Intra-oropharyngeal food transport and swallowing in white-spotted bamboo sharks

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Abstract

Despite the importance of intraoral food transport and swallowing, relatively few studies have examined the biomechanics of these behaviors in non-tetrapods, which lack a muscular tongue. Studies show that elasmobranch and teleost fishes generate water currents as a 'hydrodynamic tongue' that presumably transports food towards and into the esophagus. However, it remains largely unknown how specific musculoskeletal motions during transport correspond to food motion. Previous studies of white-spotted bamboo sharks (*Chiloscyllium plagiosum*) hypothesized that motions of the hyoid, branchial arches, and pectoral girdle, generate caudal motion of the food through the long oropharynx of modern sharks. To test these hypotheses, we measured food and cartilage motion with XROMM during intra-oropharyngeal transport and swallowing (n=3 individuals, 2-3 trials per individual). After entering the mouth, food does not move smoothly toward the esophagus, but rather moves in distinct steps with relatively little retrograde motion. Caudal food motion coincides with hyoid elevation and a closed mouth, supporting earlier studies showing that hyoid motion contributes to intra-oropharyngeal food transport by creating caudally-directed water currents. Little correspondence between pectoral girdle and food motion was found, indicating minimal contribution of pectoral girdle motion. Transport speed was fast as food entered the mouth, slower and step-wise through the pharyngeal region and then fast again as it entered the esophagus. The food's static periods in the step-wise motion and its high velocity during swallowing could not be explained by hyoid or girdle motion, suggesting these sharks may also use the branchial arches for intra-oropharyngeal transport and swallowing.

Introduction

After capturing food, there are at least two equally important steps in feeding: transport and swallowing. Intra-oropharyngeal transport is the process of moving food after initial prey capture, from the oral cavity, through the pharyngeal cavity and towards the esophagus. Food is then swallowed when it enters the esophagus. Both transport and swallowing require a force to move the food caudally. In mammals, for example, this force is provided by the tongue, which transports both liquids and solids towards the esophagus like, in the words of Hiiemae and Crompton (1985), a “conveyor belt”. The food bolus is swallowed by stereotypical activation and de-activation of muscles of the hyoid, tongue, soft palate and pharyngeal constrictors (Hiiemae and Crompton, 1985). Similar behavior has also been observed in some lissamphibians (Bemis, 1986; Reilly and Lauder, 1990), and sauropsids, unless the tongue has been adapted as a chemosensory organ, as in snakes (Kley and Brainerd, 2002). In some cases, a ‘throw-and-catch’ mechanism may be used, which involves throwing the food upward and opening the oropharyngeal cavity wide, so the food falls into the esophagus (Herrel et al., 1996; Herrel et al., 1997; Schaerlaeken et al., 2011). The throw-and-catch mechanism is considered the most basal feeding pattern of birds (Zweers et al., 1994) and occurs in birds that possess relatively small tongues with no remarkable features, such as the greater rhea (Gussekloo and Bout, 2005).

In contrast, fish do not possess a mobile, muscular tongue, and they generally do not feed in air. Feeding in water poses a quite different set of challenges and opportunities compared to feeding on land (Heiss et al., 2018). Instead of using a muscular tongue, fish can use the water to their advantage by creating a ‘hydrodynamic tongue’ (Liem, 1990). This tongue is not an anatomical structure, but rather water currents are generated inside the mouth to reposition and transport food. The water flows are generated by expansion or contraction of the oropharyngeal cavity, for example by elevation or depression of the hyoid (Dean et al., 2005; Michel et al., 2015). This hydrodynamic tongue behavior has been observed in a broad spectrum of species within the actinopterygians and lungfish (Bemis, 1986; Gillis and Lauder, 1995; Lauder, 1983; Michel et al., 2015). In addition, a hydrodynamic tongue has been observed in aquatic amphibians, turtles, and some marine mammals, even though they also possess a muscular tongue (Gillis and Lauder, 1994; Levine et al., 2004; Natchev et al., 2009; Werth, 2000). In addition to the hydrodynamic tongue, ray-finned fishes can use their pharyngeal jaws to grasp, transport and process food (Lauder, 1983; Mehta and Wainwright, 2007; Vandewalle et al., 2000; Wainwright, 2005).

Sharks, like ray-finned fishes, do not possess a muscular tongue, and they do not possess pharyngeal jaws either. Sharks also have an exceptionally long oropharyngeal cavity, spanning the space from the jaws through the hyoid region and across the five

branchial arches, which are caudal to the cranium, to the even more caudally-located pectoral girdle (Fig. 1). In contrast, in actinopterygians the branchial arches and pectoral girdle are ventral to the cranium forming a relatively short compact oropharyngeal cavity. Hence, sharks face a bigger challenge as they need to transport food a relatively longer distance than actinopterygian fishes.

Studies on fluid pressure and fluid dynamics of feeding behavior in white-spotted bamboo sharks (*Chiloscyllium plagiosum*) found they use suction to capture prey and to transport it from the jaws into the oropharyngeal cavity (Nauwelaerts et al., 2008; Wilga and Sanford, 2008), essentially using suction feeding and a hydrodynamic tongue like ray-finned fishes. Suction is generated by coordinated expansion of the oropharyngeal cavities (Ramsay and Wilga, 2017; Scott et al., 2019; Wilga, 2008; Wilga, 2010; Wilga and Sanford, 2008; Wilga et al., 2012), which results in fluid flows that move the food from the surrounding environment or jaws into the pharynx (Nauwelaerts et al., 2007; Nauwelaerts et al., 2008; Wilga and Motta, 1998a; Wilga and Motta, 1998b; Wilga and Motta, 2000; Wilga and Sanford, 2008; Wilga et al., 2007; Wilga et al., 2012). These previous studies have inferred food position within the long oropharynx, but food position has not been measured explicitly during intra-oropharyngeal transport and swallowing.

