Available online at www.sciencedirect.com
ScienceDirect
ELSEVIER
Zoology 112 (2009) 86–96
ZOOLOGY
www.elsevier.de/zool

Prey capture kinematics and four-bar linkages in the bay pipefish, *Syngnathus leptorhynchus*

Brooke E. Flammang*, Lara A. Ferry-Graham, Christopher Rinewalt, Daniele Ardizzone, Chante Davis, Tonatiuh Trejo

California State University, Moss Landing Marine Labs, 8272 Moss Landing Road, Moss Landing, CA 95039, USA

Received 13 December 2007; received in revised form 19 April 2008; accepted 24 April 2008

Abstract

Because of their modified cranial morphology, syngnathid pipefishes have been described as extreme suction feeders. The presumption is that these fishes use their elongate snout much like a pipette in capturing planktonic prey. In this study, we quantify the contribution of suction to the feeding strike and quantitatively describe the prey capture mechanics of the bay pipefish *Syngnathus leptorhynchus*, focusing specifically on the role of both cranial elevation and snout movement. We used high-speed video to capture feeding sequences from nine individuals feeding on live brine shrimp. Sequences were digitized in order to calculate kinematic variables that could be used to describe prey capture. Prey capture was very rapid, from 2 to 6 ms from the onset of cranial rotation. We found that suction contributed at most about one-eighth as much as ram to the reduction of the distance between predator and prey. This movement of the predator was due almost exclusively to movement of the snout and neurocranium rather than movement of the whole body. The body was positioned ventral and posterior to the prey and the snout was rotated dorsally by as much as 21°, thereby placing the mouth immediately behind the prey for capture. The snout did not follow the identical trajectory as the neurocranium, however, and reached a maximum angle of only about 10°. The snout consists, in part, of elongate suspensorial elements and the linkages among these elements are retained despite changes in shape. Thus, when the neurocranium is rotated, the four-bar linkage that connects this action with hyoid depression simultaneously acts to expand and straighten the snout relative to the neurocranium. We confirm the presence of a four-bar linkage that facilitates these kinematics by couplings between the pectoral girdle, urohyal, hyoid complex, and the neurocranium–suspensorium complex.

Keywords: Functional morphology; Suction feeding; Ram feeding; Cranial rotation; Snout rotation

Introduction

Suction generation is of central importance to aquatic-feeding vertebrates (Lauder 1985). Rapid expansion of the cranial elements generates a flow of water into the mouth, which serves to compensate for the forward locomotion of the predator (i.e., compensatory

---

*Corresponding author. Current address: Department of Organismic and Evolutionary Biology, Harvard University, 26 Oxford Street, MCZL 105, Cambridge, MA 02138, USA. Tel.: +1 617 496 7199; fax: +1 617 496 7205.

E-mail address: bflammang@oeb.harvard.edu (B.E. Flammang).

0944-2006/$ - see front matter © 2008 Elsevier GmbH. All rights reserved.
doi:10.1016/j.zool.2008.04.003

suction; van Damme and Aerts 1997), or to draw in prey (i.e., suction prey capture; Liem 1980).

A number of specialized morphologies thought to be advantageous for suction feeding have been described (Schaeffer and Rosen 1961; Ferry-Graham et al. 2001; Wainwright et al. 2001; de Lussanet and Muller 2007). Typically these entail some type of relatively small mouth and a region posterior to the mouth capable of expansion (Alexander 1967; Norton 1991; Norton and Brainerd 1993; Carroll et al. 2004; Higham et al. 2005, 2006; de Lussanet and Muller 2007). It has been suggested that the cranial morphologies of pipefishes, in the family Syngnathidae, are specialized for suction feeding (Branch 1966; Osse and Muller 1980; de Lussanet and Muller 2007). These fishes possess an elongate snout comprised of modified neurocranial and suspensorial elements. The jaws are located at the distal end of the snout and are turned slightly upward. Despite being heavily armored, the opercular region is capable of a notable degree of lateral expansion (Bergert and Wainwright 1997). Therefore, the head of the pipefish looks, and is thought to act, much like a pipette for drawing in prey (Alexander 1969, 1970; Osse and Muller 1980; Muller and Osse 1984; de Lussanet and Muller 2007). Like a pipette, the pipefish draws prey in through a small oral opening and up a long tubular snout via negative pressure created by expansion of the opercular region.

However, a number of purported suction specialists are ram specialists and rely on movement of the body or a body part to close the distance between them and their prey (Ferry-Graham et al. 2001; Wainwright et al. 2001; Ferry-Graham and Wainwright 2002). In comparisons with close relatives, chaetodontid and cichlid predators with specialized suction morphologies produced no more suction than their non-specialized counterparts (Ferry-Graham et al. 2001, 2003; Wainwright et al. 2001). But morphological modifications, such as a long snout, did afford these predators a unique mechanism for potentially enhanced ram prey capture (i.e., the protrusion or projection, often stealthily, of the jaws; Ferry-Graham et al. 2001).

Pipefishes, although seemingly morphologically specialized for suction, rotate their heads and long snouts towards their prey and are therefore most likely ram feeders. Rapid hyoid depression and buccal expansion, causative factors for suction feeding in fishes, are coincident with rapid cranial elevation in syngnathid fishes (Bergert and Wainwright, 1997; van Wassenbergh et al. 2008). Cranial elevation serves to position the snout nearer to the prey, a form of ram prey capture. Cranial elevation and hyoid depression are functionally coupled via a four-bar mechanism (Osse and Muller 1980). However, this mechanism may not fully explain hyoid depression during prey capture (Bergert and Wainwright, 1997). Therefore, we sought to understand the causes and consequences of cranial movements during prey capture through a kinematic and morphological study of the skeletal elements associated with prey capture and their linkages within the cranium.

Our goals in this study were specifically to: (1) determine the relative and absolute contribution of suction to prey capture in bay pipefish (*Syngnathus leptorhynchus*) as an example of what may be found in the Syngnathidae, (2) provide a detailed characterization of feeding kinematics in bay pipefish, and to (3) evaluate the mechanism underlying the potentially unique movements related to the neurocranium and the elongated snout during prey capture, with particular emphasis placed on elucidating the underlying four-bar mechanism.

