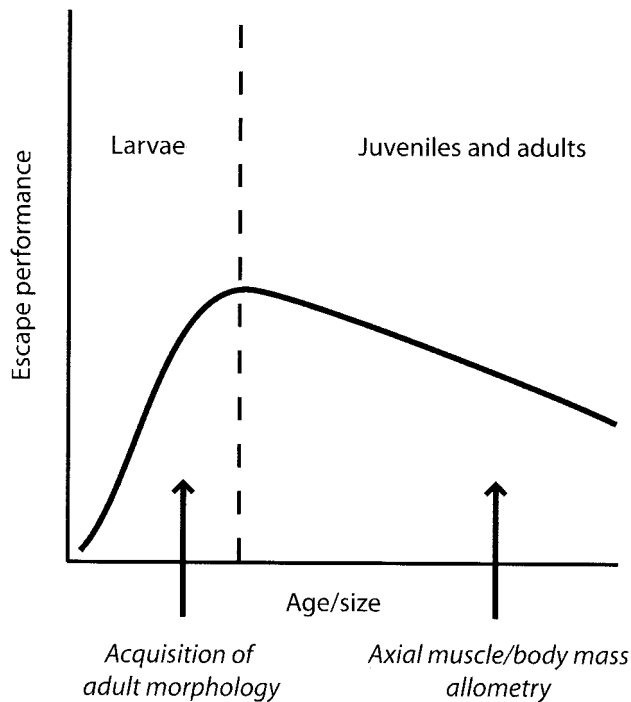


Figure 9. Model explaining performance changes across life-history stages in teleost fishes: performance increases during early development, peaks at the larva-juvenile transition, and declines in juveniles and adults.

is beyond the scope of this study (but will be considered elsewhere; Swanson and Gibb 2004). However, previous research suggests that one function of fish myomeres is to increase axial stiffness and promote elastic recoil during the escape response (Westneat et al. 1998). Thus, the formation of true myomeres during development should also serve to improve axial stiffness and allow a more rapid and effective escape response to be produced.

Conclusions

We propose that a suite of developmental changes contributes to improved escape response performance across teleost early life-history stages. We suggest that reduction in the mass of the yolk and changes in hydrodynamic regime are not the major contributors to observed improvements in performance. Rather, formation of the caudal fin and increased stiffness of the axial body are the major mechanisms that underlie improvements in escape response velocity and the time it takes to complete the escape response, respectively. The benefits gained by these developmental changes peak near the larval to juvenile transformation (Fig. 9). At this stage, the fish have achieved near-adult morphology and use this morphology to move a mass that is proportionally quite small. As juveniles become larger, performance decreases because the axial muscle

cross-sectional area cannot increase rapidly enough to match the concomitant increase in body mass (Fig. 9).

We note that this finding implies that a performance peak occurs at the beginning of juvenile life not as the result of any explicit modifications to improve performance but instead as a biomechanical consequence of interrelated developmental changes in size and shape of fish as they metamorphose from larvae into juveniles. We also note that species with intermediate or direct development will tend to encounter the environment at a larger size and an advanced state of development. Thus, individuals of species with intermediate and direct developmental strategies should have a performance advantage relative to individuals of species with an indirect developmental strategy when trying to evade predators.

Acknowledgments

This work was supported by National Science Foundation grant IBN-0002301 and Northern Arizona University (NAU) intramural funding to A.C.G., Environmental Protection Agency Science to Achieve Results fellowship U-91591901 to B.O.S., and the National Institutes of Health-funded Minority Student Development and Minority Access to Research Careers Undergraduate Student Training in Academic Research programs of NAU. We thank A. Herrell for organizing the symposium "The Ontogeny of Performance in Vertebrates" for the Seventh International Congress of Vertebrate Morphology (ICVM) in Boca Raton, Florida, in 2004 and the ICVM for funding A.C.G.'s attendance at the meeting. We also thank C. Figiel of the U.S. Fish and Wildlife Service for providing razorback sucker eggs and J. Rounds of the Redondo Beach California Halibut Hatchery for providing California halibut larvae and juveniles. K. Dickson of California State University, Fullerton, and K. Nishikawa of NAU provided facilities where these data were collected and assistance with various aspects of this research. M. Manning of NAU assisted with morphological analyses, and M. MacPhee of NAU created the illustrations of the three species used in this study. This manuscript was significantly improved by the thoughtful comments of M. McHenry, J. Long, and an anonymous reviewer.

