INTRASPECIFIC SCALING OF FEEDING MECHANICS IN AN ONTOGENETIC SERIES OF ZEBRAFISH, *DANIO RERIO*

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Summary

While a vast literature and long tradition of examining the scaling of locomotory function exists, scaling studies on feeding mechanics are relatively rare. A recent increase in research activity examining the scaling of feeding kinematics has led to conflicting results. These divergent findings may be due to the inherent differences in the biophysical systems being examined. The present study examines the role of growth in the scaling of feeding kinematics in an ontogenetic series of zebrafish, *Danio rerio*. Although many other studies have investigated aquatic feeding, this study represents the first to quantify detailed feeding kinematics in first-feeding larvae. This study examines both the effects of violating assumptions of geometric similarity when examining scaling relationships and the role water viscosity plays in molding scaling coefficients derived from feeding kinematics. The effects of Reynolds number, generally not relevant in vertebrate feeding studies, play a crucial role in determining scaling relationships in this species. Many scaling coefficients reflect the functional challenges of feeding at low Reynolds numbers. Moreover, scaling patterns in feeding mechanics often reflect allometric growth during early ontogeny. The advent of high-speed video recording ($1000\,\mathrm{frames\,s^{-1}}$) now allows the kinematics of feeding at these small sizes to be rigorously examined.

Key words: scaling, feeding, kinematics, allometry, geometric similarity, ontogeny, zebrafish, *Danio rerio*.

Introduction

Physiologists have long realized the profound effects of size on nearly all physiological parameters ranging from metabolic rate to muscle function (Schmidt-Nielsen, 1984; Calder, 1996). The effects of scaling on the mechanics of musculoskeletal systems have been addressed at two levels. Reductionist approaches examine the behavior of isolated muscle fiber bundles essential in establishing the inherent capabilities of muscle tissue from animals of different sizes (Altringham and Johnston, 1990; James et al., 1998). However, a whole-animal approach is necessary to translate these physiological properties into ecologically significant performance traits (McMahon, 1975, 1984; Dial et al., 1997a,b). Locomotion and feeding have received the most attention from the perspective of the whole organism because they are traits with obvious implications for fitness (Wassersug and Sperry, 1977; Carrier, 1987, 1996; Losos, 1990; Miller et al., 1993; Garland, 1983, 1985; Katz and Gosline, 1993).

Most scaling studies of physiological function have used an interspecific approach. For example, the well-known relationship between metabolic rate and size, illustrated by the classic mouse-to-elephant curve, covers a more than 400 000-fold increase in mass of a broad range of mammalian species (Brody, 1945). The importance of size is apparent given that such interspecific studies yield these informative relationships. In this, as in all interspecific studies, the effects of

environment, ecology, phylogeny and gross morphology are generally ignored, which may confound the effect of size on physiology. Examination of an ontogenetic series, although often more limited in size range, allows us to remove these factors to test models generated from data on the scaling of basic muscle physiology. More importantly, ontogenetic studies allow us to examine how animals maintain functional integrity as they grow (Carrier, 1996).

The aim of this study was to examine the role of growth in the scaling of feeding mechanics in an ontogenetic series of zebrafish, *Danio rerio* (Hamilton), ranging from first-feeding larvae to reproductively mature adults. This study represents an initial attempt to provide empirical data on functional changes in feeding performance associated with allometric growth during early life history stages. Three basic types of kinematic variables were examined, maximum excursions, timing variables and maximum velocity variables. No kinematic models for feeding in allometrically growing animals presently exist (but, for locomotion, see Emerson, 1978). These kinematic data, coupled with basic morphological measurements, can be used to test whether violating assumptions of geometric similarity will cause significant departures from the expected results in an isometrically growing series. The scaling of these kinematic variables is compared with the null hypothesis of geometric similarity (Hill, 1950).

The pioneering work of A. V. Hill (1950) established a theoretical framework for the scaling of muscle mechanics. Working from the behavior of whole muscles, Hill proposed a model describing the scaling of kinematic variables of the musculoskeletal system as a whole. He predicted that, for an animal growing isometrically (geometrically similar, Fig. 1A), the maximal muscle shortening velocity would not change with size. In addition, maximal excursions of skeletal elements would scale with body length (isometrically), implying that timing variables, such as gape cycle, would also scale with body length (Fig. 1C). Yet these simple predictions have rarely been tested within feeding systems. Testing this model ideally requires (i) a simple muscle lever system (Fig. 1B), (ii) isometric variation over several orders of magnitude in size, and (iii) the same behavior exhibited over the entire size range.

Research on vertebrate feeding mechanisms, with their relatively simple lever systems, has been a fertile field for functional morphologists; however, in most studies, size variation has been avoided or dealt with statistically as a confounding factor. The small body of work addressing issues of scaling in relation to feeding across an ontogenetic series has yielded contradictory results. The work of O'Reilly et al. (1993), which examined feeding in isometrically growing toads, supports the model of Hill (1950). Richard and Wainwright (1995), looking at feeding across an ontogenetic series of largemouth bass (*Micropterus salmoides*), found that relatively few kinematic variables scaled with Hill's predictions. The work of Reilly (1995) on salamander (*Salamandra salamandra*) feeding examined only two size classes and is at odds with both these studies, having found that stereotyped feeding behavior in these salamanders translated into no significant change in kinematic variables across ontogeny. Although all the animals investigated in these studies grew isometrically, inherent differences in terrestrial *versus* aquatic feeding may help explain disparate scaling variables.

Thus, to examine rigorously the scaling of feeding kinematics, it is vital to compare animals using the same basic musculoskeletal system feeding within the same medium. Reproductively mature *Danio rerio* are approximately the same size as the smallest fish used in the study of Richard and Wainwright (1995) (30 mm). Since first-feeding *Danio rerio* larvae were 4 mm in total length (*TL*), these data serve to extend the lower end of the size spectrum by an order of magnitude in length (and by several orders of magnitude in mass) while maintaining the same basic physical parameters. There exists a vast literature on feeding kinematics in adult fishes (Liem, 1978, 1980; Lauder, 1983, 1985; Muller, 1987; Hoogerhoud, 1989; Wainwright and Lauder, 1986, 1992; Wainwright, 1989; Wainwright et al., 1991; Westneat and Wainwright, 1989; Westneat, 1990, 1994; Gibb, 1995, 1997; Ferry-Graham, 1998), but variation in feeding kinematics has never been quantified over this size range because of the difficulty of working with such small organisms. This is unfortunate since larval fish are at much greater risk of starvation than are adult fishes. The purported importance of studies on feeding kinematics entails the assumption that there is a selective advantage afforded by efficient feeding. At these small sizes, this becomes increasingly important and may determine whether an animal survives to reproductive age (Carrier, 1996). The advent of high-speed video recording (1000 frames s^{-1}) now allows the kinematics of feeding at these small sizes to be rigorously examined.
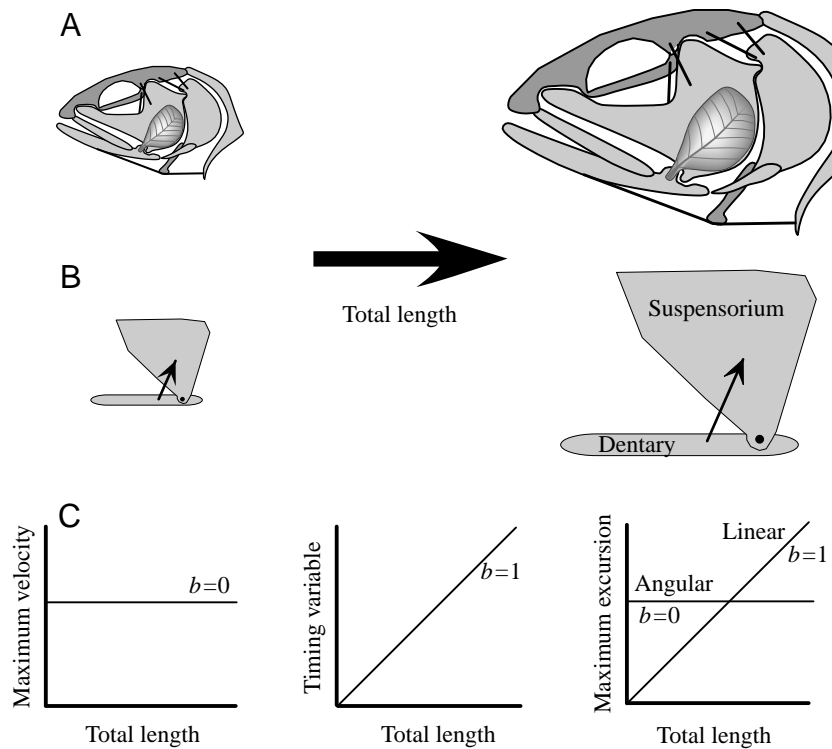

