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Unusual kinematics and jaw morphology associated with piscivory in the poeciliid, *Belonesox belizanus*

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ABSTRACT

Piscivory in fishes is often associated with the evolution of highly elongate jaws that achieve a large mouth opening, or gape. *Belonesox belizanus*, the pike killifish, has independently evolved this morphology, which is derived from short-jawed poeciliids within the Cyprinodontiformes. Using kinematic analysis of high-speed video footage, we observed a novel aspect of the elongate jaws of *Belonesox*; the premaxilla rotates dorsally during mouth opening, while the lower jaw rotates ventrally. Anatomical study revealed that this unusual motion is facilitated by the architecture of the premaxillomandibular ligament, prominent within cyprinodontiforms. In *Belonesox*, it allows force to be transferred from the lower jaw directly to the premaxilla, thereby causing it to rotate dorsally. This dorsal rotation of the premaxilla appears to be assisted by a mediolateral twisting of the maxilla during jaw opening. Twisting maxillae are found in members of the group such as *Fundulus*, but are lost in *Gambusia*. Models revealed that elongate jaws partially account for the enlarged gape, but enhanced rotation at the quadrato-mandibular joint was equally important. The large gape is therefore created by: (i) the convergent evolution of elongate jaws; (ii) enhanced jaw rotation, facilitated by loss of a characteristic cyprinodontiform trait, the lip membrane; and (iii) premaxilla rotation in a novel direction, facilitated by the retention and co-option of additional cyprinodontiform traits, the premaxillomandibular ligament and a twisting maxilla.

1. Introduction

Many fishes that feed on elusive fish prey possess highly elongate jaws (Porter and Motta, 2004). Several lineages of ray-finned fish possess this recognizable trait, including barracuda (*Sphyraena*; Grubich et al., 2008), gar (*Lepisoteus*; Kammerer et al., 2006), and pike (*Esox*; Rand and Lauder, 1981). The elongate jaw has evolved independently multiple times from the more typical ray-finned fish condition of relatively foreshortened jaws (Westneat, 2004), exhibited by species such as basses (Centrarchidae), seabasses (Serranidae), and rockfishes (Scorpeanidae). The consistent association between this particular modification and a diet primarily of fish and other highly elusive prey items (e.g., Hunt, 1953; Chapman et al., 1989; Schmidt, 1989; Porter and Motta, 2004) suggests that jaw length represents a major axis of diversity among fishes and that many piscivores occupy one end of that continuum.

Elongate jaws are presumably advantageous to elusive prey specialists because they provide for an enlarged gape, or mouth opening, even in the absence of other changes to the cephalic region. This is true because elongation of the jaws places the distal tips of the jaws farther from the quadrato-mandibular, and therefore the jaw tips will move a larger distance, even if the actual magnitude of jaw rotation is unchanged relative to short-jawed ancestors. A larger gape may be required for the capture of fish prey, in particular, which tend to be larger than other types of prey (Hoyle and Keast, 1987; Wainwright and Richard, 1995a,b; Mittelbach and Persson, 1998; Wainwright and Shaw, 1999). Further, elongate jaws should increase the chance of capturing elusive prey within the jaws, by creating a potentially larger contact area, and they may assist in the manipulation and reduction of the prey so that it can be passed into the esophagus (Grubich et al., 2008). Thus, it is perhaps not surprising that this character is associated with entire clades of predatory fishes. Species such as the aforementioned gar, pike and barracuda are members of families and orders that consist entirely of long-jawed predators (Nelson, 2006).

Belonesox belizanus exhibits convergence with other ray-finned piscivores in having elongate jaws with rows of sharp, caniform teeth (Karrer, 1967; Johnen et al., 2006; Friedman, 2009). Yet,

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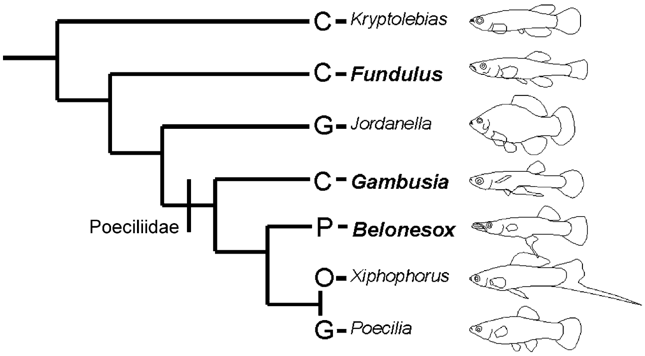

Fig. 1. Phylogenetic hypothesis of cyprinodontiform relationships based upon Hertwig (2008). Species used in this study are highlighted. Other major cyprinodontiform lineages are included for reference. Approximately 1000 species are represented by this simplified phylogeny (Nelson, 2006). Trophic guilds are inferred from published studies (Weisberg, 1986; Mansfield and McArdle, 1998; Taylor, 1992; Hargrave, 2006). *Abbreviations*: C = benthic or midwater microcarnivore; G = grazing herbivore; O = omnivore; P = piscivore.

