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Three-Dimensional Kinematics of the Jaws and Hyoid During Feeding in White-Spotted Bamboo Sharks

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THREE-DIMENSIONAL KINEMATICS OF THE JAWS
AND HYOID DURING FEEDING IN WHITE-SPOTTED
BAMBOO SHARKS

BY
BRADLEY SCOTT

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
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IN
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MASTER OF SCIENCE THESIS
OF
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2016

ABSTRACT

Elasmobranchs (sharks, skates and rays) use a distinct set of cartilaginous endochondral elements to capture and process prey using suction or bite mechanisms. In contrast, teleost use a complex linkage of dermal and endochondral bones to capture prey whether by suction or biting. This difference in complexity has, in part, enabled teleosts to radiate into a variety of different feeding styles where the jaw and hyoid arches are linked and function as a unit. However, elasmobranchs have decoupled depression of the lower jaw from depression of the hyoid arch and expand the buccal cavity using a different mechanism than teleosts. White-spotted bamboo sharks, *Chiloscyllium plagiosum*, are trophic generalists that use suction and crushing behaviors to take a wide variety of prey items. This study reconstructs the three-dimensional kinematics of the mandibular and hyoid arches during suction food capture in three bamboo sharks using X-ray Reconstruction of Moving Morphology (XROMM). In this method, two biplanar high-speed x-ray video cameras are used to record skeletal motions during feeding strikes, after which tracking data is applied to animate CT scans, from which kinematic data are compared to hypotheses regarding the feeding mechanism.

This research reveals that the mandibular and hyoid arches of bamboo sharks move in previously unsuspected ways when feeding. The hyomandibular cartilage swings backward and downward up to 58° while the ceratohyal simultaneously swings backward and downward up to 66° while suction feeding. At the same time, both cartilages undergo long-axis rotation up to 50°.: the dorsal surface of the hyomandibula rotates rostrally while the dorsal surface of the ceratohyal rotates

caudally. The lower jaw supinates as the mouth opens and pronates past resting position as the jaws close. The upper jaw mirrors this motion, pronating as the mouth opens and supinating as the mouth closes. The extra degrees of rotation in the hyoid arch elements may explain the remarkable variation in feeding performance of bamboo sharks. This variation in motion of hyoid motions is achieved without limiting expansion of the oral cavity, which could comprise suction generation. Long-axis rotation of the jaws may function to enhance grasping of prey by exposing more tooth rows to the prey when feeding, while otherwise maintaining a more hydrodynamic profile. The additional motions and mechanisms of the jaws and hyoid during feeding may provide bamboo sharks with an adaptation to take to a wide variety of prey items despite specializations for suction feeding.

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I would like to thank my advisor Cheryl Wilga for her patience and attention during my studies and for introducing me to the world of biomechanics. I would also like to thank Elizabeth Brainerd for her commitment to my studies and for giving me experience working with the fantastic XROMM facilities at Brown University. To David Fastosky, thank you for keeping me in touch with my paleontology background and for never holding back on the topic at hand. I would like to thank my family and friends for their love and support, especially my father, my brother, and in memory of my mother. I would like to thank Megan Skrip for care, patience, and understanding during my program. I would like to thank the other graduate students at the University of Rhode Island for companionship, discussion, and the beer.

PREFACE

This thesis is presented in manuscript format, compliant with the guidelines for thesis submission set forth by the Graduate school of the University of Rhode Island. Each chapter is presented as a separate manuscript, with reference to Chapter 1 in Chapter 2 as it relates comparison of data relevant to both studies. Both chapters are in preparation for submission to The Journal of Experimental Biology. Chapters are divided by mandibular and hyoid arch from the same dataset because motions of the hyoid arch are mechanically related to transport of food; but motions of the mandibular arch are related to grasping of food. Therefore anatomical divisions used to separate the chapters are also related to differences in function during feeding.

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CHAPTER 1

Three-Dimensional Motion of the Hyoid Arch of White-Spotted Bamboo Sharks,

Chiloscyllium plagiosum, using XROMM

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Summary Statement

A precise three-dimensional reconstruction of motions of the hyoid arch in a suction feeding shark.