Fig. 1. (A,B) Schematic diagrams of a fish undergoing isometric growth and of the muscle lever system involved. Note that all elements, particularly the jaw elements, are growing with geometric similarity. Given this growth pattern, I would expect linear velocities to remain constant, timing variables and maximum linear excursions to scale with a slope (*b*) of 1 and maximum angular excursions to scale with a slope of zero (C).

The aquatic environment and concomitant Reynolds number regime, particularly at these small sizes, may significantly affect feeding kinematics. Reynolds number (*Re*), a measure of the ratio between the inertial and viscous forces of a fluid, significantly affects the lives of aquatic organisms (Vogel, 1981). The overwhelming majority of aquatic feeding studies have overlooked the influence of water viscosity since viscous forces are not likely to be important at adult sizes. However, the importance of viscous forces during the early larval stages will probably influence the scaling of feeding kinematics within this ontogenetic series.

Materials and methods
Morphological analyses

Thirteen *Danio rerio* Hamilton, ranging in total length from 3.9 to 27 mm, were used in the kinematic analyses. Fish were bred in the laboratory, and different size classes were kept in separate tanks. Approximately 30 individuals were kept in 20 l tanks at 27 °C on a 12 h:12 h light:dark photoperiod. Independent samples were taken from the four age groups used. First-feeding larvae were fed a combination of *Paramecium* sp. and dry *Spirulina* sp., a protein-rich alga. After 10 days of feeding, platinum-grade (Argent) newly hatched brine shrimp (*Artemia* sp.) were added to the feeding regime. After the sixteenth day of feeding, fish were fed exclusively on newly hatched brine shrimp. Adults were also maintained on freshly hatched brine shrimp. Three or four individuals in each of four ontogenetic stages were used in this analysis. The four ontogenetic stages represent significant transformations in the life history of these fishes: first-feeding larvae (approximately 4 mm *TL*), larvae undergoing metamorphosis (approximately 6 mm *TL*), juveniles (11–12 mm *TL*) and young adults (20–27 mm *TL*).

To determine the relationship between the scaling of morphological structures and that of kinematic traits, several cranial skeletal structures were measured from 11 *Danio rerio* ranging in total length from 3 to 24 mm. Measurements were taken from cleared and stained fish, since this was the only way to discern skeletal morphology in first-feeding larvae. Morphological features from 11 individuals were measured with a microscope using an ocular micrometer. Measurements included: maximum anatomical gape, hyoid length (measured as the epihyal and ceratohyal, since these are still one solid component in early larval stages), hyoid width (across the widest portion of the ceratohyal), the length of the dentigerous arm of the premaxilla, the length of the ascending arm of the premaxilla, maxilla length, dentary length and neurocranium length. Reduced major axis regressions were used to assess growth rate in these feeding structures. A modified *t*-test was used to test whether these skeletal elements grew with a slope significantly (*P*<0.05) different from 1.

Video analyses

To quantify the scaling of kinematic variables during ontogeny, adult, juvenile and newly metamorphosed *Danio rerio* were placed individually in filming tanks and filmed while feeding. Five first-feeding larvae were placed together in one tank, and the entire tank was filmed to improve the likelihood of capturing a good lateral feeding event. First-feeding larvae and metamorphosing larvae were filmed in specially designed tanks (1.5 cm deep, 2.5 cm long and 1.0 cm wide). Juveniles and adults were fed in Plexiglas feeding tanks (10.0 cm deep, 10.0 cm long and 2.0 cm wide). In an attempt to compensate partially for changes in predator to prey size, first-feeding and metamorphosing larvae were fed *Paramecium* during feeding trials while larger size classes were fed newly hatched brine shrimp.

First-feeding larvae were filmed at a rate of 1000 frames s^{-1} while all other stages were filmed at 500 frames s^{-1} using a Kodak Ekta Pro EM1012 digital high-speed video system equipped with an SI intensified imager. Adults were filmed with a 105 mm Nikon macro lens. All other stages were filmed through a Nikon SMZ-U stereomicroscope at 7.5–75× magnification. The video camera was attached to the ocular end of the microscope, and both microscope and camera were held laterally with the aid of two boom stands. To illuminate the chamber, two high-intensity Dolan Jenner Fiber Lite illuminators were used. After a 15 min period of acclimation within the filming chamber, prey were introduced to *Danio rerio* larvae *via* a Pasteur pipette.

Thirty-five feeding sequences were analyzed. Means from individual feeding strikes were treated as independent data points on the regressions. Three feedings each from three adults and three feeding strikes from one smaller adult were used. Three feeding strikes each from three juveniles were tested. Two feedings each from three newly metamorphosed individuals were used. As mentioned above, larval tanks containing five individuals were used in filming. Three feedings from two tanks and two feedings from another tank were used for all analyses. Tanks were treated as individuals. Means from each individual were used in statistical analyses.

Only those sequences capturing a lateral view of a feeding event were used in the analyses. Because the larvae are quite transparent, only six landmarks could be consistently digitized through all ontogenetic stages (Fig. 2). The positions of these landmarks were later used to calculate 19 kinematic variables. The kinematics of the strike were measured from the video frame preceding mouth opening to the frame in which the head resumed the pre-strike conformation. Kinematic variables consisted of three basic types: maximum excursions, maximum velocities and timing variables. Maximum excursions included (i) maximum gape, the distance between the tip of the dentary and the premaxilla (or the anterior tip of the neurocranium in first-feeding larvae); (ii) maximum premaxillary protrusion, the distance between the anterior end of the neurocranium and the tip of the fully protruded premaxilla; (iii) maximum hyoid depression, the distance from the ventral edge of the eye to the ventral edge of the hyoid apparatus; and (iv) maximum head lift angle, measured between the middle of the neurocranium, the junction of the neurocranium with the
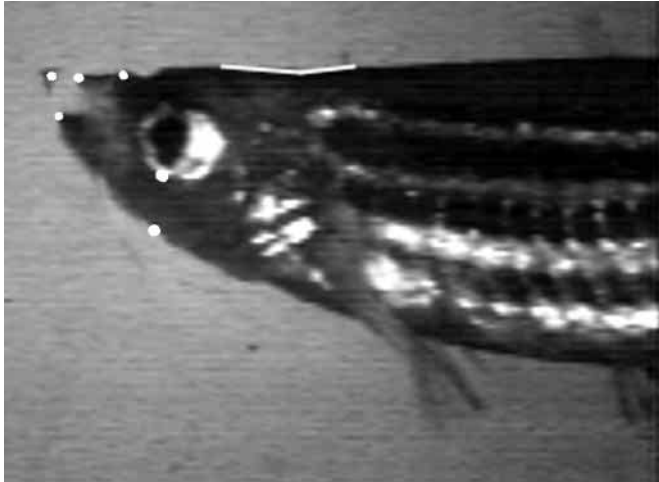
Fig. 2. Still photograph from a high-speed video recording illustrating the six anatomical landmarks measured on an adult zebrafish to generate 19 kinematic variables. Descriptions of landmarks can be found within the text.

