Copeia, 1998(4), pp. 971–984

Vertebral Column Morphology, C-Start Curvature, and the Evolution of Mechanical Defenses in Tetraodontiform Fishes

ELIZABETH L. BRAINERD AND SHEILA N. PATEK

Maximum body curvature during the initial phase of escape swimming (stage 1 of C-start) was measured in four species of tropical marine fishes. A linear correlation between maximum curvature and number of functional intervertebral joints was found (range for number of joints, 17–25). A biomechanical model of vertebral column bending predicts that, if intervertebral joint angles are held constant, increasing the number of joints should produce a linear decrease in the measured curvature coefficient (curvature coefficient is inversely related to curvature). The measured curvature coefficients fit this model closely, indicating that, within the range of 17–25 joints, vertebral number is an important determinant of vertebral column flexibility. The study species with the lowest vertebral number, a filefish, *Monacanthus hispidus*, is a member of the Tetraodontiformes, a group characterized by the lowest vertebral numbers found among fishes. Elaborate antipredator defenses, such as a carapace and the ability to inflate the body, have evolved six times within the Tetraodontiformes, and some form of mechanical defense is present in all families of this group. We propose an evolutionary scenario in which low vertebral number reduced the escape swimming performance of ancestral tetraodontiforms, thus increasing their vulnerability to predators and driving the repeated evolution of mechanical defenses in this group. Our finding that lower vertebral numbers are correlated with lower C-start curvature suggests that low vertebral number may impair escape performance; thus, one necessary condition for the proposed scenario is met.

GENERALLY, it is assumed that fishes with higher vertebral counts have more flexible bodies than those with lower vertebral counts (Breder, 1926; Nursall, 1958). For large differences in vertebral number, ranging from 26 to 200, this assumption has been tested by correlating vertebral number with measurements of propulsive wavelength during steady swimming (Long and Nipper, 1996). A significant relationship between minimum propulsive wavelength and vertebral count was found, indicating that large differences in vertebral number affect body stiffness during swimming in fishes (although not in snakes; see Jayne, 1985). It remains to be discovered, however, whether smaller differences in vertebral number, for example the difference between 20 and 25 vertebrae, have any biologically significant effect on the overall stiffness of the body in fishes.

In addition to being an important factor in determining the mechanics of steady swimming, body stiffness may also be important in limiting the maximum curvature that a fish can attain during extreme swimming maneuvers, such as escape from predators. Depending on their body form, fishes perform different types of escape maneuvers (also known as startle responses). Elongate fishes with high vertebral counts perform an S-start in which the initial curvature of the body is shaped like the letter S, with two or more inflection points. Deep-bodied fishes with lower vertebral counts perform C-starts in which the initial curve of the body is shaped like the letter C and, thus, has only one inflection point (Eaton et al., 1977; Webb, 1978). Weihs (1973) termed this initial bending of the body, during which the center of mass of the fish remains fairly stationary, stage 1 of the startle response. Stage 2 is then the propulsive stage in which the fish extends its body and moves away from the predator. To determine whether relatively small differences in vertebral number affect the maximum curvature that fishes can attain, the present study reports measurements of maximum curvature attained during stage 1 of C-starts in four species of fishes with vertebral numbers ranging from 19–26.

Two anatomical systems contribute to the bending stiffness of fishes and thus would be expected to be important in limiting maximum curvature during C-starts: (1) vertebral column stiffness; and (2) the passive and active stiffness of the interconnected muscle-tendon-skin system (Long, 1992; Long and Nipper, 1996; Long et al., 1996). However, it is not known specifically which anatomical structures are most important in limiting the maximum amount that a fish bends during escape responses. It is pos-

© 1998 by the American Society of Ichthyologists and Herpetologists

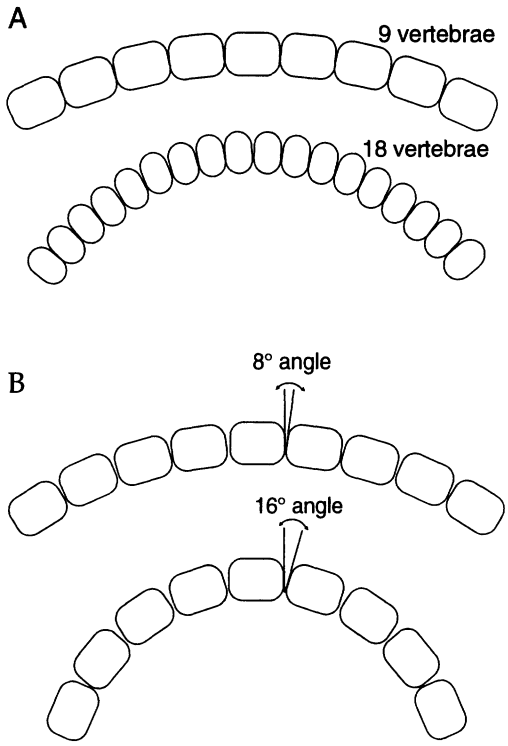


Fig. 1. Simple model of vertebral column bending. Two parameters contribute to bending: (A) increasing the number of vertebrae but keeping joint angles constant; (B) increasing the joint angle.

sible that maximum curvature is limited by the stiffness of the vertebral column, which is related to the number and shape of the vertebrae. It is also possible that maximum curvature is limited by the stiffness of the interconnected myomeric musculature, axial tendon system, and skin.

In a simple model of a vertebral column composed of equal-sized elements, maximum curvature is determined by two parameters: the total number of elements, and the maximum angle between individual elements (Fig. 1). For a given angle between vertebrae, adding more elements will increase the overall curvature (Fig. 1A). Increasing the angle between the elements will also increase curvature (Fig. 1B). These two parameters may be combined in four different ways: (1) vertebral count may vary while maximum intervertebral angles remain constant (pure Model A); (2) maximum intervertebral angles may vary while vertebral count remains constant (pure Model B); (3) intervertebral angle and vertebral number may interact synergistically; and (4) intervertebral angle and vertebral number may interact antagonistically. In the first case, curvature would be proportional to vertebral number. In the second case, vertebral number would not vary. In the third case, curvature would increase more rapidly than vertebral number. In the fourth case, curvature would increase more slowly than vertebral number, and differences in intervertebral angles could completely mask differences in vertebral number.

The Model A hypothesis implies that fishes with fewer vertebrae, and therefore fewer intervertebral joints, will exhibit lower maximum curvature during C-starts. A finding of no correlation between the number of joints and maximum curvature would refute this hypothesis. We would then conclude that vertebral number is not important in limiting overall body flexibility and that any potential effects of vertebral number are being masked by antagonistic changes intervertebral angles, which may be caused by the dominance of soft-tissue stiffness over vertebral column stiffness.

We selected one of the fish species in our study, *Monacanthus hispidus* (planehead filefish), because it is a member of the Tetraodontiformes, a group of fishes having very low vertebral numbers (Tyler, 1963, 1980). Recent descriptions of early tetraodontiform fishes, the Plectocretacicoidea, have determined that low vertebral number is a primitive character for this group (Tyler and Sorbini, 1996). The primitive condition for tetraodontiforms is to have no more than 21 vertebrae, and most members have 19 or fewer (this character is reversed only in one tetraodontid and a few monacanthids; Tyler, 1980; Tyler and Sorbini, 1996).