ABSTRACT

Elasmobranchs feed in a variety of ways. Much of their success comes from deriving different feeding mechanisms from the same basic components of the hyoid and mandibular arches. The mechanism is then dependent on movements of skeletal elements during feeding. For suction-feeding elasmobranchs (sharks, skates and rays), ventral expansion of the hyoid cavity through depression of the hyoid arch generates most of the suction force used to capture prey. Three-dimensional movements of the hyoid arch of white-spotted bamboo sharks, *Chiloscyllium plagiosum* Bennet 1830, are quantified using X-ray Reconstruction Of Moving Morphology (XROMM). Three axes of rotation and were reconstructed for the hyomandibula and ceratohyal, along with translation of the distal end of each element. Long-axis rotation in elements of the hyoid arch of an elasmobranch is observed for the first time. As the ceratohyal and hyomandibula depressed, the hyomandibula also rotated rostrally from its proximal end and the ceratohyal rotated caudally. In addition, the hyomandibula rotated around its long-axis as it depressed. The dorsal margin of the ceratohyal rotates rostrally then caudally as the ceratohyal depresses. Long-axis rotation of the hyomandibula is predominantly in the opposite direction of the ceratohyal. Rostral rotation of the hyomandibula may contribute to rostral jaw protrusion in elasmobranchs and long-axis rotation of the hyomandibula and ceratohyal may increase rotational motions,

specifically depression, of the hyoid arch by permitting reorientation of the elements during hyoid depression, but had little effect on protrusion/retraction of the hyoid arch. Bamboo sharks are able to vary the timing and extent of protrusion and retraction relative to depression of the hyoid. This can allow bamboo sharks to adjust their feeding motions according to prey size or environment, enabling them to feed on variety of prey items, despite a highly specialized suction mechanism.

INTRODUCTION

Elasmobranchs (sharks, skates and rays) are capable of feeding using a variety of strategies. The success of a feeding strategy is directly dependent on the mechanism used to obtain prey. Numerous mechanisms have been proposed for feeding, based on morphological and ecological aspects of different shark species; however, kinematic tests of feeding mechanisms remain few, and are typically limited to motions along a single plane (e.g. Motta *et al.* 2002; Wilga and Sanford 2008). The feeding mechanism of elasmobranchs consists of a mandibular arch (the jaws) and a hyoid arch (a derived gill arch that supports the jaws; Mallatt 1996). The orientation and motions of the mandibular and hyoid elements determines the feeding mechanics of different shark species (Motta *et al.* 1997; Motta and Wilga 1999; Wilga 2008). These elements are loosely connected to the cranium and highly kinetic. As such, motions of the skeletal elements of the head can be complex and three-dimensional. The skeletal motion of suction-feeding sharks is of particular interest because the mechanism appears multiple times among elasmobranchs and directly converges on the condition in

teleost fishes (Motta *et al.* 2008; Wainright *et al.* 2015) and the sequence of motions are highly stereotyped as a result of strong physical constraints (Wilga *et al.* 2012).

Suction-feeding sharks, such as the white-spotted bamboo shark, are capable of equal or even lower oral pressures than teleost fishes, but powered primarily through expansion of their hyoid region (as compared to expansion of hyoid and mandibular elements in teleost fishes, Day *et al.* 2015; Lauder 1980; Wainright *et al.* 2007; Wainright *et al.* 2015) and despite using fewer skeletal elements (Nauwelearts *et al.* 2008; Ramsay and Wilga, 2007; Wilga 2008; Wilga and Sanford 2008; Wilga *et al.* 2007). At the same time bamboo sharks, are also capable of feeding on a variety of prey (Compagno 1984; Lowry and Motta 2007). The broad diet of bamboo sharks is a possible cause in high variation in suction performance. This variation may result from different mechanisms accommodate the capture of different prey types. The hyoid arch may therefore undergo motions beyond those that expand the oral cavity including rostral/caudal (forward/backward) motions of the hyomandibula and ceratohyal. To test for these additional motions we measured the three-dimensional motions of the hyoid elements during feeding in the white-spotted bamboo shark. This study uses X-ray Reconstruction of Moving Morphology (XROMM; Brainerd *et al.* 2010) to track the rotations and translations of elements of the hyoid arch in relation to the cranium during expansion of the oral cavity.

