

Further notes on dentition and prey capture of the Pike killifish *Belonesox belizanus* (Poeciliidae)

Weitere Anmerkungen zur Bezahnung und zum Beutefang des Hechtkärpflings
Belonesox belizanus (Poeciliidae)

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Zusammenfassung: Wir ergänzen eine frühere Mitteilung zur Bezahnung und zum Beutefang von *Belonesox belizanus* anhand histologischer Bilder und REM-Aufnahmen von Ober- und Unterkiefer und stellen erste Videosequenzen (500 Bilder pro Sekunde) vom Beutefang vor. Die meisten der langen, weitgehend aufrecht stehenden Zähne von *B. belizanus* sind mit einem unmineralisierten oder schwach mineralisierten kollagenen „Scharnier“ so auf dem flachen knöchernen Sockel verankert, dass sie nach posteromedian geneigt werden können. Hechtkärpflinge ergreifen die Beute nach einem sehr raschen S-Start stets direkt von vorn, bringen also ihren Kopf nicht neben die Beute, um dann zuzustoßen. Der S-Start begann kurz, d.h. durchschnittlich weniger als die halbe Körperlänge, vor der Beute. Diese wurde innerhalb von 36 ms erreicht, dabei wurden Geschwindigkeiten von etwa elf Körperlängen pro Sekunde erreicht. Die maximale Maulöffnung bei den gefilmten Beutefangaktionen betrug durchschnittlich 44 % der Kopflänge.

The Pike killifish *Belonesox belizanus* is a small, fusiform poeciliid from southern Mexico (Yucatan), Guatemala and Honduras (ROSEN & BAILEY 1963), which exclusively feeds on fish. Prey includes fishes up to the half of the predator's body length, but shallow-bodied fishes are preferred (unpublished observations). Capture and swallowing of elusive prey is facilitated by the moveable elongate jaws and the large unicuspid teeth (for further anatomical details and functional adaptations see KARRER 1965). More recently, we presented some SEM-micrographs of premaxillae and dentaries showing several rows of unicuspid teeth of variable length, which were covered by the oral epithelium except for their tips. However, teeth appeared to be "mowed down" in our non-macerated preparations suggesting a hinge-like attachment to the underlying bones. Further, prey capture actions recorded with a conventional camcorder revealed that *B. belizanus* ambushes, stalks or pursues its prey with minimal body movement followed by an explosive S-start lunge at the prey (JOHNEN et

al. 2006). In the present note we broaden these findings by closer examination of the attachment zone of teeth and add some data on strike variables based on the analysis of a few video sequences obtained by high frequency videography.

For scanning electron microscopy (SEM), upper and lower jaw were removed from a female, placed in tap water for several weeks at room temperature. Then, the bones were stripped of macerated tissue, washed in aqua dest. and ethanol and air-dried. Dried specimens and teeth separated during the maceration procedure were mounted on aluminium stubs, gold-coated and examined using the SEM Leo 1430 VP (Fa. Zeiss). For histology (LM) the head of a female was fixed in Bouin's solution for several weeks, decalcified in EDTA for three weeks, dehydrated, embedded in paraplast, sectioned transversally at approximately 7 µm and stained with trichrom-Goldner or Azan Heidenhain (ROMEIS 1967). For videography four semiadult individuals (standard length 39 to 72 mm) were filmed with a high-speed video