Table 1. *Scaling coefficients from reduced major axis regressions of skeletal features on total length of fish*

Cranial skeletal element	Scaling coefficient	r^2
Neurocranium length	1.00±0.11	0.89
Gape	1.38±0.18*	0.85
Hyoid length	1.12±0.06	0.97
Hyoid width	1.10±0.13	0.86
Premaxilla (ascending arm)	1.33±0.15*	0.93
Premaxilla (dentigerous arm)	0.82±0.10*	0.91
Maxilla length	1.06±0.08	0.95
Dentary length	1.20±0.13*	0.90

Scaling coefficients denote slope and were measured to determine the growth trajectory of individual elements important in feeding. Since this study entails feeding kinematics, only cranial elements were measured.

Values are means ± S.E.M., N=11. An asterisk denotes that slopes were significantly different ($P<0.05$) from 1 as determined by a modified t-test.

vertebral column and the anterior insertion of the dorsal fin. Timing variables included (i) opening phase, the time from the start of mouth opening until maximum gape is achieved; (ii) time to maximum hyoid depression, the time taken for the hyoid to reach its ventral-most position; (iii) time to maximum head lift (cranial elevation); (iv) closing phase, or the duration of lower jaw (dentary) adduction; (v) gape cycle time; (vi) attack time, the entire time from frame zero until the feeding apparatus had returned to its original position; (vii) time to maximum gape; (viii) time to maximum premaxillary protrusion; and (ix) time to hyoid elevation. Maximum linear velocities included (i) hyoid depression velocity; (ii) jaw opening velocity; (iii) jaw closing velocity; (iv) premaxillary protrusion velocity; and (v) hyoid elevation velocity. The only angular velocity measured was head lift velocity.

The relationship between the total length of the fish and the kinematic variables was determined by least-squares regression of log-transformed variables. Regressions yielded scaling coefficients describing the relative allometry or isometry of the relationship. Since separate regressions were run for each kinematic variable, a Bonferroni correction was used to determine the significance of individual tests. Least-squares regressions are the appropriate statistic to use because the measurement of total length had significantly less error associated with it than the measurement of any of the kinematic variables (Sokal and Rohlf, 1995; McArdle, 1988). More importantly, previous data sets used least-squares regression, thus facilitating comparisons with the present study. Least-squares regressions on log-transformed data yielded equations of the form:

$$\log y = \log a + b \log TL,$$

where *TL* is total length, *y* is any performance variable, *a* is the *y* intercept and *b* is the scaling or allometric coefficient.

Results
Morphological analyses

For all results from this morphological analysis, a slope of 1 indicates that a given element was growing isometrically relative to total length. A slope significantly greater than 1 represented positive allometric growth, and a slope significantly less than 1 represented negative allometry relative to total length. All cranial elements measured were found to vary significantly with total length (Table 1). Dentary length, anatomical gape and the ascending arm of the premaxilla all grew with positive allometry. Gape grew at the same rate as the ascending arm of the premaxilla (Fig. 3). Thus, these structures were relatively bigger in the adults than they were at earlier ontogenetic stages. Neurocranium length, hyoid length and width and maxilla length all grew isometrically, while the dentigerous arm of the premaxilla grew with negative allometry (Fig. 3; Table 1). Thus, the assumptions of geometric similarity inherent in the model of Hill (1950) were violated. Although only cranial elements were measured here, measurements of other morphological features during ontogeny have shown that the early life history of fishes is characterized by allometric growth (Strauss and Fuiman, 1985; Osse, 1990; Weatherly, 1990; Osse and van den Boogaart, 1995).

Timing variables

Most timing variables increased significantly with increasing total length of *Danio rerio* (Fig. 4; Table 2). Gape cycle time, the time for full abduction and subsequent adduction of the jaw, which scaled with a slope not significantly different from zero (b=0.163±0.07, mean ± S.E.M., N=13), revealed less variation associated with total length than that seen in total attack time. There was, however, a difference in the components that make up the gape cycle. While both
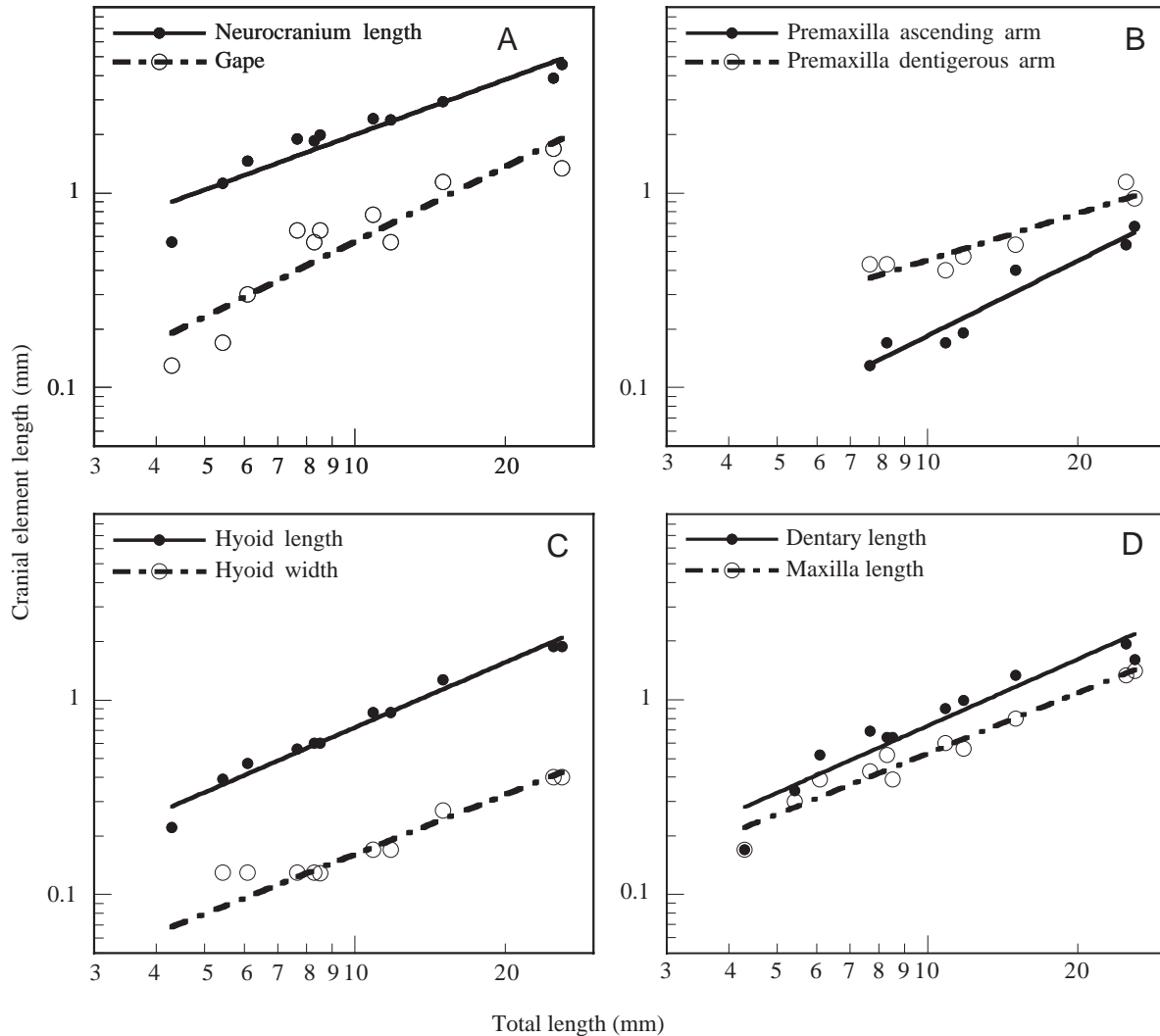
Fig. 3. (A–D) Log–log plots of cranial elements measured against total length. Differences in slope indicate that, while some elements grew isometrically (neurocranium length, hyoid length and width and maxilla length), others grew allometrically (premaxilla length, dentary length and gape). For further details, see Table 1.