Despite this evidence of sharks using a hydrodynamic tongue driven by hyoid motions to transport food initially from the jaws into the oropharynx, it remains unclear how musculoskeletal and fluid motions contribute to specific food motion within the oropharynx. Prior studies have shown that expansion and compression of the hyoid and branchial arches by their associated musculature during food processing and transport are responsible for the positive and negative pressure changes and unsteady flows in the intra-oropharyngeal cavity (Wilga, 2010; Wilga and Motta, 1998a; Wilga and Motta, 1998b; Wilga and Sanford, 2008; Wilga et al., 2012). Expansion of the hyoid arch is hypothesized to generate fluid flows, which transport the food down the center of the oropharyngeal cavity from the jaws to the esophagus (Wilga and Sanford, 2008; Wilga et al., 2012). However, the location of the food has not been measured during these behaviors, so the proposed relationship between hyoid and food motion has not been tested. The pharynx is hypothesized to function as a sink, with the branchial arches expanding to receive the incoming bolus of water and food (Wilga and Sanford, 2008; Wilga et al., 2012). A more recent study showed that the pectoral girdle is mobile and contributes to suction feeding in bamboo sharks (Camp et al., 2017). Camp et al. also hypothesized that the location of the pectoral girdle at the back of the elongated pharynx (Fig. 1) might allow caudoventral pectoral girdle motion (retraction) to contribute to pharyngeal cavity expansion and flow generation for food transport. However, the actual food motions relative to hyoid, branchial and pectoral girdle motions during food transport remain hypothetical as the head is covered with thick skin and muscle making direct, precise

measurements difficult without X-ray imaging (but see use of sonomicrometry for suction feeding, (Wilga and Sanford, 2008)).

Here, we use X-ray Reconstruction of Moving Morphology (XROMM) to test whether motions of the hyoid, pectoral girdle, or both contribute substantially to intra-oropharyngeal transport and swallowing in white-spotted bamboo sharks. XROMM is a technique that combines biplanar X-ray video and CT-scans to reconstruct *in vivo* 3D skeletal kinematics (Brainerd et al., 2010). We use an existing XROMM dataset collected for studying suction feeding (Camp et al., 2017; Scott et al., 2019) that also incidentally collected some complete sequences of transport and swallowing. Branchial arch cartilages were not marked so the hypothesized contributions of those elements cannot be tested directly, but consistent food transport in the absence of hyoid or pectoral girdle motions would lend support to contributions from motions of the branchial arches. As noted above, food transport and swallowing are equally important for nutrition and survival as food capture, and this study will test existing hypotheses for the roles of the hyoid arch and pectoral girdle in transport and swallowing in a member of a functionally and phylogenetically important vertebrate group. We hypothesize that hyoid expansion will create an unsteady flow that moves the food down the center of the oropharyngeal cavity from the jaws to the esophagus. We also hypothesize that pectoral girdle depression will assist in the creation of the flow that moves the food towards the esophagus. Sharks are functionally important because they lack the pharyngeal jaws that are thought to assist transport and swallowing in many ray-finned fishes and they are phylogenetically important as the outgroup to Osteichthyes, including lobe-finned fishes and tetrapods. These data will add to an emerging evolutionary synthesis of food transport and swallowing mechanisms in Gnathostomata that has thus far not included Chondrichthyes (Heiss et al., 2018).

Methods

Animals

Cartilage and food kinematics were quantified using XROMM for three white-spotted bamboo sharks, *Chiloscyllium plagiosum* (Bennett 1830). Total body lengths were 78.6, 79.2 and 85.0 cm for Bam02, Bam03 and Bam04, respectively. These same individuals were used in prior XROMM studies of suction feeding (Camp et al., 2017; Scott et al., 2019) and trials for all three studies were collected simultaneously. Therefore, all methods follow those two prior studies and are described here only briefly. All animal care and experiments were approved by the Institutional Animal Care and Use Committees of Brown University and the University of Rhode Island. Each shark was anaesthetized (Wilga and Sanford, 2008) and tungsten carbide conical markers (Kambic et al., 2014) were implanted in the chondrocranium,

pectoral girdle (for Bam04 only), and left palatoquadrate (upper jaw), Meckel's cartilage (lower jaw), hyomandibula and ceratohyal (Camp and Brainerd, 2014). All sharks recovered fully and resumed normal feeding behaviors prior to data collection. We follow the anatomical terminology of Wilga and Sanford (Wilga and Sanford, 2008), but we will use the term 'oral cavity' to refer to the buccal and hyoid cavities together.

Data collection

The sharks were fed small (less than half of gape width) pieces of squid or herring marked with a single tantalum or ceramic bead in the center of the prey item while being filmed within the oblique, biplanar field of view of two X-ray machines (Imaging Systems and Service, Painesville, OH, USA), which generated X-rays at 110–120 kV and 100 mA. The resulting X-ray videos were recorded at 320 or 330 frames per second by Phantom v.10 high-speed cameras (Vision Research, Wayne, NJ, USA). Video and calibration data are stored with their essential metadata on the XMAPortal (http://xmaportal.org) in accordance with best practices for video data management in organismal biology (Brainerd et al., 2017).

As noted above, we used an existing XROMM dataset collected for studying suction feeding (Camp et al., 2017; Scott et al., 2019) that also incidentally collected some complete sequences of transport and swallowing. Hence, the sample size for this study is not large; there were only 7 trials across 3 individuals (n=2 for Bam02 and Bam03, n = 3 for Bam04) in which the food was marked and the entire feeding bout—from capture to swallowing—was visible. However, given the substantial difficulty of marking animals and collecting XROMM data, it is worthwhile to make use of these data to gain insights that are unobtainable in any other way at this time.

After the first day of trials, the sharks were anesthetized and *in vivo* computed tomography (CT) scans (FIDEX CT, Animage, Pleasanton, CA, USA) were taken of all sharks (resolution = 416 x 416 or 448 x 448 pixels; slice thickness = 0.185 mm), and mesh models of the cartilages and markers were created in OsiriX (Pixmeo, Geneva, Switzerland) or Horos (horosproject.org) and Geomagic Studio (11, Geomagic, Inc., Triangle Park, NC, USA).