Literature Cited

- Balon E.K. 1999. Alternative ways to become a juvenile or a definitive phenotype (and on some persisting linguistic offenses). *Environ Biol Fishes* 56:17–38.
- Carrier D.R. 1996. Ontogenetic limits on locomotor performance. *Physiol Zool* 69:467–488.
- Cullum A.J. 1999. Didge: Image Digitizing Software. Parthenogenetic Products, Creighton University, Omaha, NE.
- Daniel T.L. 1984. Unsteady aspects of aquatic locomotion. *Am Zool* 24:121–134.

- Domenici P. and R.W. Blake. 1993. Escape trajectories in angelfish (*Pterophyllum eimekei*). *J Exp Biol* 177:253–272.
- Fuiman L.A. and D.M. Higgs. 1997. Ontogeny, growth and the recruitment process. Pp. 225–250 in R.C. Chambers and E.A. Trippel, eds. *Early Life History and Recruitment in Fish Populations*. Vol. 21. Chapman & Hall, London.
- Fuiman L.A. and P.W. Webb. 1988. Ontogeny of routine swimming activity and performance in zebra danios (Teleostei: Cyprinidae). *Alsk Fish Res Bull* 36:250–261.
- Gibb A.C. and K.A. Dickson. 2002. Functional morphology and biochemical indices of performance: is there a correlation between metabolic enzyme activity and swimming performance? *Integr Comp Biol* 42:199–207.
- Gisbert E., G. Merino, J.B. Muguet, D. Bush, R.H. Piedrahita, and D.E. Conklin. 2002. Morphological development and allometric growth patterns in hatchery-reared California halibut larvae. *J Fish Biol* 61:1217–1299.
- Hale M.E. 1996. The development of fast-start performances in fishes: escape kinematics of the chinook salmon. *Am Zool* 36:694–709.
- . 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.
- Hoff K.V. S. and R. Wassersug. 2000. Tadpole locomotion: axial movement and tail function in a largely vertebraless vertebrate. *Am Zool* 40:62–76.
- Horn M.J., P.C. Marsh, G. Mueller, and T. Burke. 1994. Predation by odonate nymphs on larval razorback suckers (*Xyrauchen texanus*) under laboratory conditions. *Southwest Nat* 39:371–374.
- Houde E.D. 1997. Patterns and consequences of selective processes in teleost early life histories. Pp. 173–196 in R.C. Chambers and E.A. Trippel, eds. *Early Life History and Recruitment in Fish Populations*. Vol. 21. Chapman & Hall, London.
- James R.S. and I.A. Johnston. 1998. Scaling of muscle performance during escape responses in the fish *Myoxocephalus scorpius*. *J Exp Biol* 201:913–923.
- Katzir G. and J.M. Camhi. 1993. Escape response of black mollies (*Poecilia sphenops*) to predatory dives of a pied kingfisher (*Ceryle rudis*). *Copeia* 1993:549–553.
- Kimmel C.B., J. Patterson, and R.O. Kimmel. 1974. The development and behavioral characteristics of the startle response in the zebrafish. *Dev Psychobiol* 7:47–60.
- Long J.H., Jr. 1992. Stiffness and damping forces in the intervertebral joints of blue marlin (*Makaira nigricans*). *J Exp Biol* 162:131–155.
- . 1995. Morphology, mechanics and locomotion: the relation between the notochord and swimming motions in sturgeon. *Environ Biol Fishes* 44:199–211.
- Long J.H., Jr., M. Koob-Emunds, B. Sinwell, and T.J. Koob. 2002. The notochord of hagfish *Myxine glutinosa*: visco-elastic properties and mechanical functions during steady swimming. *J Exp Biol* 205:3819–3831.
- Long J.H., Jr., and K.S. Nipper. 1996. The importance of body stiffness in undulatory propulsion. *Am Zool* 36:678–694.
- McHenry M.J. 2004. How do ontogenetic changes in morphology and behavior affect the swimming performance of zebrafish? *J Morphol* 260:312.
- Miller T.J., L.B. Crowder, J.A. Rice, and E.A. Marschall. 1988. Larval size and recruitment mechanisms in fishes: toward a conceptual framework. *Can J Fish Aquat Sci* 45:1657–1670.
- Minckley W.L. and E.S. Gustafson. 1982. Early development of the razorback sucker, *Xyrauchen texanus* (Abbott). *Great Basin Nat* 42:553–561.
- Nag A.C. and J.R. Nursall. 1972. Histogenesis of white and red muscle fibers of trunk muscle of a fish, *Salmo gairdneri*. *Cytobios* 6:227–246.
- Nelson J.S. 1994. *Fishes of the World*. Wiley, New York.
- Osse J.W. M. and J.G. M. van den Boogaart. 2000. Body size and swimming types in carp larvae: effects of being small. *Neth J Zool* 50:233–244.
- O'Steen S., A.J. Cullum, and A.F. Bennet. 2002. Rapid evolution of escape ability in Trinidadian guppies. *Evolution* 56:776–784.
- Seale D.B. and F.P. Binkowski. 1988. Vulnerability of early life intervals of *Coregonus hoyi* by a freshwater mysid, *Mysis relicta*. *Environ Biol Fishes* 21:117–125.
- Swain D.P. 1992a. The functional basis of natural selection for vertebral traits of larvae in the stickleback *Gasterosteus aculeatus*. *Evolution* 46:987–997.
- . 1992b. Selective predation for vertebral phenotype in *Gasterosteus aculeatus*: reversal in the direction of selection at different larval sizes. *Evolution* 46:998–1013.
- Swanson B.O. and A.C. Gibb. 2004. Ontogeny of fast starts: what are the biomechanical consequences of development in teleost fish? *Integr Comp Biol* 44:51.1A.
- Taylor W.R. 1967. An enzyme method of clearing and staining small vertebrates. *Proc US Natl Mus* 122:1–17.
- Van Buskirk J. and S. McCollum. 2000. Influence of tail shape on tadpole swimming performance. *J Exp Biol* 203:2149–2158.
- Wakeling J.M., K.M. Kemp, and I.A. Johnston. 1999. The biomechanics of fast-starts during ontogeny in the common carp *Cyprinus carpio*. *J Exp Biol* 202:3057–3067.
- Walker J.A. 1997. QuickSAND: Quick Smoothing and Numerical Differentiation for the Power Macintosh. <http://usm.maine.edu/~walker/software.html>.
- . 1998a. Estimating velocities and accelerations of animal locomotion: a simulation experiment comparing numerical differentiation algorithms. *J Exp Biol* 201:981–995.
- . 1998b. QuicKurve. <http://www.usm.maine.edu/~walker/software.html>.
- Webb P.W. 1976. The effect of size on the fast-start performance of rainbow trout *Salmo gairdneri*, and a consideration of