Materials and methods

Kinematics

Specimens of *S. leptorhynchus* Girard (1854) were captured using otter trawls towed on the borders of eelgrass beds (*Zostera marina*) in Elkhorn Slough, an estuarine region of Monterey Bay on the central California coast, USA (36°49′N latitude). This species was chosen as a generalized representative of the genus, as well as for its local availability. Once captured, the specimens were transported to the laboratory and placed in communal 34 l aquaria with flowing seawater (13 °C ± 0.5) to allow them to acclimate to captivity. Fish were acclimated for at least 2 weeks prior to experimentation. *S. leptorhynchus* were fed live, commercially collected brine shrimp (*Artemia salina*), since syngnathids show a marked preference for live food. Brine shrimp are readily available and were used in previous syngnathid feeding studies facilitating comparison (Bergert and Wainwright, 1997). Nine approximately size-matched *S. leptorhynchus* (mean total length (TL) 24.0 cm; min 22.4 cm, max 25.7 cm) were filmed capturing *Artemia* prey using Redlake PCI 8000S or 2000S MotionScope digital video camera systems filming at 1000 frames per sec (frames s^{-1}). Individuals were placed in a 122 cm × 38 cm × 38 cm glass aquarium that was subdivided into three equal-sized sections. Two individuals, easily distinguished by their relative size or coloration, were placed in each section along with plastic aquarium plants and large stones to help the animals acclimate. The field was lit with two 600 W tungsten photo lamps. A rule was filmed in the field of view for scale. Feeding events were initiated by introducing a few *Artemia* at a time via pipette into the field of view. *Artemia* continued to be added and capture events filmed until the individual was satiated. All individuals were filmed from the lateral aspect. Images were only analyzed if the individual remained perpendicular to the camera throughout the capture event. Individuals were kept in the filming tank until all sequences were collected, or until no more sequences could be collected. At least three sequences were obtained from all individuals.

Three complete prey capture sequences per individual were digitized using NIH Image (Macintosh) and Scion Image (PC) to quantify movement of pertinent features during the prey capture event. Every frame was analyzed in a subset of individuals ($n = 2$) and it was determined that no information was lost by subsampling frame rate. Therefore, digitizing at 500 frames s^{-1} provided sufficient resolution to capture the feeding event while providing the greatest efficiency in digitizing individuals. Because mouth opening is small in this species and the more remarkable aspect of syngnathid feeding is cranial elevation, time zero (t_0) was taken as the onset of rapid cranial elevation. The onset of rapid cranial movement has been defined as the expansive phase of prey capture in other syngnathid feeding studies (Bergert and Wainwright 1997; de Lussanet and Muller 2007). Also, it has been documented that little measurable movement occurs during the preparatory phase, the phase of aquatic prey capture immediately prior to the expansive phase (Bergert and Wainwright 1997; de Lussanet and Muller 2007). Time zero was determined by visually identifying the frame in which rapid elevation began. Digitizing occurred for the first ten frames at 500 frames s^{-1}, during which the prey item was always captured and all peak movements occurred. Because recovery was always very slow, digitizing continued for ten additional frames at a temporal resolution of 50 frames s^{-1}.

Nine points in the region of the head and jaws were digitized (Fig. 1): (1) the posterodorsal margin of the operculum, (2) a reference point on the ventral margin of the body directly behind the operculum, (3) the anteroventral projection of the hyoid apparatus, (4) the anterior tip of the lower jaw, (5) the "corner" of the open mouth, which is the ligamentous connection between the maxilla and the lower jaw, (6) the anterior tip of the premaxilla, (7) the posterior margin of the lacrimal, where some snout rotation appeared to occur, (8) a reference point at the anterior-dorsal margin of the eye, at the posterior junction of the frontal and prefrontal, and (9) the apex of the neurocranium. The point on the prey item closest to the predator was also digitized in every frame. These points were used to calculate three linear displacement variables: gape (point 4 to point 6), upper jaw protrusion (the straight-line distance between point 6 at t_0 and point 6 at time t), and hyoid depression (the straight-line distance between point 3 at t_0 and point 3 at time t; note that the hyoid rotates during feeding but only the ventral displacement of the distal end can be detected externally and is measured here as depression of the element). The reference point on the body (point 2) did not move in relation to the fish during the feeding event, and was used to position these displacements within the fish frame of reference. Three angular variables were also determined: change in cranial elevation relative to t_0 (the angle formed by points 1, 9, 8), snout angle of rotation (points 2, 7, 6), and gape angle relative to t_0 (points 4, 5, 6). Lastly, four kinematic variables were estimated following Wainwright et al. (2001). *Suction distance* was the distance moved by the prey from t_0 until it crossed the plane of the jaw tips, which indicated prey capture. Similarly, *ram distance* was the distance moved by the pipefish over the same time period. The contribution of both jaw protrusion and the body locomotion to ram distance were measured separately.

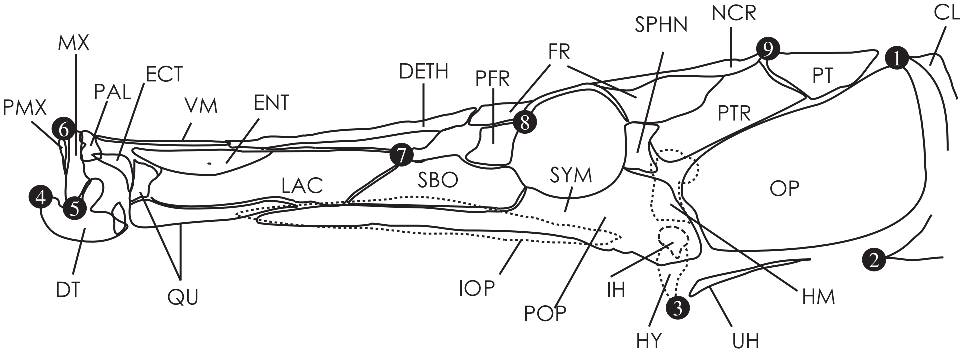

Fig. 1. Points digitized from the high-speed video images of *Syngnathus leptorhynchus* for the computation of kinematic variables, superimposed on a diagram of the skull. The branchiostegals were omitted. The hyoid complex, hyomandibula, interhyal, and interopercular (dotted lines) are deep to the preopercle (solid line). Abbrev.: CL, cleithrum; DETH, dermethmoid; DT, dentary; ECT, ectopterygiod; ENT, entopterygiod; FR, frontal; HM, hyomandibula; HY, hyoid complex. consisting of the epihyal, ceratohyal, and two hypohyals; IH, interhyal; IOP, interopercular; LAC, lacrimal; MX, maxilla; NCR, neurocranium; OP, operculum; PAL, palatine; PFR, prefrontal; PMX, premaxilla; POP, preopercular; PT, posttemporal; PTR, pterotic; QU, quadrate; SBO, suborbital; SPHN, sphenoid; SYM, symplectic; UH, urohyal; and VM, vomer.