scaled with negative allometry in relation to that expected given geometric similarity, the opening phase scaled with a slope significantly greater than zero ($b=0.314\pm0.11$, mean ± S.E.M., $N=13$; Table 2), while the slope for the closing phase was not significantly different from zero ($b=0.008\pm0.06$; $P=0.908$).

Time to maximum hyoid depression, time to maximum gape and time to maximum head lift all increased with increased total length. Moreover, all these timing variables scaled with approximately the same slope (Fig. 4). In contrast, time to hyoid elevation scaled with extreme negative allometry ($b=-0.666\pm0.08$, mean ± S.E.M., $N=13$; Table 2), illustrating that the time needed for the hyoid to return to its original position after a feeding event decreased significantly with increasing total length. As a result, attack time, here defined as the time from the frame before mouth opening until the return of the hyoid to its resting position after a feeding strike, scaled with negative allometry (Table 2), indicating

that larger fish had an absolutely shorter attack time. Mean attack time for first-feeding larvae was 172 ms, while that of adults was only 72 ms.

Maximum excursions

While all maximum excursion variables scaled significantly with total length, unlike the situation seen with the timing variables in which many shared similar scaling coefficients, allometric coefficients were markedly different for each of the four excursion variables (Figs 5, 6B). Maximum gape was the only excursion variable to scale isometrically, such that there was a tight correspondence between a change in total length and a change in functional gape ($b=0.94\pm0.09$, mean ± S.E.M., $N=13$). Whereas maximum hyoid depression scaled with moderate negative allometry ($b=0.423$) with regard to predictions based on geometric similarity, premaxillary protrusion scaled with extreme positive allometry ($b=1.613$). This result attests to the increasing importance of premaxillary
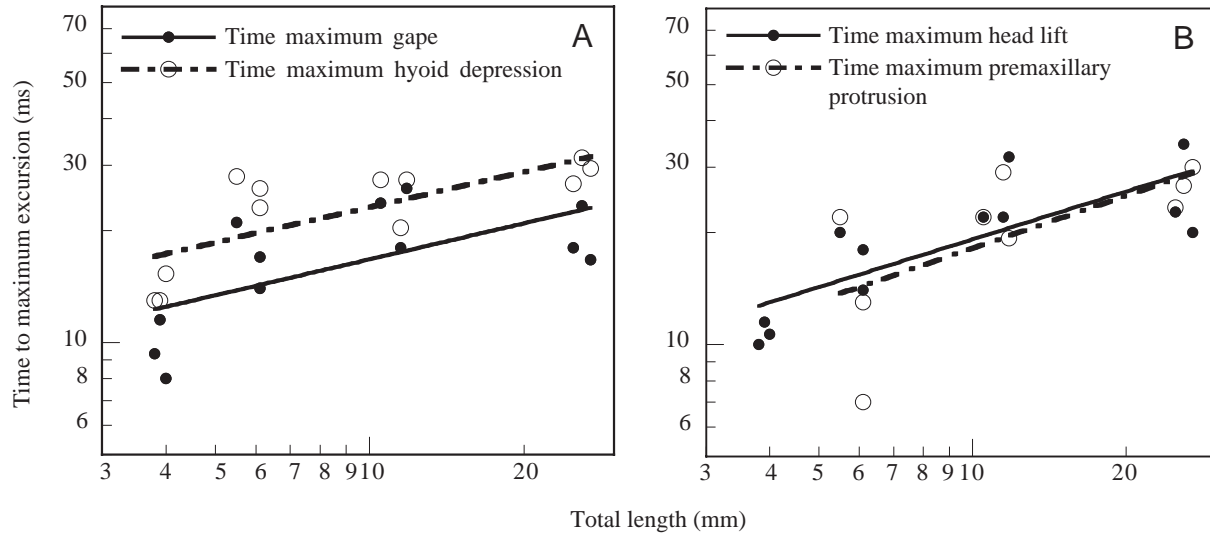
Fig. 4. (A,B) Log–log plots of four timing variables *versus* total length. Note the similar slopes for these variables. None of these kinematic variables increased as would be predicted given geometric similarity (*b*=1). For further details, see Table 2.

Table 2. *Least-squares regression statistics for log-transformed kinematic variables regressed on total length of fish*

Kinematic variables	Scaling coefficient	r^2	P	Geometric similarity	Modified *t*-test P	Richard and Wainwright (1995)
Timing variables						
Attack time	−0.449±0.06**	0.83	<0.0001	1	<0.001**	
Gape cycle time	0.163±0.07	0.34	0.0475	1	<0.001**	0.43±0.07
Opening phase	0.314±0.11**	0.43	0.0201	1	<0.001**	
Closing phase	0.008±0.06	0	0.9084	1	<0.001**	
Time to maximum hyoid depression	0.315±0.09	0.55	0.0058	1	<0.001**	0.31±0.06
Time to maximum gape	0.321±0.12	0.42	0.0238	1	<0.001**	0.31±0.07
Time to maximum premaxillary protrusion	0.468±0.20	0.43	0.0562	1	<0.001**	0.21±0.08
Time to maximum head lift	0.426±0.10**	0.62	0.0022	1	<0.001**	0.30±0.07
Time to hyoid elevation	−0.666±0.08**	0.88	<0.0001	1	<0.001**	
Maximum excursions						
Maximum gape	0.940±0.09**	0.92	<0.0001	1	<0.001**	1.10±0.08
Maximum hyoid depression	0.423±0.05**	0.88	<0.0001	1	<0.001**	1.19±0.06
Maximum premaxillary protrusion	1.613±0.18**	0.92	<0.0001	1	<0.001**	0.93±0.02
Maximum head lift angle	−0.466±0.11**	0.65	0.0016	0	<0.001**	−0.01±0.06
Maximum velocities						
Maximum jaw opening velocity	0.559±0.12**	0.69	0.0008	0		0.76±0.09
Maximum jaw closing velocity	0.708±0.16**	0.66	0.0014	0		0.64±0.05
Maximum hyoid depression velocity	0.400±0.14	0.44	0.0179	0		0.75±0.10
Maximum hyoid elevation velocity	1.061±0.11**	0.90	<0.0001	0		
Maximum premaxillary protrusion velocity	1.275±0.08**	0.98	<0.0001	0		0.39±0.08
Maximum head lift velocity	−0.591±0.12**	0.71	0.0006	0		−0.24±0.05

Values are means ± S.E.M. (*N*=13). Note the similarity between the scaling coefficients (slopes) for kinematic variables from this study and those of Richard and Wainwright (1995), especially when compared with predictions based on geometric similarity.