The biplanar X-ray videos were undistorted, calibrated, and all markers in the cartilages and food were tracked in XMALab (Knörlein et al., 2016) with a precision of 0.15 mm. This precision of marker tracking was calculated by taking the mean of the standard deviations of marker-to-marker distance pairs for markers within each rigid body of every trial, and subsequently calculating the mean across all trials (Brainerd et al., 2010; Knörlein et al., 2016). Using the XYZ coordinates of the cartilage markers from the X-ray videos, and the anatomical location of each marker from the CT scan, rigid body transformations were

calculated and filtered (low-pass Butterworth, 50 Hz cut-off frequency) for each cartilage. In addition, XYZ coordinates of the food marker were exported from XMALab.

Data visualization and analysis

For each feeding trial, the mesh models of the cartilages were animated with the rigid body transformations in Maya (2016, Autodesk, San Rafael, CA, USA) to create an XROMM animation. The unmarked pectoral girdles in Bam02 and Bam03 were animated by Scientific Rotoscoping (Gatesy et al., 2010). The pectoral girdle was clearly visible in the X-ray images (Camp et al., 2017) and a mesh model of the pectoral girdle was aligned with the image of its position in the two X-ray videos. The result was a single skeletal animation combining marker-based (Brainerd et al, 2010) and markerless (Gatesy et al., 2010) XROMM for each feeding trial.

Within each animated feeding trial, virtual landmarks were selected (by parent constraining a locator to the mesh cartilage model) at the rostroventral tips of the upper jaw, lower jaw, ceratohyal and the ventral tip of the pectoral girdle. An anatomical coordinate system (ACS) was placed in the middle of the chondrocranium with the X-axis aligned rostrocaudally, the Y-axis aligned medio-laterally (left-right) and the Z-axis aligned ventrodorsally. This ACS served as a frame of reference for measuring food translation and cartilage landmark displacements relative to the cranium.

Kinematic measurements

The XYZ coordinates of the food were re-calculated relative to the chondrocranial ACS. Translations in the rostrocaudal axis were normalized by the distance between the jaw tips and the pectoral girdle to correct for size differences among individuals. This distance represents the length of the entire oropharyngeal cavity, and therefore allowed us to express food motion relative to how much of the cavity it had travelled. The oropharyngeal cavity length (mouth-pectoral girdle distance) was calculated for each trial as the difference between the rostral position of the food when it entered the mouth and the position of the food when it passed the pectoral girdle and then averaged for each shark. Dissection of Bam04 confirmed that the opening to the esophagus lies within the plane of the pectoral girdle, i.e. medial to both scapulae and slightly dorsal to the coracoid (Fig. S1), so we used the position of the pectoral girdle as a proxy for the location of the entrance to the esophagus. Thus, a normalized rostrocaudal translation value of 0 indicates the food is at the rostral tip of the jaws and about to be captured, and a value of 1 indicates that the food is passing the pectoral girdle, entering the esophagus and being swallowed. Non-normalized

rostrocaudal translations of the food were used to calculate the velocity of the food motion toward the esophagus.

Cartilage motions were described by the displacement of virtual landmarks, relative to the chondrocranial ACS. Rostrocaudal cartilage displacements were normalized for mouth-pectoral girdle distance, as described above for the food. The normalized displacements allowed us to more directly compare motions of the cartilages to those of the food. Gape was calculated as the distance between the upper and lower jaw landmarks. We confirmed that rotation of the pectoral girdle relative to the body plane (as measured previously in Camp et al., 2017) and the dorsoventral displacement of the coracoid bar (relative to the chondrocranium ACS) showed the same pattern.

Results

Across the seven trials in this study, all sharks used suction feeding to draw food directly into the oral cavity; none of the sharks captured the food between the teeth or manipulated food with the jaws, likely because the food pieces were deliberately cut to no more than half-gape width for the suction-feeding studies (Camp et al., 2017; Scott et al., 2019). We observed no difference in transport and swallowing between herring and squid pieces.

After the food entered the mouth ($x = 0$ in Fig. 2), it initially moved caudally through the oral cavity in a smooth trajectory, with very little lateral or dorsoventral motion in the first 30% of oropharyngeal length (the length from the jaw tip to the pectoral girdle) ($x \leq 0.3$ in Fig. 2); approximately at the level of the hyomandibula-cranial articulation (Figs. 1-2). Then the food continued to move caudally as well as laterally in most trials (Fig. 2B) and ventrally in some trials (Fig. 2A). However, motions in both the lateral and the dorsoventral axes were relatively small during this period. After the food reached 80% of oropharyngeal length ($x \geq 0.8$ in Fig. 2), it moved back toward the mid-sagittal plane, and in all trials there was a small rostral translation just before or after the food was swallowed ($x = 1$ in Fig. 2). For an example of a trial, see Movie 1 and 2.

When we isolated the rostrocaudal translations of the food, we observed a step-wise movement (Fig. 3). The food moved rapidly in a caudal direction during the initial suction capture event, and then continued to move in a series of smaller, discontinuous motions where it moved caudally, then stopped or moved slightly rostrally, and then moved caudally again until the food reached the esophagus. During the relatively stationary phases, the food moved slightly anteriorly in most cycles. In one case (Bam03, Trial 02), the food moved nearly equally in the rostral and caudal directions through several cycles, making no progress toward the esophagus until about 80% of the duration of the feeding bout, at which

time it began the step-wise motion seen in the other trials and progressed into the esophagus (Fig. 3).

During feeding, rostrocaudal translation of the food was accompanied by dorsoventral motion of the ceratohyal and the coracoid bar and changes in gape (Fig. 4), as measured by virtual landmark displacements. During prey capture, all sharks depressed (i.e., ventrally displaced relative to the chondrocranium) the ceratohyal as the gape closed and the food accelerated into the oral cavity. One shark, Bam02, slightly elevated the coracoid bar and then depressed it, and the gape closed after the food moved caudally.