In addition to possessing low vertebral counts, tetraodontiform fishes are remarkable for having some of the most elaborate antipredator defenses among teleost fishes. Two elaborate mechanical defenses found in members of the Tetraodontiformes are the presence of a hard carapace of enlarged scale plates sutured together, and the ability of pufferfishes and some filefishes to inflate their bodies (Fig. 2; tetraodontiform phylogeny from Winterbottom, 1974; Leis, 1984; Tyler and Sorbini, 1996). A hard carapace covering all or part of the body has evolved at least four times within the tetraodontiforms (Fig. 2; Tyler and Bannikov, 1992). A carapace is best developed in the clade Ostraciidae + Aracanidae (Ostracioidea), in which the entire body and head are covered with hexagonal, sutured scale plates, with only the mouth, caudal peduncle, and fins retaining mobility. A partial carapace is also found in the extinct clade Plectocretacicidae + Protriacanthidae, in a molid *Ranzania*, and in a pufferfish, *Ephippion* (covering only the dorsal and lateral

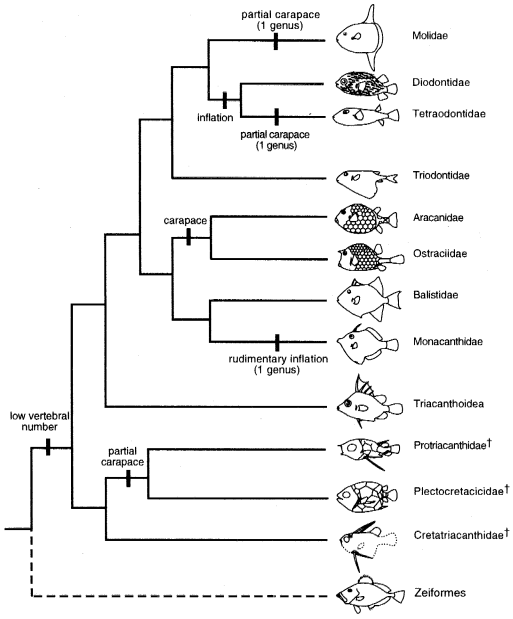


Fig. 2. Phylogeny of Tetraodontiformes (Winterbottom, 1974; Tyler and Sorbini, 1996). All extant families are shown, but some extinct families have been omitted. Low vertebral number (21 or fewer) is a primitive character for the Tetraodontiformes. Elaborate mechanical defenses have evolved six times within the order: a carapace four times and puffing twice (although the form of puffing found in one genus of monacanthid is much less specialized than that found in pufferfishes). Outlines of fishes adapted from Nelson (1994), Tyler (1980), and Tyler and Sorbini (1996). There is some uncertainty about the outgroup for Tetraodontiformes, and thus the sister relationship with the Zeiformes is indicated with a dashed line (Johnson and Patterson, 1993; Nelson, 1994)

aspects). In addition to these four, independent derivations, a carapace was also present in two extinct balistoids, *Probalistum* and *Eospinus*. Tyler and Bannikov (1992) place these two genera in an unresolved trichotomy with the clade Monacanthidae + Balistidae. This placement does not resolve the issue of whether the carapaces of these extinct genera are independently derived or whether they might be homologous with each other and possibly with the ostracioid carapace (Tyler and Bannikov, 1992). Thus we do not find that there is enough evidence to conclude that the carapaces of *Probalistum* and *Eospinus* are independently derived, and we shall use a conservative estimate of four independent derivations of a carapace within the tetraodontiforms for the purposes of this study.

Pufferfishes (clade Tetraodontidae + Diodontidae) exhibit perhaps the most extreme mechanical defense mechanism of any fishes. When attacked by a predator, pufferfishes are able to swallow water and thereby inflate their bodies up to four times their original volume (Brainerd, 1994). This ability to inflate is correlated with remarkable anatomical features that are unique to pufferfishes (Brainerd, 1994; Wainwright et al., 1995). In addition, a much less anatomically specialized form of inflation is present in one genus of monacanthid filefish (*Brachaluteres*; Clark and Gohar, 1953). Thus, elaborate mechanical defenses have evolved six times within the tetraodontiforms (a carapace has evolved four times and puffing twice).

Why have mechanical defenses evolved repeatedly within the Tetraodontiformes, whereas similarly elaborate mechanical defenses are relatively rare in other groups of fishes occupying similar habitats? This paper proposes an evolutionary scenario in which low vertebral number and the evolution of mechanical defenses are linked. Low vertebral number in ancestral tetraodontiforms may have impaired their escape swimming performance, and poor escape performance may have driven the repeated evolution of mechanical defenses in this group.

Measurements of maximum curvature during C-starts will provide one test of this scenario. Reduced curvature during C-starts is the most likely mechanism by which low vertebral number might affect escape performance. Previous studies of C-starts in sticklebacks have found that lower curvature results in lower instantaneous velocity, acceleration, and total distance traveled within 0.10 sec after a simulated attack (Andraso and Barron, 1995; Andraso, 1997). If we find no correlation between vertebral number and maximum curvature in this study, then reduced vertebral number probably does not affect C-start performance, and we shall conclude that our scenario for the evolution of mechanical defenses in tetraodontiforms is probably false. If we find that low vertebral number is correlated with curvature, then it remains a possibility that low vertebral number and the evolution of mechanical defenses are linked.

Materials and Methods

The fishes.—The original design of this study included three species of tetraodontiform fishes and three species of nontetraodontiform fishes, all of which occupy structured, tropical marine habitats such as coral reefs or grass beds. Tetraodontiform species studied were *Monacanthus hispidus, Canthigaster rostrata,* and *Diodon holocanthus.* Nontetraodontiform species studied were *Acanthurus chirurgus, Abudefduf saxatilis,* and ju-

TABLE 1. SPECIES AND NUMBERS OF INDIVIDUALS USED FOR THIS STUDY.

	SL (mm) of individuals	Number of C-Starts per individual
Nontetraodontiform species		
Acanthurus chirurgus (Acanthuridae)	93, 102, 103	10, 17, 50
Abudefduf saxatilis (Pomacentridae)	87, 98, 100	19, 12, 14
Scarus coeruleus (Scaridae)	63, 68, 99, 126	11, 13, 15, 12
Tetraodontiform species		
Monacanthus hispidus (Monacanthidae)	78, 80, 80, 123	6, 15, 18, 14
Canthigaster rostrata (Tetraodontidae)	62, 67, 68	No C-starts could be induced
Diodon holocanthus (Diodontidae)	60, 180	No C-starts could be induced

venile *Scarus coeruleus* (species determined from juvenile parrotfish key in Böhlke and Chaplin, 1968).

Numerous C-starts were recorded from the three nontetraodontiform species and from *M. hispidus* (Table 1). We were not able, however, to induce pufferfishes, *C. rostrata* and *D. holocanthus*, to perform startle responses. Thus we did not include the puffers in our analysis, and the remaining four species became our study species. Voucher specimens of the study species have been deposited in the University of Massachusetts Museum of Natural History (UMA F10001-UMA F10009).

Most of the fishes were collected, and videos of the fishes were recorded, at the Discovery Bay Marine Lab (DBML) in Discovery Bay, Jamaica. One species, *M. hispidus*, was collected in Florida and shipped to Massachusetts. In Jamaica, all fishes were kept between one and three days after collection in glass aquaria with flowing sea water before video recordings began. In Massachusetts, the filefish were kept between one week and one month in glass aquaria filled with artificial sea water before video recordings began.

