

# 1 Intra-oropharyngeal food transport and 2 swallowing in white-spotted bamboo 3 sharks

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5 Noraly M.M.E. van Meer<sup>1\*</sup>, Hannah I. Weller<sup>2</sup>, Armita R. Manafzadeh<sup>2</sup>, Elska B.  
6 Kaczmarek<sup>2</sup>, Bradley Scott<sup>3</sup>, Sander W.S. Gussekloo<sup>1</sup>, Cheryl D. Wilga<sup>4</sup>, Elizabeth L.  
7 Brainerd<sup>2</sup> and Ariel L. Camp<sup>2,5</sup>

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9 <sup>1</sup>Experimental Zoology Group, Wageningen University, Wageningen, The Netherlands

10 <sup>2</sup>Department of Ecology and Evolutionary Biology, Brown University, Providence, RI, USA

11 <sup>3</sup>Department of Animal Biology, University of Illinois, Urbana-Champaign, IL, USA

12 <sup>4</sup>Department of Biological Sciences, University of Alaska Anchorage, Anchorage, AK, USA

13 <sup>5</sup>Institute of Ageing and Chronic Disease, University of Liverpool, Liverpool, United Kingdom

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15 \*Author for correspondence (e-mail: [noralymmevanmeer@gmail.com](mailto:noralymmevanmeer@gmail.com))

## 16 Abstract

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

## 37 Introduction

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

55 In contrast, fish do not possess a mobile, muscular tongue, and they generally do not  
56 feed in air. Feeding in water poses a quite different set of challenges and opportunities  
57 compared to feeding on land (Heiss et al., 2018). Instead of using a muscular tongue, fish  
58 can use the water to their advantage by creating a ‘hydrodynamic tongue’ (Liem, 1990). This  
59 tongue is not an anatomical structure, but rather water currents are generated inside the  
60 mouth to reposition and transport food. The water flows are generated by expansion or  
61 contraction of the oropharyngeal cavity, for example by elevation or depression of the hyoid  
62 (Dean et al., 2005; Michel et al., 2015). This hydrodynamic tongue behavior has been  
63 observed in a broad spectrum of species within the actinopterygians and lungfish (Bemis,  
64 1986; Gillis and Lauder, 1995; Lauder, 1983; Michel et al., 2015). In addition, a  
65 hydrodynamic tongue has been observed in aquatic amphibians, turtles, and some marine  
66 mammals, even though they also possess a muscular tongue (Gillis and Lauder, 1994;  
67 Levine et al., 2004; Natchev et al., 2009; Werth, 2000). In addition to the hydrodynamic  
68 tongue, ray-finned fishes can use their pharyngeal jaws to grasp, transport and process food  
69 (Lauder, 1983; Mehta and Wainwright, 2007; Vandewalle et al., 2000; Wainwright, 2005).

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

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

78         Studies on fluid pressure and fluid dynamics of feeding behavior in white-spotted  
79 bamboo sharks (*Chiloscyllium plagiosum*) found they use suction to capture prey and to  
80 transport it from the jaws into the oropharyngeal cavity (Nauwelaerts et al., 2008; Wilga and  
81 Sanford, 2008), essentially using suction feeding and a hydrodynamic tongue like ray-finned  
82 fishes. Suction is generated by coordinated expansion of the oropharyngeal cavities  
83 (Ramsay and Wilga, 2017; Scott et al., 2019; Wilga, 2008; Wilga, 2010; Wilga and Sanford,  
84 2008; Wilga et al., 2012), which results in fluid flows that move the food from the surrounding  
85 environment or jaws into the pharynx (Nauwelaerts et al., 2007; Nauwelaerts et al., 2008;  
86 Wilga and Motta, 1998a; Wilga and Motta, 1998b; Wilga and Motta, 2000; Wilga and  
87 Sanford, 2008; Wilga et al., 2007; Wilga et al., 2012). These previous studies have inferred  
88 food position within the long oropharynx, but food position has not been measured explicitly  
89 during intra-oropharyngeal transport and swallowing.