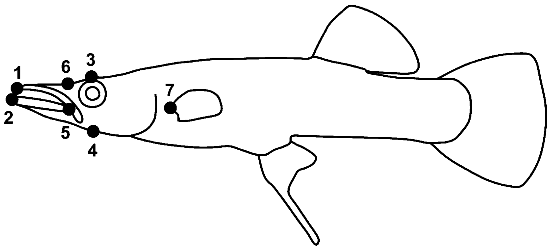

Fig. 2. Schematic image of digitized points from high-speed video images of *Belonesox belizanus* from the lateral aspect. For a detailed explanation of points 1–7 see Section 2.1.

although there are nearly 1000 cyprinodontiform species (Nelson, 2006), *Belonesox* is the only cyprinodontiform with these particular features. *Belonesox* is descended from a lineage largely modified for picking food such as insect larvae and small crustaceans from out of the water column or off the substrate, and occasionally from the water surface (Weisberg, 1986; Taylor, 1992; Mansfield and McArdle, 1998). As a group, cyprinodontiforms possess short jaws that protrude largely ventrally during jaw opening, leading to a characteristic beak-like appearance when the jaws are protruded (Ferry-Graham et al., 2008; Hernandez et al., 2008). In addition, within the poeciliids, many lineages are secondarily modified for a grazing, herbivorous habit (Gibb et al., 2008). *Belonesox* is the only poeciliid with a predominantly piscivorous diet (Fig. 1), and a diet that appears to include exclusively elusive prey (Villa, 1970; Turner and Snelson, 1984). Piscivory within cyprinodontiforms as a whole is very rare, tending to occur only in isolated species possessing concomitant changes to the jaw structures (i.e., specialized morphs of *Cyprinodon*; Turner et al., 2008).

Thus, this is an unusual system where a long-jawed piscivore has been recently derived from short-jawed, omnivorous ancestors—with an apparently larger gape being produced in the piscivore, *Belonesox* (Karrer, 1967; Johnen et al., 2006). Therefore, we ask the following questions: Do long and short jaws move differently during prey capture? Or, more specifically, are the prey capture kinematics of *Belonesox* different from those of its short-jawed, omnivorous relatives, *Gambusia* and *Fundulus*? Assuming gape is larger in *Belonesox*, do elongate jaws alone account for increased gape or are there other essential changes to the kinematics? And, finally, is the underlying anatomy specialized for pronounced jaw kinesis in *Belonesox*?

2. Materials and methods

2.1. Kinematics

Live specimens of juvenile *Belonesox* were obtained from commercial suppliers and were maintained in captivity on a diet of live fish according to Northern Arizona University IACUC approved protocols (NAU IACUC # 04-007). A total of four individuals were recorded capturing live, commercially obtained, feeder guppies using a Redlake PCI 1000S MotionScope digital-imaging system recording at 500 frames per second. For the feeding trials, individuals were placed in small (~15 cm × 15 cm × 20 cm) chambers and allowed to acclimate for approximately 30 min, after which they would readily feed. The feeding arena was illuminated by one or two 500 W tungsten photo lamps and a grid was placed in the background of the chamber for scale. Feeding events were initiated by introducing fish prey into the feeding arena; prey offered varied in body depth between 0.25 and 0.6 of predator gape and thus represented an appropriate and ecologically relevant prey size for piscivores (e.g., Hoyle and Keast, 1987; Wainwright and Richard, 1995a,b). Multiple capture events were recorded during a given feeding trial until the individual was satiated (i.e., the fish showed no interest in eating additional food items when presented) or at least four sequences were obtained from each individual.

Two to three prey capture sequences per individual were selected and digitized using QuickImage (Walker, 1998) to quantify movement of particular cranial elements during the prey capture event. Images were only analyzed if the individual remained perpendicular to the camera throughout the capture event. Time zero (t_0) was defined as the onset of mouth opening. The seven points digitized from the lateral aspect of the fish were as follows: the anterior tips of (1) the upper (premaxilla) and (2) the lower (dentary) jaws, (3) a point dorsal to the eye on the neurocranium and (4) a corresponding point ventral to the eye on the anterior ventral margin of the preopercular bone, (5) the vertex of the mouth, as created by the junction of the upper and lower jaws (as the actual quadrato-mandibular is not visible externally), (6) the anterior margin of the neurocranium (and nasal bone), and (7) the anterior margin of the pectoral fin (Fig. 2). These points were used to calculate kinematic variables that characterize particular aspects of the feeding mechanism of *Belonesox*, and to facilitate comparisons with other cyprinodontiform species. Two linear displacement variables were calculated: gape (the distance between points 1 and 2), and premaxillary protrusion (the anterior displacement of point 1, relative to point 7). Several angular variables were also calculated: gape angle (the angle formed by points 1, 5 and 2), premaxillary rotation (the angle formed by points 1, 5 and 7), and neurocranial rotation (the angle formed by points 6, 3 and 7); these variables were expressed relative to their starting position at time zero (t_0). The time to maximum gape, time to maximum premaxillary protrusion and time to jaw closure (typically on the prey item) were also determined for each prey capture event.

Kinematic variables from *Belonesox* were compared with two omnivorous cyprinodontiform species, *Fundulus rubrifrons* and *Gambusia affinis* (see also Ferry-Graham et al., 2008). These two taxa were selected for comparison with *Belonesox* because they are of similar size, and provide an interesting phylogenetic comparison. Additionally, they are very similar, in terms of the magnitude of jaw movements, to other cyprinodontiform species that have been studied, such as *Jordanella floridae*, *Kryptolebias marmoratus*, and *Poecilia sphenops*; total gape in these species ranges from slightly less than 0.1 cm to slightly more than 0.2 cm (Ferry-Graham et al., 2008; Gibb et al., 2008). *Fundulus* is an omnivore, taking primarily micro-crustaceans (Weisberg, 1986), while *Gambusia* feeds primarily on similar invertebrates in the water column, but is known to additionally include the occasional small fish in the diet (Harrington and Harrington, 1961; Mansfield and McArdle, 1998). Therefore, both of these species were fed live brine shrimp as a representative of a natural prey item. Because *Gambusia* will also occasionally take small fish, we collected data from four additional similarly sized *Gambusia* individuals feeding on small fish as described for *Belonesox* (above). The use of additional individuals of *Gambusia* in this way allowed us to avoid a repeated measures design for a single species.