After capture, the step-wise food motions began as all sharks closed the gape and elevated (i.e., dorsally displaced relative to the chondrocranium) the ceratohyal while the food was transported caudally. The coracoid bar was either depressed or elevated with the ceratohyal; the direction of motion varied between individuals. In general, Bam03 and Bam04 elevated, while Bam02 depressed the coracoid bar. The ceratohyal also elevated during swallowing as the mouth was closed, but ceratohyal and coracoid bar translations were generally smaller than during food transport.

In Trial 02 from Bam02, the shark depressed the ceratohyal and coracoid bar during intra-oropharyngeal transport while the gape was open, as it did during capture (Fig. S2). When the shark combined a closed gape and hyoid elevation, the step-wise food transport was successful, and the food was swallowed.

In all trials, the food particle made an additional rostrally directed, high-velocity movement when it was near or inside the esophagus, before it continued caudally down the esophagus towards the stomach (Fig. 4, 5). As the position of the esophageal sphincter was not marked in the X-ray video, it is unclear whether this movement occurred just before or after the food entered the esophagus.

The velocities of the food trajectories through the oropharynx show four phases of food motion (Fig. 5). The first phase, prey capture, was the fastest, with peak velocities of 55-270 cm s^{-1} (mean of 145 cm s^{-1}), as the food moved through about the first half of the oropharynx (up to x = 0.5). Food velocity then dropped to a mean of 5.4 cm s^{-1} (range of 0.2-71 cm s^{-1}) between x = 0.5 and 0.8 during intra-oropharyngeal transport in Phase 2, after which it increased again during swallowing in Phase 3, reaching local peaks of 29-130 cm s^{-1} (mean of 74 cm s^{-1}) near the opening to the esophagus (x = 1.0). Peak velocities in Phase 3 were in between those of Phase 2 and Phase 1. In Phase 4, when the food has been swallowed, it slowed down inside the esophagus to a velocity comparable to those seen in the middle of the pharynx in Phase 2.

Discussion

Until now it was unclear how food motion corresponds to the musculoskeletal motions that sharks use to transport food through the long oropharynx, without either a muscular tongue or pharyngeal jaws. We show that white-spotted bamboo sharks transport food items in a series of distinct steps, where the food alternates between phases of caudal motion and relative immobility (Fig. 3). This step-wise food transport has not previously been observed in sharks, as the muscles and skin surrounding the oropharynx make it difficult to directly and precisely measure food location without X-ray imaging. Our results support the hypothesis that motions of the hyoid—and not the pectoral girdle—generate caudally-directed unsteady water currents to move food towards the esophagus. The branchial arches may be responsible for the food's relatively static periods during step-wise transport and contributing to its relatively high velocity during swallowing, as neither hyoid nor pectoral girdle motions could account for these. While this hypothesized contribution of the branchial arches remains to be tested, our study demonstrates how sharks use coordinated cartilage motions to control the motion of food through the oropharynx so that it can be successfully transported and swallowed.

Hyoid motion during transport

Caudal food motion consistently corresponded with hyoid motion during the transport behaviors observed in this study. Although the exact mechanism cannot be directly determined from our data, our results are consistent with the food being moved by caudally-directed water currents, generated by hyoid motion. In most trials, the food travelled caudally towards the esophagus as the hyoid elevated with the mouth (gape) closed (Fig. 4). Hyoid elevation compresses the oral cavity, and since the jaws are closed water—and food—will be pushed caudally through the oropharynx and out of the opened fifth gill slit, which remains open throughout most of the feeding events (Wilga and Sanford, 2008). Such compressive transport behaviors occur in several elasmobranch species where the closed jaws, hyoid and hypobranchial regions are elevated by nearly simultaneous activation of cranial muscles that reduce the volume of the oropharyngeal cavity (Wilga and Motta, 1998a; Wilga and Motta, 1998b; Wilga et al., 2012). Thus, our results support the hypothesis that hyoid motion drives food transport, via caudally-directed water flows within the oropharyngeal cavity (Dean et al., 2005).

While all the sharks in this study used a step-wise food transport behavior, we did observe some variation in the relationship between caudal food motion and hyoid motion. In two of the seven trials, the food moved caudally as the hyoid depressed with the mouth open in the first cycle of transport, and then switched to the pattern of caudal motion with hyoid

elevation and closed gape once the food had moved past the hyoid area (Fig. 4C, D). In one trial (Fig. S2, Bam03 Trial02) the food remained in the hyoid region of the oropharyngeal cavity for several seconds—moving caudally as the hyoid depressed and the mouth opened, then rostrally with hyoid elevation for several cycles—before moving step-wise towards the esophagus. This variation is likely due to the changing position of the food: while the food is in the oral or hyoid region of the oropharynx (rostral to the hyoid), the food moves caudally with hyoid depression (i.e., towards the hyoid). After moving into the pharynx (caudal to the hyoid), food moves in a caudal direction (i.e., away from the hyoid) during hyoid elevation. These patterns also suggest that sharks use a coordinated combination of hyoid and gape motion to control the position and motion of food throughout the oropharynx.

Pectoral girdle motion during transport

We did not find evidence that motion of the pectoral girdle contributes substantially to food transport in these sharks, as was hypothesized by Camp et al. (2017). First, pectoral girdle depression and elevation motions during transport were relatively small—both compared to the ceratohyal and to the pectoral girdle motions during the initial suction capture event—suggesting its motion would contribute little to volume changes and therefore fluid flows in the pharynx. Second, the relationship between pectoral girdle and food motion is not consistent. During transport and swallowing, the coracoid bar elevated in or out of phase with the motion of the hyoid and the food. This differed among individuals, and also within some trials, and all individuals used both in and out of phase pectoral girdle rotation at least once. While both in and out of phase hyoid and pectoral girdle compression could theoretically drive anterior-to-posterior flows in the pharynx, it seems unlikely that a shark would switch between these strategies during a single transport event. Coracoid bar depression did not appear to hinder ceratohyal elevation even though these cartilages are connected by two muscles in-series, the coracohyoideus and the coracoarcualis (Ramsay and Wilga, 2017). While the pectoral girdle was mobile during food transport, the inconsistency of the phase relationship between the hyoid and the pectoral girdle suggest that the pectoral girdle does not drive caudal food motion, although it is possible that both of these motions could make some contribution to food transport.