A coarse estimate of strike velocity was determined for each pipefish by dividing the average maximum ram distance by the average time taken to reach the maximum and termed *average strike velocity*. *Predator-prey distance* was the distance measured between a pipefish and their prey at t_0.

Morphology and mechanical linkages

Movements of skeletal elements, in particular the elongate snout, were further investigated through anatomical study. Specimens were cleared using a trypsin and double-stain method (Dingerkus and Uhler 1977). The cranial skeletal anatomy was examined with the aid of an AmScope 7*x*–45*x* stereomicroscope. Cranial element terminology followed Bergert and Wainwright (1997) to facilitate direct comparison. The potential roles of elements associated with prey capture were determined through direct manipulation of the stained specimens as well as fresh dead specimens.

To best identify and illustrate the location of the four-bar linkage elements in situ, computed microtomography (μCT) scans of the head of a 21 cm TL specimen were collected using a Skyscan 1172 (MicroPhotonics, Inc., Allentown, PA, USA, software version 1.4) at 4.4 μm pixel resolution. The scan resulted in 4670 slices 1500×1500 pixels in size, which were reconstructed in three dimensions using Amira 4.1 (Mercury Computer Systems, Inc., Chelmsford, MA, USA).

These morphological and manipulation data were used to infer mechanical linkages and function with respect to the postulated four-bar mechanism proposed for pipefishes (Osse and Muller 1980; Muller 1987). This mechanism consists of a set of links between the neurocranium plus suspensorium, the hyoid complex, the urohyal, and the pectoral girdle and serves to functionally couple hyoid depression with cranial elevation.

Results

Kinematics

S. leptorhynchus tended to swim slowly about the tank during feeding trials and speed remained unchanged as individual prey items were captured. The body was held at an angle typically between 30° and 60° relative to vertical with the head higher than the tail. An individual almost always initiated the strike with its head oriented beneath the prey (Fig. 2), accelerating the jaw towards the prey via rapid cranial elevation and rotation. The body moved little during the strike, maintaining a small amount of inertia from locomoting about the tank. Prey capture was ram dominated but with little contribution from the body. The contribution of combined body plus jaw ram distance to the strike was eight times greater than the contribution of suction distance (Table 1).

Prey capture occurred quickly, within a range of 2–6 ms for all individuals, and average strike velocity was $256.3\,\mathrm{cm\,s^{-1}}$ (Table 1). At the time of prey capture, kinematic variables that showed a displacement were approximately half of their maxima (Fig. 2). Maximum gape, hyoid depression, snout rotation, and cranial elevation occurred nearly simultaneously and more than 10 ms after prey capture (Table 1). Mouth opening typically was quite minimal, averaging 0.4 cm, and individuals tended to hold the mouth slightly open even when prey capture was not occurring, therefore the movement due to prey capture was slight. Upper jaw protrusion was also very small, <0.2 cm. Instead, cranial elevation appeared to contribute primarily to prey capture, with rotation of the order of 20° (Table 1). Snout rotation was largely synchronized with cranial elevation but achieved only about 10°, which is about half of the maximum angle (Fig. 2). Recovery from prey capture was slow and kinematic variables had not returned to their resting positions even after 150 ms.

Morphology

With the exception of the elongate suspensorium, the cranial morphology of *S. leptorhynchus* is not very different from most other members of the order Gasterosteiformes (Branch, 1966; Anker 1974; Pietsch 1978; Britz and Johnson 2002; Takata and Sasaki 2005; Fig. 1). The elongate bones of the suspensorium were very thin and flexible. The vomer, dermethmoid, entopterygoid, lacrimal, and quadrate were not fused to each other, and instead were sheathed in the thin tubular sheets of tissue that line the snout. There was little overlap between the bones of the snout. Spaces between the dermethmoid and frontal and the lacrimal and suborbital bones were filled by the continuous sheath of connective tissue. The interopercule was medial to the thin, broad anterior process of the preopercule and both bones were ventrolateral to the suspensorium. The posterior portion of the preopercle was fused to the symplectic ventral to the orbit, and broadened dorsally and ventrally posterior to the orbit. The hyomandibula was medial to the preopercle-symplectic complex. The ventrolateral portion of the hyomandibula fit into a groove on the medial aspect of the preopercle (Fig. 3). The dorsal aspect of the hyomandibula had three distinct projections. The anterodorsal-most of these fit into a socket in the sphenoid bone. The medial-most projection was cup-shaped and articulated with a condyle of the pterotic bone. The posterolateral-most projection was also cup-shaped and was the insertion site for a ball-in-socket