The cranial skeleton of elasmobranchs (sharks, skates and rays) such as bamboo sharks consists of a chondrocranium, a mandibular arch consisting of a palatoquadrate and Meckel's cartilage, and a hyoid arch (Fig. 1A). The palatoquadrate is suspended from the cranium by a ligament at the rostral end and from the distal end

of the hyomandibula at the jaw joint, allowing the upper and lower jaws to protrude away from the cranium (Fig. 1A, Moss 1972; Motta and Wilga 1999). The hyoid arch consists of a hyomandibula and ceratohyal on both sides and a single median basihyal between the ceratohyals (Fig. 1A, C; Moss 1972; Motta and Wilga 1999).

For suction-feeding sharks the motions of the hyoid arch generate the forces that draw water and prey into the mouth (Motta *et al.* 2002; Wilga 2008; Wilga and Sanford 2008). The mandibular and hyoid arches are able to move independently, connected only at the hyoidiomandibular joint through a series of ligaments. Transverse motions of the hyoid elements are responsible for expanding the buccal cavity during suction-feeding (Wilga 2008; Wilga and Sanford 2008); however, other mechanisms may be involved in feeding beyond suction. In bamboo sharks the buccal cavity compresses laterally by adduction of laterally directed hyomandibulae (Fig. 1C, D; Wilga 2008; Wilga and Sanford 2008). At the same time the buccal cavity is expanded ventrally by depression of the ceratohyal (Wilga 2008; Wilga and Sanford 2008). Longitudinal motions of the hyomandibula and ceratohyal have been observed in other shark species, including those closely related to bamboo sharks (Wu 1994; Ramsay 2012). These motions consist of a backwards rotation (retraction) of the ceratohyal and basihyal and a forward rotation (protrusion) of the hyomandibuloceratohyal joint (Fig. 1B; Ramsay 2012; Wu 1994). Protrusion of the hyomandibula may then protrude the lower jaws, a possible mechanism for moving the jaws closer to prey during feeding (Wu 1994).

Transverse motions of the hyoid elements relate to expansion of the buccal cavity and have been well studied because of this; however, the rostro-caudal motions,

particularly at the hyomandibuloceratohyal joint (Fig. 1A) are linked to protrusion of the jaws (Wu 1994). Jaw protrusion follows prey capture in *Orectolobiformes* and does not appear to relate to suction in elasmobranchs (Motta *et al.* 2008). As prior studies on bamboo sharks have focused on the effects of hyoid mechanics on suction, these rostrocaudal motions have gone largely unexamined.

Based on manipulation of deceased specimens the hyomandibuloceratohyal joint of *Chiloscyllium plagiosum* may move rostrally simultaneous to ceratohyal depression (Fig. 1B; Ramsay 2012) as in other orectolobiforms (Wu 1994; Motta *et al.* 2008), contributing to rostral jaw protrusion and jaw depression (Wu 1994). In *Orectolobus* rostral protrusion of the proximal end of the ceratohyal depresses and protrudes the lower jaw rostrally via an articulation with the mandibular knob (Wu 1994). A similar mechanism could be present in *Chiloscyllium*; however, the mandibular knob is smaller than in *Orectolobus* and the hyomandibula, rather than the ceratohyal, articulates directly with the Meckel's cartilage (Ramsay, 2012). In the morphologically similar nurse shark, ceratohyal depression follows jaw depression (Motta *et al.* 2002; Motta *et al.* 2008) and any connected hyomandibula protrusion would not contribute to leverage of the lower jaw during depression. Instead hyomandibula motion would contribute rostral protrusion of the jaws.

The way that longitudinal and transverse motions of the hyoid arch interact during feeding has not been measured for any elasmobranch. Motion of hyoid elements through longitudinal and transverse planes is complex, both as a result of operating in three-dimensions, but also as a result of articulations at multiple joints. Complex multi-planar motions can result in long-axis rotation of skeletal elements, an

additional degree of motion that has not been described or hypothesized for hyoid elements (Kambic *et al.* 2014). Long-axis rotation of cranial elements has not been studied for aquatic vertebrates; however, the limbs of walking birds exhibit long-axis rotation during complex multiplanar motions (Kambic *et al.* 2014). Long-axis rotation can increase range of motion of skeletal elements, as well as permit complex maneuvering behavior (Kambic *et al.* 2014). Depression of the ceratohyal not only reorients it relative to the cranium, but creates a complex series of changes in the orientation of the basihyal and hyomandibula (Fig. 1). Long-axis rotation in the hyomandibula and ceratohyal may act to maintain pitch of the basihyal, the insertion for muscles that depress the hyoid arch (Motta and Wilga 1999; Ramsay 2012) relative to the cranium, or increase the possible range of motion during ceratohyal depression.