A modified *t*-test was used to test whether the scaling coefficient was significantly different from the slope predicted by geometric similarity (when that slope was different from zero).

**Indicates that the relationship was significant after Bonferroni correction.

Values from Richard and Wainwright are means ± S.E.M. (*N*=10).

protrusion during growth. First-feeding larvae showed no premaxillary protrusion, whereas adult *Danio rerio* achieved protrusions of between 7 and 21 % of head length. Maximum

hyoid depression (*b*=0.423±0.05, mean ± S.E.M., *N*=13) scaled with a slope considerably less than that of maximum gape, indicating that larval fish produced a hyoid depression
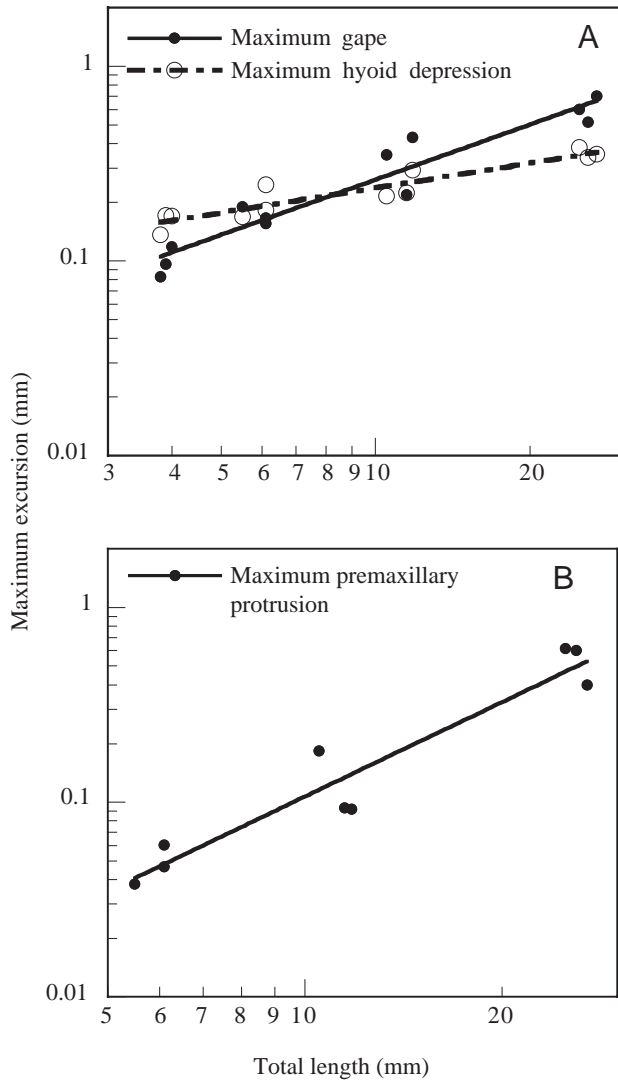
Fig. 5. (A,B) Log–log plots of maximum linear excursion variables *versus* total length. Note that these excursion variables all scale with different slopes. The scaling coefficient in Table 2 for maximum premaxillary protrusion considers all stages, while B illustrates only those stages at which a premaxilla is present. However, both data sets can be interpreted as characterizing strong positive allometry as regards isometry (*b*=1). For further details, see Table 2.
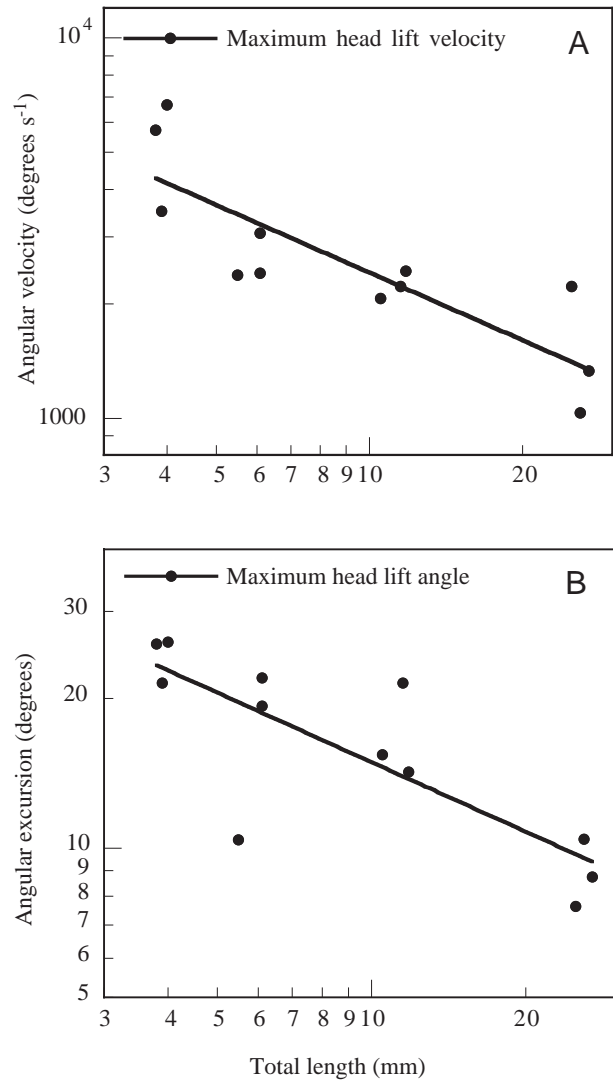

Fig. 6. (A,B) Log–log plots of kinematic variables associated with cranial elevation. Note the decreases in both maximum head lift angle and head lift velocity with ontogeny. For further details, see Table 2.

considerably larger than what would be expected given isometry. In contrast to these positive scaling patterns, maximum head lift angle scaled with extreme negative allometry (Fig. 6B; *b*=−0.466±0.11, mean ± s.e.m., *N*=13), such that larger fish achieved a considerably smaller head angle during feeding than did larval and juvenile fish. While larval fish often produced head elevations of greater than 24 °, adult *Danio rerio* were found to achieve mean head elevations of only 9 °, with intermediate stages producing mean angles of approximately 17 °.

Maximum velocities

All maximum velocity variables except maximum hyoid depression velocity scaled with slopes significantly different from zero. Most maximum linear velocities increased with increasing length, although three of the six (Table 2) scaled with negative allometry with regard to isometry (*b*=1). Maximum jaw opening and closing velocities scaled similarly, showing a significant increase in jaw velocity with increased body length (Fig. 7A; Table 2). Scaling values for maximum hyoid depression velocity indicated that, as fish grew, they were able to achieve a greater velocity of hyoid depression, although significantly less than that predicted by isometry. This trend was most pronounced for maximum hyoid elevation and premaxillary protrusion velocities, which scaled with positive allometry (Table 2).