The data met the assumptions of parametric statistics, so MANOVA (SPSS 16.0 for Mac OSX) was used to determine if there were differences among the four treatment combinations, with treatment considered as a single, fixed effect. We employed a conservative approach by using the mean for each variable and each individual; thus, our sample size for each test was $n = 4$, which reflects the true level of replication. This did not result in a loss of statistical power, as the MANOVA revealed that significant differences existed. ANOVA was performed as a post hoc test to determine which variables demonstrated differences among treatments. The *F*-values were significant in all cases, so Fisher's PLSD post hoc tests were used to determine which treatments were different from one another.

2.2. Modeling

The jaw movements of *Belonesox* were modeled to determine if gape, as measured in the kinematic analyses, was the result of jaw length alone, or if other changes to the kinematics or underlying morphology were responsible. We used *Fundulus* as a short-jawed species for comparison because the kinematic profile was closest to *Belonesox* in terms of the maxima achieved, and represented the least extreme difference in movements and, therefore, the most conservative comparison. The upper and lower jaw of *Belonesox* and *Fundulus* were modeled as isosceles triangles, wherein the jaws form the two sides of a triangle with equal lengths, with an imaginary line connecting the upper and lower jaw (equivalent to gape distance) that forms the base of this triangle. Using this simple geometric model, we could mathematically predict the length of the base of the triangle, or gape distance (output), given various changes to the length of the jaws and/or the angular rotation of the upper and lower jaws (input). Through these iterations, we assessed the relative contributions of increased jaw length, increased dorsally directed premaxillary rotation, and increased ventrally directed lower jaw rotation to generating a large gape. The natural movements of these elements were determined from the video sequences, as described in the previous section, for setting input parameters and limits in the models. Lower jaw rotation, which was not included previously, was measured analogous to premaxillary rotation.

2.3. Morphology

Individuals of *Belonesox* used for the kinematic analysis and several additional specimens were sacrificed in accordance with IACUC procedures, preserved in 10% formalin, and stored in 70% ethanol. A subset of these preserved specimens were dissected and stained with iodine to facilitate identification of muscle fiber orientation, and musculoskeletal and ligamentous architecture. The remaining specimens were cleared and stained using a protocol presented in Dingerkus and Uhler (1977) with modifications by Pothoff (1984). Cleared and stained specimens were used to describe osteological elements within the anterior jaws. Additional specimens, both in alcohol and cleared and stained (USNM 134597), were kindly provided by Lynne Parenti.

Line drawings of specimens were made from photographs taken using an Olympus DP12 digital camera attached to an Olympus SZX12 dissecting microscope, or by use of an Olympus SZX-DA camera lucida attached to the aforementioned dissecting microscope. Digital illustrations of skeletal and muscle morphology were constructed by creating vector drawings from the camera lucida drawings using technical illustration programs (Canvas X and Adobe Illustrator).

The cranial anatomy of *Fundulus* and *Gambusia* has previously been described (Hernandez et al., 2008, 2009). We used information from these studies to make qualitative comparisons with the anatomy observed here for *Belonesox*. As suggested previously, these two species are generally like other cyprinodontiforms in their cranial morphology. *Fundulus*, in particular, retains a condition apparently unchanged from the most ancestral cyprinodontiform species (Hernandez et al., 2008, 2009). Aspects of the cranial anatomy of *Belonesox* have been described (Karrer, 1967; Gunther, 1970); however, key features of the jaw musculature are elucidated here.

3. Results

3.1. Kinematics

When feeding on live fish, an individual *Belonesox* slowly approached the prey, then S-started toward the prey while rapidly opening its jaws (Fig. 3). During the feeding trials, the attacks on the prey fish were always forward-oriented attacks; gar-like sideways snaps (see also Porter and Motta, 2004) were never observed. During an attack the prey item was trapped within the jaws (Fig. 3). Although little anteriorly directed premaxillary protrusion occurred during prey capture (Fig. 4, Table 1), dorsal rotation of the premaxilla and ventral rotation of the lower jaw appeared to contribute to a large gape, which approached 90° (Table 1). During mouth opening, the angle between the premaxilla and the neuro-

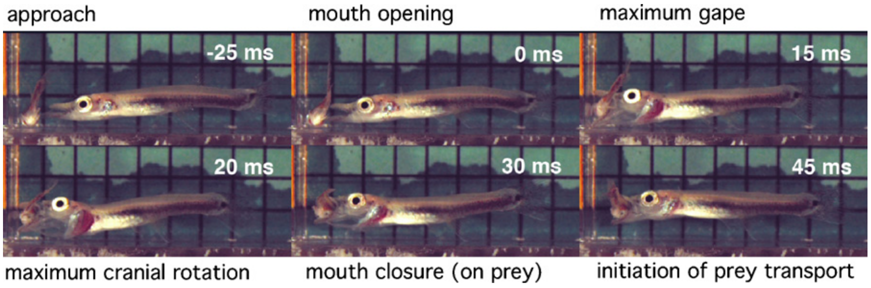

Fig. 3. Composite images extracted from high-resolution, high-speed video footage illustrating key kinematic events during *Belonesox* prey capture. The event depicted is noted next to each frame, as is the relative timing of the event (ms). Background grid with 0.5 cm squares.