Measuring three-dimensional motions of the hyoid arch is difficult. Thick muscle and skin of the head prevents the use of video (Motta *et al.* 2002). Sonomicrometry can measure internal motions with high precision (Wilga and Sanford 2008); however, measurements beyond a single plane require implantation of numerous crystals and complex calculations to infer direction simply from changes in distance. The low density of cartilage relative to water has also inhibited radiocinematographic analysis of hyoid elements (Wu 1994). X-ray Reconstruction of Moving Morphology (XROMM, Brainerd *et al.* 2010) tracks skeletal elements, here using dense markers in the hyoid and jaw elements, and biplanar high speed x-ray video to record and reconstruct motions in three dimensions (Camp *et al.* 2015; Camp and Brainerd 2015; Gidmark *et al.* 2012).

Bamboo sharks move the hyoid elements transverse to the cranium to generate suction; however, bamboo sharks feed on a broad variety of prey and show a high degree of variation in suction performance. Additional mechanisms, specifically protrusion of the hyomandibuloceratohyal joint for prey capture may account for variation in diet and performance. Three-dimensional kinematics of the hyomandibula and ceratohyal of a suction-feeding elasmobranch are recorded in-vivo for the first time, including the magnitude and timing of rotation and translation. XROMM was used to reconstruct three-dimensional motions of the hyoid elements. Kinematic data was used to test for protrusion of the hyomandibula and long-axis rotation of the hyomandibula and ceratohyal during suction. If protrusion of the hyomandibula corresponds with prey capture then it is predicted to occur during or prior to peak gape. Long-axis rotation is predicted to result from protrusion/retraction and depression of the hyomandibula and ceratohyal and greater long-axis rotation is predicted to correspond to increased range of motion, including greater magnitude protrusion/retraction and depression. In addition variation in hyoid motions is compared across multiple individuals, variation is predicted to be high, corresponding to previous reports of variation in suction performance in bamboo sharks (Wilga and Sanford 2008; Wilga *et al.* 2012).

MATERIALS AND METHODS

Implantation

Three conical tungsten carbide markers (Kambic *et al.* 2014) were implanted in each of the cranium, palatoquadrate, Meckel's, hyomandibula, and ceratohyal on the left

side of the head (Fig. 2). Three individuals of *Chiloscyllium plagiosum* (78.6 cm, 79.2 cm, 85 cm; craniums 6.47 cm, 6.35 cm, 6.58 cm long respectively) were implanted. The sharks were anaesthetized with 0.033 gL⁻¹ of tricaine methanesulfonate (MS-222) in a recirculating fish anaesthesia system (Wilga and Sanford 2008). All experimental procedures were approved by Institutional Animal Care and Use Committees of Brown University and the University of Rhode Island. All sharks recovered to feed normally following implantation. Prior to feeding trials food was withheld from individuals for one week.

CT Scans

Each shark was CT scanned to determine marker positions and create models of the cranium and each element in the mandibular and hyoid arches (the basihyal was unmarked). Each individual was anaesthetized with 0.033g/L of Tricaine methanesulfonate (MS-222) in seawater. Individuals were then placed in a FIDEX CT scanner (Animage) at Brown University and scanned at a resolution of 448 X 448 pixels (416 X 416 pixels for Bamboo 3) and a slice thickness of 0.185 mm. CT image series were compiled and reconstructed using Osirix (Pixmeo, Geneva, Switzerland). Three-dimensional surface meshes of the cranial, Meckel's, palatoquadrate, hyomandibula, and ceratohyal cartilages with implanted markers were exported to Maya (2015; Autodesk). The centroid of the vertices of each marker was calculated using the vertAvg tool in the XROMM_tools shelf add-on for Maya (2015; Autodesk). The tool shelf is available at xromm.org. Centroids of markers are exported from Maya as CT coordinates (CTex tool).