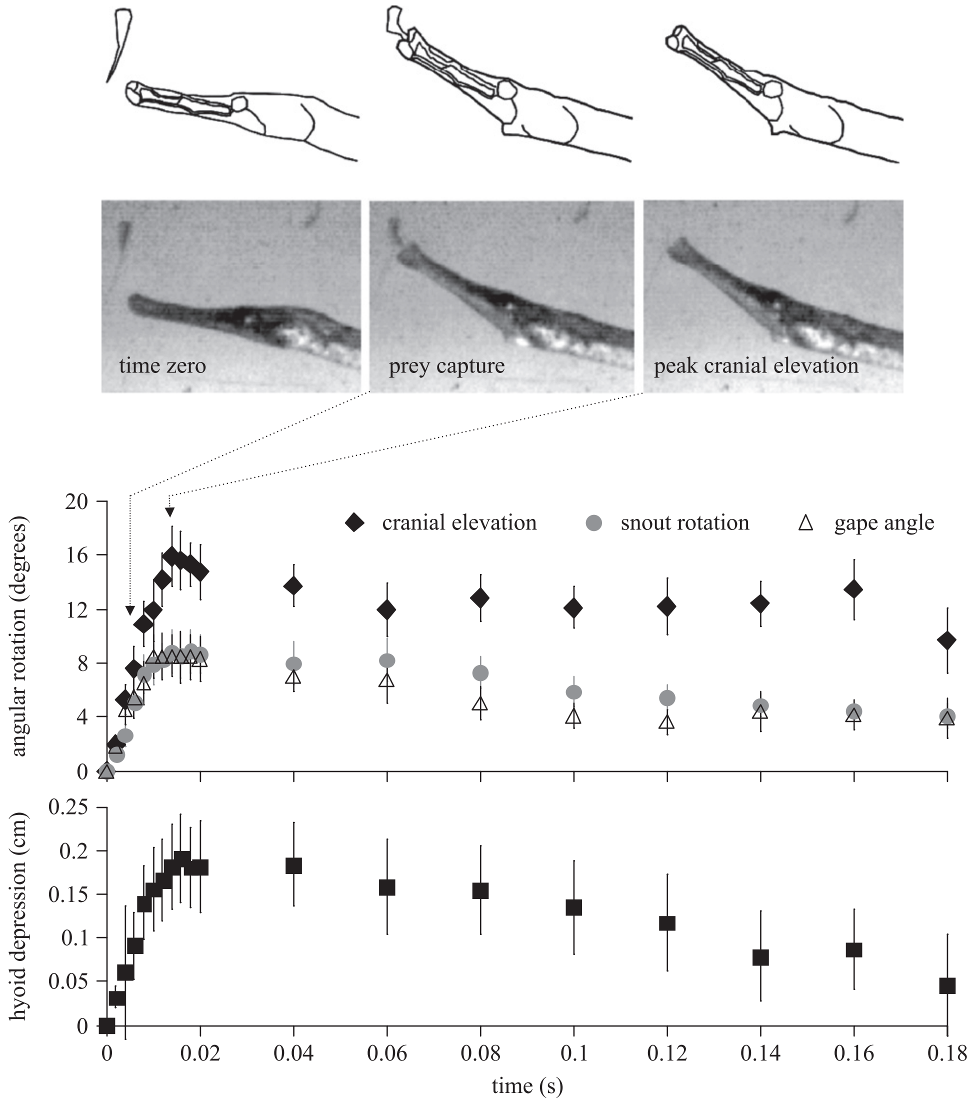

Fig. 2. Kinematic plots of angular and displacement variables for the duration of the feeding event digitized, paired with composite images from high-speed video footage of *Syngnathus leptorhynchus*. Time of image is indicated on each frame. In this sequence, prey capture occurs at 6 ms with an appendage of the prey item crossing the plane of the open mouth. Maximum cranial elevation and hyoid depression are achieved by 16 ms. Note that the variables had not returned to their starting positions even after 100 ms into the strike. Subsequent increases in cranial elevation during this time are suggestive of prey transport events. Points are means for each individual, and then a grand mean taken for all individuals.

articulation on the medial aspect of the operculum. Ventrally, the hyomandibula fused with the preopercle anterior to the connection to the hyoid complex. The ventral edge of the preopercle formed an acetabular-like cup, into which the ball-shaped lateral end of the interhyal inserted. The saddle-shaped, medial end of the interhyal was connected by a short ligament to the epihyal and had a short triangular projection that extended ventrally, medial to the hyoid complex (Fig. 4). The hyoid complex consisted of the epihyal, ceratohyal and two (dorsal and ventral) fused hypohyals, all of which were bound tightly together by connective tissue into one functional unit. A long, thin band of connective tissue extended anteriorly from the ceratohyal and inserted onto the posterior edge of the thin interopercle. Attached to the posteroventral edge of each epihyal were two branchiostegal rays that extended posteriorly, ventral to the operculum. The ventral hypohyal connected to the urohyal via a short, thick ligament.

The major muscles assumed to be involved in the abduction of the cranium and suspensorium and depression of the hyoid of *S. leptorhynchus* were the m. adductor arcus palatini, m. levator arcus palatini, m. adductor hyomandibulae, m. epaxialis, m. protractor pectoralis, and m. sternohyoideus. The m. adductor arcus palatini was a relatively large muscle that

Table 1. Means (SE) for kinematic variables from this and other kinematic studies of pipefish

Variables	This study	*S. acus*[a]	*S. floridae*[b]
Time of prey capture (ms)	4.2 (0.5)	6–8	7.9 (2.2)
Time of maximum snout rotation (ms)	14.8 (2.2)		
Time of maximum gape (ms)	16.3 (4.6)		6.8 (2.8)
Time of maximum cranial elevation (ms)	17.4 (2.7)	10–13[c]	7.5 (2.9)
Time of maximum hyoid depression (ms)	16.0 (5.0)	8.5[c]	6.1 (2.0)
Maximum snout rotation (deg.)	10.7 (1.2)		
Maximum gape angle (deg.)	12.1 (2.1)		
Maximum cranial elevation (deg.)	19.2 (2.2)	~27[c]	29.2 (8.5)
Maximum gape distance (cm)	0.36 (0.08)		0.33[c]
Maximum hyoid depression (cm)	0.19 (0.06)	~0.3[c]	0.17[c]
Suction distance (cm)	0.09 (0.03)		
Ram_{body} distance (cm)	0.19 (0.05)		
$\text{Ram}_{\text{body+jaw}}$ distance (cm)	0.80 (0.2)		
Initial predator-prey distance (cm)	0.79 (0.1)		0.88[c]
Average strike velocity (cm s^{-1})	256.3 (62.9)		

Note that suction and ram distance may not sum to predator-prey distance as the jaws pass through an arc during prey capture while predator-prey distance is a straight-line measurement.

[a] de Lussanet and Muller (2007); $n = 1$ individual, 2 strikes, filmed at ~1000 frames s^{-1}.

[b] Bergert and Wainwright (1997); $n = 3$ individuals, 7 strikes, filmed at 200–400 frames s^{-1}.