- piscivorous predator-prey interactions. *J Exp Biol* 65:157–177.
- . 1977. Effects of median-fin amputation on fast-start performance of rainbow trout (*Salmo gairdneri*). *J Exp Biol* 68:123–135.
- . 1978. Fast-start performance and body form in seven species of the teleost fish. *J Exp Biol* 74:211–226.
- Webb P.W. and D. Weihs. 1986. Functional locomotor morphology of early life history stages of fishes. *Trans Am Fish Soc* 115:115–127.
- Weihs D. 1973. The mechanism of rapid starting in slender fish. *Biorheology* 10:343–350.
- . 1980. Energetic significance of changes in swimming modes during growth of anchovy larvae, *Engraulis mordax*. *US Natl Mar Fish Serv Fish Bull* 77:597–604.
- Werner E.E. and J.F. Gilliam. 1984. The ontogenetic niche and species interactions in size-structured populations. *Annu Rev Ecol Syst* 15:393–425.
- Wesp H.M. and A.C. Gibb. 2003. Do endangered razorback suckers have poor larval escape performance relative to introduced rainbow trout? *Trans Am Fish Soc* 132:1166–1178.
- Westneat M., M. Hale, M. McHenry, and J. Long. 1998. Mechanics of the fast-start: muscle function and the role of intramuscular pressure in the escape behavior of *Amia calva* and *Polypterus palmas*. *J Exp Biol* 201:3041–3055.