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

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

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

134

## 135 Methods

### 136 Animals

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

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

151

## 152 Data collection

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

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

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

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

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

184

## 185 Data visualization and analysis

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

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

201

## 202 Kinematic measurements

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

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

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

227

## 228 Results

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

234 After the food entered the mouth ( $x = 0$  in Fig. 2), it initially moved caudally through  
235 the oral cavity in a smooth trajectory, with very little lateral or dorsoventral motion in the first  
236 30% of oropharyngeal length (the length from the jaw tip to the pectoral girdle) ( $x \leq 0.3$  in Fig.  
237 2); approximately at the level of the hyomandibula-cranial articulation (Figs. 1-2). Then the  
238 food continued to move caudally as well as laterally in most trials (Fig. 2B) and ventrally in  
239 some trials (Fig. 2A). However, motions in both the lateral and the dorsoventral axes were  
240 relatively small during this period. After the food reached 80% of oropharyngeal length ( $x \geq$   
241 0.8 in Fig. 2), it moved back toward the mid-sagittal plane, and in all trials there was a small  
242 rostral translation just before or after the food was swallowed ( $x = 1$  in Fig. 2). For an  
243 example of a trial, see Movie 1 and 2.

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



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

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

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

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

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

276 The velocities of the food trajectories through the oropharynx show four phases of  
277 food motion (Fig. 5). The first phase, prey capture, was the fastest, with peak velocities of 55-  
278 270 cm s<sup>-1</sup> (mean of 145 cm s<sup>-1</sup>), as the food moved through about the first half of the  
279 oropharynx (up to  $x = 0.5$ ). Food velocity then dropped to a mean of 5.4 cm s<sup>-1</sup> (range of 0.2-  
280 71 cm s<sup>-1</sup>) between  $x = 0.5$  and 0.8 during intra-oropharyngeal transport in Phase 2, after  
281 which it increased again during swallowing in Phase 3, reaching local peaks of 29-130 cm s<sup>-1</sup>  
282 (mean of 74 cm s<sup>-1</sup>) near the opening to the esophagus ( $x = 1.0$ ). Peak velocities in Phase 3  
283 were in between those of Phase 2 and Phase 1. In Phase 4, when the food has been  
284 swallowed, it slowed down inside the esophagus to a velocity comparable to those seen in  
285 the middle of the pharynx in Phase 2.

286

## 287 Discussion

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

303

### 304 **Hyoid motion during transport**

305 Caudal food motion consistently corresponded with hyoid motion during the transport  
306 behaviors observed in this study. Although the exact mechanism cannot be directly  
307 determined from our data, our results are consistent with the food being moved by caudally-  
308 directed water currents, generated by hyoid motion. In most trials, the food travelled caudally  
309 towards the esophagus as the hyoid elevated with the mouth (gape) closed (Fig. 4). Hyoid  
310 elevation compresses the oral cavity, and since the jaws are closed water—and food—will be  
311 pushed caudally through the oropharynx and out of the opened fifth gill slit, which remains  
312 open throughout most of the feeding events (Wilga and Sanford, 2008). Such compressive  
313 transport behaviors occur in several elasmobranch species where the closed jaws, hyoid and  
314 hypobranchial regions are elevated by nearly simultaneous activation of cranial muscles that  
315 reduce the volume of the oropharyngeal cavity (Wilga and Motta, 1998a; Wilga and Motta,  
316 1998b; Wilga et al., 2012). Thus, our results support the hypothesis that hyoid motion drives  
317 food transport, via caudally-directed water flows within the oropharyngeal cavity (Dean et al.,  
318 2005).

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

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

333

### 334 **Pectoral girdle motion during transport**

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

353

### 354 **Role of branchial arches in transport**

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

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

365         Although we have no data on the branchial arches, it might be possible that these  
366 cartilages adduct to hold the food between the basibranchial and hypobranchial cartilages in  
367 the floor of the pharynx and the roof of the pharynx (Fig. 6). Vertical distance in the  
368 pharyngeal cavity of white spotted bamboo sharks show that the pharyngobranchials and  
369 basibranchials compress down to 2-4 mm apart during processing and transport events (C.J.  
370 Wilga, unpublished data). While Fig. 6 show all the branchial arches compressed at the  
371 same time, the gill slits and branchial arches can move independently (Dolce and Wilga,  
372 2005; Karch et al., 2006; Wilga and Sanford, 2008) and could also compress in a wave-like  
373 pattern. Hence, we hypothesize that the pharyngeal roof and floor compress to momentarily  
374 stop the food. During this compression, the hyoid arch can depress again to start another  
375 cycle of food transport without drawing the food rostrally, thus creating the step-wise motion  
376 of the food toward the esophagus. In support of this theory, the epithelium lining the  
377 oropharynx is studded with denticles (Atkinson et al., 2016) that could help increase friction  
378 to grip the food. While we cannot directly test this hypothesis with the current dataset, the  
379 lack of consistent hyoid or pectoral girdle motion to explain these relatively immobile phases  
380 does support the branchial arches playing a role in food transport.

