

Feeding with speed: prey capture evolution in cichlids

T. E. HIGHAM,* C. D. HULSEY,† O. ŘÍČAN‡§ & A. M. CARROLL¶

*Section of Evolution and Ecology, University of California, One Shields Avenue, Davis, CA, USA

†Department of Biology, Georgia Institute of Technology, Atlanta, GA, USA

‡Department of Zoology, Faculty of Biological Sciences, University of South Bohemia, Bohemia, Czech Republic

§Institute of Animal Physiology and Genetics of the Academy of Sciences of the Czech Republic, Liběchov, Czech Republic

¶Department of Organismic and Evolutionary Biology, Concord Field Station, Harvard University, Cambridge, MA, USA

Keywords:

Cichlidae;
comparative methods;
ecomorphology;
feeding;
functional morphology;
gape;
predation;
prey capture;
ram speed;
swimming.

Abstract

The diversity of both the locomotor and feeding systems in fish is extensive, although little is known about the integrated evolution of the two systems. Virtually, all fish swim to ingest prey and all open their buccal cavity during prey capture, but the relationship between these two ubiquitous components of fish feeding strikes is unknown. We predicted that there should be a positive correlation between ram speed (RS) and maximum gape (MG) because the accuracy of a predatory strike goes down with an increase in RS and fish with larger mouths eat larger, more evasive prey. For 18 species of neotropical cichlids, we used phylogenetic-independent contrasts to study the relationship between the predator closing speed (RS) and mouth size (MG) during prey capture. To provide a robust comparative framework, we augmented existing phylogenetic information available from the mitochondrial cytochrome *b* gene with sequences from the S7 nuclear ribosomal intron for these species. Then, we captured high-speed (500 images per second), lateral view feeding sequences of each species by using a digital video camera and measured both RS and MG. Uncorrected species values of MG and RS were positively and significantly correlated. When accounting for any of the set of phylogenetic relationships recovered, the independent contrasts of RS and MG remained significantly, and positively, correlated. This tight evolutionary coupling highlights what is likely a common relationship between locomotor behaviour and feeding kinematics in many organisms.

Introduction

Locomotion is highly integrated with prey capture in diverse groups of predators such as snakes (e.g. Alfaro, 2003), lizards (e.g. Irschick & Losos, 1998), fish (e.g. Higham *et al.*, 2005a), carnivorous mammals (e.g. Iwaniuk *et al.*, 1999), primates (e.g. Dunbar & Badam, 2000) and spiders (e.g. Gorb & Barth, 1994; Schmid, 1997). These relationships persist on multiple levels, including physiological, morphological and behavioural, suggesting that the co-evolution between these levels of organization is extensive. Given how ubiquitous these interrelationships are likely to be, identifying commonalities

among and across groups of organisms will provide considerable insight into general rules that constrain and facilitate the co-evolution of what have traditionally been treated as distinct classes of organismal performance.

The locomotor and feeding systems of fishes are extremely diverse, and have been the focus of numerous functional (reviewed in Ferry-Graham & Lauder, 2001; Blake, 2004; Lauder, 2005) and evolutionary (Wainwright *et al.*, 1989; Jayne & Bennett, 1990; Hale *et al.*, 2002; Wainwright, 2002; McHenry & Patek, 2004; Westneat, 2004; Hulsey & Garcia de Leon, 2005) studies. Despite an ever-increasing understanding of the evolution of both locomotion and feeding, there is a paucity of studies that examine locomotion and feeding concurrently in an evolutionary context. This is surprising given the potential interdependence of these systems. For

Correspondence: Timothy E. Higham, Section of Evolution and Ecology, University of California, One Shields Avenue, Davis, CA 95616, USA. Tel.: 530 752 6784; fax: 530 752 1449; e-mail: tehigham@ucdavis.edu

example, suction feeding, which involves expansion of the mouth cavity resulting in an influx of water towards the predator (Day *et al.*, 2005; Higham *et al.*, 2006a,b), is the most common feeding mode among fishes (Lauder, 1980; Muller & Osse, 1984; Carroll *et al.*, 2004), but the body must be positioned close to the prey (within one mouth diameter) for suction to be effective (Wainwright *et al.*, 2001; Day *et al.*, 2005; Higham *et al.*, 2006a). Also, predator locomotion is clearly intertwined with feeding modes (e.g. ambush vs. roving predator) and specialization on particular prey types (e.g. evasive vs. sessile).

A common theme stemming from studies of both locomotion and feeding kinematics is that fish exhibit a wide range of ram speeds (RS) during prey capture (Webb, 1984; Norton & Brainerd, 1993; Domenici & Blake, 1997; Webb & Gerstner, 2000; Wainwright *et al.*, 2001; Higham *et al.*, 2005a, 2006a). The possible consequences of swimming faster at the time of prey capture include the ingestion of a more narrow and elongated volume of water, decreased suction performance, and an increase in overall closing speed (Higham *et al.*, 2005a, 2006a). However, it seems that a relatively low RS at the time of prey capture enables the predator to maintain the optimal suction performance, and at the same time better focus the flow of water anteriorly, thus increasing the suction feeding efficiency (Higham *et al.*, 2005a). There are advantages to both fast and slow RSs that generate tradeoffs with respect to suction feeding, and these tradeoffs should generate negative correlations between the locomotor and feeding behaviours that influence suction feeding during evolution.

Slower attack speeds are also advantageous because they increase strike accuracy as predators have more time to modulate the position of their mouths relative to prey when attacking (Webb & Skadsen, 1980; Higham *et al.*, 2006a). Fish frequently miss prey when they open their mouths too soon or too late (e.g. Nyberg, 1971). Despite the possible cost of decreased accuracy, species must attack faster when feeding on rapidly escaping evasive prey items (Nemeth, 1997; Wainwright *et al.*, 2001). Given the likely importance of higher attack speeds for capturing evasive prey, a predator could offset the decrease in accuracy by ingesting a larger volume of water through an increase in mouth (gape) size (Higham *et al.*, 2006a) rather than decelerating prior to prey capture to maintain accuracy (Lauder & Drucker, 2004; Higham *et al.*, 2005a,b). Thus, average attack speeds should evolve in tandem with the size of the mouth opening used to capture prey.