Morphology.—One individual from each of the four study species was cleared and double-stained for drawing, determination of vertebral count, and measurement of vertebral segment lengths (Dingerkus and Uhler, 1977). Dried skeletons of one or more additional individuals collected from the same locations were used for comparison, and no variability in vertebral counts was observed. Counts included the vertebral centrum fused in the hypural plate; thus, the number of vertebrae is the same as the number of intervertebral joints. In addition to counts of the actual numbers of vertebrae, we also counted the number of functional intervertebral joints. All species except *S. coeruleus* exhibited highly reduced vertebrae and reduced intervertebral discs at the cranial end of the column. In the three species in which manipulation of specimens demonstrated limited mobility in one or more of the intervertebral joints, these joints were excluded from counts of functional intervertebral joints. Thus, we defined functional intervertebral joints as those which, from appearance and manipulation, did not exhibit a marked reduction in mobility.

Video recordings.—For video recordings of C-starts, we positioned a camera above an inflatable wading pool or a large aquarium. In the center of the pool was a clear acrylic corral (30 cm × 30 cm). Each fish was placed in the corral and allowed to acclimate for at least one hour. We elicited C-starts by thrusting a small net toward the head of the fish. The net remained outside the corral, and the walls of the corral prevented waves from obscuring the dorsal view of the fishes. We constructed a blind so that the fish did not anticipate the stimulus, and at least 10 min were allowed to pass between attempts to elicit starts to prevent fatigue and habituation.

We generally tested fishes individually, although the juvenile parrotfish, *S. coeruleus*, were tested in small schools of three or four individuals. We attempted to film parrotfish individually, but they did not acclimate to the corral and instead lay on the bottom and would not perform C-starts. Testing them in small schools seemed to reduce their stress and permitted us to induce C-starts.

C-starts were filmed in dorsal view with a Sony Hi-8 camcorder. High-speed film or video were not available; thus, we recorded and analyzed the C-starts at 60 fields per second (60 Hz) time resolution.

For each start, we identified the field of video in which maximum curvature was reached. The low time resolution of our video recordings (60 Hz) may have caused us to miss the moment of

maximum curvature in some starts and, thus, to underestimate the mean maximum curvature for all of the species. The effect of slow film speeds on estimating acceleration in C-starts has been quantified (Harper and Blake, 1989a, 1989b), but the effect on a simple measure such as maximum curvature has not been determined empirically. Based on results from the literature, however, we have been able to estimate that the error in our curvature measurements was certainly not more than 10% and probably not more than 5%. A survey of C-starts depicted diagrammatically in the literature indicates that teleost fishes spend an average of 18 milliseconds (msec) within approximately 10% of maximum curvature [as estimated from diagrams of 13 C-starts from 10 species: *Carassius auratus* (Eaton et al., 1977; Nissanov and Eaton, 1989); *Salmo gairdneri* (Eaton et al., 1977; Webb, 1976, 1982); *Brachydanio rerio* (Eaton et al., 1977); *Ictalurus nebulosus* (Eaton et al., 1977); *Hydrocinos maculatus* (Eaton et al., 1977); *Xenetodon cancila* (Eaton et al., 1977); *Esox lucius* (Harper and Blake, 1990); *Xenomystus nigri* (Kasapi et al., 1993); *Pterophyllum eimekei* (Domenici and Blake, 1991); *Citharichtys stigmaeus* (Webb, 1981)]. Thus, although startle responses do produce rapid movements, fishes spend a relatively long time near maximum curvature because they must decelerate and then accelerate their body segments to change direction between stages 1 and 2 of the escape response. Small species, such as *B. rerio,* spend only 5–10 msec near maximum curvature compared with larger species, such as *S. gairdneri,* that spend 25–35 msec. Our fishes were intermediate in size between these extremes; thus, with a video camera recording one field every 17 msec, we are not likely to have underestimated the maximum curvature in any individual start by more than 10%; and we estimate the mean error to be 5%. Because our fishes were all approximately the same size (Table 1), we have no reason to believe that our study species were differentially affected by the underestimate.

Curvature analysis.—We used a curvature coefficient (CC) defined as the ratio of the chord length of the bent vertebral column divided by its straight length as an approximation of whole body curvature (Fig. 3; method modified from Webb, 1978). From the video of each start we first measured the straight length of the vertebral column when the fish was at rest. The vertebral straight length (VSL) was the distance from the caudal edge of the neurocranium to the cranial edge of the caudal fin, as determined by external appearance. We then identified the video field in which maximum curvature was reached and measured the vertebral bent length (VBL) as the chord connecting the same two points as in the straight length measurement. The ratio of these, VBL/VSL, yields a curvature coefficient which is an inverse measure of the overall vertebral column curvature.

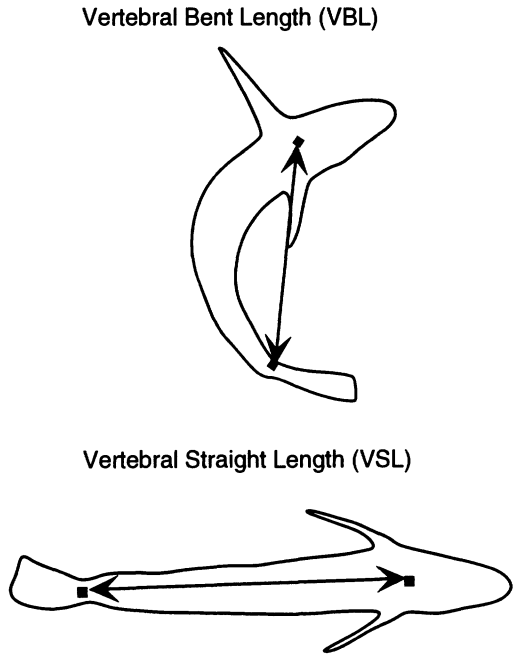


Fig. 3. Method for measuring curvature coefficient (CC) during fast starts. The vertebral bent length (VBL) was measured as the chord connecting the ends of the vertebral column in the field of video depicting maximum curvature during fast starts. The vertebral straight length (VSL) of the fish was measured from a field of video in which the fish was at rest, just before or after the start. The curvature coefficient was calculated as ratio of the vertebral bent length; CC = VBL/VSL (modified from Webb, 1978).

We also used the segment-angle analysis method of Jayne and Lauder (1993, 1995) to estimate the angles between adjacent vertebral segments. For each species, we measured the length between each functional intervertebral joint from cleared-and-stained specimens. We then selected the 10 starts for each species that exhibited the greatest curvature as measured by the curvature coefficient method above. On the field of video with maximum curvature, we traced the outline of the fish. A custom computer program then calculated the midline of the fish and estimated the angles between adjacent vertebral segments (Jayne and Lauder, 1993). We calculated the mean of the angles along the length of the fish to produce a mean intersegmental angle for each start, and we then

calculated means for each species and an overall mean.

Statistical analysis.—Curvature coefficient was measured for as large a number of starts as possible for three or four individuals of each species (Table 1). The mean and minimum CC for each individual were then used for least-squares regression against vertebral number. In addition, a nested ANOVA with individuals as a random factor was used to test for significant effect of vertebral number on CC. Because CC is a ratio, there is a priori reason to suspect that the variable may not be normally distributed. Shapiro-Wilk W-tests on both the raw data for each individual and the means found no significant deviation from normality, except for one of 14 fish. Nonetheless, all data were natural log transformed, and R^2 and *P*-values are reported for transformed data. The equations for regression lines, however, are reported for untransformed data.

Mathematical model.—To visualize the theoretically expected relationship between the curvature coefficient and the number of functional intervertebral joints, we developed a simple model of vertebral bending (Fig. 1A). In this model, the angle between individual vertebral segments remains constant, such that adding more segments increases overall curvature. This model includes only the effect of vertebral number on curvature. It does not include the effect of different maximum intervertebral angles on curvature (Fig. 1B).