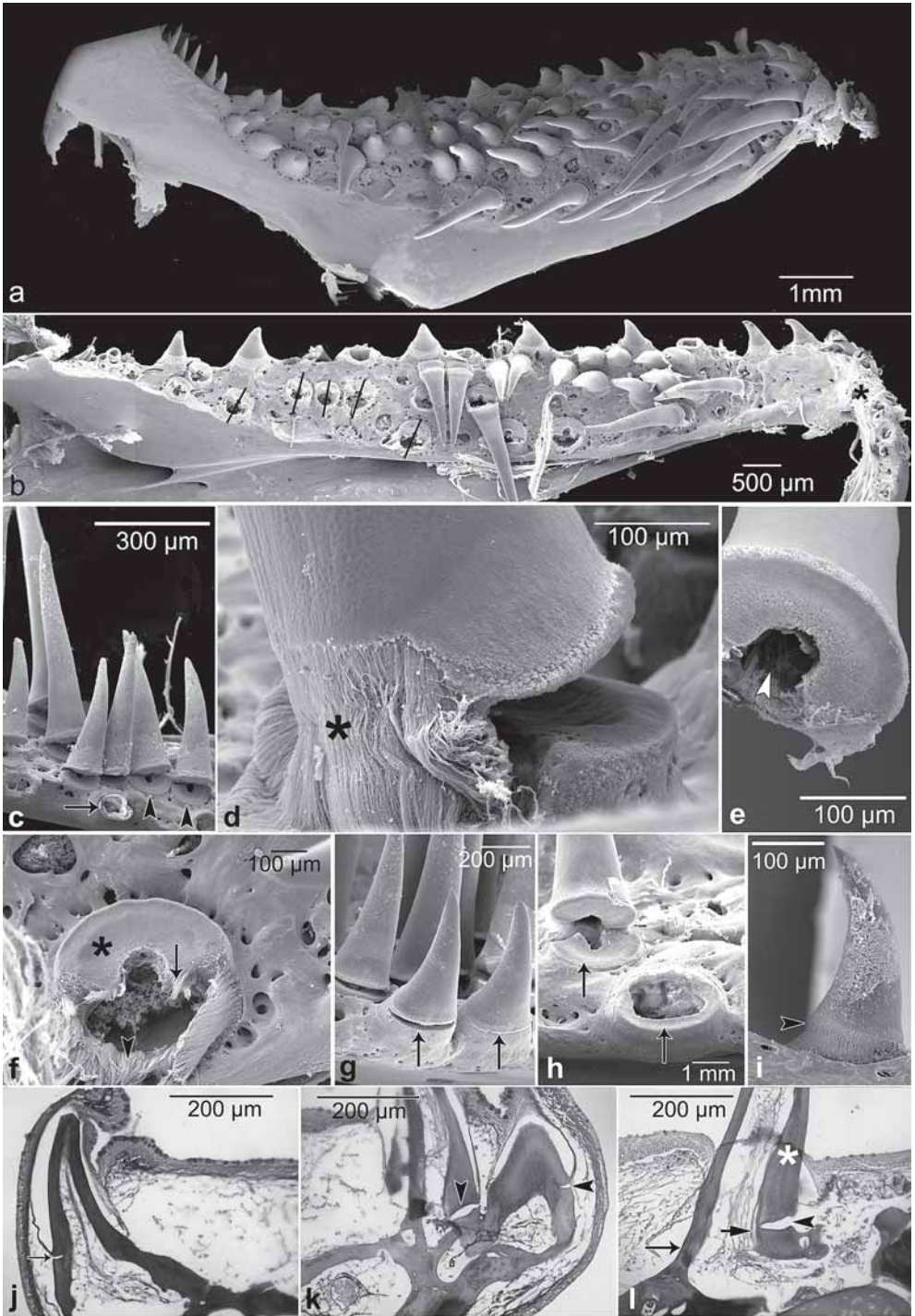
system (Motion Pro 500; Fa. Redlake, objective 65-200 mm focal length, Fa. Olympus; illumination: Primalux 2500 Watt) at a rate of 500 frames per second. During filming, a hungry fish was placed in an aquarium (about 35 l), which was equipped with a background scale (ruler), and fed one male female guppy (standard length approximately 1-2 cm). Due to the jumpiness of *B. belizanus*, the prey was introduced relatively far from the predator. In this way, six strikes were recorded from lateral. Video sequences were converted to stacks of digital images (Redlake MIDAS, version 2.1.4R) and analysed frame by frame with MIDAS and Adobe Photoshop. According to PORTER & MOTTA (2004) we defined start initiation as the time immediately before the S-start as starting point (0 ms) and determined (1) strike distance (the distance from the tip of the predator's upper jaw to the approximate mass centre of the prey at strike onset converted to percent body length), (2) strike duration (the total time from strike onset to the time of prey contact with the jaws), (3) strike velocity (the strike distance divided by the strike duration converted to body lengths s⁻¹ using standard length), (4) time to mouth opening (the time