Cichlid fishes are an ideal group to study the evolution of predators that vary in both locomotor and feeding abilities. For example, even subsets of the cichlid radiation such as the neotropical Heroine cichlids are incredibly diverse in their jaw morphology and dietary specialization (Eaton, 1943; Winemiller, 1989; Winemiller *et al.*, 1995; Waltzek & Wainwright, 2003; Hulsey & Garcia de Leon, 2005). Mitochondrial DNA phylogenies

suggest that even within this relatively small cichlid clade there is convergence in the morphological specializations used for obtaining prey with escape abilities as disparate as shrimp and molluscs (Winemiller *et al.*, 1995; Hulsey, 2006). Given that some of these cichlid species capture predominantly large, evasive fish and other species ingest predominantly small, nonevasive prey, locomotion during prey capture should differ among these cichlid species. It would be ideal to more extensively evaluate the phylogenetic relationships of Heroine cichlids using information obtained from their nuclear genomes in order to robustly examine the co-evolution of trophic characteristics such as gape and attack speed.

We hypothesized that there would be a positive evolutionary correlation between RS and maximum gape (MG) in Heroine cichlids. To test this, we first reconstructed the relationships of numerous Heroines by using the S7 ribosomal intron and mitochondrial DNA sequences of the cytochrome *b* gene. Then, we examined the variation in MG and attack speed among Heroine cichlids. Finally, we determined the evolutionary relationships between feeding kinematics and RS in the 18 species by using the phylogenetic-independent contrasts.

Materials and methods

Sequencing

The S7 ribosomal intron, one of 18 Heroine cichlid species (Table 1) and the outgroup *Mesonauta festivus* (Farias *et al.*, 2001), was sequenced and analysed in conjunction with cytochrome *b* sequences previously

Table 1 The species used in this study and their average gape (+SEM) and ram speed (RS) (+SEM).

Species	Standard length (mm)	Maximum gape (mm)	RS (cm s ⁻¹)
<i>Amphilophus citrinellus</i>	85	11.2 ± 0.5	102.1 ± 8.2
<i>Archocentrus nigrofasciatus</i>	64	5.8 ± 0.3	40.6 ± 8.1
<i>Caquetaia kraussii</i>	83	11.7 ± 0.3	146.3 ± 18.6
<i>Caquetaia myersi</i>	94	14.9 ± 0.4	96.7 ± 11.0
' <i>Cichlasoma</i> ' <i>octofasciatum</i>	69	7.7 ± 0.7	80.5 ± 5.1
' <i>Cichlasoma</i> ' <i>salvini</i>	77	8.9 ± 0.6	108.9 ± 18.0
' <i>Cichlasoma</i> ' <i>trimaculatum</i>	76	8.1 ± 0.3	82.5 ± 3.5
<i>Caquetaia umbrifera</i>	65	8.1 ± 0.4	57.9 ± 5.6
<i>Nandopsis haitiensis</i>	65	8.9 ± 0.4	105.6 ± 11.9
<i>Nandopsis tetracanthus</i>	62	7.9 ± 0.3	49.9 ± 3.3
' <i>Cichlasoma</i> ' <i>festae</i>	67	8.2 ± 0.5	46.7 ± 5.1
<i>Herichthys pearsei</i>	83	10.2 ± 0.8	96.2 ± 12.1
<i>Herichthys minckleyi</i>	77	8.1 ± 0.7	39.6 ± 10.0
<i>Hypsophrys nicaraguensis</i>	79	5.5 ± 0.4	23.1 ± 5.8
<i>Parachromis managuensis</i>	61	8.9 ± 0.3	73.9 ± 3.0
<i>Petenia splendida</i>	67	9.6 ± 0.6	105.0 ± 2.5
<i>Vieja maculicauda</i>	69	4.9 ± 0.4	25.6 ± 6.4
<i>Vieja synspila</i>	81	7.0 ± 0.4	50.5 ± 2.3

Ram speed includes the speed of the jaw during protrusion.

generated. The individuals sequenced for the S7 intron were collected from sites reported in previous studies (e.g. Hulsey *et al.*, 2004). For sequencing, total genomic DNA was isolated from axial muscle by using Puregene® extraction at the University of California, Davis. A 1- μ L aliquot of this solution was used to provide a DNA template for the polymerase chain reaction (PCR). The entire S7 protein intron one was amplified (Chow & Hazama, 1998). Amplifications were carried out in a Perkin-Elmer DNA thermocycler (Perkin-Elmer, Wellesley, MA, USA). The PCR reaction volume was 50 μ L [32 μ L of H₂O, 5 μ L 10 \times MgCl₂ PCR buffer, 2.5 μ L MgCl₂, 4 μ L dNTPs (10 mM), 2.5 μ L of each primer (10 μ M), 0.5 μ L of TAQ, and 1 μ L DNA (~15–20 ng)]. Thermal cycling conditions consisted of an initial denaturation step of 94 °C (30 s), 55 °C (30 s) and 72 °C (1.5 min). A final incubation of 72 °C for 5 min was added to ensure the complete extension of amplified products. Subsequently, the 1.3-kb PCR products were electrophoretically separated from unincorporated primers and dNTPs by using electrophoresis in low-melting point agarose gel run in Tris–acetate buffer (pH 7.8). Gels were stained in ethidium bromide (1 mg μ L⁻¹) for 5 min and destained in de-ionized water for 15 min. Positively amplified DNA was then purified by using an enzymatic combination of 1 μ L of Exonuclease I (10.0 U μ L⁻¹) and 1 μ L shrimp alkaline phosphatase (2.0 U μ L⁻¹) per 10 μ L of PCR product. Treated PCR products were used as templates for Big Dye sequencing reactions (Applied Biosystems terminator cycle sequencing reactions; Applied Biosystems, Foster City, CA, USA). Sequences were read with an ABI377-automated sequencer at the Automated DNA Sequencing Facility at the University of California, Davis. Complete gene sequences were assembled from individual sequencing reactions by using the program SEQUENCHER version 4.1 (Gene Codes, Ann Arbor, MI, USA). For analyses, sequences were aligned using CLUSTAL X (Thompson *et al.*, 1999). All sequences for the S7 ribosomal intron one have been submitted to Genbank (DQ836805–DQ836823).