If we assume that the vertebral curvature approximates a circular arc (not an unreasonable assumption for our fishes; see outline in Fig. 3), the curvature coefficient (CC) is then equal to the ratio of the length of the chord to the length of the arc subtended by a given angle (θ). This ratio, as a function of θ, is given by (see Appendix 1 for derivation):

$$CC = \frac{2 \sin\left(\frac{\theta}{2}\right)}{\theta} \qquad (1)$$

The mean intervertebral angle for all species, estimated with the segment-angle technique (see above), was used to convert the angle θ to number of intervertebral joints. The angle θ, in degrees, was divided by the number of degrees per joint. Thus, we were able to visualize the expected curve for the curvature coefficient by graphing the ratio of chord to arc length for a circle versus intervertebral joint number.

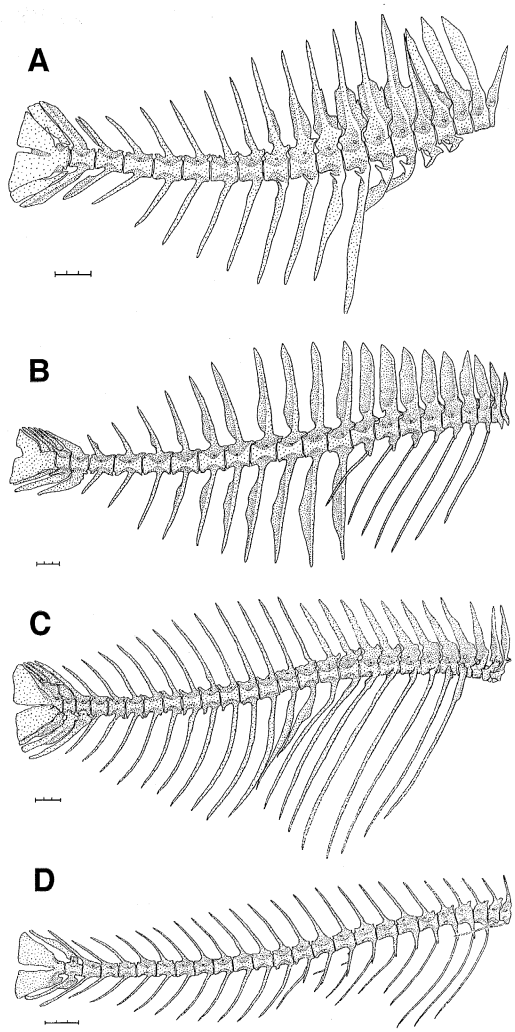


Fig. 4. Vertebral columns of the four study species. (A) *Monacanthus hispidus* (19 vertebrae, 17 functional intervertebral joints). (B) *Acanthurus chirurgus* (22 vertebrae, 20 functional intervertebral joints). (C) *Abudefduf saxatilis* (26 vertebrae, 23 functional intervertebral joints). (D) *Scarus coeruleus* (25 vertebrae, 25 functional intervertebral joints).

Results

Morphology.—The anterior vertebrae of three of the four study species are reduced in size and exhibit reduced joint mobility (Fig. 4). *Monacanthus hispidus* (Fig. 4A) has 19 vertebrae but only 17 functional intervertebral joints, because the first vertebra is reduced and immovably fixed to the neurocranium and second vertebra. *Acanthurus chirurgus* (Fig. 4B) has 22 vertebrae with the first vertebra reduced and its joints immobilized, leaving 20 functional intervertebral joints. *Abudefduf saxatilis* (Fig. 4C) has 26 verte-

TABLE 2. RESULTS OF A TWO-LEVEL, NESTED ANOVA OF CURVATURE COEFFICIENT. Three or four individuals nested inside four species.

	Degrees of freedom	*F*-value	*P*-value
Individual	10	2.06	< 0.05
Species	3	18.0	< 0.0005
Error	213		

brae within which the first two are reduced and have large caudally pointing processes that restrict mobility, leaving 23 functional intervertebral joints. *Scarus coeruleus* (Fig. 4D) has no immobilized joints and thus has 25 vertebrae and 25 functional intervertebral joints.

C-start curvature.—Four of six of the species that we attempted to study performed C-starts readily in response to the stimulus (Table 1). Two pufferfishes, *Diodon holocanthus* and *Canthigaster rostrata,* would not perform C-starts. A 60-mm SL *D. holocanthus* tended to inflate when threatened, and a 180-mm SL *D. holocanthus* simply swam slowly away by pectoral sculling. Specimens of *C. rostrata* would often use their pectoral fins to swim around the filming arena while holding their caudal fins bent at a right angle. When startled, these fish would rapidly straighten their tails, thus performing stage 2 of a C-start without having performed stage 1 as part of the same movement.

For the four species that did perform C-starts, we found that fishes with a larger number of functional intervertebral joints achieved greater overall curvature than those with fewer. A nested ANOVA yielded significant effects of both species and individuals on curvature coefficient (Table 2). Linear regression of the mean CC for each individual versus number of functional intervertebral joints yielded a significant inverse relationship ($y = -0.02x + 0.99$; $R^2 = 0.70$; $P < 0.001$), indicating that 70% of the variability in CC is explained by differences in the number of functional joints (Fig. 5). Linear regression on the actual number of joints, as opposed to the functional number, also yielded a significant relationship, with actual number explaining 57% of the variability ($R^2 = 0.57$; $P < 0.005$).

Silhouettes traced from videos of the maximum curvature recorded for each species indicate that the differences observed in curvature coefficient translate into large differences in the shape of the fishes during the start (Fig. 5; actual CC values for the illustrated starts: *M. hispidus,* 0.48; *A. chirurgus,* 0.47; *A. saxatilis,* 0.32; *S. coeruleus,* 0.25). A CC of 0.4–0.5 translates into

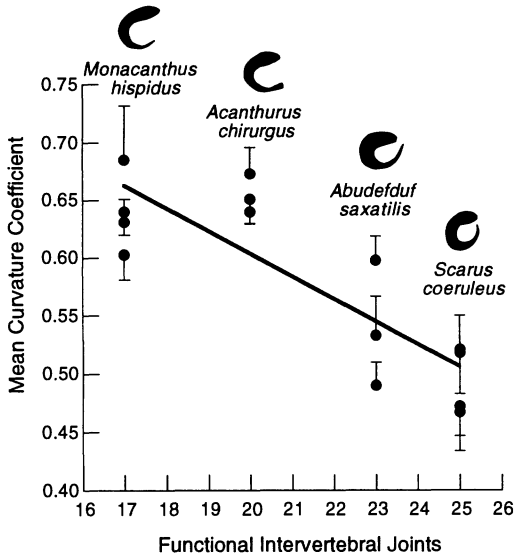


Fig. 5. Regression of mean curvature coefficient on number of functional intervertebral joints for three or four individuals of each species. Curvature coefficient is the ratio of vertebral column bent length to vertebral column straight length. Note that smaller numbers indicate greater curvature. The silhouette for each species was traced from the video of the individual C-start in which minimum curvature coefficient for each species was achieved. The points represent the mean curvature coefficient for each individual within each species. Error bars are standard error. Number of starts analyzed per individual may be found in Table 1. Mean square regression line: $y = -0.02x + 0.99$; $R^2 = 0.70$; $P < 0.001$.

an open C-shape, whereas a CC of 0.2–0.3 corresponds to a closed O-shape in which the caudal fin nearly touches the head. Linear regression of maximum curvature (minimum CC) attained by each individual (as opposed to mean CC as in Fig. 5) yielded a significant relationship with functional vertebral number ($R^2 = 0.72$; $P < 0.005$).