Role of branchial arches in transport

Although the caudal motion phases of food transport appear to be driven by hyoid elevation (as described above), neither hyoid nor pectoral girdle motions can fully account for the relatively immobile phases. In the pauses between caudal food motions, the hyoid depresses. This should expand the oropharyngeal cavity and tend to pull water (and food) back rostrally. However, the food is relatively stationary as the hyoid depresses, and we

observed minimal rostral translation of the food during this phase (Fig. 4). Pectoral girdle motion is variable during these relatively immobile phases—either elevating or depressing—and therefore unlikely to be stabilizing the food at this time. This suggests that the shark uses some other structure or motion in these phases to prevent the food from being sucked back rostrally.

Although we have no data on the branchial arches, it might be possible that these cartilages adduct to hold the food between the basibranchial and hypobranchial cartilages in the floor of the pharynx and the roof of the pharynx (Fig. 6). Vertical distance in the pharyngeal cavity of white spotted bamboo sharks show that the pharyngobranchials and basibranchials compress down to 2-4 mm apart during processing and transport events (C.J. Wilga, unpublished data). While Fig. 6 show all the branchial arches compressed at the same time, the gill slits and branchial arches can move independently (Dolce and Wilga, 2005; Karch et al., 2006; Wilga and Sanford, 2008) and could also compress in a wave-like pattern. Hence, we hypothesize that the pharyngeal roof and floor compress to momentarily stop the food. During this compression, the hyoid arch can depress again to start another cycle of food transport without drawing the food rostrally, thus creating the step-wise motion of the food toward the esophagus. In support of this theory, the epithelium lining the oropharynx is studded with denticles (Atkinson et al., 2016) that could help increase friction to grip the food. While we cannot directly test this hypothesis with the current dataset, the lack of consistent hyoid or pectoral girdle motion to explain these relatively immobile phases does support the branchial arches playing a role in food transport.

Cartilage and food motion during swallowing

It is clear that hyoid motion drives food transport through the oropharyngeal cavity, but additional structures are likely contributing to swallowing. The velocity of the food during swallowing is relatively high compared to the transport phase (Fig. 5). This high velocity might suggest that food is carried to the esophagus by a water current (Fig. 5), although we cannot test this hypothesis with our data as water flows were not measured. For example, during the compressive transport of Atlantic guitarfish jaw elevation is proposed to generate positive pressure and push food and water from the pharynx and presumably into the esophagus (Wilga and Motta, 1998b). We did observe hyoid elevation just before swallowing, but with a substantially smaller magnitude than during transport or capture (Fig. 4), suggesting that hyoid motion alone is insufficient to explain the high velocity of food just before swallowing. There was also little motion of the pectoral girdle during swallowing, so we hypothesize that compression of the pharyngeal region could generate the water flow that produces relatively high food velocities in the swallowing phase (Fig. 6), similar to that of other elasmobranch species during compression transport (Wilga et al., 2012). However, as

the branchial arches were not visible in the X-ray videos and their motion could not be measured, this hypothesis remains to be tested.

Concluding Remarks

Although based on a limited sample size, our results show how food is moved through the oropharyngeal cavity and support previous studies by demonstrating that white-spotted bamboo sharks can use coordinated motion of cartilages—from the jaws to the branchial arches—to transport food. The step-wise motion of food via multiple cycles of hyoid elevation may have been used by these sharks because of the relatively small size of the food items (less than one half gape width). While larger food items may not elicit this step-wise food transport, it could be used in other sharks that bite off small pieces of prey during feeding and use compressive transport (Motta and Wilga, 2001; Wilga and Motta, 2000). Our results lend further support to previous studies showing that hyoid-generated water currents drive intraoral food transport in sharks, but also raise new hypotheses about the contribution of branchial arch motion (especially dorsoventral compression) to food transport and swallowing. Additional detailed studies of these structures are needed to determine their specific role in allowing sharks to meet the challenge of transporting food through a relatively long oropharyngeal cavity (compared to actinopterygians) without a muscular tongue or pharyngeal jaws. Revealing the specific mechanisms of this step-wise motion of food during transport and swallowing in Chondrichthyes will fill a major gap in our understanding the functional diversity and evolution of these essential behaviors in gnathostome vertebrates (Heiss et al., 2018).

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Competing interests

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Data availability

Data for this publication have been deposited and opened for public use in the XMAPortal (xmaportal.org), in the study 'Bamboo Shark Feeding,' with the permanent identifier URI1. Video data are stored with their essential metadata in accordance with best practices for video data management in organismal biology (Brainerd et al., 2017).

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Figure legends

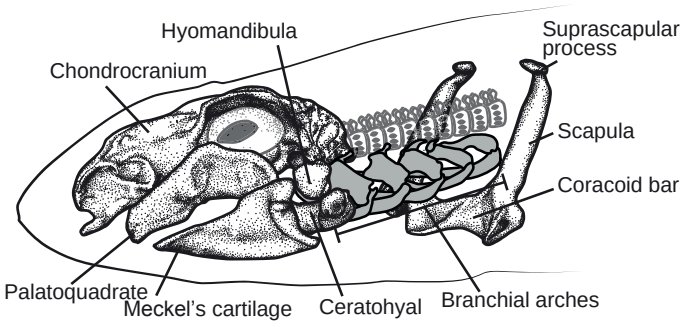

Figure 1: The feeding apparatus of a white-spotted bamboo shark, *Chiloscyllium plagiosum*. The coracoid bar, scapulae, and suprascapular processes together form the scapulocoracoid or pectoral girdle. The muscles and most of the right-side cartilages have been left out for clarity. The grey branchial arches are in a natural, dorsoventrally compressed posture in this image, based on CT scans. Figure modified from (Camp et al., 2017).