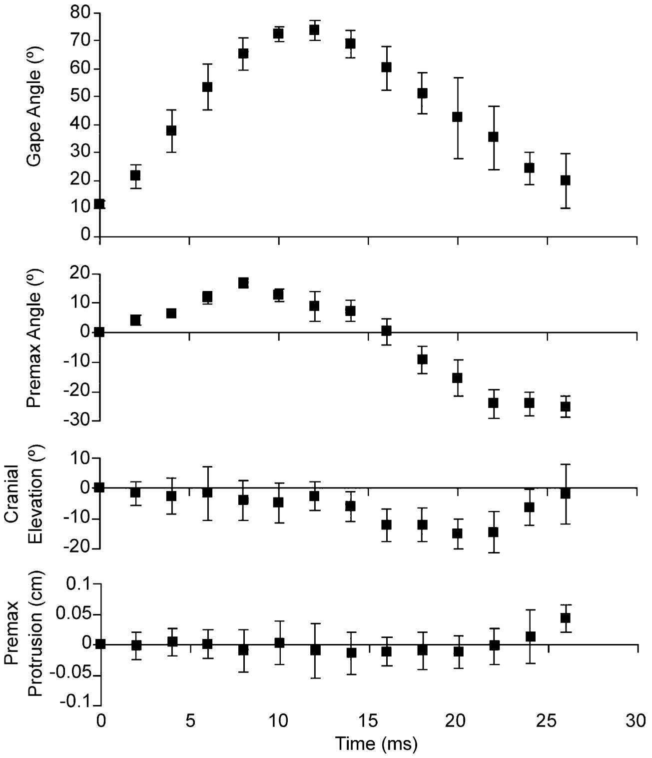

Fig. 4. Select kinematic variables quantified from high-speed video footage of *Belonesox*. Values are means of individual means (±SE) for all events analyzed. Note that the maxima plotted here are typically smaller than the maxima reported in Table 1, as these plots represent the mean position of the element at any time *t*, and strikes differed slightly in the timing of the maxima (also reported in Table 1).

cranium changed by an average of 20° as the anterior tip of the premaxilla rotated dorsally. This means, by subtraction, that the lower jaw rotated ventrally by as much as 70°. During mouth closing, the premaxilla rotated ventrally, beyond the resting position. This was indicated as a negative angle between the premaxilla and the neurocranium, which averaged −13° at mouth closure (Table 1). The total rotational displacement of the premaxilla exceeded 50° in the most extreme feeding events.

In contrast, dorsal rotation of the neurocranium did not appear to consistently contribute to prey capture. Rotation of the neurocranium during prey capture varied among individuals, and within individuals it varied among feeding events. A slight dorsal rotation occurred in 50% of feeding events; in these it averaged at 6° of rotation. During the other feeding events, the neurocranium rotated ventrally by as much as −28°, with an average of −20°. Due to this bimodal distribution of behaviors, this variable was not compared statistically among species. However, these sequences strongly influenced the mean kinematic profile shown in Fig. 4. We note anecdotally that additional head and jaw movements were required to move the prey item from the buccal cavity into the pharynx and digestive tract during prey transport, during which dorsal rotation of the neurocranium was consistently observed.

Compared with *Fundulus* and *Gambusia* feeding on live brine shrimp, strikes on live fish prey by both *Gambusia* and *Belonesox* were significantly more rapid (MANOVA and post hoc pair-wise comparisons; all $p < 0.007$), as indicated by time to peak gape. The time to peak gape, and time to mouth closure, was approximately three to eight times faster for fish predators relative to brine shrimp predators (Table 1). Even when feeding on a common prey type, *Belonesox* and *Gambusia* exhibited different patterns in the relative timing of maximum anteriorly directed premaxillary protrusion, with *Belonesox* demonstrating maximum anteriorly directed protrusion prior to maximum gape, and *Gambusia* demonstrating maximum anteriorly directed protrusion post-maximum gape. Gape was significantly different among all four treatments (all $p < 0.002$), with *Belonesox* achieving the largest gape distance (or angle) of any of the species studied by 50% or more (Table 1). Note that gape angle followed this pattern exactly, but was not examined statistically because it is statistically confounded with gape distance. The amount of anteriorly directed premaxillary protrusion during prey capture in *Belonesox* is small, and indistinguishable from *Gambusia* feeding on either prey item, but significantly smaller than in *Fundulus* ($p < 0.004$). The dorsal rotation of the premaxilla during mouth opening, whereby the premaxilla rotates about 20° relative to its starting position, is found only in *Belonesox* (Table 1). *Gambusia* and *Fundulus*, like the other cyprinodontiforms studied to date, rotate the premaxilla ventrally (toward the lower jaw) during prey capture, although this is less pronounced in *Gambusia* when feeding on live fish prey. However, ventral rotation of the premaxilla did occur during mouth *closing* in *Belonesox* (Table 1).