Estimates of the angle between individual vertebrae showed that the angles were not constant along the length of the vertebral column (Fig. 6). The angle was largest between the head and the vertebral column and was generally small just behind the head, increased toward the middle of the vertebral column, and then decreased again toward the tail. *Monacanthus hispidus* showed a different pattern in which the angle remained fairly constant behind the cranial joint. The large angle between the head and first functional intervertebral joint is not an artifact, and probably results from the great length of the first segment, which includes the

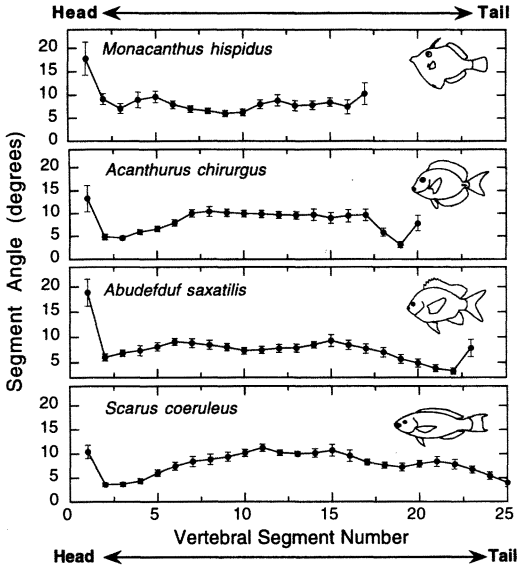


Fig. 6. Mean intersegmental angles versus segment number along the body as counted from cranial toward caudal. Means are for 10 starts from each of the four study species, and error bars are standard error. Angles were estimated from a midline fit to the outline of the fishes at maximum curvature combined with anatomical measurements of segment lengths from cleared-and-stained specimens (Jayne and Lauder, 1993; percent lengths may be found in Appendix 2).

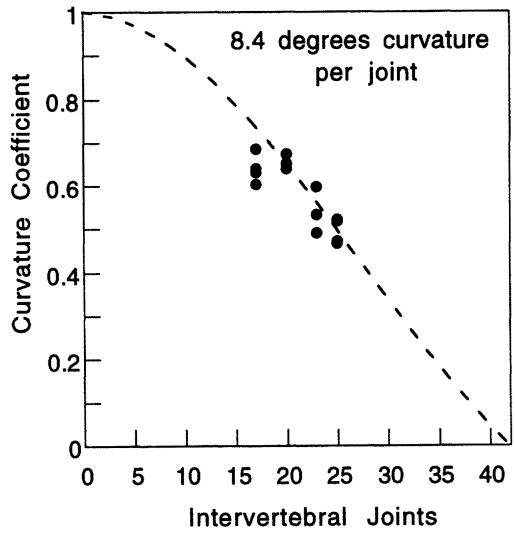


Fig. 7. Predicted curvature coefficient plotted with measured curvature coefficient for individuals from the four study species. The line represents the predicted curvature coefficient for a mean intervertebral angle of 8.4° (for derivation of the model, see Materials and Methods and Appendix 1). The points are the mean curvature coefficient values from Figure 5.

length of the head plus any fused vertebrae (Appendix 2).

For each C-start analyzed, the mean intervertebral angle of all the segments combined was calculated. Means and standard errors for the 10 starts from each species were: *M. hispidus*, 8.5 ± 0.35; *Acanthurus chirurgus* 8.7 ± 0.26; *Abudefduf saxatilis* 8.3 ± 0.37; *S. coeruleus*, 7.9 ± 0.25. The overall mean for all species combined was 8.4°. A regression of mean angle on vertebral number yielded no significant relationship ($R^2 = 0.07$; $P > 0.1$).

Mathematical model.—We used the overall intervertebral angle mean (8.4°) to calculate the expected relationship between curvature coefficient and the number of intervertebral joints (Fig. 7). With zero joints, the chord and arc are the same length, and the predicted curvature coefficient is 1. With 43 joints, the angle subtended by the arc exceeds 360° (43*8.4°), and the cranial and caudal ends of the vertebral column come together and the curvature coefficient is zero. The relationship is curved toward the extremes but linear in the range of vertebral numbers of our species. The measured mean curvature coefficients (from Fig. 5) are plotted for comparison and follow the predicted relationship fairly closely.

Discussion

The primary goal of this study was to determine whether maximum body curvature during C-starts is correlated with the number of functional intervertebral joints. A strong correlation between maximum curvature and number of joints was found, with 70% of the variability in curvature between species explained by differences in the number of functional intervertebral joints (Fig. 5).

A biomechanical model of the vertebral column (Fig. 1A, Appendix 1) provides an explicit hypothesis for the causal basis of the observed correlation. If maximum curvature is determined entirely by the number of intervertebral joints, and not by differences in bending angles of these joints or the stiffness of the muscle-tendon-skin system, then vertebral column curvature should increase linearly with the number of functional intervertebral joints (within the range of joint numbers studied; Fig. 7). A good fit between the predictions of the model and the actual measurements of curvature coefficient was found (Fig. 7), thus supporting a causal relationship between intervertebral joint

number and maximum curvature attained in C-starts.

Estimates of intervertebral joint angles also support the conclusion that differences in vertebral number are more important in determining maximum curvature than differences in intervertebral joint angles. We found no significant difference in mean intervertebral angles between species, although we observed a trend toward larger angles in fishes with fewer vertebrae. If further study shows this trend to be statistically significant, then we would conclude that fishes with fewer vertebrae compensate for a reduced number of joints by increasing the angle between joints (an antagonistic relationship between Models A and B presented in Fig. 1) but that this compensation is not sufficient to overcome the effects of reduced joint number.

It is unclear why the mean curvature coefficient for *M. hispidus* is lower than predicted by the model. The estimated mean intervertebral angle for this species is 8.5°, which is close to the overall mean (8.4°) used to calculate the predicted relationship. Furthermore, we would expect *S. coeruleus*, which has an estimated mean intervertebral angle of 7.9, to have a slightly higher curvature coefficient than the model, and yet this species falls on the line. Thus, although there is a qualitatively good fit between model and measured values, there are enough simplifications and sources of error introduced by both the curvature coefficient and the segment angle measurement methods to produce some disagreement between model and measured values.

One possible source of error in this study is the level of effort expended by each species to attain maximum curvature during escape responses. It is well known that fishes are capable of a range of escape responses, from slow starts to true fast starts, depending on the motivation of the fish (Domenici and Blake, 1997). However, in this study, we recorded a large number of starts from each individual, and we would expect differences in motivation between species to be reflected in differences in the variation about the mean curvature for each individual. Fishes expending less than maximal effort should exhibit greater variation about the mean curvature than fishes that are consistently approaching their true maximum curvature. No consistent difference between species was found (see error bars on mean values in Fig. 5). Furthermore, we expect that, given the large number of starts collected for each individual, we would have measured at least one start that represented maximal effort for each individual. A regression of the maximum curvature (minimum CC) attained by each individual also yielded a significant relationship with the number of functional intervertebral joints. Thus no evidence for consistent differences in motivation between the study species was observed.