Phylogenetic analysis

For the Bayesian analyses, the S7 ribosomal intron and the cytochrome *b* gene were analysed independently and also in combination. The S7 intron was treated as a single partition and indels were treated as missing data. The cytochrome *b* gene was partitioned into its three-codon sites by using MACCLADE 4.0 (Maddison & Maddison, 2000) for the analyses. MODELTEST 3.06 (Prosada & Crandall, 1998) was used to identify the best model of molecular evolution for each cytochrome *b* codon site and the entire S7 partition. MRBAYES 3.1 (Ronquist & Huelsenbeck, 2003) was used to perform the Bayesian analyses in order to find the best approximations of the maximum likelihood tree. The analyses treated the transition-transversion matrices, number of invariant

sites and gamma shape parameters as unlinked and independent for each codon site. Flat prior probability distributions for all parameters were assumed before analysis. We ran five separate Bayesian analyses for 1 000 000 generations with four Markov chains in each run for the cytochrome *b* gene alone, the S7 intron alone and with both genes together. We sampled trees from the Markov–Chain–Monte–Carlo search algorithm every 100 generations. At the end of each analysis, the log-likelihood scores were plotted against generation time to identify the point at which log likelihood values reached a stable equilibrium. In all analyses, the equilibrium appeared to be reached at approximately 50 000 generations, and therefore, sample points prior to generation 100 000 in each run were discarded as ‘burn-in’ samples. The remaining samples from all runs combined were used to produce a single majority rule consensus tree in PAUP* 4.0B10 (Swofford, 2002) for each of the three separate genetic partitions. The percentages of all trees recovered for a particular clade (the clade’s posterior probability) were depicted on the single lowest likelihood tree topology found during each of the three different partitioned Bayesian analyses. Comparative analyses were performed on the three lowest likelihood topologies obtained from each of the three gene partitions analysed.

Experimental protocol

All cichlids used in the kinematic analyses were purchased commercially and housed at the University of California, Davis. During experiments, all fish were fed similarly sized (approximately 2–3-cm long) guppies (*Poecilia reticulata*). The prey item that we used in this study represents a relatively evasive prey species (Wainwright *et al.*, 2001). Using a single prey type is important in comparative studies because the strike behaviour of a predator will vary depending on the type of prey (Nemeth, 1997). One individual from each species was tested, and a minimum of four sequences per individual were obtained. Although one individual provides minimal intraspecific replication and could potentially increase the noise in our analysis, we feel that the most extensive variation in prey capture likely lies among the species examined and not within species. Furthermore, any significant results recovered would suggest that this level of within species replication provides enough power to test the hypotheses examined. All maintenance and experimental procedures used in this research complied with guidelines for the use and care of animals in research at the University of California, Davis.

We recorded the body, jaw and mouth movements in lateral view from each fish by using a high-speed NAC Memrecam ci digital system (Tokyo, Japan) operating at 500 images per second. Grids (1 \times 1 cm) were placed behind the fish in order to provide a distance scale. Additionally, we took a picture of a ruler inside the tank

at the location of the prey in order to verify the scaling. Two floodlights (600 W) on either side of the camera illuminated the experimental tank during the experiment. Following experiments, the fish were killed with an overdose of anaesthetic (MS-222) and the standard length (SL) of the fish was measured.

Kinematic measurements

For the frame-by-frame analysis, we chose only those trials where the trajectory of the fish was straight, parallel to the long axis of the tank, and where the prey was completely consumed. All sequences were converted to stacks of digital images and imported into IMAGE J version 1.33 (NIH, Washington, DC, USA) for digitizing. For each frame, we digitized the anterior tips of the upper and lower jaws. We then quantified the displacement of the anterior tip of the upper jaw throughout the feeding event, which started at the onset of mouth opening and ended when the mouth was closed. Ram speed was the first derivative of the fish's displacement. As we were primarily interested in the overall closing speed of the predator, we refer to RS as the speed including jaw protrusion. During prey capture, we measured gape as the vertical distance between the tip of the lower jaw and the tip of the upper jaw. Maximum gape was defined as the maximum distance between these two points.

Comparative analyses

Correlations between MG and RS were examined both without taking phylogenetic relationships into account and also in a phylogenetic context. Ram speeds were uncorrelated ($P = 0.19$, $r = 0.33$) with SL, so we did not scale this variable. Because MG frequently scales with SL (e.g. Wainwright *et al.*, 2006), we scaled MG using the residuals from a log-log least squares regression of MG and SL for both analyses.

Correlations among species values are not statistically independent because of the shared evolutionary history

of species (Felsenstein, 1985) and comparative methods are widely used to explore the relationships between functional traits (e.g. Lauder, 1990; Garland *et al.*, 2005; Herrel *et al.*, 2005; Johnson *et al.*, 2005). Thus, we calculated the phylogenetic independent contrast correlations between RS and maximum gape by using the S7 ribosomal intron phylogeny, the cytochrome *b* phylogeny, and the phylogenetic tree obtained from analysing both the sets of sequences combined. For the independent contrasts, the phylogenetic topology with branch lengths was imported into MESQUITE version 1.06 (Maddison & Maddison, 2005) and we then used the PDAP package implemented in MESQUITE (Midford *et al.*, 2005) to obtain the independent contrasts (Garland *et al.*, 1992). The relationship between the phylogenetic independent contrasts was then determined by using a reduced major axis (RMA) regression as there is considerable variation in both variables.

Results

Phylogenetics

Although the cytochrome *b* analyses suggested that *Nandopsis haitiensis* and *N. tetracanthus* are phylogenetically located at the base of the Heroine radiation, the analyses based on the S7 intron suggest that these species may have affinities with the clade containing '*Cichlasoma*' *festae* and species such as *Herichthys minckleyi* and the genus *Vieja* (Fig. 1). Also, in the cytochrome *b* phylogeny, *Caquetaia kraussi* and *C. myersi* group together but *C. umbrifera* appears to be phylogenetically distinct. However, *C. umbrifera* is clearly nested with other *Caquetaia* based on the S7 intron (90% posteriors). This species has been placed in the genus *Caquetaia* in previous taxonomic groupings because of its elongate jaws and our results from the S7 analysis support this previous morphologically based hypothesis. The close grouping of '*Cichlasoma*' *salvini*, *Herichthys pearsei*, *H. minckleyi*, *Vieja synspilus* and *V. maculicauda* is recovered in both the trees although the