3.2. Modeling

By 'reverse engineering' of the *Belonesox* jaw using our geometric model, we could ascertain the relative contributions of increased jaw length, dorsal rotation of the upper jaw, and increased ventral rotation of the lower jaw to maximum gape. We began with a *Belonesox* head model with an input gape angle of 50°, which is the average gape angle achieved for *Gambusia* feeding on fish prey (see Table 1). This was inserted into the model as ventrally directed motion, as is typical of cyprinodontiforms. By comparing Fig. 5A1 and B1, it is clear that when maximum gape angle is constrained to 50°, and in the direction typical of a cyprinodontiform, a simple doubling of jaw length (another input) confers an approximate

Table 1
Kinematic data expressed as the average (means of individual means ± SE) for each treatment in the analysis.

	Fundulus	*Gambusia*	*Gambusia*	*Belonesox*
Total length (range; cm)	3.7–4.4	2.4–3.9	4.4–5.0	2.6–4.3
Prey item	Brine shrimp	Brine shrimp	Guppy	Guppy
Max. premaxilla protrusion dist. (cm)	0.12 (0.012)	0.06 (0.001)	0.06 (0.014)	0.06 (0.010)
Max. gape distance (cm)	0.16 (0.026)	0.08 (0.013)	0.34 (0.020)	0.49 (0.010)
Max. gape angle (°)	35.32 (3.36)	25.01 (3.29)	50.40 (3.08)	89.69 (4.14)
Premaxilla angle at max. gape (°)[a,b]	−5.84 (1.82)	−8.92 (1.30)	−0.50 (1.38)	19.57 (3.70)
Premaxilla angle at closure (°)[a,b]	−18.00 (2.95)	−11.07 (2.93)	−23.63 (7.47)	−12.80 (6.03)
Time to max. gape (ms)	30 (8)	67 (14)	11 (1)	11 (1)
Time to max. premaxilla distance (ms)[c]	45 (12)	84 (11)	15 (1)	8 (3)
Time to jaw closure (ms)	59 (10)	125 (17)	19 (3)	21 (3)

[a] Angles are expressed as a change in angle relative to the starting angle of the premaxilla at t_0.
[b] Angles <0° refer to movement in a ventral direction relative to the starting position.
[c] This is anteriorly directed premaxilla protrusion in *Fundulus* and *Gambusia* and dorsally directed premaxilla rotation in *Belonesox*.

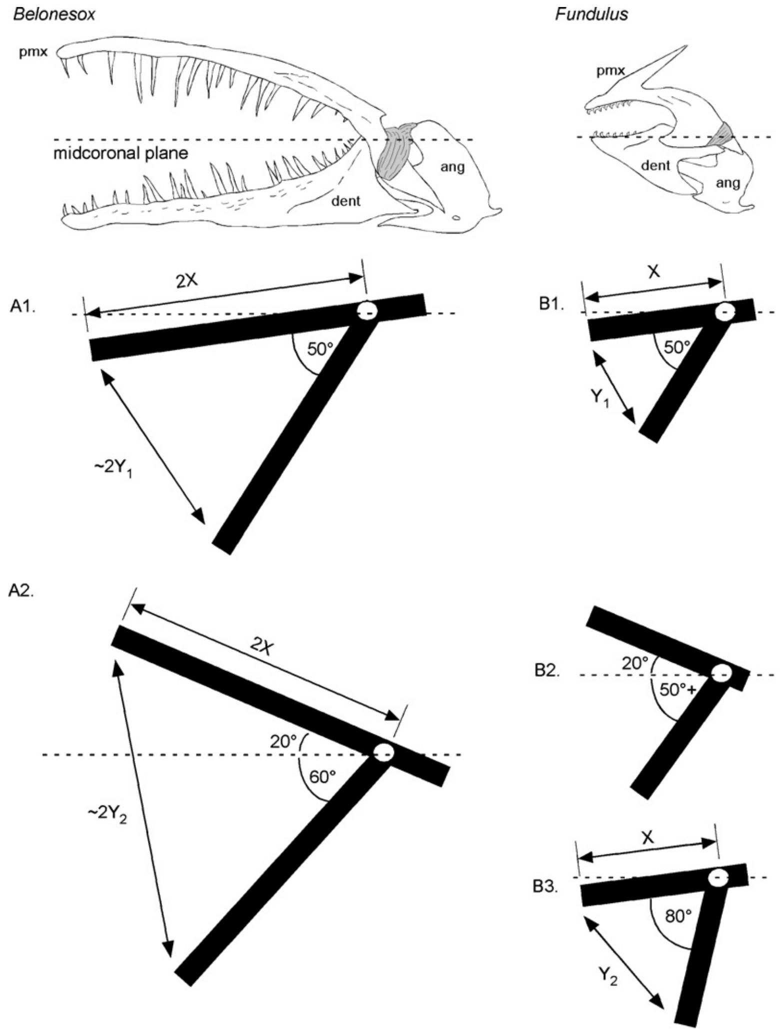

Fig. 5. Models of *Belonesox* and *Fundulus* (identical to *Gambusia* in this context) jaws evaluating the relative advantage of long jaws vs. enhanced rotation at the quadratomandibular due to dorsally directed premaxillary rotation. Shown are the upper and lower jaws, and the premaxillomandibular ligament (shaded in grey). The jaws are modeled as straight rods in subsequent images. The relative lengths of the jaws are indicated in each image, along with hypothetical gape angle and the plane of the quadratomandibular (dashed lines). Hypothetical gape angles are based upon real (i.e., measured) gape angles as well as experimental angles. Explanations and outcomes of each condition illustrated are found in the text. *Abbreviations*: ang, angular; dent, dentary; pmx, premaxilla.

doubling in gape distance (shown as $2Y_1$ vs. Y_1). Under this scenario, *Belonesox* could produce a gape distance of approximately half of what was observed from the kinematic data: 0.25 cm (from the model) vs. 0.49 cm (empirical; Table 1). This suggests that the added rotation of the premaxilla is fundamentally important for increasing gape distance.