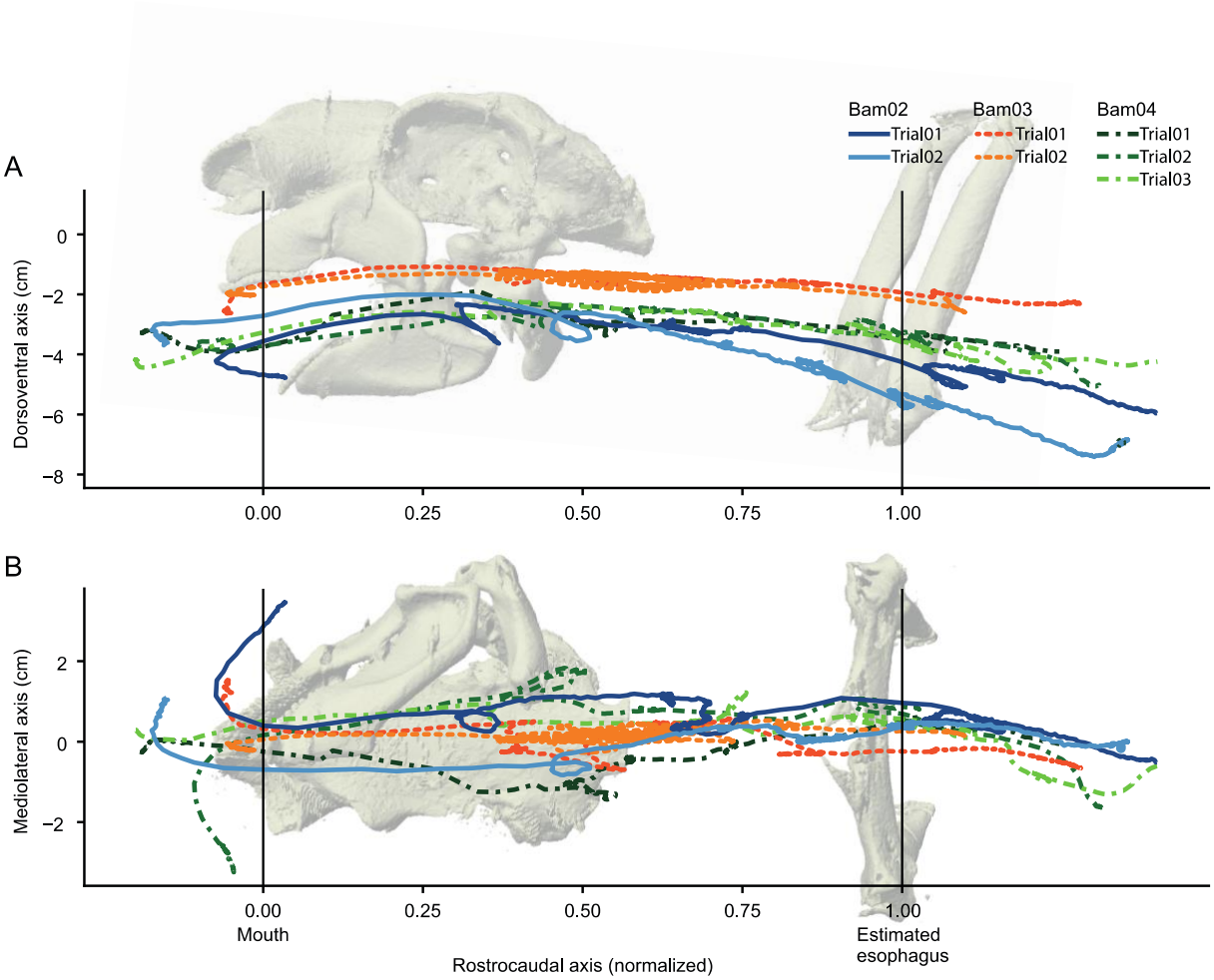

Figure 2: Food trajectories measured relative to the chondrocranium from a (A) lateral view and (B) ventral view. The colors correspond to individual trials (see legend), with trials from Bam02 in blues, Bam03 in reds, and Bam04 in greens (total n = 7). The x-axis represents the food's position along the rostrocaudal axis where x = 0.0 and x = 1.0 represent the mouth and pectoral girdle/esophagus, respectively. Images of the marked cartilages (including only the left-side mandibular and hyoid arches) of Bam04 at peak gape are included as an approximate guide to the food's position. Because sharks have flexibility in the relative positions of their chondrocranium and pectoral girdle from trial to trial, it appears in A as if the opening to the esophagus is very large, but this is not the case. The dorsoventral range of food location as it passes the pectoral girdle is an artifact of plotting these trajectories relative to the chondrocranium; plotting food motion relative to the pectoral girdle would show the opening to the esophagus more clearly but produce artifacts at the mouth.