from the onset of the strike until the time the jaws began to open), (5) duration of mouth opening (the time the jaws began to open until maximum gape was reached), (6) maximum gape (the maximum distance between the anterior tip of the predator's upper jaw and the anterior tip of the dentary converted to percent head length), (7) duration of mouth closing (the time from the end of maximum gape until the jaws were closed on the prey), (8) time to hyoid depression (the time from the onset of the strike until the time the hyoid began to depress), (9) duration of hyoid depression (the time from the onset of hyoid depression until maximal hyoid depression).

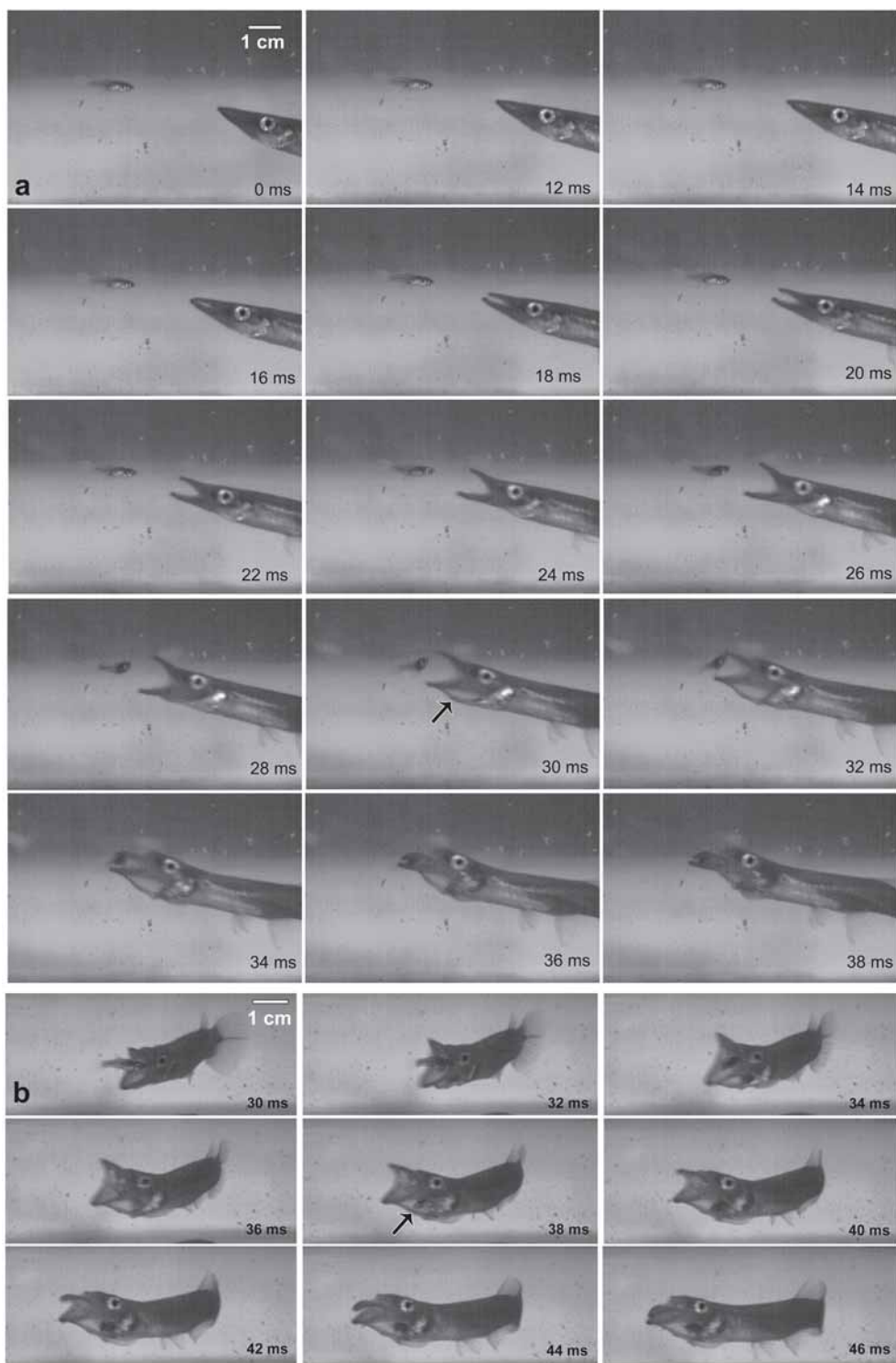
SEM shows that teeth on the premaxillae and dentaries teeth are unicuspid, conical and relatively straight. They are arranged in 4 to 5 parallel rows in the upper jaw and in 2 to 3 rows in the lower jaw (figs. 1 a, b). The dark discoloration of the tips (not shown) is probably associated with iron deposits in the enameloid layer. The symphyseal zones of the jaws are edentulous (fig. 2 b). Teeth are of various sizes depending on their location, but generally decrease in size posteriorly. The smallest and most recurved teeth are found on the outer