[c] Value inferred from graph provided in publication.

originated from the groove between the hyomandibular and symplectic bones and from the prefrontal bones and inserted on the parasphenoid bone between the orbits. In addition, a long, thin aponeurosis extended anteriorly from this muscle, attached to the ectopterygoid and entopterygoid via connective tissue. The m. levator arcus palatini originated from the ventral edge of the sphenoid and inserted into a deep groove created by the symplectic and preopercle. An aponeurosis extended ventrally from the lateral midsection of this muscle, and inserted onto the small triangular-shaped medial process of the interhyal and around the acetabular structure of the preopercle, into which the interhyal inserts. A second aponeurosis from the medial side of the m. levator arcus palatini at its attachment at the anterior edge of the hyomandibula extended anteriorly and was attached to the m. adductor arcus palatini. The m. adductor hyomandibulae originated on the ventral edge of the pterotic and inserted onto the medial side of the hyomandibula, as well as to the posterior-most hyomandibular projection. The m. epaxialis and m. protractor pectoralis muscles lay in close association

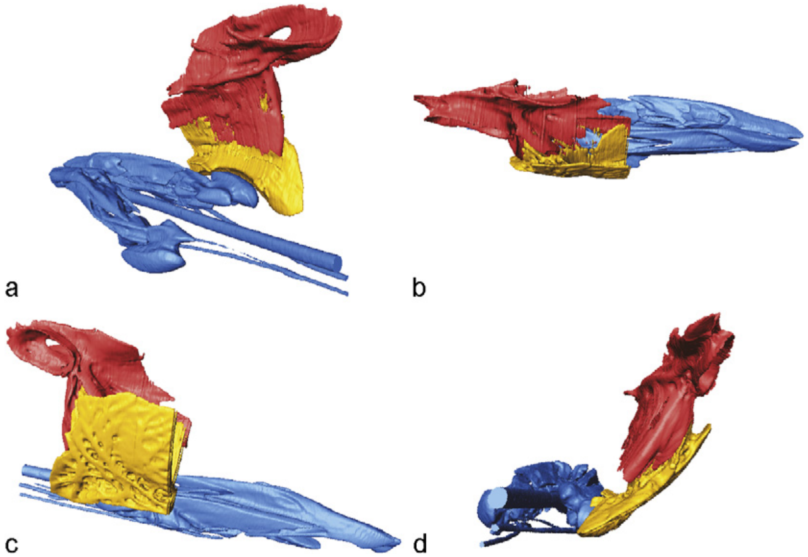

Fig. 3. Three-dimensional reconstruction of computed microtomography (μCT) scan of *Syngnathus leptorhynchus* (21 cm TL) hyoid complex (blue) and articulations with the preopercle (POP, yellow) and hyomandibula (HM, red). The reconstructed image is shown in four different orientations, in postero-medial (a), dorsal (b), lateral (c), and posterior (d) views.

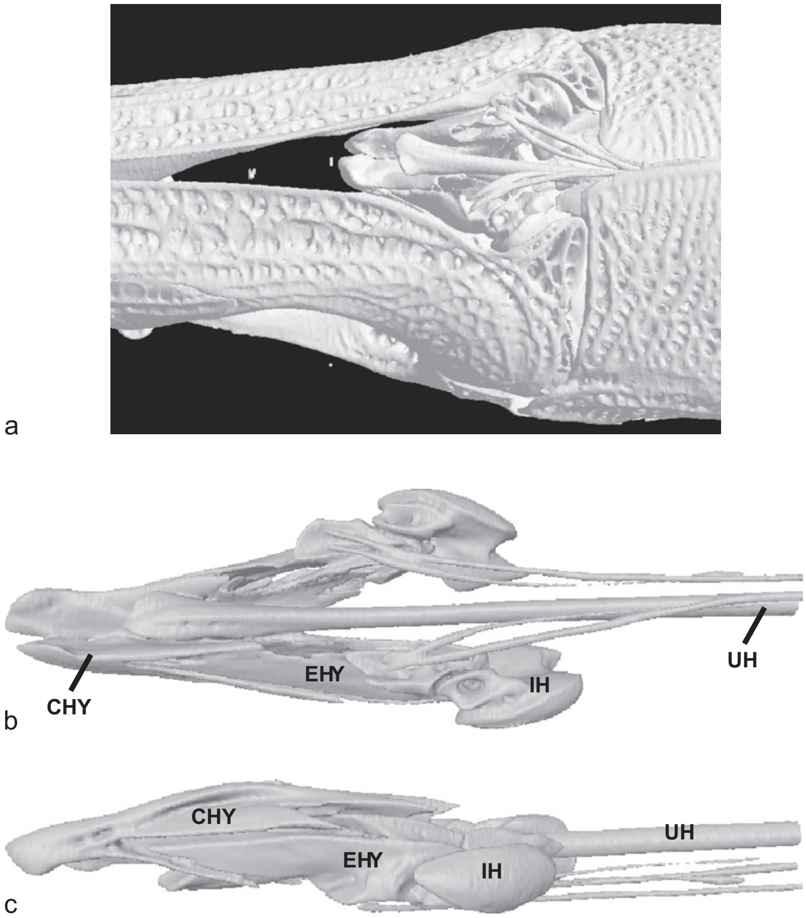

Fig. 4. (a) Ventral view of computed microtomography (μCT) scan of *Syngnathus leptorhynchus* (21 cm TL) cranium. (b, c) Three-dimensional reconstruction of μCT scan of the hyoid complex of *S. leptorhynchus* in ventral (b) and lateral (c) views. Abbrev.: CHY, ceratohyal; EHY, epihyal; IH, interhyal; UH, urohyal.

with one another, postero-dorsally to the cranium. The m. epaxialis, which continued posteriorly for the length of the fish, was attached to the neurocranium by a tendon. The m. protractor pectoralis originated on the dorsal aspect of the cleithrum and inserted ventral to the posttemporal at the posterior edge of the neurocranium. The m. sternohyoideus was paired but shared a tendon that projected anteriorly and inserted onto the dorsal surface of the urohyal; posteriorly, this muscle lay to both sides of the urohyal and attached to the coracoid of the pectoral girdle.

Investigations of cleared and stained specimens indicated that the mechanism allowing for snout rotation relative to the neurocranium was a linkage system similar to the hyoid four-bar mechanism. Manual rotation of the cranium resulted in depression of the hyoid. Conversely, manual rotation of the hyoid did not result in elevation of the cranium, but in rotation of the lower jaw. The interopercle is located immediately medial to the preopercle and extends from the ceratohyal to the angular-articular bones thereby transferring force from the hyoid to the lower jaw. Lateral motion was also observed at the ventral ends of the hyomandibulae during manipulation. Removal of the preopercle from cleared and stained specimens, however, also indicated that movement of the hyomandibulae is constrained by the position of the preopercle bones. The lateral motion of the ventral ends of the hyomandibulae presumably transfers force laterally to the preopercles, which form the more posterior region of the ventral snout elements. The skin covering the snout, in which the suspensorial bones of the snout are encased, becomes tensed and may transfer forces produced by the hyomandibulae to create the lateral spreading of the suspensorium. Lateral expansion of the snout was observed to occur simultaneously with this lateral motion of the preopercles.