Dorsal rotation of the premaxilla contributes approximately 25% of the total gape angle in *Belonesox* (see also Fig. 4); the remaining 75% is produced by ventral rotation of the lower jaw. The degree of ventral rotation of the lower jaw is comparable between the two species shown (Fig. 5A2 vs. B2). Dorsal rotation of the premaxilla is apparently facilitated by the elimination of the lip membrane characteristic of other cyprinodontiforms. If the premaxilla of *Fundulus* rotated dorsally by $\sim 20^\circ$, as in *Belonesox*, and the lower jaw maintained its position, a total gape angle of 70° or more could be attained, depending upon the relative change in position of the premaxilla (Fig. 5B2). While dorsally directed movement of the premaxilla may not be strictly required for producing a larger gape, it will enhance gape angle without hyper-depression of the lower jaw, which is shown in Fig. 5B3 where the jaws are oriented at a *Belonesox*-like 70°+ angle.

3.3. *Morphology*

In *Belonesox* and other cyprinodontiforms, the bony elements forming the anterior jaws are the premaxilla and maxilla (upper jaw), and the dentary and anguloarticular (lower jaw). However, in *Belonesox* the ascending arm of the premaxilla is eliminated entirely (Fig. 6); this is not the case for most other cyprinodontiforms, although it is also eliminated in some herbivorous poeciliids (Hernandez et al., 2009). However, the descending arm of the premaxilla is highly elongate, as is the dentary of the lower jaw. As in other poeciliids, in *Belonesox* the maxilla extends ventrally to the articulation of the dentary and the anguloarticular within the lower jaw. Thus, in *Belonesox*, the maxilla is even more elongate relative to other poeciliids to accommodate the increased length of the lower jaw. The ventral end of the maxilla is affixed firmly to the ventral end of the premaxilla. A large Y-shaped cartilage connects the heads of the right and left maxillae, the base of which extends anteriorly to the heads of the premaxillae. Thus, tension placed on the maxillae can be transferred to the premaxillae through this cartilage. The heads of the maxillae possess hook-like extensions for articulating with this cartilage, in a

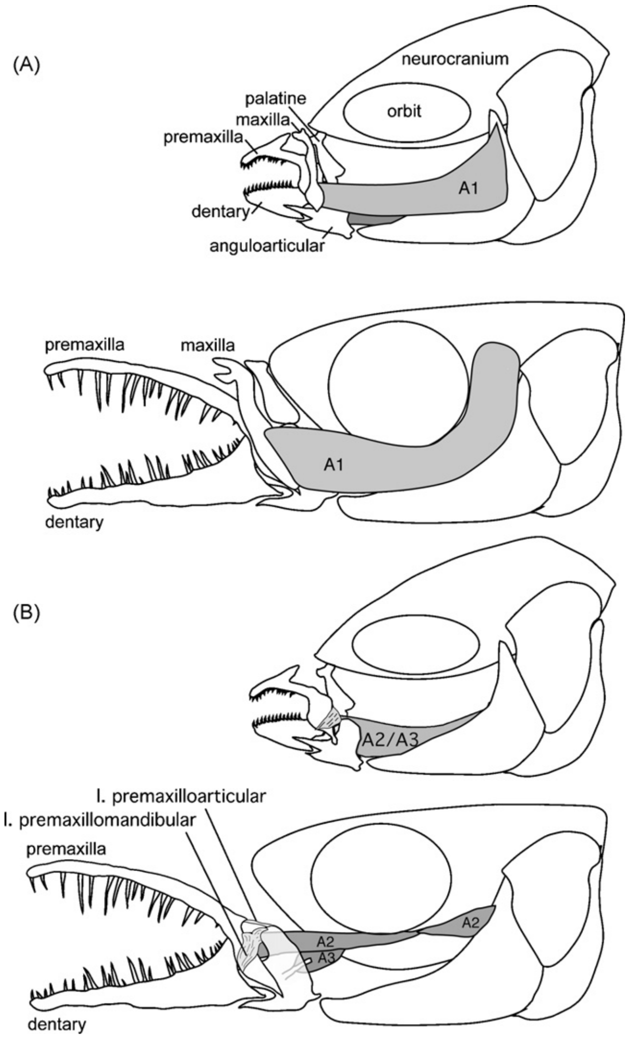

Fig. 6. Cranial anatomy of *Gambusia* (above) and *Belonesox* (below) showing details primarily of the upper and lower jaws and adductor mandibulae muscles. Elements of the neurocranium, suspensorium, and opercular series have been simplified for clarity. Adductor mandibulae A1 is shown in (A), and has been removed in (B) along with the maxilla to show the insertions of A2 and A3. The more lateral portions are slightly transparent in this image to allow for visualization of the underlying sections.

configuration similar to that of *Fundulus* (Hernandez et al., 2008, 2009).