The four species of fishes in this study formed their bodies into C-shapes or even O-shapes during escape responses (Figs. 3,5). Recent work on C-starts in a centrarchid fish, *Lepomis macrochirus*, indicates that this moderately deep-bodied species does not form a C-shape during escape but rather tends toward an abbreviated S-shape in which the caudal axial skeleton and caudal fin bend in the opposite direction from the rest of the body (Jayne and Lauder, 1993, 1996). This S-shape was not observed in the four species studied here, although estimates of segment angles show that the angles become smaller toward the head and the tail. These smaller angles indicate a flattening of the curve in these areas, but the angles never reverse in sign as was seen in *L. macrochirus*. Centrarchid fishes have 28–32 vertebrae (Johnson, 1984), whereas the fishes in this study have only 19–25 vertebrae, and the difference observed in body-shape during C-starts may be due to differences in vertebral column flexibility.

The pufferfishes in our study did not exhibit typical escape responses when subjected to the same stimuli as the other fishes in the study. In most fishes, two large neurons in the brain stem, the Mauthner cells, are important in the initiation of fast-startle responses. In agreement with our finding that filefishes readily perform C-starts whereas puffers do not, Mauthner cells have been found in filefishes and triggerfishes, but they are absent in some or all puffers (Zottoli, 1978). Other fishes have been shown to initiate fast starts through alternate, non-Mauthner neural pathways (Eaton et al., 1982), but we were not able to induce pufferfishes to do so.

The conclusion of this study that reduced vertebral number results in less curvature during C-starts provides one necessary condition for the proposed scenario, in which the repeated evolution of elaborate mechanical defenses in tetraodontiform fishes is driven by poor escape performance and increased vulnerability to predators. Four postulates comprise the evolutionary scenario: (1) the primitive antipredator defense for tetraodontiforms is to escape by performing a C-start; (2) low vertebral number is a synapomorphy of the Tetraodontiformes; (3) possession of fewer intervertebral joints limits the maximum curvature the body can attain during C-starts, thus impairing escape performance; and (4) relatively poor escape perfor-

mance in the tetraodontiform ancestor created a bias toward the evolution of elaborate mechanical defenses in the group, such as a carapace and body inflation.

Postulate 1 is supported by the observation that the basal groups of tetraodontiform fishes that have not evolved a carapace or body inflation, such as the Triacanthoidea, Balistidae, and Monacanthidae, rely on C-starts as their primary means of defense (monacanthids and balistids, ELB, pers. obs.; triacanthids, P.C. Wainwright, pers. comm.). Balistids and monacanthids retain the Mauthner neurons used by most fishes for initiating fast-startle responses, whereas pufferfishes have lost Mauthner neurons (Zottoli, 1978; triacanthoids and ostracioids have not been examined for Mauthner cells).

Postulate 2 is well supported by the literature. The primitive condition for tetraodontiform fishes is to have 21 or fewer vertebrae, and most tetraodontiforms have 19 or fewer (Tyler, 1963, 1980; Tyler and Sorbini, 1996). The proposed sister group to the Tetraodontiformes, the Zeiformes (Rosen, 1984), have vertebral counts ranging from 25–46, indicating that very low vertebral number is a synapomorphy of tetraodontiforms (Fig. 2).

There is some uncertainty, however, about the sister relationship between the zeiforms and the tetraodontiforms. Prior to the work of Rosen (1984), in which he proposed the zeiforms as the sister group to tetraodontiforms, the prevailing view favored a sister relationship between acanthuroids and tetraodontiforms (e.g., Tyler 1980). Recent studies of percomorph relationships, however, have rejected such a relationship (Johnson and Patterson, 1993). Rosen (1984) also proposed that capriods are the sister group to the clade Tetraodontiformes + Zeiformes, but a recent study of percomorph relationships strongly opposes this relationship (Johnson and Paterson, 1993). The zeiform + tetraodontiform relationship has been supported in recent phylogenetic studies of extinct, basal tetraodontiforms (Tyler et al., 1993; Tyler and Sorbini, 1996), and other recent treatments of the higher relationships of fishes have declined to take a stand on the outgroup of the tetraodontiforms (Johnson and Paterson, 1993; Nelson, 1994).

The validity of Postulate 2 does not, however, depend critically on which group of fishes is the correct outgroup. Vertebral numbers for most groups of fishes are available in Moser et al. (1984), and these data indicate that the vast majority of fishes have at least 24 vertebrae. Notable exceptions include numerous members of the Lophiiformes with 18–23 vertebrae (Pietsch, 1984), and the Acanthuroidei with 22–23 vertebrae (Leis and Richards, 1984). Thus, unless the Lophiiformes are the sister group to the Tetraodontiformes, the possession of 21 or fewer vertebrae is a tetraodontiform synapomorphy.

Postulate 3 generates two testable hypotheses: first, that fishes with fewer vertebrae will not be able to form as deep a C-shape when compared with fishes having more vertebrae; and second, that reduced curvature in C-starts leads to impaired escape performance. In this study, we tested the first hypothesis and found that the number of intervertebral joints may indeed limit curvature in tetraodontiforms. We did not test the second hypothesis in this study, but recent studies of sticklebacks have found that lower curvature during C-starts is correlated with lower instantaneous velocity, lower acceleration, and a reduction in distance traveled within the first 0.10 sec of the startle response (Andraso and Barron, 1995; Andraso, 1997).

A requirement of Postulate 4 is that low vertebral number must have evolved before elaborate mechanical defenses. It is possible to imagine the reverse scenario in which the evolution of mechanical defenses impairs escape ability, and thus high vertebral counts become unnecessary and are lost. In tetraodontiform fishes, however, it is clear that the evolution of low vertebral number preceded the evolution of carapaces and body inflation (Fig. 2), and there is no evidence that primitive tetraodontiforms had mechanical defenses that were more elaborate than those found in other groups of acanthomorph fishes occupying similar habitats. From the phylogenetic distribution of defense characters, it is likely that the ancestor of tetraodontiforms had an elongate first dorsal-fin spine and elongate pelvic-fin spines (Fig. 2; Tyler and Sorbini, 1996). Extant balistoids exhibit a trigger-type locking mechanism for the first dorsal spine, and triacanthoids can lock the pelvic spines (Tyler, 1980). Evidence from fossil tetraodontiforms, however, does not support the presence of a firm locking mechanism for either the dorsal- or pelvic-fin spines in the tetraodontiform ancestor (Tyler and Sorbini, 1996). A less well-developed frictional locking mechanism for the dorsal spines, similar to that found in triacanthids and many other acanthomorphs, may be primitive for tetraodontiforms, but even the presence of this mechanism is uncertain (Tyler and Sorbini, 1996). Thus there is currently no evidence to support the hypothesis that early tetraodontiforms had more elaborate mechanical defenses than did other deep-bodied fishes occupying similar habitats. It seems

likely that all of the mechanical defenses seen in modern tetraodontiforms, including spine-locking mechanisms, a carapace, and puffing, evolved within the group, and we conclude that low vertebral number evolved before mechanical defenses.

Thus one necessary requirement for Postulate 4 is met, but an actual test of this postulate would require historical analysis of other lineages that have also developed extreme mechanical defenses. Studies of other lineages of animals might reveal parallel patterns of character acquisition. If we find other lineages in which poor escape ability is a primitive character and mechanical defenses have evolved within the group, then our scenario for the evolution of mechanical defenses in tetraodontiform fishes would be supported.

The above scenario proposes that elaborate mechanical defenses may have evolved in response to low vertebral number in early tetraodontiforms. In light of this scenario, it is interesting to consider what might have caused the evolution and retention of these extremely low vertebral counts. It is possible that moderate mechanical defenses, such as the dorsal- and pelvic-fin spines that are thought to have been present in early tetraodontiforms, were effective enough to permit the initial reduction in vertebral number and escape ability, perhaps in an environment with low predation pressure. These reductions in turn may have caused greater elaboration of mechanical defenses, perhaps when predation pressure increased again, and increased mechanical defenses may have further reduced body flexibility. Thus a positive feedback loop could have been produced, in which body flexibility decreased and mechanical defenses increased. This leaves an interesting question for future investigations. Why have tetraodontiforms become trapped in this feedback loop, whereas other groups of fishes with moderate mechanical defenses (such as the Acanthuridae and Chaetodontidae) have not?