Video data collection

Feeding trials were performed at the W.M. Keck Foundation XROMM Facility at Brown University. Individuals were fed pieces of either squid or herring for each trial. Trials began with food placed in front of an individual. X-ray recording would begin as the individual moved toward the food. Feeding trials are recorded by a pair of Phantom v. 10 X-ray high speed video camera (Vision Research), each with a linked X-ray emitter and fluoroscopic image intensifier (Imaging Systems and Service; Brainerd *et al.* 2010). The pair of fluoroscopes recorded the motion of implanted markers at 320 Hz or 330 Hz, with X-ray energies of 110 or 120 kV and 100mA.

For marker-based XROMM animation we followed previously published methods (Brainerd *et al.* 2010; Kambic *et al.* 2014). Marker position was tracked for both cameras, across all trials using XMALab (open-sourced software authored by Ben Knörlein; bitbucket.org/xromm/xmalab). Three-dimensional translation of markers was calculated in XMALab. CT coordinates were imported into XMALab (impdata tool) and assigned to the appropriate markers. Based on the translation of each marker in an element the translation and rotation of the element was calculated and exported assuming the element was a rigid body (i.e. there was no strain). Rigid-body transformations exported from XMALab were then imported into Maya and applied to the meshes of elements rendered from the CT scans.

Analysis

A joint coordinate system (JCS, Brainerd *et al.* 2010; Gidmark *et al.* 2012; Kambic *et al.* 2014; jointAxes tool, XROMM_tool_shelf for Maya, xromm.org) was used to calculate the rotation of each element during a feeding trial. Joint coordinate systems consist of two sets of x, y, and z axes (Fig. 3). One set of axes tracked the motion

rotation along the x, y, and z axes of the proximal element (here the cranium) and the other axis tracks the motion of the distal element (here the hyomandibula, Fig. 3A and ceratohyal, Fig. 3B). Together the difference in motion between the two axes gives the motion of the distal element relative to the proximal element (Fig. 3). Motion of the hyomandibula and the ceratohyal relative to the cranium was calculated. The x-axis is through the long-axis, the y-axis is vertical, so that rotations around it represent rostral/caudal rotations of the distal end of the element. Rotations around the z-axis are depression (negative) or elevation (positive) of the distal end of the ceratohyal or hyomandibula.

The long-axes (x-axis) of the hyomandibula and the ceratohyal pointed in opposite directions. Joint coordinate systems of both elements were set at the proximal end of each element. For the hyomandibula this was at the cranium, with the long-axis pointing laterally. For the ceratohyal this was the hyomandibuloceratohyal joint, long-axis pointing medially towards the basihyal. The difference in direction of the long axes would give opposite directions for long axis rotations and protrusion/retraction, such that a positive rotation of the long-axis, of the hyomandibula was dorsal margin rostrally while a positive rotation of the long-axis of the ceratohyal was dorsal margin caudally. To maintain consistency of direction, polarities were reversed for the x and y axes of the hyomandibula, so that protrusion is positive and positive long-axis rotation is a caudal rotation of the dorsal margin for both hyoid elements. For each element the timing in milliseconds and magnitude of rotation in degrees along all three axes relative to peak gape was measured. For the hyomandibula, timing and magnitude of peaks were recorded for negative x-axis rotation, positive y-axis rotation, and negative

z-axis rotations. Peaks in timing and magnitude of negative and positive x-axis rotation, negative y-axis rotation, and negative z-axis rotation were recorded for the ceratohyal. Four trials were analyzed for each individual for a total of 12 feeding trials. Timings are recorded relative to peak gape as the majority of hyoid expansion takes place during gape compression, compared to teleosts where gape and hyoid expansion are directly linked and can both be encompassed simply as expansive and compressive phases.

An Anatomical Coordinate System (ACS) was used to measure translation (Brainerd *et al.* 2010; Gidmark *et al.* 2012) of the distal ends of the left hyomandibula and left ceratohyal. Translational motions of these elements were recorded along a single set of x, y, and z axes aligned orthogonal to the cranium. Locators at the distal ends of the left hyomandibula and left ceratohyal along those axes relative to the cranium. Positive x-axis motion is rostral, while negative motion is caudal. The y-axis points dorsally, so that positive motions are dorsal, and negative motions are ventral. Medial translations are positive along the z-axis. Negative z-axis translations represent lateral translation of the locators. Peak timing and magnitude of translation of the distal end of the hyomandibula was measured in rostral, ventral, and medial directions. For the distal end of the ceratohyal, rostral and ventral translation was measured. Medial translation of the distal end of the ceratohyal was also measured, but only varied from zero in some trials, and medial translations were small because the distal end of the ceratohyal is directly limited by articulation with the basihyal.