In contrast to the increasing linear velocities of skull movements at larger total lengths, the angular maximum velocity of head lift scaled with extreme negative allometry;
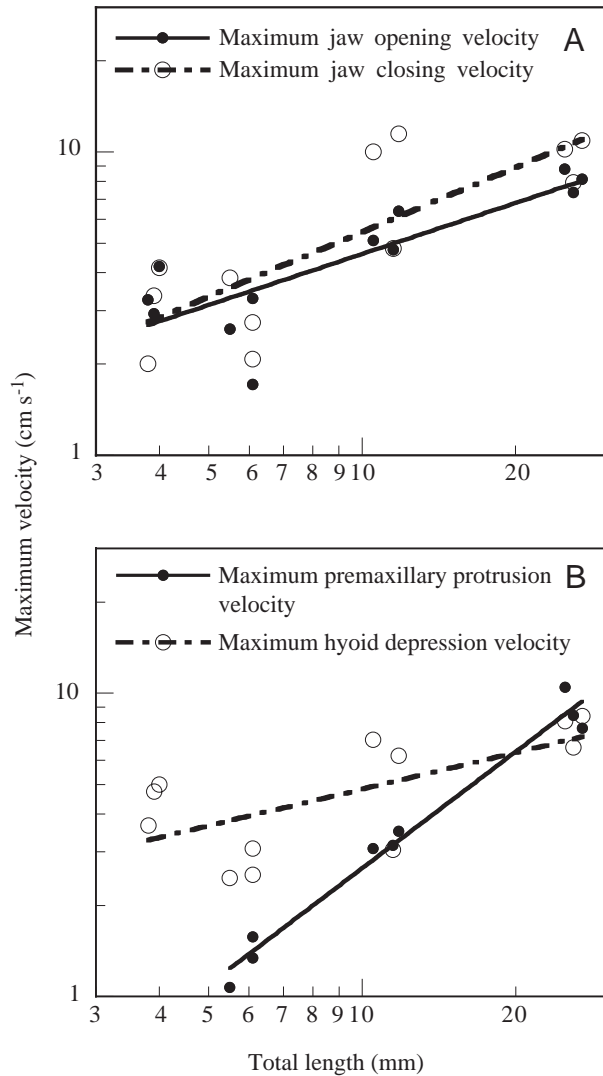
Fig. 7. (A,B) Log–log plots of maximum velocity variables measured against total length. Note that for all variables there is a trend towards increased velocity with ontogeny. However, except for maximum premaxillary protrusion velocity, other velocities shown here scale with negative allometry as regards isometry ($b=1$), in other words they do not increase as rapidly as would be expected given geometric similarity. For further details, see Table 2.

larvae therefore produced an absolutely greater angular velocity than that produced by adult fish (Fig. 6A). While first-feeding larvae typically elevated their cranium through a rotation of 24 ° in 12 ms, adults generally took 20 ms to attain a maximum angle of 10 °.

Discussion

The kinematics of feeding mechanics in *Danio rerio* were significantly affected by total length. Values for most kinematic variables (74 %) increased with increasing total length, although most scaled with negative allometry in relation to expected slopes given geometric similarity. Given

that 95 % of the variables examined here showed a significant relationship with total length, it is important that we consider the implications of size in kinematic studies. Moreover, noncompliance with assumptions of geometric similarity in the model of Hill (1950) results in poor predictions of scaling coefficients, such that morphological allometry has profound effects on kinematic scaling variables.

Comparison with other data sets

A basic assumption of the model of Hill (1950) is that the animals being compared are geometrically similar. Allometric growth typifies early growth trajectories in fishes (Strauss and Fuiman, 1985; Osse, 1990; Weatherly, 1990; Osse and van den Boogaart, 1995). Moreover, data from this ontogenetic series, in which many key cranial elements undergo allometric growth (Table 2), violate these assumptions. Consequently, Hill's model is not appropriate to predict the scaling relationships observed for nearly all the kinematic variables associated with feeding. Of the 19 variables examined, only one scaled according to geometric similarity. Work on feeding in bass by Richard and Wainwright (1995) found that only six of 24 variables matched Hill's predictions. Scaling coefficients for the present study did not agree with the findings of either Reilly (1995) or O'Reilly et al. (1995). In contrast, of the 14 common variables examined in this study and that of Richard and Wainwright (1995), there was a much greater correspondence (Table 2).

In the present study, six of nine timing variables showed a significant positive relationship with total length (Table 2). Of these six variables, three (time to maximum hyoid depression, time to maximum gape and time to maximum head lift) were quite similar to the values of Richard and Wainwright (1995). Even with markedly different growth patterns, allometric growth of *Danio rerio versus* isometric growth of bass, there was considerable overlap in some scaling coefficients.

Maximum linear excursions and maximum angular excursions from Richard and Wainwright (1995) all scaled with a slope of 1 and zero, respectively, as would be expected for geometric similarity. The four maximum excursion variables measured in *Danio rerio* yielded significantly different scaling coefficients (Table 2). Of the linear excursions measured here, only maximum gape scaled with a slope similar to that observed in bass. Maximum premaxillary protrusion scaled with a remarkably high slope ($b=1.613$), showing that premaxillary protrusion becomes increasingly important as *Danio rerio* mature and grow. The scaling value for maximum hyoid depression ($b=0.423$), particularly when compared with that of Richard and Wainwright (1995) ($b=1.19\pm0.06$), suggests that extreme hyoid depression is more important in larval *Danio rerio* than in younger stages of bass. The only angular excursion measured in this study was head lift angle, which scaled with extreme negative allometry ($b=-0.466\pm0.11$). Comparison with the scaling coefficient of Richard and Wainwright (1995) ($b=-0.01\pm0.06$) reveals that there was a significant decrease in importance of head lift angle with ontogeny in *Danio rerio* not seen in bass.

Six maximum velocity variables were measured (Table 2). Two kinematic values (maximum jaw opening and closing velocities) were close to the values of Richard and Wainwright (1995). Such similarity may be due to the general consistency seen in scaling of maximum velocity within aquatic systems, as is illustrated by comparable slopes for maximum or burst performance in several studies. Gibson and Johnston (1995) found that maximum burst-swimming speed (U_{max}) for turbot (*Scophthalmus maximus*) scaled as $U_{max}=aTL^{0.74}$, in fish ranging from 0.88 to 8.0 cm *TL*. Moreover, even in situations of significantly different temperature regimes, such as in the Antarctic fish *Notothenia corriceps*, Archer and Johnston (1989) found that maximum velocity scaled as $TL^{0.66}$.

While there was an absolute increase in the maximum velocities generated, they did not increase as expected given predictions of geometric similarity; thus, relative velocity decreased with ontogeny. The results presented here and by Richard and Wainwright (1995) agree with the results of James et al. (1998), in which increases in total length were associated with a slowing of contractile properties of muscle bundles. This might explain the relative slowing seen in maximum velocities.