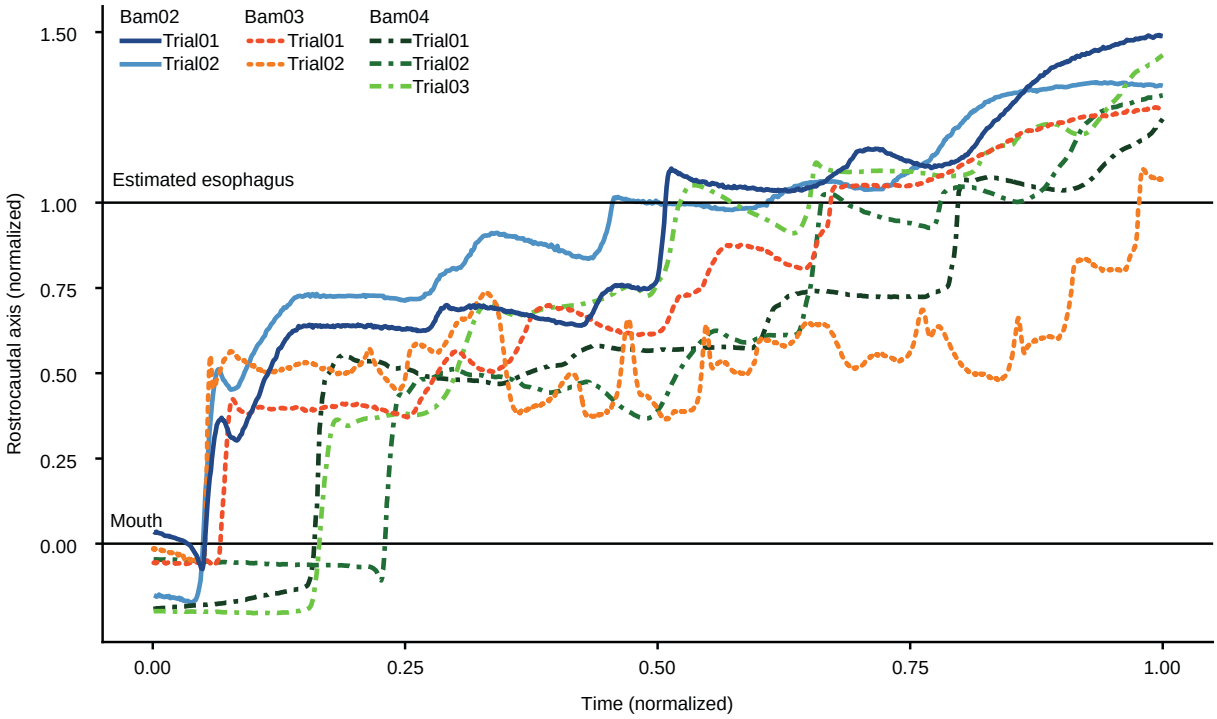

Figure 3: Rostrocaudal translation of the food relative to the cranium as a function of normalized time. The y-axis represents the food's position along the rostrocaudal axis where y=0.0 and y=1.0 represent the mouth and pectoral girdle, respectively, as in the x-axis of Fig. 2. Time was normalized to trial length for comparison among trials. Line colors correspond to trials and individuals, following Fig 2.

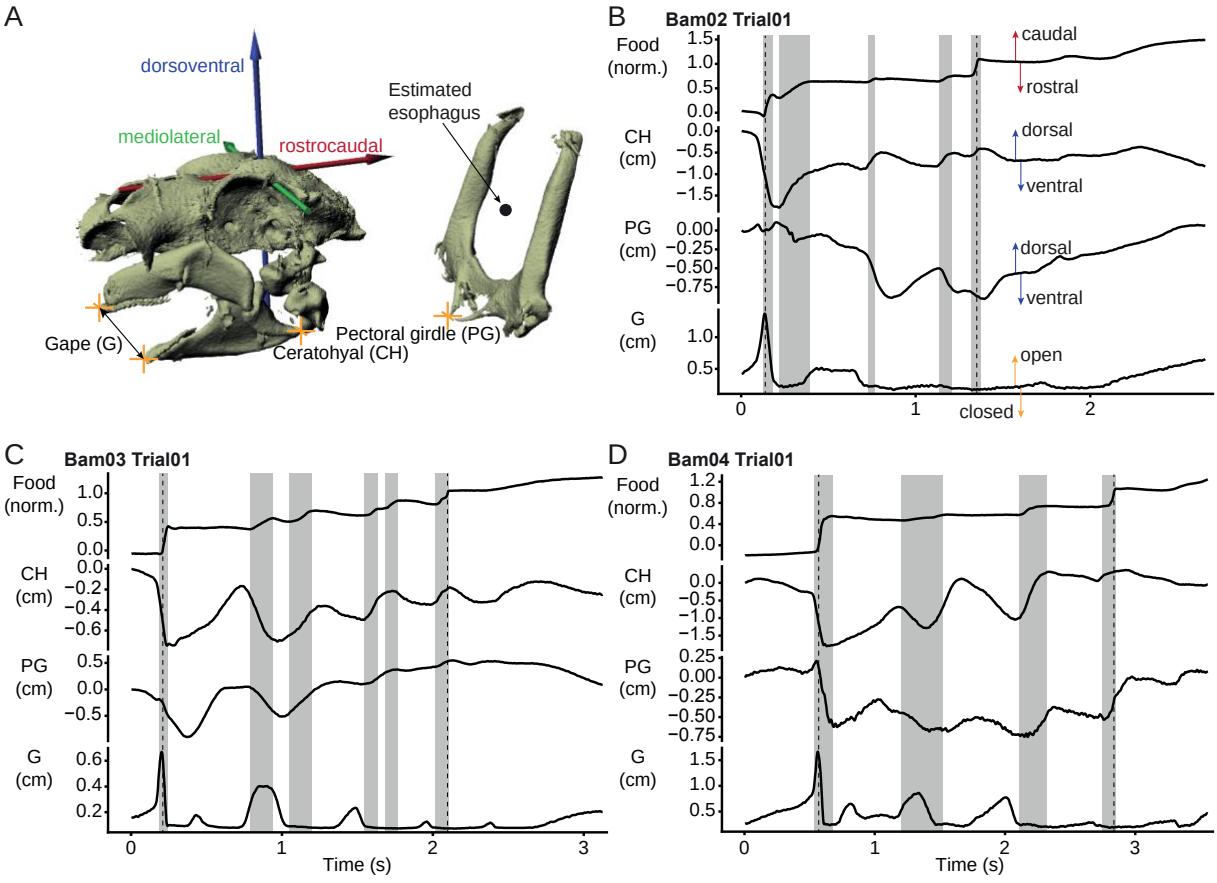

Figure 4: Rostrocaudal translations of food, displacements of ceratohyal and pectoral girdle, and gape width over time in a sample trial from each individual. A) Cartilages of Bam04 at peak gape, showing the