Statistical Analysis

To test for intraspecific differences in rotations for both the hyomandibula and the ceratohyal, single factor ANOVAs were performed using the General Linear Model in SAS 9.4 software (SAS Institute, Cary NC). The ANOVAs compared timing of the peak for rotational axes of each element, and comparing magnitude of the peak rotation for axes of each element. Individuals were the factor and time and magnitude were the measured variables for each axis of rotation of the ceratohyal and the hyomandibula. Specifically based on observed direction of peak motion, the significant differences between individuals were tested for time and magnitude of: negative peak in long-axis rotation, protrusion (positive) peak rotation, and peak depression (negative) of the hyomandibula and ceratohyal, as well as negative long-axis rotation of the ceratohyal (Table 1). ANOVAs were also used to assess peak rostral and ventral translations of the distal ends of the hyomandibula and ceratohyal and medial translation of the hyomandibula. For trials with significant ($p < 0.05$) individual differences a Tukey adjusted multiple comparisons test was used to determine which individuals varied significantly (Table 2).

Multiple linear regression analyses were used to test for effects of ceratohyal long-axis rotation on ventral rotation of the ceratohyal, caudal rotation of the ceratohyal, and ventral translation of the distal end of the ceratohyal. Magnitude of long-axis rotation was the independent variable while magnitude of depression was the dependent variable. Only the 8 trials with distinct positive long axis rotations were used for regression analysis of ceratohyal motions. Regression analysis tested effects of long-axis rotation of the hyomandibula on ventral rotation and rostral rotation of the

hyomandibula. All twelve trials were used in the regression analysis of the hyomandibula.

Four trials that did not exhibit positive long-axis rotation, the dominant direction during depression in all other trials, were excluded from the regression analysis of ceratohyal motions. Three of the trials for one individual exhibited a greatly reduced positive long-axis rotation of the ceratohyal (see discussion). Two post-hoc single tailed t-test adjusted for heteroscedasticity (Welch 1947) with a Bonferonni adjusted $\alpha=0.025$ determined whether this also significantly reduced depression of the ceratohyal. Another trial for Bamboo 1 is excluded from the t-tests because although there is a positive peak during the compressive phase it also does not exceed the resting position. Levene's test and the Shapiro-Wilke test were used to test for homogeneity of variance and normality of the distribution respectively, for each ANOVA.

RESULTS

Hyoid motions during feeding

Bamboo sharks captured food using inertial suction in all trials and transported the food to the back of the mouth in a single motion in eleven of the twelve trials. For a single trial prey was sucked to the mouth, grasped and then transported in a second motion. Very little cranial elevation was observed during feeding strikes, but the head was positioned so that the food was directly in front of the mouth.

Feeding began with the mouth opening by depression of the Meckel's cartilage (Fig. 4 between -100 and 0 ms). As the Meckel's cartilage depressed and gape increased the ceratohyal began to retract and depress, and the hyomandibula began to

rotate such that its distal end depressed ventrally and protruded rostrally (most clear in Fig. 4B, at -22 and 0 ms). At peak gape (Fig. 4, 0 ms), the palatoquadrate and Meckel's cartilage were somewhat protruded and the hyomandibula had not yet reached their peak rotations. As the hyomandibula and ceratohyal continued to depress after peak gape the distal end of the hyomandibula and the proximal end of the ceratohyal translated medially, constricting the hyoid cavity as depression of the ceratohyal expanded the cavity ventrally (Fig. 4B, C at 56 ms). The distal end of the ceratohyal moved caudally as it depressed, retracting past the hyomandibuloceratohyal joint (Fig. 4A, B at 56 ms). At the same time that the ceratohyal retracted, the distal end of the hyomandibula and the proximal end of the ceratohyal protruded rostrad, pushing the jaws to their most protruded position at 56 ms. Retraction and depression of the ceratohyal, and rotation of the hyomandibula peaked following peak gape (Fig. 4, at 56 ms), typically beginning their recovery just before the jaws were fully closed and continuing after the jaws are closed (Fig. 4 at 288 ms).