381

### 382 **Cartilage and food motion during swallowing**

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

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

399

## 400 Concluding Remarks

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

419

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426

## 427 Competing interests

428 The authors declare no competing or financial interests.

429

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437

## 438 Data availability

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

443

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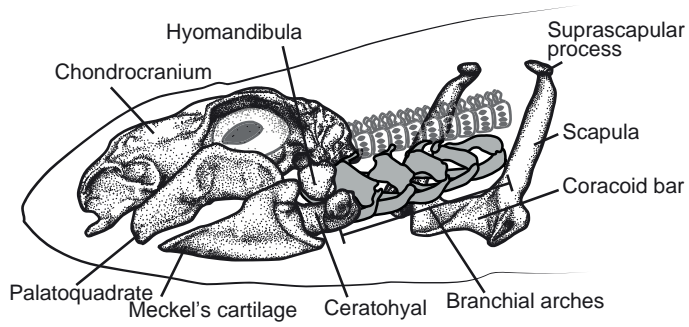
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551 Figure legends

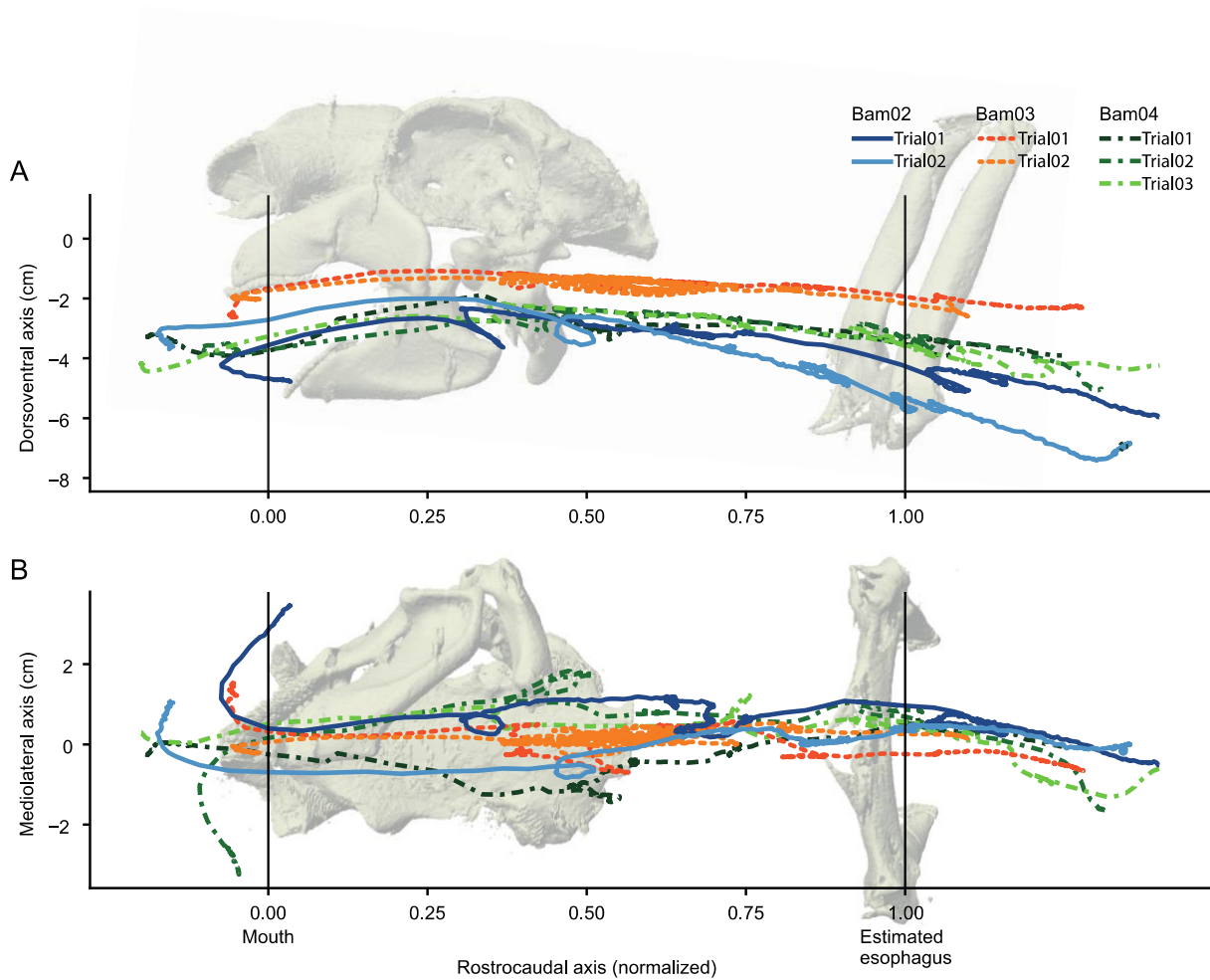


552

553 **Figure 1: The feeding apparatus of a white-spotted bamboo shark, *Chiloscyllium plagiosum*.** The coracoid  
554 bar, scapulae, and suprascapular processes together form the scapulocoracoid or pectoral girdle. The muscles  
555 and most of the right-side cartilages have been left out for clarity. The grey branchial arches are in a natural,  
556 dorsoventrally compressed posture in this image, based on CT scans. Figure modified from (Camp et al., 2017).  
557

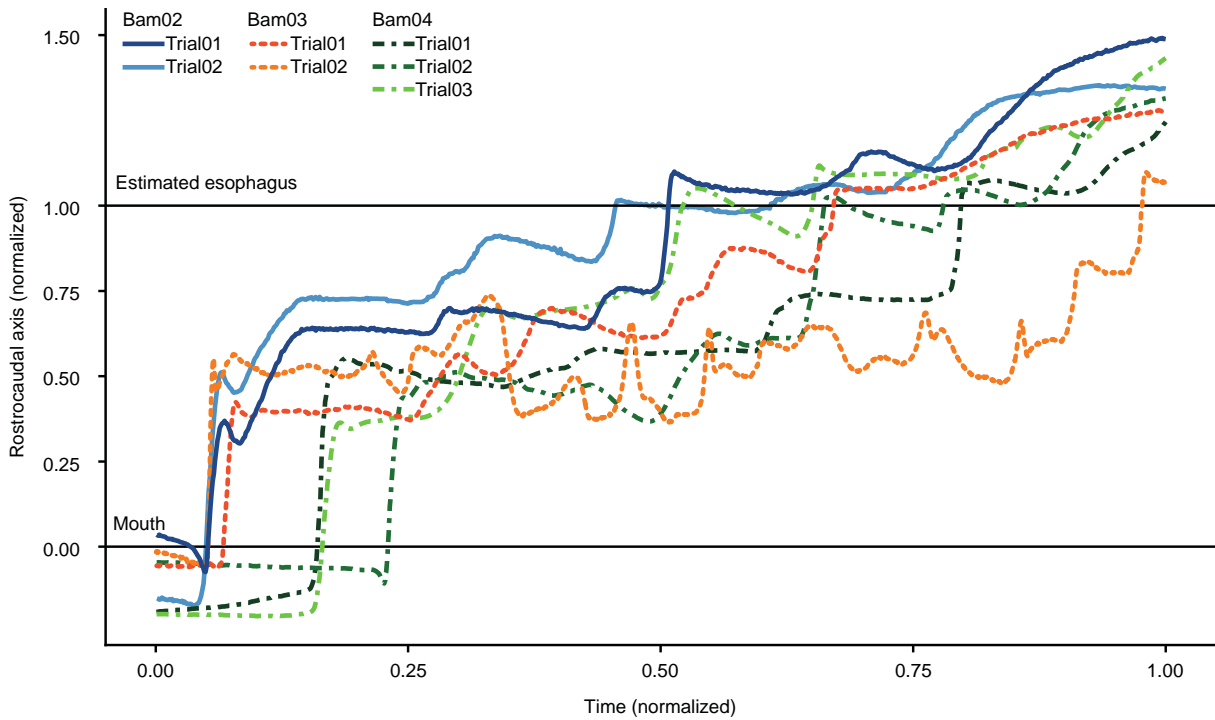
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560