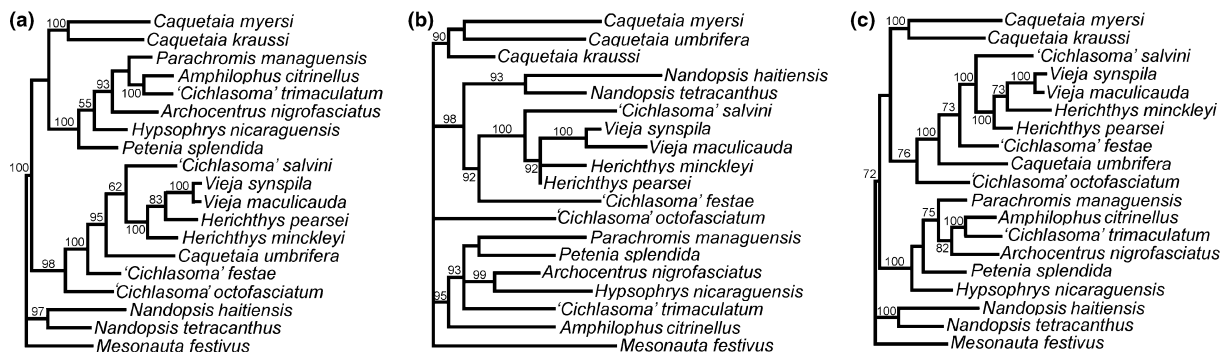


Fig. 1 The phylogenetic relationships reconstructed from the cytochrome *b* gene alone (a), the S7 intron (b), and both genes analysed simultaneously (c) for 19 species of neotropical cichlids. The Bayesian posterior probabilities are shown to the left of nodes recovered in >50% of the phylogenetic trees once the first 100 000 burn-in trees were excluded.

exact relationships of *H. minckleyi* and *H. pearsei* are not consistent. '*Cichlasoma*' *festae* groups with this clade in all three topologies. The phylogenetic affinities of '*Cichlasoma*' *octofasciatum* are generally nebulous. Both gene partitions support a clade containing, *Petenia splendida*, *Hypsophrys nicaraguensis*, *Archocentrus nigrofasciatus*, *Nandopsis managuensis*, '*Cichlasoma*' *trimaculatum* and *Amphilophus citrinellus* (Fig. 1). However, the two genes support very distinct relationships among these morphologically disparate species. The combined analysis of both genes is largely congruent with the cytochrome *b* tree. One difference is the rearrangement of *Caquetaia umbrifera* and '*C.*' *festae* as compared to the single mitochondrial

gene tree. *Parachromis managuensis* and *A. nigrofasciatus* also switch their topological locations in the combined analysis. Although exhibiting less support than the other two rearrangements, *P. splendida* and *H. nicaraguensis* also have slightly different relationships.

Ram speed and mouth size

Ram speed and maximum gape, at the time of prey capture, varied considerably among the 18 species in this study (Table 1; Fig. 2). Ram speeds ranged from $23.1 \pm 5.8 \text{ cm s}^{-1}$ (*H. nicaraguensis*) to $146.3 \pm 18.6 \text{ cm s}^{-1}$ (*C. kraussii*). The highest and lowest varia-