Fig. 1 a-l: Dentition of *Belonesox belizanus*, premaxilla (a, d, g, i, j, k), dentary (b, c, e, f, h, l), SEM. (a-i), LM (j-l). (a) Outer margin of the jaw on the top, anterior tip left. (b) Outer margin of the jaw on the top. Slight posterolingual orientation of the teeth (oblique lines); symphyseal region (asterisk). (c) Pedestals of teeth are asymmetrical in larger (arrowheads), but not in smaller teeth (arrow), labial view. (d) Large tooth with collagen attached to the pedestal (asterisk), lateral view. (e) Isolated tooth with narrow pulp (arrow) and asymmetrical dentine shaft. (f) Asymmetrical pedestal; collagen (arrowhead) and broad labial wall (asterisk) with only a few collagen fibres (arrow). (g) Variable labial attachment zone of the outer small teeth (arrows). (h) Different pedestals in outer (in front) and inner teeth (arrows). (i) Small posterior tooth with a rugose attachment zone (arrowhead). (j) Marginal tooth with a labial collagen free incision (arrow) between dentine shaft and pedestal. (k) Edge of the jaw with two adjacent teeth; incision between dentine shaft and pedestal (arrowheads). (l) Larger tooth with collageneous hinge lingually (thick arrow) and incision labially (arrowhead). Note the thin collagen sheet (thin arrow) and the thick labial dentine shaft (asterisk).

Abb. 1 a-l: Bezahnung von *Belonesox belizanus*. Praemaxillare (a, d, g, i, j, k), Dentale (b, c, e, f, h, l), REM (a-i), LM (j-l). (a) Kieferrand oben, Kieferspitze rechts. (b) Kieferrand oben. Der Symphysenbereich ist zahnfrei (Stern). (c) Die Zahnsockel der größeren Zähne sind asymmetrisch (Pfeilköpfe), nicht aber die der kleineren (Pfeil), labial. (d) Großer Zahn; Kollagen (Stern) verbindet Sockel und Zahn, lateral. (e) Isolierter Zahn mit enger Pulpa (Pfeilkopf) und asymmetrischem Dentinschaft. (f), Asymmetrischer Sockel; Kollagen (Pfeilkopf) und breiter labialer Wall (Stern) mit nur wenigen Kollagenfasern (Pfeil). (g) Variable Anheftungszone bei den kleineren Zähnen (Pfeile). (h) Unterschiedliche Sockel bei außen stehenden (vorn) und innen stehenden Zähnen (Pfeile). (i) Kleiner hinterer Zahn mit rauer Verankerungszone. (j) Am Kieferrand gelegener Zahn; Sockel und Zahn sind labialseits durch einen Einschnitt getrennt (Pfeil). (k) Kieferrand mit



zwei benachbarten Zähnen. Einschnitt zwischen Zahn und Sockel (Pfeilspitzen). (l) Größerer Zahn mit kollagenem Scharnier (Pfeil) und kollagenfreiem Einschnitt (Pfeilkopf) zwischen Sockel und Zahn. Man beachte die dünne Kollagenverbindung (dünner Pfeil) und die labial dickere Zahnwand (Stern).



margins (figs. 2 a, b) and most posterior of the jaws (fig. 1 a). Manipulation of the larger teeth *in situ* with a needle reveals that most can be depressed at least 45 degrees from vertical in posterolingual direction (see fig. 1 b), but largely resist depression in an opposite or lateral direction. Teeth return to their original position after depression.