virtual landmarks (yellow), the chondrocranium ACS (green, blue, and red arrows), and estimated esophagus location (black circle). B-D) Plots of food and cartilage movements and gape. With the exception of gape, all movements were calculated relative to the chondrocranium ACS. The shaded bars represent periods of caudally-directed food translation, and the vertical dotted lines represent the times when the food passes the jaw tips and the pectoral girdle (on the food y-axis, where y = 0.0 and y = 1.0, respectively). The directional arrow colors in B correspond to the arrow colors of the ACS in A. All trials are shown in Fig. S2. Abbreviations: Food (norm), normalized translation of food on the rostrocaudal axis; CH, displacement of the rostroventral tip of the ceratohyal in the dorsoventral direction (cm); PG, displacement of the ventral tip of the pectoral girdle (cm) in the dorsoventral direction; G, gape width, calculated from the distance between the jaw tips (cm).

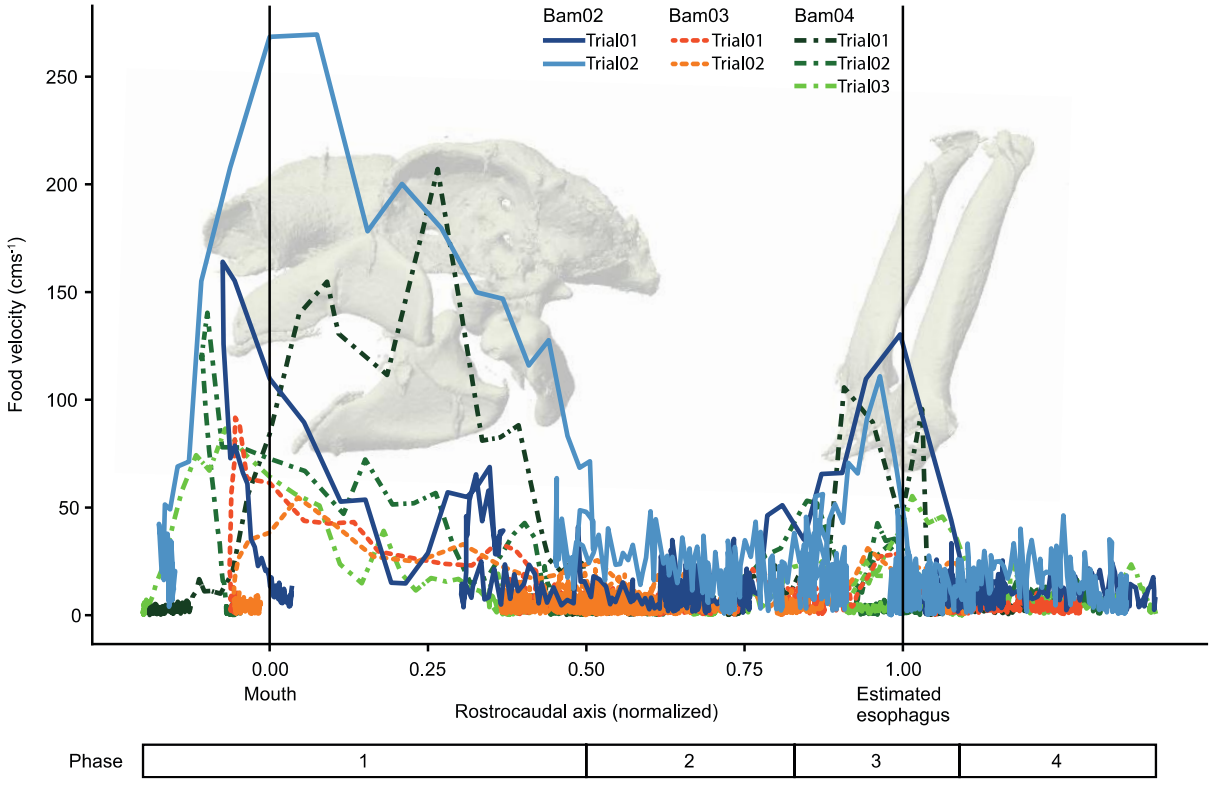

Figure 5: Rostrocaudal velocity of the food relative to its position within the oropharynx. The marked cartilages in the background serve as an indicator of the approximate position of the food within the animal. As in Fig. 2, the x-axis represents the food's position along the rostrocaudal axis where x=0.0 and x=1.0 represent the mouth and pectoral girdle, respectively. Line colors correspond to trials and individuals, following Fig. 2. Food motion occurred in four phases: Phase 1: prey capture, phase 2: oropharyngeal transport, phase 3: swallowing, phase 4: after swallowing.

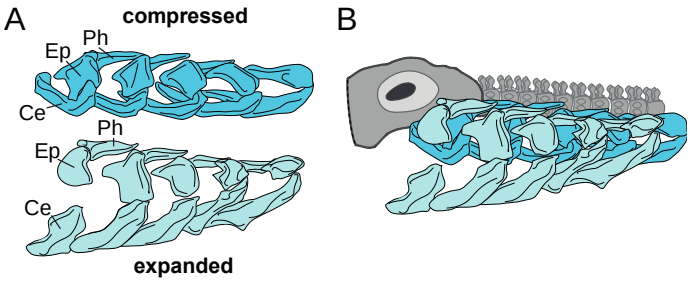

Figure 6: Lateral-view diagram of the branchial arch anatomy and positions. (A) Left-side of the branchial arches in the compressed (dark blue, top) and expanded (light blue) positions from CT scans. Shown in lateral view with rostral to the left. Ph: Pharyngobranchials, Ep: Epibranchials, Ce: Ceratobranchials. The ventralmost elements of the arches that make up the floor of the pharynx, the basibranichials and hypobranchials, are not visible. (B) Lateral view of the branchial arches (in blues), relative to the cranium and vertebral column (in grey).