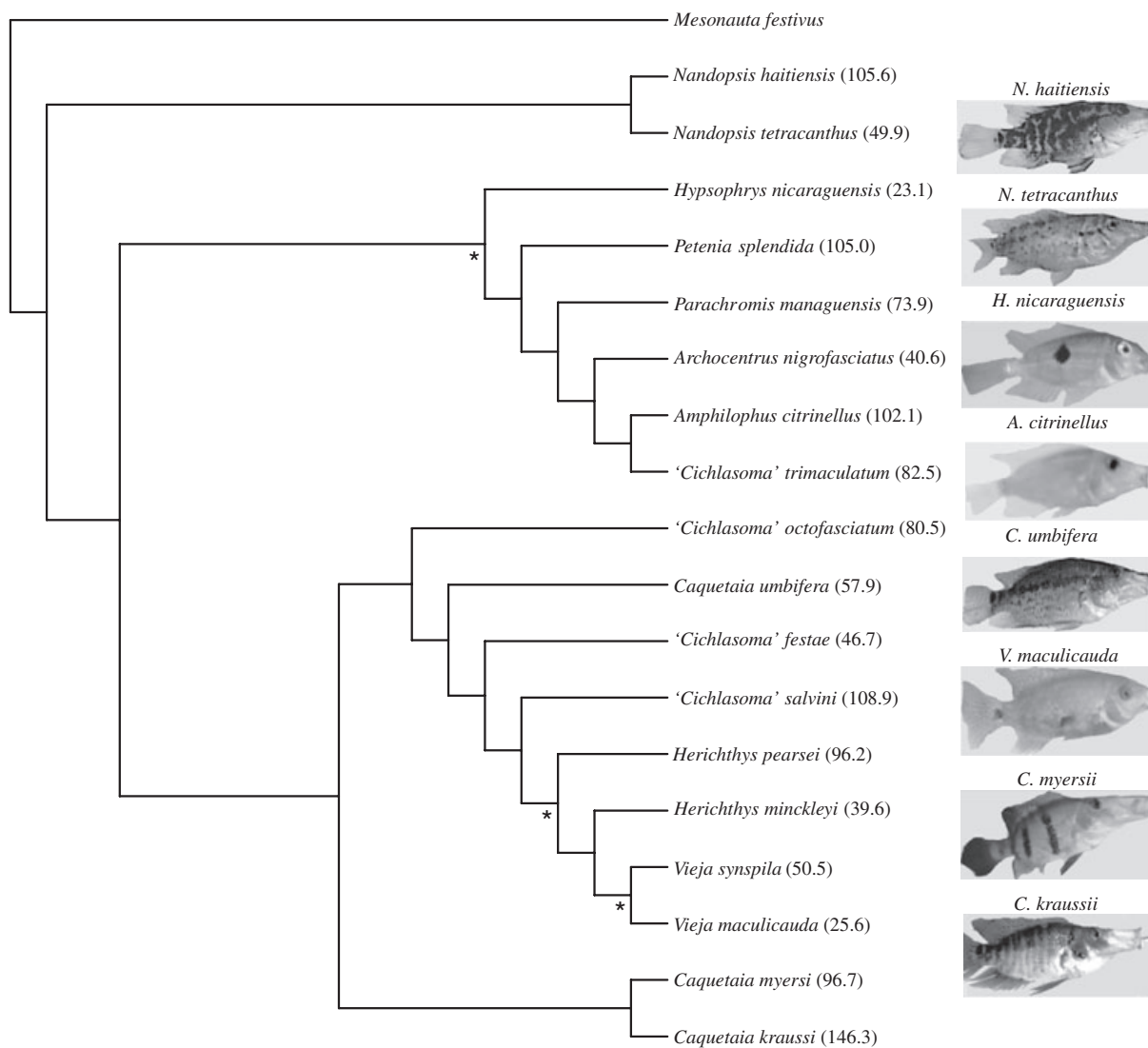


Fig. 2 The phylogenetic relationships among the Heroines examined in this study. In parentheses are the average ram speeds (cm s^{-1}) for each species, with the exception of *Mesonauta festivus* for which we did not have experimental data. The topology shown is from the combined cytochrome *b* and *S7* sequence data. Several species representing the diversity in the species examined are depicted in the pictures to the right of the tree. The three nodes with asterisks represent the three contrasts with the largest values (see Fig. 3c).

bility in RSs was for *H. nicaraguensis* ($SEM = 18.6 \text{ cm s}^{-1}$) and *Vieja synspila* ($SEM = 2.3 \text{ cm s}^{-1}$) respectively (Table 1). Ram speed (at the time of prey capture), and maximum gape (scaled to the average SL from all species) were significantly and positively correlated using a RMA regression (Fig. 3b; $P < 0.001$, $r = 0.73$). After correcting for phylogenetic relationships, the independent contrasts of maximum gape were significantly, and positively, correlated with the independent contrasts of RS at the time of prey capture using the S7 ribosomal intron sequences ($P < 0.001$, $r = 0.89$), the cytochrome *b* sequences ($P < 0.001$, $r = 0.81$), and both genes analysed together (Fig. 3c; $P < 0.001$, $r = 0.80$). Three independent contrasts (see Figs 2 and 3c) were substantially higher than the other 14 independent contrasts, and two of these were well nested within the phylogeny making it unlikely they were simply due to motivational differences between two closely related species.

Discussion

Heroine phylogeny

The phylogenies recovered for the Heroines based on genetic data from both the mitochondrial and nuclear genomes provide some consistent results but also differ substantially in several respects. The clade of cichlids containing *H. minckleyi*, *H. pearsei*, and the *Vieja* species appears to be robustly supported. Likewise the generally close affinities of *P. splendida*, *H. nicaraguensis*, *A. nigrofasciatus*, *Parachromis managuensis*, '*C.*' *trimaculatum* and *A. citrinellus* are clear. However, the transient relationships among these species and the indeterminate relationships of *C. umbrifera*, *Nandopsis tetracanthus*, *N. haitiensis* and *C. octofasciatus* suggest that in order to make the most robust conclusions possible concerning the trophic evolution of this group that the phylogenetic relationships of the entire radiation need to be more extensively assessed. Because cichlids are known to hybridize in nature (Streelman *et al.*, 2004), and gene trees and species trees can frequently be incongruent (Maddison, 1997), these results may not be surprising. The inconsistencies recovered here between cytochrome *b* and the S7 intron indicate that it would be ideal to examine the relationships of these species with the inclusion of many more closely related Heroines and other cichlid outgroups with further genetic partitions. Nevertheless, our study suggests that, despite some phylogenetic uncertainty, attack speed and maximum gape during prey capture clearly change in a predictable manner during evolution.

Maximum gape and ram speed

Using phylogenetic-independent contrasts, we found that RS and maximum gape were significantly, and positively, correlated for the 18 species of cichlids we

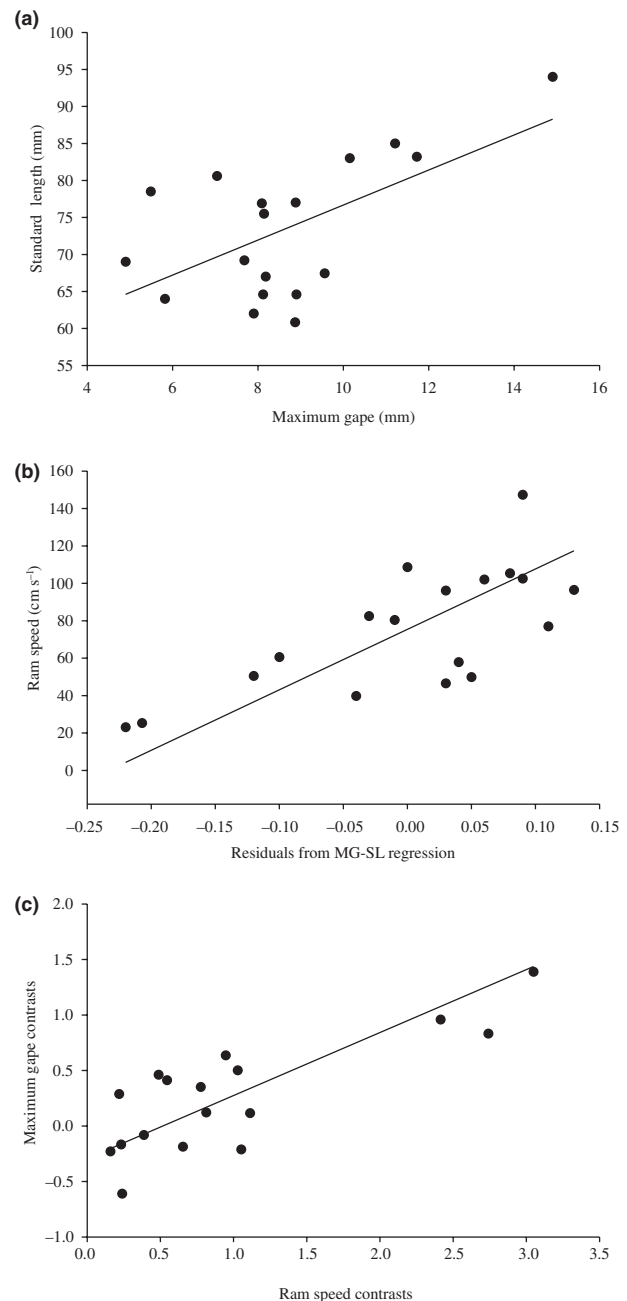


Fig. 3 The relationships between maximum gape and standard length (a), ram speed (RS) and maximum gape (b) and the RS (cm s^{-1}) contrasts and maximum gape (cm) contrasts (c). For panel A, the relationship was determined by using a least squares regression. For panels B and C, reduced major axis regressions were used. For panel C, both of the variables were \log_{10} transformed. The values of r in panels A, B and C were 0.63, 0.70 and 0.80, respectively. The contrasts in C are those from the analysis using both the cytochrome *b* and S7 sequence data, and the relationship was statistically significant ($P < 0.001$).