As a final note, it is interesting that the two extant groups of tetraodontiforms with the most elaborate mechanical defenses may also have chemical defenses. Pufferfishes (Tetraodontidae + Diodontidae) contain tetrodotoxin, a potent neurotoxin that is found concentrated in the liver, skin, and ovary of adults and in eggs and larvae (Halstead, 1978). Tetrodotoxin is made by bacteria that are present both in the intestine of puffers and in sinking particles and sediments (Noguchi et al., 1987; Hamasake et al., 1993). It is not clear whether puffers derive most of their toxicity through resident intestinal bacteria or through food-chain concentration. Ostraciids contain the toxic substance ostracitoxin. When under stress, ostraciids can release ostracitoxin into the water, thus harming other fishes kept with them (Halstead, 1978). Thus both pufferfishes and boxfishes contain toxins, but it has never been demonstrated that these toxins are actually used for defense against predators.

Acknowledgments

For help building equipment and recording videos, we thank B. Page and A. Dumka. For help collecting fishes in Jamaica, we thank C. Curran; for advice and loan of collecting equipment, we thank P. Lobel and L. Kaufman. Thanks to G. Lauder for the use of his segment angle analysis system and to G. Gillis for help learning to use the segment angle system. For reading and commenting on the manuscript, we thank P. Wainwright, P. Morris, N. Kley, A. Summers, and T. Hiebert. Figure 4 was drawn by L. Meszoly. Thanks to P. Wainwright for collecting and sending us filefish from Florida. Collecting permits, as necessary, were held by the Discovery Bay Marine Lab, P. C. Wainwright, and the Gulf Specimen Marine Laboratory, Inc., Panacea, Florida. Work on live animals was approved by the Institutional Animal Care and Use Committees of the University of Massachusetts Amherst and Harvard University. This work was partially funded by National Science Foundation grant BSR-8914618 to K. Liem and ELB and by National Science Foundation IBN-9419892 to ELB. Discovery Bay Marine Lab submission number 588.

Literature Cited

Andraso, G. M. 1997. A comparison of the startle response in two morphs of the brook stickleback (*Culaea inconstans*): further evidence for a trade-off between defensive morphology and swimming ability. Evol. Ecol. 11:83–90.

———, and J. N. Barron. 1995. Evidence for a trade-off between defensive morphology and startle response performance in the brook stickleback (*Culaea inconstans*). Can. J. Zool. 73:1147–1153.

Böhlke, J. E., and C. C. G. Chaplin. 1968. Fishes of the Bahamas and adjacent tropical waters. Livingston Publishing Co., Wynnewood, PA.

Brainerd, E. L. 1994. Pufferfish inflation: functional morphology of postcranial structures in *Diodon holocanthus* (Tetraodontiformes). J. Morphol. 220:243–261.

Breder, C. M. 1926. The locomotion of fishes. Zoologica 4:159–297.

Clark, E., and H. A. F. Gohar. 1953. The fishes of

the Red Sea: order Plectognathi. Mar. Biol. Sta., Ghardaqa, Red Sea, Publ. 8:1–80.

DINGERKUS, G., AND L. D. UHLER. 1977. Enzyme clearing of alcian blue stained whole small vertebrates for demonstration of cartilage. Stain Technol. 52: 229–232.

DOMENICI, P., AND R. W. BLAKE. 1991. The kinematics and performance of the escape response in the angelfish (*Pterophyllum eimekei*). J. Exp. Biol. 156:187–205.

EATON, R. C., R. A. BOMBARDIERI, AND D. L. MEYER. 1977. The Mauthner-initiated startle response in teleost fish. *Ibid.* 66:65–81.

———, W. A. LAVENDER, C. M. WIELAND. 1982. Alternate neural pathways initiate fast-start responses following lesion of the Mauthner neuron in goldfish. J. Comp. Physiol. 145:485–496.

HALSTEAD, B. W. 1978. Poisonous and venomous marine animals of the world. Darwin Press, Princeton, NJ.

HAMASAKE, K., K. KOGURE, T. NOGUCHI, Y. SHIDA, AND K. OHWADA. 1993. Tetrodotoxin in sinking particles from coastal waters. Mar. Biol. 118:761–765.

HARPER, D. G., AND R. W. BLAKE. 1989a. A critical analysis of the use of high-speed film to determine maximum accelerations of fish. J. Exp. Biol. 142: 465–471.

———, AND ———. 1989b. On the error involved in high-speed film when used to evaluate maximum accelerations of fish. Can J. Zool. 67:1929–1936.

———, AND ———. 1990. Fast-start performance of rainbow trout *Salmo gairdneri* and northern pike *Esox lucius*. J. Exp. Biol. 150:321–342.

JAYNE, B. C. 1985. Swimming in constricting (*Elaphe g. guttata*) and nonconstricting (*Nerodia fasciata pictiventris*) colubrid snakes. Copeia 1985:195–208.

———, AND G. V. LAUDER. 1993. Red and white muscle activity and kinematics of the escape response of the bluegill sunfish during swimming. J. Comp. Physiol. A 173:495–508.

———, AND ———. 1995. Speed effects on midline kinematics during steady undulatory swimming of largemouth bass, *Micropterus salmoides*. J. Exp. Biol. 198:585–602.

———, AND ———. 1996. New data on axial locomotion in fishes: how speed affects diversity of kinematics and motor patterns. Am. Zool. 36:642–655.

JOHNSON, G. D. 1984. Percoidei: development and relationships, p. 464–498. *In:* Ontogeny and systematics of fishes. H. G. Moser, W. J. Richards, D. M. Cohen, M. P. Fahay, A. W. Kendall Jr., and S. L. Richards (eds.). American Society of Ichthyologists and Herpetologists, Spec. Publ. 1. Allen Press, Lawrence, KS.

———, AND C. PATTERSON. 1993. Percomorph phylogeny: a survey of acanthomorphs and a new proposal. Bull. Mar. Sci. 52:554–626.

KASAPI, M. A., P. DOMENICI, R. W. BLAKE, AND D. HARPER. 1993. The kinematics and performance of escape responses of the knifefish *Xenomystus nigri*. Can. J. Zool. 71:189–195.

LEIS, J. M. 1984. Tetraodontiformes: relationships, p. 459–463. *In:* Ontogeny and systematics of fishes. H. G. Moser, W. J. Richards, D. M. Cohen, M. P. Fahay, A. W. Kendall Jr., and S. L. Richards (eds.). American Society of Ichthyologists and Herpetologists, Spec. Publ. 1. Allen Press, Lawrence, KS.

———, AND W. J. RICHARDS. 1984. Acanthuroidei: development and relationships, p. 547–551. *In:* Ontogeny and systematics of fishes. H. G. Moser, W. J. Richards, D. M. Cohen, M. P. Fahay, A. W. Kendall Jr., and S. L. Richards (eds.). American Society of Ichthyologists and Herpetologists, Spec. Publ. 1. Allen Press, Lawrence, KS.

LONG, J. H., JR. 1992. Stiffness and damping forces in the intervertebral joints of blue marlin (*Makaira nigricans*). J. Exp. Biol. 162:131–155.