As has been previously described for other cyprinodontiforms (Hernandez et al., 2008), a discrete premaxillomandibular ligament serves to connect the anguloarticular, dentary and premaxilla (Fig. 6). In *Belonesox*, this ligament is similar to that seen in other more derived poeciliids, such as *Poecilia* (Hernandez et al., 2008, 2009): a discrete ligamentous band originates on the rostrolateral face of the premaxilla, wraps around the posterior aspect of the premaxilla and then attaches to the dentary and anguloarticular—this ligament appears to couple movements of the upper and lower jaw (Fig. 6). A second thick, discrete ligament characteristic of some poeciliids, the premaxilloarticular ligament (sensu Karrer, 1967), connects a dorsal notch in the premaxilla to the anguloarticular, also serving to connect these elements (Fig. 6).

The musculature of the anterior jaws is similar in gross morphology to that of *Fundulus* and *Gambusia*. The first division of the adductor mandibulae (A1) extends from the suspensorium and preopercle to an insertion on the ventral third of the maxilla in all three species. However, in *Belonesox*, A1 is more dorsocaudally expanded when compared with other cyprinodontiforms. While in other cyprinodontiforms the adductores mandibulae A2 and A3 form one largely united mass, in *Belonesox* A2 and A3 are more distinct, almost bipartite, and much more complex than modeled by Gunther (1970). A2 originates from the caudal portion of the suspensorium, deep to A1, and fans out to insert muscularly along the dorsocaudal edge of the dentary. A3 inserts on the medial side of the anguloarticular. We note that in *Gambusia* only a small dorsal section of A2 inserts on loose ligamentous tissue that invests the premaxilla, with the remainder of A2 inserting muscularly on the dorsocaudal edge (coronoid process) of the dentary. In *Fundulus*, A2 inserts only on the coronoid process of the dentary. In *Fundulus* and *Gambusia*, the bulk of A3 is found deep to A2 and inserts on the medial face of the anguloarticular via a discrete tendon, while in *Belonesox* most of A3 is ventral to the bulk of A2 (Fig. 6).

4. Discussion

The kinematics of prey capture in *B. belizanus* are distinct when compared with other poeciliids and cyprinodontiforms studied to date (Ferry-Graham et al., 2008). As might be expected for a piscivore, prey capture was rapid, and characterized by a very large gape. Most cyprinodontiforms, in contrast, are micro-carnivores and omnivores: picker-type feeders, typically characterized by a slower prey capture event with a small "nipping" gape, both of which have been associated with dexterity and precision (Ferry-Graham et al., 2008). Numerous kinematic studies have posited that feeding on elusive prey will result in a prey type effect whereby faster and larger prey capture kinematics are elicited, even from the same fish species (Norton, 1991; Norton and Brainerd, 1993; Nemeth, 1997; Ferry-Graham et al., 2001; Wainwright et al., 2001), and indeed, *Gambusia* demonstrated this when foraging on prey comparable to the prey offered to *Belonesox*. However, even relatively large, fish-eating *Gambusia* were unable to match the gape achieved by *Belonesox*. As far as we know, *Belonesox* is the only example of an acanthopterygian (ray-finned) fish in which an enlarged gape is achieved, at least in part, by a premaxilla that rotates dorsally. In most species, such as gar and pike, the lower jaw rotates ventrally and there is no rotation of the premaxilla; species such as needlefish rotate the premaxilla dorsally, but the lower jaw is immobile.

Our models suggest that the dorsal rotation of the premaxilla is essential in *Belonesox* for producing the large gape observed, and that jaw elongation alone could not produce such movements. Increasing the gape angle from those characteristic of *Gambusia* and *Fundulus* to that observed for *Belonesox* contributes nearly 50% of the resultant increase in gape. The loss of the lip membrane, a characteristic of most other cyprinodontiforms responsible for the "beak-like" jaw protrusion in this group (Alexander, 1967a), likely facilitates both increased rotation of the lower jaw and dorsal rotation of the premaxilla. We note that premaxillary protrusion is anteriorly and ventrally directed in outgroup species (even in *Gambusia* feeding on fish prey) and in all other cyprinodontiform species studied to date (Ferry-Graham et al., 2008). Indeed, simply protruding the premaxilla anteriorly, without a ventral contribution, results in a significant increase in gape distance in *Gambusia* when feeding on fish prey. Rotation of the premaxilla dorsally, while retaining the ventrally rotating lower jaw, additionally enhances gape.

Because of their cyprinodontiform ancestry, *Belonesox* may be uniquely predisposed to this particular solution. Previous work (Hernandez et al., 2008, 2009) has shown that many cyprinodontiforms possess a distinct premaxillomandibular ligament. While the transfer of force via depression of the lower jaw, through the maxilla, to the premaxilla is fairly widespread as a mechanism of premaxillary protrusion in teleosts (as reviewed in Hernandez et al.,