examined (Fig. 3). This supports the hypothesis that a larger gape in predators that attack faster could offset the decrease in accuracy and enable cichlids with larger mouths to maintain a greater probability of successful prey capture. This idea is supported by Higham *et al.* (2006a) in which strike accuracy, measured as the location of the prey item relative to the centre of the ingested volume of water, was lower in the species of centrarchid with higher RS. Predators like Heroine cichlids must balance the effort they expend during an attack with the potential reward gained from the probabilistic success of actually capturing a prey item (Bolnick & Ferry-Graham, 2002). An increase in ram is costly due to increased energy expenditure arising from the greater amount of axial muscle recruitment necessary for increased swimming speed (Johnson *et al.*, 1994; Jayne & Lauder, 1995, 1996; Peake & Farrell, 2004). Energy return from eating a larger, more evasive prey item should exceed the amount of energy expended when swimming faster. Future experiments that measure RS and accuracy while manipulating prey size, prey evasiveness and energy content of the prey will be useful for teasing apart the causal relationships between prey and predator behaviour recovered here.

Mouth and prey size

Fish with larger mouths eat larger, more evasive, prey items (Keast & Webb, 1966; Wainwright & Richard, 1995; Mittelbach & Persson, 1998). Thus, the higher RSs in fish with larger mouths, as in cichlids, is potentially linked to catching larger, more evasive prey. However, the higher RSs that we observed might be a compensation for a reduced ability to generate a suction-induced flow of water that results from having a larger mouth (Van Leeuwen & Muller, 1984; Higham *et al.*, 2006a). This explanation and the previous one are not necessarily exclusive of one another. Eating larger prey should require a larger mouth and perhaps a higher attack velocity and afford the predator more flexibility with regard to accuracy.

Preliminary evidence suggests that cichlids exhibiting higher attack speeds and larger gapes consume a larger quantity of evasive prey items as part of their normal diet (C.D. Hulsey, unpubl. data). For example, 96% of the diet of *P. splendida* (relatively high RS and large gape) is comprised of evasive prey, whereas only 1% of the diet of *H. nicaraguensis* (relatively low RS and small gape) is comprised of evasive prey. Thus, a large gape and high RS at the time of prey capture are likely adaptations to feeding on evasive prey.

The evolution of locomotion and feeding

Phylogenetic independent contrasts remove the effects of shared history on the correlations of characters, but the results are not always easy to interpret biologically.

In our analyses of gape and RS, there is a cluster with relatively low contrast values that presumably exhibit a relatively slow change in both RS and maximum gape. Conversely, there are three contrasts with higher values that represent three nodes in the tree where these two traits have evolved extensively. In most ahistoric analyses of trait correlation, these large 'outliers' might lead to the quick inference that a few errant and nongeneralizable contrasts are completely responsible for a trend. However, this kind of result in an independent contrast analysis is potentially very interesting evolutionarily because it suggests that a few major concordant changes in RS and gape punctuated throughout Heroine evolution may be responsible for the general pattern in the group. For example, the contrast with the highest values in Fig. 3c is for a node that resides relatively deep in the tree (see Fig. 2). Thus, the high value for this independent contrast is a result of a major change that, once it occurred, continued to influence without much change the association of RS and gape within sister lineages that contain numerous species. Coupling comparative phylogenetic analyses with nonphylogenetic analyses allows one to understand not only the correlations between traits (e.g. Fig. 3b,c), but also permits the identification of major, qualitative-like changes in the relationships among the quantitative characters such as RS and gape.

Our finding that RS is positively correlated with mouth size has implications for the co-evolution of locomotor and feeding abilities in fishes as well as other aquatic and terrestrial organisms. We predict that species that are more flexible with regard to the accuracy required during a feeding strike, because of their ability to exhibit a larger gape, will generally exhibit greater attack speeds. Our prediction that these two abilities will often be correlated is powerful and exceedingly testable because both attack speeds and mouth sizes are readily measured in mobile predators (e.g. Wainwright *et al.*, 2001; Rice & Westneat, 2005; Higham *et al.*, 2006a). Our understanding of what constrains and facilitates the evolution of novel trophic habits will be greatly increased through additional integrative analyses of both feeding and locomotor behaviours in a phylogenetic context.

Acknowledgments

D. Collar provided valuable discussion regarding the comparative methods. M. Higham, P. Wainwright and E. Brainerd provided valuable comments on the manuscript. Funding sources included a grant for Research and Exploration from the National Geographic Society to C.D.H., NSF grants IBN-0076436, IBN-0326968 and IOB-0444554 to P. Wainwright, and SYNTHESYS (Spain), Grant Agency of the University of South Bohemia 04-GAJU-58 (Czech Republic) and the Helge Ax:son Johnsons Stiftelse (Sweden) grants to OŘ.