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



571

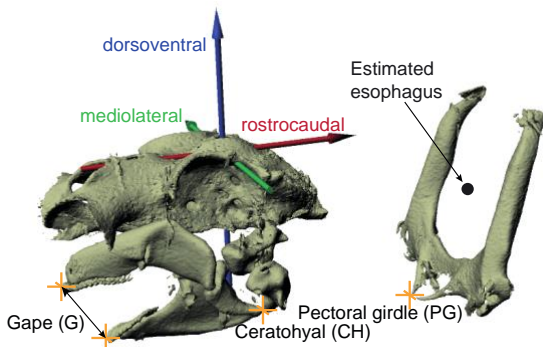
572 **Figure 3: Rostrocaudal translation of the food relative to the cranium as a function of normalized time.**

573 The y-axis represents the food's position along the rostrocaudal axis where  $y=0.0$  and  $y=1.0$  represent the mouth

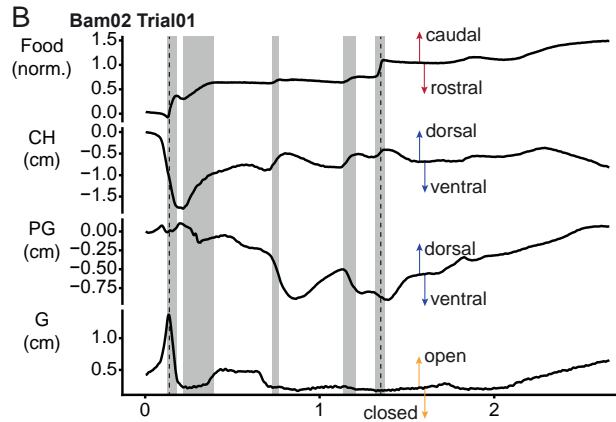
574 and pectoral girdle, respectively, as in the x-axis of Fig. 2. Time was normalized to trial length for comparison

575 among trials. Line colors correspond to trials and individuals, following Fig 2.

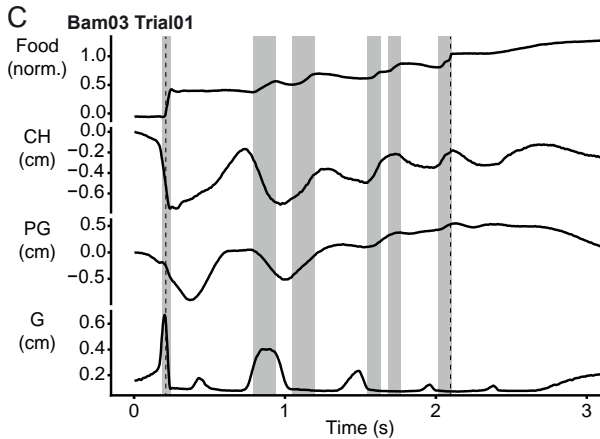
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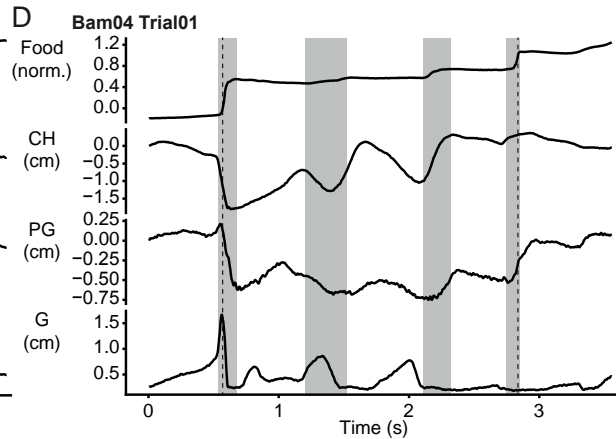
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D