Rotations from joint coordinate systems (JCSs)

The hyomandibula and ceratohyal elements are nearly cylindrical making it difficult to visualize long-axis rotation in the XROMM animations. Data from the JCSs, however, show substantial long-axis rotation in both elements (Fig. 5). The hyomandibula and ceratohyal rotated in opposite directions, with the dorsal margin of the hyomandibula moving rostrally and the dorsal margin of the ceratohyal moving caudally. The magnitude of long-axis rotation of the hyomandibula is greater than protrusion and depression, with means ($\pm$ standard error of mean, s.e.m.) of -34.4 ± 3.2 , 29.8 ± 1.9 and -28.2 ± 3.6 degrees respectively (Table 1). Initially the dorsal margin of

the ceratohyal rotated slightly rostrally, but then made a greater caudal rotation (Fig. 5B). Rostral long-axis rotation of the ceratohyal was small, -12.22 ± 2.0 deg, and caudal long-axis rotation was greater, 21.2 ± 5.0 deg, but smaller than retraction and depression at -43.4 ± 2.0 and -47.0 ± 4.4 deg, respectively.

The mouth began to open substantially before the hyoid elements began to depress, and the majority of ceratohyal and hyomandibula motion occurred after peak gape (Fig. 5). All three rotations of both the ceratohyal and hyomandibula peaked at about 50 ms after peak gape, but with a wide range of variation (Table 1).

Translations of points in an anatomical coordinate system (ACS)

The rotations of the hyoid elements generated a corresponding translation of the distal end of the ceratohyal and the distal end of the hyomandibula where it articulates with the ceratohyal at the hyomandibuloceratohyal joint (Fig. 6). Protrusion of the hyomandibula (Fig. 5A rotation about Y-axis) resulted in a rostral translation of the distal end of the hyomandibula (green trace, Fig. 6A) by a mean of 7.2 ± 0.5 mm (Table 2). At the same time depression moved the distal end of the hyomandibula -6.2 ± 0.7 mm ventrally and 6.8 ± 0.6 mm medially, constricting the buccal cavity as the distal end of the ceratohyal translated -12.8 ± 0.7 mm ventrally to expand the buccal cavity (Table 2). Ceratohyal rotation about its Y-axis moved the distal end caudally by 13.3 ± 1.1 mm. In the trial shown there is also a lateral translation of the distal end of the ceratohyal (Fig. 6B) that is not seen in all trials.

Variation among individuals

Individual variation was common for motions of the hyoid arch. Significant levels of individual variation can be seen as p-values in Tables 1 (rotations) and 2

(translations) and how individuals vary in timing and magnitude of motions is shown in Table 3 as tukey-adjusted multiple comparisons. Presence or absence of positive, or dorsal to caudal, long-axis rotation not only affected results for individual variation of ceratohyal long-axis rotation, but may have affected other factors. Trials where positive long-axis rotation of the ceratohyal was well past resting position had greater depression of the ceratohyal ($p=0.0002$) than trials with no positive rotation of the ceratohyal past resting position. Most of the trials without dorsal-to-caudal long-axis rotation of the ceratohyal were by Bamboo 3. As a result variance was high for timing of maximum positive long-axis rotation (28738, Table 1), and timing of peak negative long-axis rotation was later (ANOVA p -value=0.0029) for Bamboo 3 than the other individuals (Table 3). Magnitudes of ceratohyal long-axis rotation also differed significantly, as positive long-axis rotation was greater in Bamboo 2 than Bamboo 3 and negative long-axis rotation was higher magnitude in Bamboo 3 than either of the other individuals. Bamboo 1 retracted and caudally translated the ceratohyal earlier than other individuals, and non-long-axis rotations were typically of lower magnitude in Bamboo 1 and Bamboo 2 than rotations of Bamboo 3 (Table 3).