References

- Alfaro, M.E. 2003. Sweeping and striking: a kinematic study of the trunk during prey capture in three thamnophiine snakes. *J. Exp. Biol.* **206**: 2381–2392.
- Blake, R.W. 2004. Fish functional design and swimming performance. *J. Fish Biol.* **65**: 1193–1222.
- Bolnick, D.I. & Ferry-Graham, L.A. 2002. Optimizing prey-capture behavior to maximize expected net benefit. *Evol. Ecol. Res.* **4**: 843–855.
- Carroll, A.M., Wainwright, P.C., Huskey, S.H., Collar, D.C. & Turingan, R.G. 2004. Morphology predicts suction feeding performance in centrarchid fishes. *J. Exp. Biol.* **207**: 3873–3881.
- Chow, S. & Hazama, K. 1998. Universal PCR primers for S7 ribosomal protein gene introns in fish. *Mol. Ecol.* **7**: 1247–1263.
- Day, S.W., Higham, T.E., Cheer, A.Y. & Wainwright, P.C. 2005. Spatial and temporal patterns of water flow generated by suction feeding bluegill sunfish *Lepomis macrochirus* resolved by Particle Image Velocimetry. *J. Exp. Biol.* **208**: 2661–2671.
- Domenici, P. & Blake, R.W. 1997. The kinematics and performance of fish fast-start swimming. *J. Exp. Biol.* **200**: 1165–1178.
- Dunbar, D.C. & Badam, G.L. 2000. Locomotion and posture during terminal branch feeding. *Int. J. Primatol.* **21**: 649–669.
- Eaton, T.H. 1943. An adaptive series of protractile jaws in cichlid fishes. *J. Morphol.* **72**: 183–190.
- Farias, I.P., Orti, G., Sampaio, I., Schneider, H. & Meyer, A. 2001. The cytochrome *b* gene as a phylogenetic marker: the limits of resolution for analyzing relationships among cichlid fishes. *J. Mol. Evol.* **53**: 89–103.
- Felsenstein, J. 1985. Phylogenies and the comparative method. *Am. Nat.* **125**: 1–15.
- Ferry-Graham, L.A. & Lauder, G.V. 2001. Aquatic prey capture in ray-finned fishes: a century of progress and new directions. *J. Morphol.* **248**: 99–119.
- Garland, T. Jr., Harvey, P.H. & Ives, A.R. 1992. Procedures for the analysis of comparative data using phylogenetically independent contrasts. *Syst. Biol.* **41**: 18–32.
- Garland, T. Jr., Bennett, A.F. & Rezende, E.L. 2005. Phylogenetic approaches in comparative physiology. *J. Exp. Biol.* **208**: 3015–3035.
- Gorb, S.N. & Barth, F.G. 1994. Locomotor behavior during prey-capture of a fishing spider, *Dolomedes plantarius* (Araneae, Araneidae) – galloping and stopping. *J. Arachnol.* **22**: 89–93.
- Hale, M.E., Long, J.H., McHenry, M.J. & Westneat, M.W. 2002. Evolution of behavior and neural control of the fast-start escape response. *Evolution* **56**: 993–1007.
- Herrel, A., Podos, J., Huber, S.K. & Hendry, A.P. 2005. Evolution of bite force in Darwin's finches: a key role for head width. *J. Evol. Biol.* **18**: 669–675.
- Higham, T.E., Day, S.W. & Wainwright, P.C. 2005a. Sucking while swimming: evaluating the effects of ram speed on suction generation in bluegill sunfish *Lepomis macrochirus* using digital particle image velocimetry. *J. Exp. Biol.* **208**: 2653–2660.
- Higham, T.E., Malas, B., Jayne, B.C. & Lauder, G.V. 2005b. Constraints on starting and stopping: behavior compensates for reduced pectoral fin area during braking of the bluegill sunfish *Lepomis macrochirus*. *J. Exp. Biol.* **208**: 4735–4746.
- Higham, T.E., Day, S.W. & Wainwright, P.C. 2006a. Multi-dimensional analysis of suction feeding performance in fishes: fluid speed, acceleration, strike accuracy and the ingested volume of water. *J. Exp. Biol.* **209**: 2713–2725.
- Higham, T.E., Day, S.W. & Wainwright, P.C. 2006b. The pressures of suction feeding: the relation between buccal pressure and induced fluid speed in centrarchid fishes. *J. Exp. Biol.* **209**: 3281–3287.
- Ronquist, F. & Huelsenbeck, J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572–1574.
- Hulseley, C.D. 2006. Function of a key morphological innovation: fusion of the cichlid pharyngeal jaw. *Proc. Roy. Soc. B.* **273**: 669–675.
- Hulseley, C.D. & Garcia de Leon, F.J. 2005. Cichlid jaw mechanics: linking morphology to feeding specialization. *Func. Ecol.* **19**: 487–494.
- Hulseley, C.D., Garcia de Leon, F.J., Johnson, Y.S., Hendrickson, D.A. & Near, T.J. 2004. Temporal diversification of Mesoamerican cichlid fishes across a major biogeographic boundary. *Mol. Phylogen. Evol.* **31**: 754–764.
- Irschick, D.J. & Losos, J.B. 1998. A comparative analysis of the ecological significance of maximal locomotor performance in Caribbean *Anolis* lizards. *Evolution* **52**: 219–226.
- Iwaniuk, A.N., Pellis, S.M. & Winshaw, I.Q. 1999. The relationship between forelimb morphology and behaviour in North American carnivores (Carnivora). *Can. J. Zool.* **77**: 1064–1074.
- Jayne, B.C. & Bennett, A.F. 1990. Selection on locomotor performance capacity in a natural population of garter snakes. *Evolution* **44**: 1204–1229.
- Jayne, B.C. & Lauder, G.V. 1995. Red muscle motor patterns during steady swimming in largemouth bass: effects of speed and correlations with axial kinematics. *J. Exp. Biol.* **198**: 1575–1587.
- Jayne, B.C. & Lauder, G.V. 1996. New data on axial locomotion in fishes: how speed affects diversity of kinematics and motor patterns. *Am. Zool.* **36**: 642–655.
- Johnson, T.P., Syme, D.A., Jayne, B.C., Lauder, G.V. & Bennett, A.F. 1994. Modeling red muscle power output during steady and unsteady swimming in largemouth bass. *Am. J. Physiol.* **267**: R481–R488.
- Johnson, M.K., Russell, A.P. & Bauer, A.M. 2005. Locomotor morphometry of the *Pachydactylus* radiation of lizards (Geckota: Geckkonidae): a phylogenetically and ecologically informed analysis. *Can. J. Zool.* **83**: 1511–1524.
- Keast, A. & Webb, D. 1966. Mouth and body form relative to feeding ecology in the fish fauna of a small lake, Lake Opinicon, Ontario. *J. Fish. Res. Board Can.* **23**: 1133–1144.
- Lauder, G.V. 1980. The suction feeding mechanism in sunfishes (*Lepomis*): an experimental analysis. *J. Exp. Biol.* **88**: 49–72.
- Lauder, G.V. 1990. Functional morphology and systematics: studying functional patterns in an historical context. *Ann. Rev. Ecol. Syst.* **21**: 317–340.
- Lauder, G.V. 2005. Locomotion. In: *The Physiology of Fishes*, 3rd edn (D.H. Evans & J.B. Claiborne, eds), pp. 3–46. CRC Press, Boca Raton.
- Lauder, G.V. & Drucker, E.G. 2004. Morphology and experimental hydrodynamics of fish fin control surfaces. *IEEE J. Oceanic Eng.* **29**: 556–571.
- Maddison, W.P. 1997. Gene trees in species trees. *Syst. Biol.* **46**: 523–536.