Geometric similarity – allometry versus *isometry*

Models based on geometric similarity assume that, with ontogeny, morphological elements will grow isometrically or in direct proportion to one another. While relatively few morphological measurements were made (Fig. 3; Table 1), allometric growth of skeletal elements characterizes the early life history stages of fishes (Fuiman, 1983; Strauss and Fuiman, 1985; Osse, 1990; Weatherly, 1990; Osse and van den Boogaart, 1995). Indeed, metamorphosis can be defined as the point at which fish assume adult morphology through a series of dramatic shifts in growth trajectories, changing from allometric to isometric growth.

Whereas the largemouth bass examined by Richard and Wainwright (1995) underwent isometric growth, as determined by measurement of key cranial elements, morphological measurements of *Danio rerio* revealed that many elements grew allometrically. Such divergent growth patterns may explain differences in scaling coefficients for *Danio rerio* and largemouth bass. Nearly all the kinematic variables that differed between the present study and that of Richard and Wainwright (1995) probably reflect significant departures from isometric growth in *Danio rerio*. Moreover, such departures from the scaling values of Richard and Wainwright (1995) may indicate qualitative differences between larval and post-larval feeding (L. P. Hernández, in preparation). It is reasonable to assume that maximum excursions will be affected most by differential growth of skeletal elements, such that positive allometric growth of the premaxilla would logically lead to a large scaling coefficient for maximum premaxillary protrusion. In the present study, maximum hyoid depression, predicted to increase in direct proportion to total length, scaled with a slope markedly less than expected, suggesting that at early stages the hyoid may be larger than expected. However, the

morphological data do not bear this out. Both hyoid length and width increased proportionately with total length (Table 1); thus, some other explanation must exist for the pattern seen in hyoid depression.

The scaling coefficients for maximum head lift angle and head lift velocity indicate that cranial elevation is more important in larvae than in adults. This may allow first-feeding larvae with extremely small mouths to increase their functional gape. This is supported by the fact that cranial elevations in larvae were consistently greater than in adults. While the maximum head lift angle seen for any of the adults was 11.9°, the minimum head lift angle for any larvae was 19.4°, and values ranged up to 31.1°.

Variables associated with gape (time to maximum gape, maximum gape and velocity of mouth opening and closing) all scaled similar to an isometrically growing series of largemouth bass (Richard and Wainwright, 1995). Thus, it it is possible that, although allometric growth is viewed as one of the defining characteristics in fish early life histories, muscles effecting mouth opening in *Danio rerio* may scale isometrically. Future studies examining the basic muscle physiology of larvae coupled with a scaling study of muscular anatomy may be able to answer some of these basic questions.

Predictions based on geometric similarity suggest that maximum linear velocities should not increase with increases in size because per sarcomere rate of shortening varies inversely with length (Hill, 1950). Because of the extreme difficulty in measuring muscle mass in these larvae, an examination of kinematic variables may provide insight into patterns of larval muscle growth. Scaling coefficients indicate that adults are generating greater velocities than larvae. This scaling pattern may be due to the allometric growth of muscles associated with feeding. Alternatively, either learning effects or sensorimotor limitations during the early stages may be affecting maximum potential (Carrier, 1996). Moreover, different Reynolds number regimes may influence the speed at which cranial elements can move through the viscous medium.

Hydrodynamic concerns

An area that has received little attention in aquatic feeding studies of vertebrates involves the effect of low Reynolds numbers on feeding (but, for larval fishes, see Osse, 1990; Osse and van den Boogaart, 1995). Typical kinematic studies on aquatic feeding use relatively large animals for which the effects of viscous forces can be ignored. Larval fish, however, function in a different hydrodynamic world, where viscosity problems are assuredly more important.

Although relatively few such studies have been undertaken, the greatest similarity in scaling coefficients of feeding behavior in vertebrates has been between the results in the present study and those of Richard and Wainwright (1995). Since both species share an aquatic environment, similar scaling variables may be explained by the similar functional challenges of suction feeding. However, it must be taken into account that, at the smallest larval sizes, the Reynolds number regime (Re≈5; L. P. Hernández, unpublished data) is markedly

different from that encountered at adult stages (Re≈120; L. P. Hernández, unpublished data). Three main zones of Reynolds number regime are generally accepted (Fuiman and Webb, 1988). Reynolds numbers less than 1 suggest a situation in which viscous forces predominate, although previous researchers have suggested that even at Re=20 fish larvae are certainly affected by the relative viscosity of the water (Webb and Weihs, 1986). Re>200 indicates that inertial forces are more important. Both viscous and inertial forces must be taken into account at intermediate Reynolds numbers, defined as 20<Re<200. Given these conditions, previous researchers have found that most larval fish are affected to some degree by the viscous forces of water (Fuiman and Webb, 1988). Indeed, Fuiman and Webb (1988) found that *Danio rerio* larvae spent 98 % of their time within the viscous and intermediate regimes. Such important hydrodynamic differences, legitimately ignored in studies of adult fishes, probably play a vital role in molding feeding kinematics at these early stages.

Viscosity factors probably affected several of the kinematic variables examined here. Attack time was highest for first-feeding larvae and decreased with total length, resulting in a negative scaling coefficient (b=−0.449). Time to hyoid elevation was also greatest for larvae and decreased with total length, with a slope of −0.666 (Table 2). Both variables have a significant portion of their variation explained by total length. But this is not likely to be a straightforward relationship because the factor that probably determines these scaling values is relative water viscosity, as represented by the Reynolds number. Attack time is so much longer for larvae because the time to hyoid elevation is so long at these early stages. Hyoid elevation is certain to be one of the variables most affected by the relative viscosity of the water. During these early stages, when the operculum is very poorly developed, an important constraint determining timing variables will probably be the time it takes a first-feeding larva to force this viscous fluid out of its buccal chamber.

Most anamniote larvae must pass through an ontogenetic stage in which aquatic feeding at small sizes is necessary. Functional morphologists have largely ignored the concomitant increase in viscous forces associated with feeding at small sizes. Technological advances in video recording equipment now make it possible to examine critically first feeding events in a variety of larvae. Detailed analyses of early feeding mechanics, coupled with the data that exist for adult feeding, will provide one means of investigating how animals are able to maintain functional integrity while undergoing rapid growth.

References

Altringham, J. D. and Johnston, I. A. (1990). Modelling muscle power output in a swimming fish. *J. Exp. Biol.* **148**, 395–402.

Archer, S. D. and Johnston, I. A. (1989). Kinematics of labriform and subcarangiform swimming in the Antarctic fish *Notothenia neglecta*. *J. Exp. Biol.* **143**, 195–210.

Brody, S. (1945). *Bioenergetics and Growth*. New York: Reinhold.

Calder, W. A. (1996). *Size, Function and Life History*. Cambridge, MA: Harvard Press.

Carrier, D. R. (1987). The evolution of locomotor stamina in tetrapods circumventing a mechanical constraint. *Paleobiology* **13**, 326–341.

Carrier, D. R. (1996). Ontogenetic limits on locomotor performance. *Physiol. Zool.* **69**, 467–488.

Dial, K. P., Biewener, A. A., Tobalske, B. W. and Warrick, D. R. (1997a). Mechanical power output of bird flight. *Nature* **390**, 67–70.

Dial, K. P., Tobalske, B. W. and Biewener, A. A. (1997b). Experimental studies in avian flight: The importance of studying a range of behaviours. *J. Morph.* **232**, 247.

Emerson, S. B. (1978). Allometry and jumping in frogs: helping the twain meet. *Evolution* **32**, 551–564.