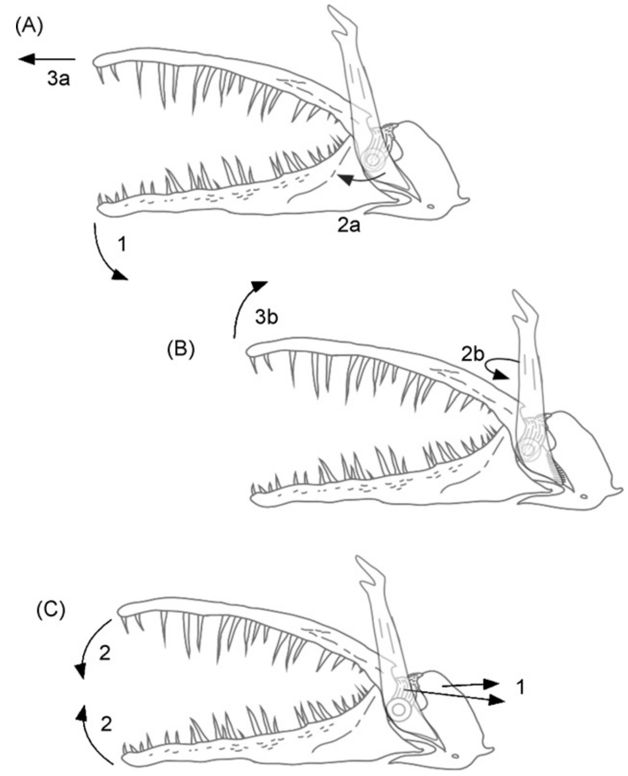

Fig. 7. Hypothetical representation of how forces are transferred within the jaws of *Belonesox* and the resultant motions. Shown are two pictorial representations of complementary and simultaneous jaw opening mechanisms. In (A), forces are transferred from the lower jaw (1), which is being depressed, through the maxilla (2a), to the premaxilla (3a), which protrudes slightly because of the tension created at the premaxillomandibular ligament. In (B), the addition of the twisting maxilla is represented, with the maxilla rotating out of the plane of the page (2b) and the premaxilla subsequently being pulled dorsally (3b) because of the cartilage elements (not shown) that connect the head of the maxilla to the dorsal aspect of the premaxilla. In (C), putative mechanisms of jaw closure are suggested, with extensions of the adductor mandibulae complex placing tension on the posterior aspect of the jaw elements, thereby pulling them into a closed position.

2008), a distinct cyprinodontiform premaxillomandibular ligament is lacking in these groups. The unusual architecture of this premaxillomandibular ligament links ventral rotation of the lower jaw directly with dorsal rotation of the premaxilla, allowing the articulation to function as a novel joint relative to other well-studied teleostean groups. In *Belonesox*, as the lower jaw is depressed, tension from this ligament pulls the posterior end of the premaxilla ventrally. As this action is on the caudal side of the quadratomandibular, the elongate end of the premaxilla is subsequently protruded slightly (Fig. 7A), placing it into position to be rotated dorsally. This mechanism of force transfer has been described for other cyprinodontiforms, however, as noted above, in those species the premaxilla is prevented from rotating dorsally (Hernandez et al., 2008, 2009).

The twisting maxilla (sensu Eaton, 1935; Alexander, 1967b; Motta, 1984) provides the torque (or moment) necessary to fully rotate the premaxilla dorsally (Fig. 7B). During lower jaw depression, there is a lateral expansion at the distal ends of the maxilla/premaxilla, visible in video footage of the ventral aspect (LFG, pers. obs.). As the maxillae and premaxillae are firmly connected at their caudoventral ends, this places both elements in tension. The head of the maxilla articulates with a rostral cartilage via a hook-like structure that is very similar to the structures seen in *Fundulus*, which also utilize the twisting maxilla model to protrude the premaxilla (Hernandez et al., 2008). In *Belonesox*, lower jaw depression generates rotation of the maxilla laterally and caudally, visible from the dorsal aspect in manipulated specimens (LFG, pers. obs.). This torque pulls the head of the premaxilla (which lacks an ascending process) dorsally via the rostral cartilage. The twisting maxilla as a mechanism contributing to premaxillary protrusion is lost in other poeciliids studied to date, but present in sister taxa such as *Fundulus* and *Kryptolebias* (Hernandez et al., 2008, 2009). The jaws are subsequently closed by extensions of the adductor mandibulae complex that work to collapse the bony elements back into a closed position (Fig. 7C).

Belonesox is characterized by a functional solution involving both novelty and ancestry, with both contributing to feeding performance in the form of an enlarged gape relative to related species. Novelty appears in the form of elongate jaws, grossly convergent with many other piscivores, and an associated loss of the lip membrane that appears to limit lower jaw depression within closely related cyprinodontiforms. Within this clade, elongate jaws are unique to *Belonesox*, and the lip membrane is an otherwise prominent cyprinodontiform trait. Both character states must be present for the production of an enlarged gape. Ancestry contributes the underlying mechanism for moving these modified elements and generates a jaw opening mechanism that appears to be distinct to this species (relative to other piscivores studied to date). The premaxillomandibular ligament is required to transfer forces to the elongate premaxilla, and the twisting maxilla then assists in transferring forces to move the premaxilla into a dorsally protruded position.

While the direct advantages of an enlarged gape have yet to be determined empirically, a large gape has a number of theoretical advantages, including the ability to capture larger, more elusive prey and the ability to process and transport these large items once they have been captured (Hoyle and Keast, 1987; Wainwright and Richard, 1995a,b; Mittelbach and Persson, 1998; Wainwright and Shaw, 1999; Grubich et al., 2008). Remarkably, *Belonesox* is essentially piscivorous at parturition, readily consuming other small fish such as co-occurring cyprinodontiform species (Lachner et al., 1970; Lee et al., 1980), and other *Belonesox* including siblings (Turner and Snelson, 1984). While most piscivorous fishes typically must grow into this niche (Wainwright and Richard, 1995a,b; Mittelbach and Persson, 1998), the elongate upper and lower jaw, combined with the unique and extreme degree of rotation of these elements, provides for a large gape relative to body size and facilitates the ability of *Belonesox* to be predatory essentially from birth onward.

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