- Maddison, D.R. & Maddison, W.P. 2000. *MacClade 4.0*. Sinauer, Sunderland, MA.
- Maddison, W.P. & Maddison, D.R. 2005. *Mesquite: A Modular System for Evolutionary Analysis*. Version 1.06. <http://mesquite-project.org>.
- McHenry, M.J. & Patek, S.N. 2004. The evolution of larval morphology and swimming performance in ascidians. *Evolution* **58**: 1209–1224.
- Midford, P.E., Garland, T.J. & Maddison, W.P. 2005. *PDAP Package of Mesquite*. Version 1.07. <http://mesquiteproject.org>.
- Mittelbach, G.G. & Persson, L. 1998. The ontogeny of piscivory and its ecological consequences. *Can. J. Fish. Aquat. Sci.* **55**: 1454–1465.
- Muller, M. & Osse, J.W.M. 1984. Hydrodynamics of suction feeding in fish. *Trans. Zool. Soc. Lond.* **37**: 51–135.
- Nemeth, D.H. 1997. Modulation of attack behavior and its effect on feeding performance in a trophic generalist fish, *Hexagrammos decagrammus*. *J. Exp. Biol.* **200**: 2155–2164.
- Norton, S.F. & Brainerd, E.L. 1993. Convergence in the feeding mechanics of ecomorphologically similar species in the Centrarchidae and Cichlidae. *J. Exp. Biol.* **176**: 11–29.
- Nyberg, D.W. 1971. Prey capture in largemouth bass. *Am. Mid. Nat.* **86**: 128–144.
- Peake, S.J. & Farrell, A.P. 2004. Locomotory behaviour and post-exercise physiology in relation to swimming speed, gait transition and metabolism in free-swimming smallmouth bass (*Micropterus dolomieu*). *J. Exp. Biol.* **207**: 1563–1575.
- Prosada, D. & Crandall, K.A. 1998. MODEL TEST: testing the model of DNA substitution. *Bioinformatics* **14**: 817–818.
- Rice, A.N. & Westneat, M.W. 2005. Coordination of feeding, locomotor and visual systems in parrotfishes (Teleostei: Labridae). *J. Exp. Biol.* **208**: 3503–3518.
- Schmid, A. 1997. A visually induced switch in mode of locomotion of a spider. *Z. Naturforsch.* **52c**: 124–128.
- Streelman, J.T., Gmyrek, S.L., Kidd, M.R., Kidd, C., Robinson, R.L., Hert, E., Ambali, A.J. & Kocher, T.D. 2004. Hybridization and contemporary evolution in an introduced cichlid fish from Lake Malawi National Park. *Mol. Ecol.* **13**: 2471–2479.
- Swofford, D.L. 2002. *PAUP*: Phylogenetic Analyses Using Parsimony (* and other methods) Beta Version 4.0*. Sinauer, Sunderland, MA.
- Thompson, J.D., Plewniak, F. & Poch, O. 1999. A comprehensive comparison of multiple sequence alignment programs. *Nucleic Acid Res.* **27**: 2682–2690.
- Van Leeuwen, J.L. & Muller, M. 1984. Optimum sucking techniques for predatory fish. *Trans. Zool. Soc. Lond.* **37**: 137–169.
- Wainwright, P.C. 2002. The evolution of feeding motor patterns in vertebrates. *Curr. Op. Neurobiol.* **12**: 691–695.
- Wainwright, P.C. & Richard, B.A. 1995. Predicting patterns of prey use from morphology with fishes. *Env. Biol. Fish.* **44**: 97–113.
- Wainwright, P.C., Sanford, C.P., Reilly, S.M. & Lauder, G.V. 1989. Evolution of motor patterns: aquatic feeding in salamanders and ray-finned fishes. *Brain Beh. Evol.* **34**: 329–341.
- Wainwright, P.C., Ferry-Graham, L.A., Waltzek, T.B., Carroll, A.M., Hulsey, C.D. & Grubich, J.R. 2001. Evaluating the use of ram and suction during prey capture by cichlid fishes. *J. Exp. Biol.* **204**: 3039–3051.
- Wainwright, P.C., Huskey, S.H., Turingan, R.G. & Carroll, A.M. 2006. Ontogeny of suction feeding capacity in snook, *Centropomus undecimalis*. *J. Exp. Zool.* **305A**: 246–252.
- Waltzek, T.B. & Wainwright, P.C. 2003. Functional morphology of extreme jaw protrusion in neotropical cichlids. *J. Morphol.* **257**: 96–106.
- Webb, P.W. 1984. Body form, locomotion and foraging in aquatic vertebrates. *Amer. Zool.* **24**: 107–120.
- Webb, P.W. & Gerstner, C.L. 2000. Fish swimming behaviour: predictions from physical principles. In: *Biomechanics in Animal Behaviour* (P. Domenici & R.W. Blake, eds), pp. 59–77. BIOS Scientific, Oxford.
- Webb, P.W. & Skadsen, J.M. 1980. Strike tactics of *Esox*. *Can. J. Zool.* **58**: 1462–1469.
- Westneat, M.W. 2004. Evolution of levers and linkages in the feeding mechanisms of fishes. *Integr. Comp. Biol.* **44**: 378–389.
- Winemiller, K.O. 1989. Ontogenetic diet shifts and resource partitioning among piscivorous fishes in the Venezuelan llanos. *Env. Biol. Fish.* **26**: 177–200.
- Winemiller, K.O., Kelso-Winemiller, L.C. & Brenkert, A.L. 1995. Ecological diversification and convergence in fluvial cichlid fishes. *Env. Biol. Fish.* **44**: 235–261.

Received 27 April 2006; revised 11 July 2006; accepted 25 July 2006