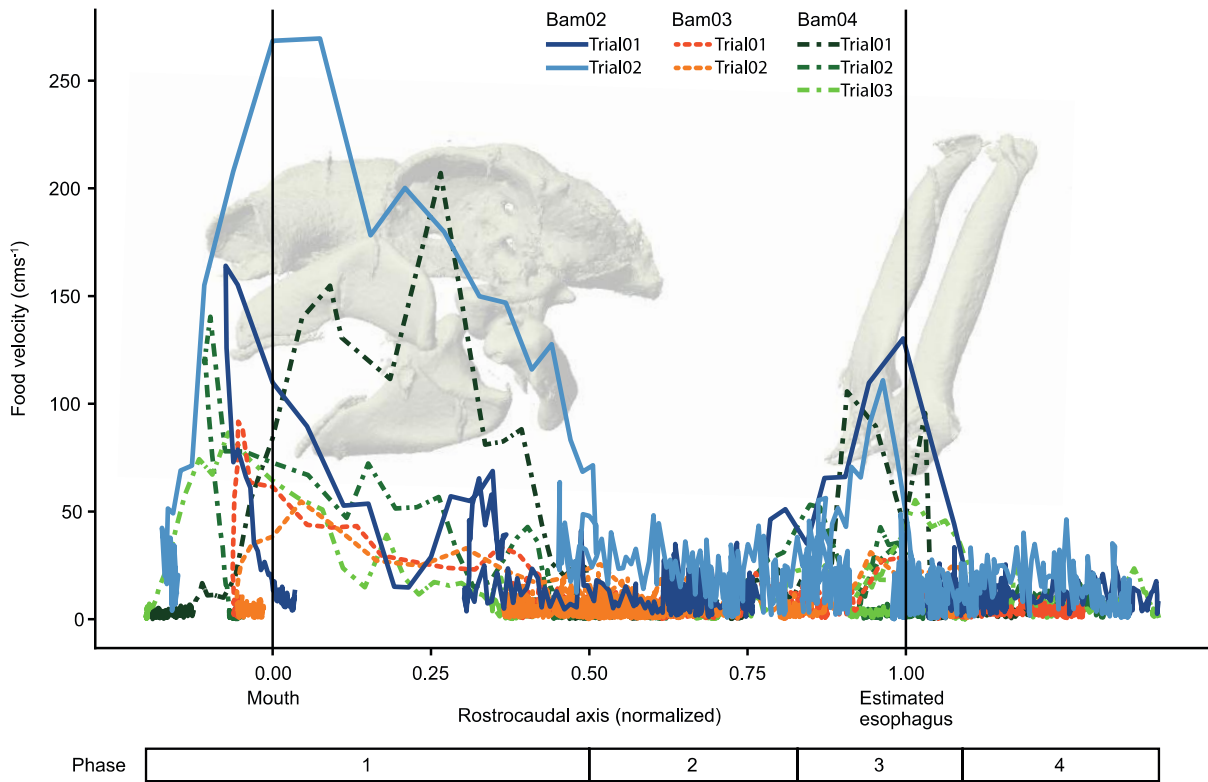


576

577 **Figure 4: Rostrocaudal translations of food, displacements of ceratohyal and pectoral girdle, and gape**

578 **width over time in a sample trial from each individual. A) Cartilages of Bam04 at peak gape, showing the**

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

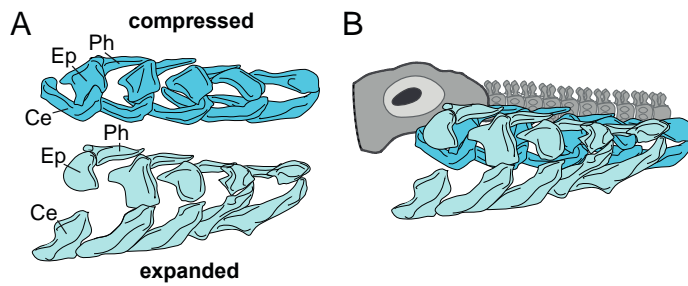


588 Phase 

1	2	3	4
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589 **Figure 5: Rostrocaudal velocity of the food relative to its position within the oropharynx.** The marked  
 590 cartilages in the background serve as an indicator of the approximate position of the food within the animal. As in  
 591 Fig. 2, the x-axis represents the food's position along the rostrocaudal axis where  $x=0.0$  and  $x=1.0$  represent the  
 592 mouth and pectoral girdle, respectively. Line colors correspond to trials and individuals, following Fig. 2. Food  
 593 motion occurred in four phases: Phase 1: prey capture, phase 2: oropharyngeal transport, phase 3: swallowing,  
 594 phase 4: after swallowing.  
 595

596



597

598 **Figure 6: Lateral-view diagram of the branchial arch anatomy and positions.** (A) Left-side of the branchial  
599 arches in the compressed (dark blue, top) and expanded (light blue) positions from CT scans. Shown in lateral  
600 view with rostral to the left. Ph: Pharyngobranchials, Ep: Epibranchials, Ce: Ceratobranchials. The ventralmost  
601 elements of the arches that make up the floor of the pharynx, the basibranchials and hypobranchials, are not  
602 visible. (B) Lateral view of the branchial arches (in blues), relative to the cranium and vertebral column (in grey).  
603