Discussion

Prey capture in *S. leptorhynchus* happens primarily by jaw ram (Table 1). The jaws are rapidly rotated up to the prey item and suction appears to affect the prey only when the jaws are very close, within a few millimeters.

Prey items were captured quickly from the onset of cranial rotation. Mean time to capture was a little over 4 ms, which is consistent with other syngnathids (Bergert and Wainwright, 1997; de Lussanet and Muller 2007), and is among the fastest on record (see also Grobecker and Pietsch 1979). Despite this rapid capture speed, the absolute contribution of suction is similar to values reported for teleosts without elongate snouts (Ferry-Graham et al. 2001; Wainwright et al. 2001). More impressive is the relative contribution of ram, which exceeds that recorded for long-jawed chaetodontids (which use twice as much ram as suction; Ferry-Graham et al. 2001). The relative contribution of ram places *S. leptorhynchus* on par with some of the most highly modified cichlids, which use 2–8 times more ram than suction (Ferry-Graham and Wainwright, 2002; van Wassenbergh et al. 2008; Wainwright et al. 2001).

Timing of expansion of the oral region tended to be slow when compared with the timing reported for other syngnathids (Table 1), further contradicting the theory of suction generation as dominant in prey capture. It took more than twice as long for *S. leptorhynchus* (this study) to reach displacement maxima than for *S. floridae* (Bergert and Wainwright, 1997). The limited data for *S. acus* places them somewhat intermediate between these two species (de Lussanet and Muller 2007).

There are several potential explanations for the differences in the timing variables between the two pipefish studies. We note that the three *S. floridae* used in Bergert and Wainwright (1997) ranged in size from 12.6 to 15.4 cm; these were much smaller than the animals used in our study and differences may be explained by scaling effects (Richard and Wainwright 1995). Our single 17 cm individual that we dropped from the analysis in the event of scaling differences, however, was not different from the group means reported here for *S. leptorhynchus*. It is possible also that suction is more important for prey capture in *S. floridae* than in *S. leptorhynchus*, and thus expansion of the buccal cavity is more rapid in *S. floridae*. Cranial elevation is similarly more extreme in *S. floridae*.

However, it is equally likely that these timing differences, while seemingly large, are actually unimportant kinematically. Timing differences could be attributable to the techniques used by different researchers. In *S. leptorhynchus* alone, the difference between points of maximum cranial elevation, for example, at 8–10 ms and at 16 ms is negligible. It is worth noting that the feeding events were captured at a greater time resolution in our study (recorded at 1000 frames s^{-1} and digitized at 500 frames s^{-1}), as opposed to the feeding events filmed at 200 frames s^{-1} in the study of *S. floridae*. The resulting time-resolution of 5 ms between subsequent frames is actually greater than some of the values reported, and easily enough time to account for some of the differences noted here. Insufficient filming speed increases the likelihood of missing a maximum point and underestimating timing variables (Walker 1998).

Ram seems to be the most important component of prey capture in *S. leptorhynchus*; but this does not suggest that suction is altogether unimportant. Suction is certainly crucial for moving prey along the tubular snout. Muller and Osse (1984) suggested that syngnathids possess supportive structural modifications for dealing with tremendous suction generation during prey transport, primarily to the gill and opercular regions. Suction pressures generated during prey transport have not been measured but may well reach values necessitating supportive modifications such as united gill arches (via connective tissue), fused branchiostegal rays, and heavily ossified operculae (Osse and Muller 1980). Muller and Osse (1984) noted that the operculae are more robust in species with longer snouts. Increasing snout length has been correlated with increasing speed of the capture event and a concomitant reduction in time for prey capture (de Lussanet and Muller 2007). Therefore, pipefish may very well possess modifications for both enhanced ram prey capture and suction prey transport.

There is circumstantial evidence from all three *Syngnathus* species that supports the hypothesis that more suction is produced during prey transport than during prey capture. We found that there was a second hyoid depression long after the prey was captured in *S. leptorhynchus*. This peak was larger (0.33 cm), and occurred on average 60 ms into the feeding event although the timing was also variable (SE ± 21.5 ms). This large variation in timing is probably related to strike-to-strike deviation associated with positioning the prey properly for transport, and prevented an obvious peak from appearing in graphs of hyoid depression. Multiple additional increases in cranial elevation, however, were observable during this time period (Fig. 2). In limited observations of *S. acus* ($n = 1$), a second, larger peak in cranial elevation is found at 25–50 ms into the feeding event (de Lussanet and Muller, 2007), and in *S. floridae* a second, larger (0.22 cm) hyoid depression event occurs about 50 ms into the strike (Bergert and Wainwright, 1997). We suggest that these second peaks are tied to strong suction transport and underscore the role of the hyoid in generating suction.

Cranial elevation, which is perhaps the most important element of prey capture kinematics, is linked to hyoid depression via a four-bar mechanism (Muller 1987; de Lussanet and Muller 2007; Fig. 5). When the cranium is rotated dorsoposteriorly, the hyoid complex is simultaneously depressed via connections through the suspensorium and retraction of the sternohyoideus