Ferry-Graham, L. A. (1998). Effects of prey size and mobility on prey-capture kinematics in leopard sharks *Triakis semifasciata*. *J. Exp. Biol.* **201**, 2433–2444.

Fuiman, L. A. (1983). Growth gradients in fish larvae. *J. Fish Biol.* **23**, 117–123.

Fuiman, L. A. and Webb, P. A. (1988). Ontogeny of routine swimming activity and performance in zebra danios (Teleostei: Cyprinidae). *Anim. Behav.* **36**, 250–261.

Garland, T., Jr (1983). The relation between maximal running speed and body mass in terrestrial mammals. *J. Zool., Lond.* **199**, 157–170.

Garland, T., Jr (1985). Ontogenetic and individual variation in size, shape and speed in the Australian agamid lizard *Amphibolurus nuchalis*. *J. Zool., Lond.* **207**, 425–439.

Gibb, A. C. (1995). Kinematics of prey capture in a flatfish, *Pleuronichthys verticalis*. *J. Exp. Biol.* **198**, 1173–1183.

Gibb, A. C. (1997). Do flatfish feed like other fishes? A comparative study of percomorph prey-capture kinematics. *J. Exp. Biol.* **200**, 2841–2859.

Gibson, S. and Johnston, I. A. (1995). Scaling relationships, individual variation and the influence of temperature on maximum swimming speed in early settled stages of the turbot *Scophthalmus maximus*. *Mar. Biol.* **121**, 401–408.

Hill, A. V. (1950). The dimensions of animals and their muscular dynamics. *Scient. Prog.* **38**, 209–230.

Hoogerhoud, R. J. C. (1989). Prey processing and predatory morphology in mollscivoous cichlid fishes. *Trends Vertebrate Morph.* **35**, 19–21.

James, R. S., Cole, N. J., Davies, M. L. F. and Johnston, I. A. (1998). Scaling of intrinsic contractile properties and myofibrillar protein composition of fast muscle in the fish *Myoxocephalus scorpius*. *J. Exp. Biol.* **201**, 901–912.

Katz, S. L. and Gosline, J. M. (1993). Ontogenetic scaling of jump

performance in the African desert locust (*Schistocerca gregaria*). *J. Exp. Biol*. **177**, 81–111.

Lauder, G. V. (1983). Food capture. In *Fish Biomechanics* (ed. P. W. Webb and D. Weihs), pp. 280–311. New York: Praeger Publishers.

Lauder, G. V. (1985). Aquatic feeding in lower vertebrates. In *Functional Vertebrate Morphology* (ed. M. Hildebrand, D. M. Bramble, K. F. Liem and D. B. Wake), pp. 210–229. Cambridge: Cambridge University Press.

Liem, K. F. (1978). Modulatory multiplicity in the functional repertoire of the feeding mechanism in cichlid fishes. *J. Morph*. **158**, 323–360.

Liem, K. F. (1980). Adaptive significance of intra- and interspecific differences in the feeding repertoires of cichlid fishes. *Am. Zool*. **20**, 295–314.

Losos, J. B. (1990). The evolution of form and function: Morphology and locomotor performance in West Indian *Anolis* lizards. *Evolution* **44**, 1189–1203.

McArdle, B. H. (1988). The structural relationship: regression in biology. *Can. J. Zool*. **66**, 2329–2339.

McMahon, T. A. (1975). Using body size to understand the structural design of animals: quadrupedal locomotion. *J. Appl. Physiol*. **39**, 619–627.

McMahon, T. A. (1984). *Muscles, Reflexes and Locomotion*. Princeton: Princeton University Press.

Miller, K., Monteforte, P. B. and Laudis, L. F. (1993). Scaling of locomotor performance and enzyme activity in the leopard frog, *Rana pipiens*. *Herpetologica* **49**, 383–392.

Muller, M. (1987). Optimization principles applied to the mechanism of neurocranial levation and mouth bottom depression in bony fishes (Halecostomi). *J. Theor. Biol*. **126**, 343–368.

O'Reilly, J. C., Linstedt, S. L. and Nishikawa, K. C. (1993). The scaling of feeding kinematics in toads (Anura: Bufonidae). *Am. Zool*. **33**, 147A.

Osse, J. W. M. (1990). Form changes in fish larvae in relation to changing demands of function. *Neth. J. Zool*. **40**, 362–385.

Osse, J. W. M. and van den Boogaart, J. G. M. (1995). Fish larvae, development, allometric growth and the aquatic environment. *ICES J. Mar. Sci*. **201**, 21–34.

Reilly, S. M. (1995). The ontogeny of aquatic feeding behavior in *Salamandra salamandra*: stereotypy and isometry in feeding kinematics. *J. Exp. Biol*. **198**, 701–708.

Richard, B. A. and Wainwright, P. C. (1995). Scaling of the feeding mechanism of largemouth bass (*Micropterus salmoides*). I. Kinematics of prey capture. *J. Exp. Biol*. **198**, 419–433.

Schmidt-Nielsen, K. (1984). *Scaling: Why is Animal Size so Important?* Cambridge: Cambridge University Press.

Sokal, R. R. and Rohlf, F. J. (1995). *Biometry*. New York: W. H. Freeman & Company.

Strauss, R. E. and Fuiman, L. A. (1985). Quantitative comparisons of body form and allometry in larval and adult Pacific sculpins (Teleostei: Cottidae). *Can. J. Zool*. **63**, 1582–1589.

Vogel, S. (1981). *Life in Moving Fluids: The Physical Biology of Flow*. Princeton, NJ: Princeton University Press.

Wainwright, P. C. (1989). Functional morphology of the pharyngeal jaw apparatus in perciform fishes: An experimental analysis in the Haemulidae. *J. Morph*. **200**, 231–245.

Wainwright, P. C. and Lauder, G. V. (1986). Feeding biology of sunfishes: patterns of variation in the feeding mechanism. *Zool. J. Linn. Soc*. **88**, 217–228.

Wainwright, P. C. and Lauder, G. V. (1992). The evolution of feeding biology in sunfishes (Centrarchidae). In *Systematics, Historical Ecology and North American Freshwater Fishes* (ed. R. L. Mayden), pp. 472–491. Stanford: Stanford University Press.

Wainwright, P. C., Osenberg, C. W. and Mittlebach, G. G. (1991). Trophic polymorphism in the pumpkinseed sunfish (*Lepomis gibbosus*): effects of environmental on ontogeny. *Funct. Ecol*. **5**, 40–55.

Wassersug, R. J. and Sperry, D. G. (1977). The relationship of locomotion to differential predation of *Pseudacris triseriata* (Anura: Hylidae). *Ecology* **58**, 830–839.

Weatherly, A. H. (1990). Approaches to understanding fish growth. *Trans. Am. Fish. Soc*. **119**, 662–672.

Webb, P. W. and Weihs, D. (1986). Functional locomotor morphology of early life history stages of fishes. *Trans. Am. Fish. Soc*. **115**, 115–127.

Westneat, M. W. (1990). Feeding mechanics of teleost fishes: A test of four-bar linkage models. *J. Morph*. **205**, 269–295.

Westneat, M. W. (1994). Transmission of force and velocity in the feeding mechanisms of labrid fishes (Teleostei, Perciformes). *Zoomorph*. **114**, 103–118.

Westneat, M. W. and Wainwright, P. C. (1989). Feeding mechanism of *Epibulus insidiator*: Evolution of a novel functional system. *J. Morph*. **202**, 129–150.