Teeth are attached to a very flat bony pedestal. The tooth base and the pedicel are slightly inclined (figs. 1 c, g, i). In the larger teeth the dentine shaft as well as the pedestal is remarkable thick at the labial face (figs. 1 c-f, h, l). Here, the pedestals only abut the bases of teeth leaving a conspicuous collagen-free gap (figs. 1 c f, h, j-l). SEM and especially histology reveal that here only a thin sheath of collagen (probably unmineralised or slightly mineralised) extends from the internal surface of the pedestal to the internal surface of the tooth base (figs. 1 f, l). At the lingual face, however, unmineralised (stained blue by Azan) or largely unmineralised collagen fibres occupy the entire width of the dentine and pedestal wall (see figs. 1 d, f, l). Thus, the collagen passes completely around the tooth base, but is thicker and more pronounced in the half directed posterolingual. Such teeth correspond to the “type 4” teeth (*sensu* FINK 1981), in which “collagen is lacking between anterior dentine border and attachment bone” (FINK 1981: 174). In the outer smaller teeth the collagen-free gap is more variable (see fig. 1 h) and the width of the pedestals and dentine walls appears more uniform (fig. 1 g, h, j, k). In the smallest posterior teeth the labial face shows only a rough texture and longitudinal

fibres indicating the zone of attachment (fig. 1 i). These smaller teeth may correspond to FINK’s types 2 and/or 3, which are characterised by a complete collagenous ring at base (type 2) or a collagenous ring with mineralised collagen (dentine) anteriorly (FINK 1981). However, this has to be verified by other techniques.

Surely, teeth of *B. belizanus* are involved in prey capture and the flexible zone of collagen in the longer teeth may act as hinge. Teeth hold struggling prey, but depressibility (derived from artificial manipulation) may also hinder anterior escape of prey and facilitate entry of large prey items into the mouth. In our video sequences we could not see any depression of teeth during prey capture, which, however, is due to the limited image solution of high-speed videography and probably to the smallness of the prey items. When swallowing very large and struggling prey, the collagenous hinge may also serve as a “buffer” to reduce tooth wear and the risk of breaking off the teeth.

B. belizanus is exclusively piscivorous since birth. Feeding of newborn and juveniles with alternative prey items is a temporary solution at best. Although jaws are proportionally shorter, dentition of newborns resembles that of adults in relative size, shape and arrangement of teeth (unpublished observations).

To capture prey, fish use: (inertial) suction feeding, ram feeding and biting (e.g., NORTON & BRAINERD 1993, PORTER & MOTTA 2004). In most cases hungry *B. belizanus* approached the guppies immediately after detection and oriented to the prey. At a varying distance of body lengths, the fish used an explosive S-start

Figs. 2 a and b: Feeding sequence of *Belonesax belizanus*. (a) Lateral view from strike initiation (at 0 ms) until closure of the jaws close, lateral view. The mouth begins to open (at 14 ms). Maximal mouth opening (at 28 ms). Depression of the hyoid (30 ms; arrow). Maximal depression of the hyoid (at 32 ms). The mouth is closed and the hyoid are largely elevated (at >38 ms). (b) Laterofrontal view. Note the large opening of the mouth and the prey in the expanded oral cavity (arrow).

Abb. 2 a und b: Videosequenzen des Beutefangs von *Belonesax belizanus*. (a) Lateralansicht, vom Beginn (bei 0 ms) bis zum Schließen des Mauls. Das Maul beginnt sich zu öffnen (bei 14 ms). Das Maul ist maximal geöffnet bei 28 ms). Absenkung des Hyoids (bei 30 ms, Pfeil). Maximale Absenkung des Hyoids (bei 32 ms). Das Maul ist geschlossen und das Hyoid wieder weitgehend in Normalstellung (bei >38 ms). (b) Ansicht von seitlich-vorn. Man beachte die weite Öffnung der Kiefer sowie die Beute in der erweiterten Mundhöhle (Pfeil).