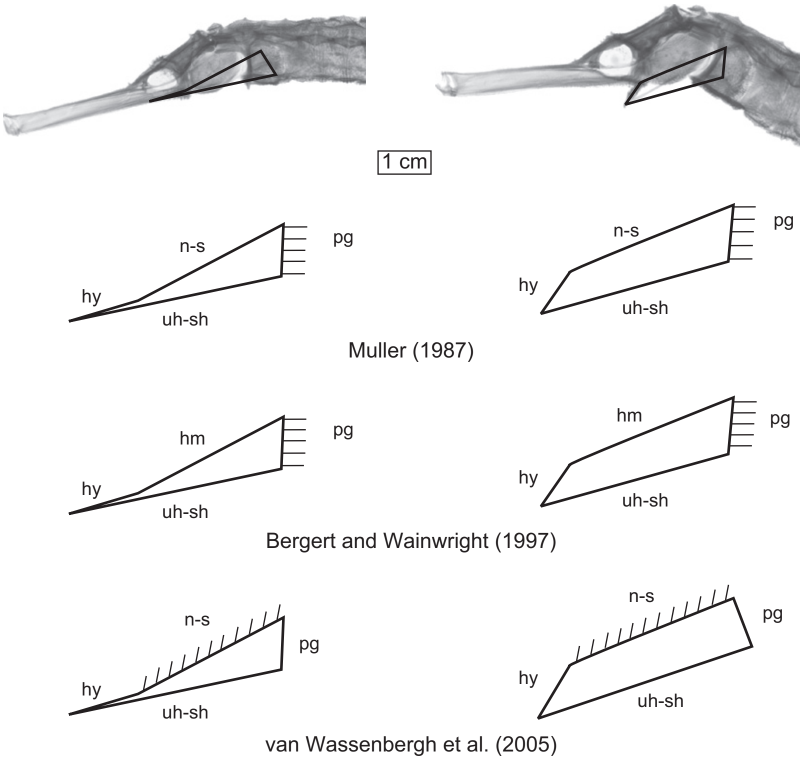

Fig. 5. The four-bar linkage as described by Muller (1987), Bergert and Wainwright (1997), and van Wassenbergh et al. (2005). The hatched lines denote the stationary bar of the mechanical linkage. The linkage exhibited in pipefish (top, MCZ 154078) most closely resembles that defined by van Wassenbergh et al. (2005). Radiograph image courtesy of Andrew Williston, Museum of Comparative Zoology, Harvard University. Abbrev.: hm, hyomandibula; hy, hyoid complex; n-s, neurocranium-suspensorium complex; pg, pectoral girdle; uh-sh, urohyal-sternohyoideus complex.

muscle, as demonstrated in other fishes (Branch 1966; Westneat 1994; van Wassenbergh et al. 2005, 2008). However, it is the precise articulation of the hyoid complex with the suspensorium, and possibly the misrepresentation of this articulation, that has led to two distinctly different models being purported as the four-bar linkage mechanism of the hyoid complex in syngnathids. The four-bar linkage, as originally described by Muller (1987), kinematically links the pectoral girdle, urohyal and sternohyoideus, hyoid complex, and neurocranium–suspensorium complex. Conversely, the four-bar linkage interpreted by Bergert and Wainwright (1997) identifies the hyomandibula as the input bar for the mechanism, and removes the neurocranium–suspensorium complex from the linkage (Fig. 5). In *S. leptorhynchus* at least, it is clear that the hyomandibula does not directly articulate with the hyoid complex at all (Fig. 3), which in turn means that the length of the input bar in Bergert and Wainwright (1997) model would have been underestimated. This could explain Bergert and Wainwright, (1997) finding that this model describes the linkages present in the seahorse *Hippocampus erectus*, but overestimates the amount of hyoid depression when cranial elevation angle is used as an input. Using the hyomandibula as a bar in the model also excludes the insertion of the epaxial muscles to the neurocranium–suspensorium complex and underestimates the role of cranial elevation in the feeding mechanism. The elements of the four-bar linkage as defined by Muller (1987) are consistent with the anatomy of the pipefish; however, Muller (1987) assumed that the pectoral girdle was the stationary bar of the linkage. Contrary to this, the retraction of the pectoral girdle by hypaxial muscle contraction is considered to be a contributing factor to hyoid depression in a number of fishes (Anker 1974; Lauder 1980; Motta 1982; Aerts 1991; Carroll 2004). Recent work by van Wassenbergh et al. (2005) suggests the most applicable four-bar linkage mechanism includes the neurocranium–suspensorium complex, hyoid complex, urohyal, and pectoral girdle. This mechanism follows Muller (1987) except that the neurocranium–suspensorium bar is stationary, not the pectoral girdle (Fig. 5). Their model allows for both cranial elevation through contraction of the epaxial muscles and hyoid depression through contraction of the hypaxial muscles and retraction of the pectoral girdles, sternohyoideus, and urohyal to produce hyoid depression.

This four-bar mechanism is also ultimately linked with the snout bending that we observed. The hyoid is linked to the lower jaw such that when the hyoid is manually depressed or rotated, the lower jaw is depressed. This is consistent with observations on seahorses by Bergert and Wainwright (1997). Presumably, this force is transmitted not only through the protractor hyoideus muscle which connects the hyoid to the lower jaw, but also through the modified suspensorial bones within the ventral portion of the snout. Despite being modified to create a tubular opening at the distal end of the snout, the suspensorial bones still possess most of the anatomical connections typical of a teleost fish. This means that while the dorsal portion of the snout is rotated postero-dorsally during cranial elevation, the sides of the snout are also being laterally expanded through the action of hyoid depression. These two seemingly opposing forces cause the snout to expand and the anterior region rotates ventrally. Kinesis occurs at the region between the dermethmoid and the frontal bones. These two bones are not continuous, giving the appearance of a clear demarcation between snout (dermethmoid, lacrimal, and quadrate) and cranium (frontal, prefrontal, the sinus, suborbital, preopercle) (Fig. 1). As the snout expands laterally, the spaces between the snout and cranial portions are closed by the snout bones pushing dorsolaterally. Therefore, the snout rotates ventrally with respect to the cranium. This reduces the maximum angle traversed by the snout, which serves to explain our kinematic observation that during prey capture the maximum angle achieved by the snout was less than the maximum angle of cranial elevation. The snout gives the appearance of elongating just as prey capture is occurring and the angle relative to the body is reduced. Bergert and Wainwright (1997) found a similar result for the seahorse *Hippocampus erectus* and noted that lateral expansion also occurs in this region during prey capture, as well as at the distal ends of the paired hyomandibulae. Indeed, the timing of maximum snout rotation (this study; 14 ms) coincides tightly with the timing of maximum snout width reported by de Lussanet and Muller (2007; 18 ms).

The movement of the neurocranium documented here is impressive but is not the most extreme on record. Other pipefish species and some flatfish species have been reported to meet or exceed these values (Gibb 1995, 1996; Bergert and Wainwright, 1997). However, this is the first description of snout rotation disassociated from neurocranial rotation. This kinesis may well be a consequence of retained linkages among suspensorial elements, and probably does not serve to enhance or direct suction to any degree. Indeed, the suction generated during the prey capture portion of the feeding event appears to be unexceptional when compared with other species morphologically specialized for suction. Studies of prey transport are necessary in this species, as well as other generalized species, to quantitatively determine the role suction plays during that portion of the feeding event.