———, AND K. S. NIPPER. 1996. The importance of body stiffness in undulatory propulsion. Am. Zool. 36:678–694.

———, M. E. HALE, M. J. MCHENRY, AND M. W. WESTNEAT. 1996. Functions of fish skin: flexural stiffness and steady swimming of longnose gar, *Lepisosteus osseus*. J. Exp. Biol. 199:2139–2151.

MOSER, H. G., W. J. RICHARDS, D. M. COHEN, M. P. FAHAY, A. W. KENDALL JR., AND S. L. RICHARDS. 1984. Ontogeny and systematics of fishes. American Society of Ichthyologists and Herpetologists, Spec. Publ. 1. Allen Press, Lawrence, KS.

NELSON, J. S. 1994. Fishes of the world. John Wiley and Sons, New York.

NISSANOV, J., AND R. C. EATON. 1989. Reticulospinal control of rapid escape turning maneuvers in fishes. Am. Zool. 29:103–121.

NOGUCHI, T., D. F. HWANG, O. ARAKAWA, H. SUGITA, Y. DEGUCHI, Y. SHIDA, AND K. HASIMOTO. 1987. *Vibro alginolyticus*, a tetrodotoxin-producing bacterium, in the intestines of the fish *Fugu vermicularis vermicularis*. Mar. Biol. 94:625–630.

NURSALL, J. R. 1958. The caudal fin as a hydrofoil. Evolution 12:116–120.

PIETSCH, T. W. 1984. Lophiiformes: development and relationships, p. 320–325. *In:* Ontogeny and systematics of fishes. H. G. Moser, W. J. Richards, D. M. Cohen, M. P. Fahay, A. W. Kendall Jr., and S. L. Richards (eds.). American Society of Ichthyologists and Herpetologists, Spec. Publ. 1. Allen Press, Lawrence, KS.

ROSEN, D. E. 1984. Zeiforms as primitive plectognath fishes. Am. Mus. Novit. 2782:1–45.

TYLER, J. C. 1963. The apparent reduction in number of caudal vertebrae in trunkfishes (Ostraciontoidea, Plectognathi). Proc. Acad. Nat. Sci. Phila. 115: 153–190.

———. 1980. Osteology, phylogeny, and higher classification of the fishes of the order Plectognathi (Tetraodontiformes). NOAA Tech. Rept., NMFS Circ. 434:1–422.

———, AND A. F. BANNIKOV. 1992. A remarkable new genus of tetraodontiform fish with features of both balistids and ostraciids from the Eocene of Turkmenistan. Smith. Contr. Paleobiol. 72:1–14.

———, AND L. SORBINI. 1996. New superfamily and three new families of tetraodontiform fishes from the upper Cretaceous: the earliest and most morphologically primitive plectognaths. *Ibid.* 83:1–59.

———, A. JERZMANSKA, A. F. BANNIKOV, AND J. SWIDNICKI. 1993. Two new genera and species of oli-

gocene spikefishes (Tetraodontiformes: Triacanthodidae), the first fossils of the Hollardiinae and Triacanthodinae. *Ibid.* 75:1–27.

WAINWRIGHT, P. C., R. G. TURINGAN, AND E. L. BRAINERD. 1995. Functional morphology of pufferfish inflation: mechanism of the buccal pump. Copeia 1995:614–625.

WEBB, P. W. 1976. The effect of size on the fast-start performance of a rainbow trout *Salmo gairdneri*, and a consideration of piscivorous predator-prey interactions. J. Exp. Biol. 65:157–177.

———. 1978. Fast-start performance and body form in seven species of teleost fish. *Ibid.* 74:221–226.

———. 1981. The effect of the bottom on the fast start of flatfish *Citharichthys stigmaeus*. Fish. Bull. 9: 271–276.

———. 1982. Fast-start resistance of trout. J. Exp. Biol. 96:93–106.

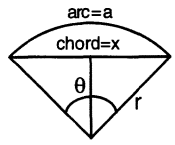
WEIHS, D. 1973. The mechanism of rapid starting of slender fish. Biorheology 10:343–350.

WINTERBOTTOM, R. 1974. The familial phylogeny of the Tetraodontiformes (Acanthopterygii: Pisces) as evidenced by their comparative myology. Smith. Contr. Zool. 155:1–201.

ZOTTOLI, S. J. 1978. Comparative studies on the Mauthner cell in fish and amphibians, p. 13–45. *In:* Neurobiology of the Mauthner cell. D. Faber and H. Korn (eds.). Raven Press, New York.

(ELB) DEPARTMENT OF BIOLOGY AND GRADUATE PROGRAM IN ORGANISMIC AND EVOLUTIONARY BIOLOGY, UNIVERSITY OF MASSACHUSETTS, AMHERST, MASSACHUSETTS 01003; AND (SNP) DEPARTMENT OF ZOOLOGY, DUKE UNIVERSITY, DURHAM, NORTH CAROLINA 27708. E-mail: (ELB) brainerd@bio.umass.edu. Send reprint requests to ELB. Submitted: 26 May 1997. Accepted: 31 March 1998. Section editor: J. D. McEachran.

APPENDIX 1. DERIVATION OF RELATIONSHIP BETWEEN CURVATURE COEFFICIENT (CC) AND ANGLE SUBTENDED BY ARC OF BODY.



$$a = r\theta \qquad \frac{x}{2r} = \sin\left(\frac{\theta}{2}\right)$$

$$x = (2r)\sin\left(\frac{\theta}{2}\right)$$

$$CC = \frac{\text{chord}}{\text{arc}} = \frac{x}{a} = \frac{2\sin\left(\frac{\theta}{2}\right)}{\theta}$$

APPENDIX 2. SEGMENT LENGTHS USED TO ESTIMATE INTERSEGMENTAL ANGLES USING THE SEGMENT-ANGLE ANALYSIS METHOD (JAYNE AND LAUDER, 1993). Segment 1 is the head plus the first few fused vertebrae (if any). The last segment is the caudal fin. Lengths of segments are expressed as a running sum of the position of the caudal edge of the segment, expressed as a percentage of the total body length. These data may be used, along with data on intersegmental angles in Figure 6, to reconstruct the shape of the fishes at maximum curvature.

M. hispidus		*A. chirurgus*		*A. saxatilis*		*S. coeruleus*	
Seg.	% length	Seg.	% length	Seg.	% length	Seg.	% length
1	46.4	1	36.1	1	35.7	1	22.8
2	48.7	2	37.6	2	38.3	2	25.0
3	51.4	3	40.1	3	41.4	3	27.1
4	54.2	4	42.9	4	44.3	4	30.0
5	57.3	5	45.9	5	47.4	5	33.0
6	60.2	6	49.5	6	50.3	6	36.4
7	63.3	7	52.8	7	53.2	7	39.8
8	66.3	8	56.3	8	56.2	8	43.0
9	69.5	9	59.6	9	59.0	9	46.2
10	72.9	10	63.0	10	61.8	10	49.6
11	76.4	11	66.3	11	64.8	11	53.0
12	79.8	12	70.0	12	67.7	12	56.3
13	83.3	13	73.5	13	70.6	13	59.6
14	86.9	14	77.1	14	73.2	14	63.0
15	90.4	15	81.1	15	76.0	15	66.3
16	94.1	16	85.1	16	78.6	16	69.5
17	100	17	88.6	17	81.3	17	72.9
		18	91.1	18	84.0	18	76.0
		19	92.8	19	86.8	19	79.1
		20	100	20	89.4	20	82.2
				21	91.7	21	85.2
				22	93.6	22	88.4
				23	100	23	91.5
						24	93.8
						25	100