Tab. 1: Strike variables of *Belonesox belizanus* (this article) and of three other ram feeders (means from PORTER & MOTTA 2004).

Tab. 1: Beutestoß-Variable von *Belonesox belizanus* (diese Arbeit) und von drei anderen Stoßräubern (Mittelwerte aus PORTER & MOTTA 2004).

Variables	<i>Belonesox belizanus</i>	<i>Lepisosteus platyrhincus</i>	<i>Strongylura notata</i>	<i>Sphyaena barracuda</i>
Strike distance (mm / % body length)	26±12.6 / 39.7±16.5 (n=6)	11.7	68.9	102.4
Strike duration (ms)	36±8 (n=6)	32	455	147
Mean velocity (cm s ⁻¹ / body lengths s ⁻¹)	67.5±27.1 / 10.7±3.3 (n=6)	--/3.6	--/1.7	--/7.5
Time to mouth opening (ms)	17.3±6.3 (n=6)	3	264	87
Duration of mouth opening (ms)	14.7±2.4 (n=6)	16	141	25
Maximum gape (mm / % head length)	9.7±2.5 / 44±14.1 n=6	36/--	42/--	30/--
Duration of mouth closing (ms)	11.2±2.7 (n=5)	7	19	8
Time to hyoid depression (ms)	37.6±19.7 (n=5)	14	385	105
Duration of hyoid depression (ms)	5.5±5.7 (n=4)	10	22	19

(not shown) and the prey was grasped by head-on lunge towards the prey (see fig. 2 a, tab. 1). The mouth was opened milliseconds after strike initiation. However, the lateral gap formed by the slightly curved edges of the dentate jaws, which do not meet when the mouth is closed, seems to enlarge first (fig. 2 a, 14 and 16 ms). The prey was contacted after 36 ms and captured at velocities of approximately 11 body length's s⁻¹. Hyoid depression coincided with maximum gape, which reached 44 % of head length. The relatively small prey used by us was overtaken by the large gape before the jaws close and prey seemed to be engulfed during or shortly after maximum gape. Further variables are given in table 1. Especially values given for hyoid depression varied considerably and may be accepted with reservation. Total prey capture duration, i.e. the time from the onset of jaw opening until retraction of hyoid apparatus was approximately 25 ms. The relatively small guppies fed to *B. belizanus* were directly transported through the mouth cavity, and can be seen in the oral cavity after the mouth was closed (fig. 2 b). Prey, which was

seized by the jaws at the tail or in the midst of the body and hold with the teeth, was reoriented by jerky movements of the head and rapid up and down movements of the jaws to bring the prey in the right position, i.e. often tail, but occasionally also head first (not shown).

Our analysis of strike variables is still incomplete and the presented data are based on a few strikes only. However, our observations show that *B. belizanus* uses ram feeding, i.e. a dominance of the predator movement towards the prey starting with a head-on lunge towards the prey and the mouth open. Further, kinematic sequences (expansive, compressive, and recovery phases) occurred in a posteriorly directed sequence as in other aquatic feeding vertebrates (LAUDER 1985). There are several ram feeding fishes that have a similar morphology as *B. belizanus* including posteriorly placed dorsal and anal fins, e.g. pikes (*Esox* spp., e.g. RAND & LAUDER 1980, WEBB & SKADSEN 1980, HARPER & BLAKE 1991), Florida gar (*Lepisosteus platyrhincus*), redfin needle fish (*Strongylura notata*) and great barracuda (*Sphyaena barracuda*) (PORTER & MOTTA 2004).

They all show considerable species-specific differences in their prestrike, strike and poststrike behavior as exemplified for some strike variables in table 1 (for further data and readings see PORTER & MOTTA 2004). Data of most variables measured for *B. belizanus* lie in between those of *L. platyrinchus* and *S. notata* (table 1).

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