Acknowledgements

All animals were handled ethically in accordance with IACUC standards under SJSU protocol #814 to LFG, and collected under California Fish and Game permit 801043-01 to LFG. Equipment used in this study was purchased via NSF MRI Grant #0320972 to LFG and co-investigators. T. Sledz and MicroPhotonics, Inc. generously contributed the μCT scan. M. McDonald assisted with high-speed video data collection. K. Hartel and A. Williston (Museum of Comparative Zoology, Harvard University) supplied comparative specimens and assisted in radiography of specimens, respectively. We are also grateful to S. van Wassenbergh for his review of an earlier version of this manuscript and for insightful discussions regarding pipefish.

References

Aerts, P., 1991. Hyoid morphology and movements relative to abducting forces during feeding in *Astatotilapia elegans* (Telestei: Cichlidae). J. Morphol. 208, 323–345.

Alexander, R.M., 1967. The functions and mechanisms of the protrusible upper jaws of some acathopterygian fish. J. Zool. London 151, 43–64.

Alexander, R.M., 1969. Mechanisms of the feeding action of a cyprinid fish. J. Zool. London 159, 1–15.

Alexander, R.M., 1970. Mechanisms of the feeding action of various teleost fishes. J. Zool. London 162, 145–156.

Anker, G.C., 1974. Morphology and kinetics of the head of the stickleback, *Gasterosteus aculeatus*. Trans. Zool. Soc. London 32, 311–416.

Bergert, B.A., Wainwright, P.C., 1997. Morphology and kinematics of prey capture in the syngnathid fishes *Hippocampus erectus* and *Syngnathus floridae*. Mar. Biol. 127, 563–570.

Branch, G.M., 1966. Contributions to the functional morphology of fishes. Part III, the feeding mechanism of *Syngnathus acus* Linnaeus. Zool. Afr. 2, 69–89.

Britz, R., Johnson, G.D., 2002. “Paradox lost”: skeletal ontogeny of *Indostomus paradoxus* and its significance for the phylogenetic relationships of Indostomidae (Teleostei, Gasterosteiformes). Am. Mus. Nov. 3383, 1–43.

Carroll, A.M., 2004. Muscle activation and strain during suction feeding in the largemouth bass *Micropterus salmoides*. J. Exp. Biol. 207, 983–991.

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.

de Lussanet, M.H.E., Muller, M., 2007. The smaller your mouth, the longer your snout: predicting the snout length of *Syngnathus acus*, *Centriscus scutatus* and other pipette feeders. J. R. Soc. Interface 4, 561–573.

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

Ferry-Graham, L.A., Wainwright, P.C., 2002. Evaluating suction feeding performance in fishes: implications for evolutionary diversification. In: Bels, V.L., Gasc, J.P., Casinos, A. (Eds.), Vertebrate Biomechanics and Evolution. BIOS Scientific Publishing Ltd., Oxford, UK, pp. 101–116.

Ferry-Graham, L.A., Wainwright, P.C., Bellwood, D.R., 2001. Prey capture in long-jawed butterflyfishes (Chaetodontidae): the functional basis of novel feeding habits. J. Exp. Mar. Biol. Ecol. 256, 167–184.

Ferry-Graham, L.A., Wainwright, P.C., Lauder, G.V., 2003. Quantification of flow during suction feeding in bluegill sunfish. Zoology 106, 159–168.

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

Gibb, A.C., 1996. The kinematics of prey capture in *Xystreurys liolepis*: do all flatfish feed asymmetrically? J. Exp. Biol. 199, 2269–2283.

Grobecker, D.B., Pietsch, T.W., 1979. High speed cinematographic evidence for ultrafast feeding in antennariid anglerfishes. Science 205, 1161–1162.

Higham, T.E., Day, S.W., Wainwright, P.C., 2005. 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., Day, S.W., Wainwright, P.C., 2006. Multidimensional analysis of suction feeding performance in fishes: fluid speed, acceleration, strike accuracy and the ingested volume of water. J. Exp. Biol. 209, 2713–2725.

Lauder, G.V., 1980. The suction feeding mechanism in sunfishes (*Lepomis*): an experimental analysis. J. Exp. Biol. 88, 49–72.

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

Liem, K.F., 1980. Acquisition of energy by teleosts: adaptive mechanisms and evolutionary patterns. In: Ali, M.A. (Ed.), Environmental Physiology of Fishes. Plenum Publishing Corporation, NY, USA, pp. 299–334.

Motta, P.J., 1982. Functional morphology of the head of the inertial suction feeding butterflyfish, *Chaetodon miliaris* (Perciformes, Chaetodontidae). J. Morphol. 174, 283–312.

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

Muller, M., Osse, J.W.M., 1984. Hydrodynamics of suction feeding in fish. Trans. Zool. Soc. London 37, 51–135.

Norton, S.F., 1991. Capture success and diet of cottid fishes: the role of predator morphology and attack kinematics. Ecology 72, 1807–1819.

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.

Osse, J.W.M., Muller, M., 1980. A model of suction feeding in teleostean fishes with some implications for ventilation. In: Ali, M.A. (Ed.), Environmental Physiology of Fishes. Plenum Publishing Corp., NY, USA, pp. 335–352.

Pietsch, T.W., 1978. Evolutionary relationships of the sea moths (Teleostei: Pegasidae) with a classification of Gasterosteiform families. Copeia 1978, 517–529.

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

Schaeffer, B., Rosen, D.E., 1961. Major adaptive levels in the evolution of the actinopterygian feeding mechanism. Am. Zool. 1, 187–204.

Takata, Y., Sasaki, K., 2005. Branchial structures in the Gasterosteiformes, with special reference to myology and phylogenetic implications. Ichthyol. Res. 52, 33–49.

van Damme, J., Aerts, P., 1997. Kinematics and functional morphology of aquatic feeding snake-necked turtles (Pleurodira; *Chelodina*). J. Morphol. 233, 113–125.

van Wassenbergh, S., Herrel, A., Adriaens, D., Aerts, P., 2005. A test of mouth-opening and hyoid-depression mechanisms during prey capture in a catfish using high-speed cineradiography. J. Exp. Biol. 208, 4627–4639.

van Wassenbergh, S., Strother, J.A., Flammang, B.E., Ferry-Graham, L.A., Aerts, P., 2008. Extremely fast prey capture in pipefish is powered by elastic recoil. J. R. Soc. Interface 5, 285–296.

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 in cichlid fishes. J. Exp. Biol. 204, 3039–3051.

Walker, J.A., 1998. Estimating velocities and accelerations of animal locomotion: s simulation experiment comparing numerical differentiation algorithms. J. Exp. Biol. 201, 981–995.

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