Regression

Least squares linear regression analysis (Table 3) showed a strong relationship between magnitude of ceratohyal long-axis rotation and ceratohyal depression ($R^2=0.83$, p -value=0.002, coefficient=-0.64) but ceratohyal retraction had no relationship with either long-axis rotation ($R^2=0.10$, p -value=0.44, coefficient=-0.16) or depression ($R^2=0.17$, p -value=0.31, coefficient=0.57). Hyomandibula long axis rotation varied strongly with hyomandibula depression ($R^2= 0.93$, p -value<0.0001,

coefficient=-1.08), but not protrusion (rostral rotation, $R^2=0.001$, p-value=0.922, coefficient=0.02) and protrusion had little to no relationship with hyomandibula depression ($R^2=0.021$, p-value=0.948, coefficient=0.04)

DISCUSSION

The three-dimensional motions of the hyoid arch of white-spotted bamboo sharks extend beyond those previously associated with suction. Motions along a plane transverse to the cranium are attributed to generating suction; XROMM analysis confirms compression of the hyoid arch through adduction of the hyomandibula (Fig. 1D, 4C) simultaneous to depression of the ceratohyal (Wilga 2008; Wilga and Sanford 2008), as well as additional degrees of motion of the hyoid elements. This study also finds longitudinal motions including protrusion of the hyomandibula and retraction of the ceratohyal (Wu 1994; Ramsay 2012) are present during feeding. Long-axis rotations of the hyoid elements result from these complex motions and are linked to an increased depression of the hyomandibula and ceratohyal. This study provides the first report of long-axis rotation of the hyoid elements of any vertebrate. Protrusion of the hyomandibula does not appear to correspond to prey capture, occurring too late in the feeding sequence; however, the extra degrees of motion are highly variable along with ceratohyal depression. The high variability in both direction and magnitude of motion of the hyoid arch may explain the high variation observed in suction performance of bamboo sharks (Wilga and Sanford 2008; Wilga *et al.* 2012).

Protrusion

The hyomandibula of *C. plagiosum* protrudes as the ceratohyal depresses and retracts (Wu 1994; Ramsay 2012), as in other orectolobiform shark species. Protrusion of the hyomandibula pushes the mandibular knob and the jaw joint rostrally (Fig. 1B); however, protrusion of the hyomandibula follows lower jaw depression, and therefore does not contribute to jaw opening mechanics during feeding. As in other elasmobranchs the timing of protrusion is also too late to enhance suction forces (Motta *et al.* 2008; Wilga and Sanford 2008) through rostral protrusion of the jaws (Holzman *et al.* 2007).

Protrusion of the hyomandibula coincides with the retraction and depression of the ceratohyal, and may be an important component of the buccal expansion mechanism of *C. plagiosum*. By protruding the hyomandibuloceratohyal joint rostrally the horizontal rotational axis (Z) of the ceratohyal is shifted so that as the ceratohyal retracts the distal end of the ceratohyal is being constantly depressed. If there was no protrusion then, as the ceratohyal retracted, it would achieve peak depression ventral to the hyomandibuloceratohyal joint, elevating or remaining level as the ceratohyal continued to retract. Instead retraction and depression peak at nearly the same time in all trials. Simultaneous peak in retraction and depression allows bamboo sharks to increase buccal volume throughout the contraction of the hyoid depressors connecting the ceratohyal and basihyal to the pectoral girdle. While hyoid and buccal pressure peaks at or before peak gape (Wilga and Sanford 2008), water continues to flow into the buccal cavity until the jaws close. Continuous expansion of the buccal cavity can prevent backflow and any subsequent prey escape until the jaws are closed (Muller and Osse 1984).

Long-axis rotation

Long-axis rotation of the hyoid elements had not been recorded for any other vertebrate, but based on this study may be a key component of the rotational motions of the hyoid arch during expansion and compression of the buccal cavity of elasmobranchs. The degree to which dorsal margin of the hyomandibula rotates rostrally is directly related to the degree of depression of the hyomandibula (slope= 1.08, Table 4), so that long-axis rotation is an integral part of the depression mechanism and therefore adduction of the hyomandibula. A similar relationship is seen with the depression and dorsal to caudal long-axis rotation of the ceratohyal, though less extreme (Table 4), and more varied across all trials and the proximal articulation of the ceratohyal is less constrained by hyomandibula than the proximal end of the hyomandibula is by the cranium. At the joint with the chondrocranium the hyomandibula has a pair of articulations (Motta and Wilga 1999); it may be that the hyomandibula must rotate around its long-axis to maintain contact with both theses articulations during depression.

Positive Long-axis rotation of the ceratohyal, where the dorsal margin rotates caudally (Fig. 7), has a strong relationship with depression of the ceratohyal (Table 4). While the ceratohyal will still depress in the absence of positive long-axis rotation, depression is stronger when long-axis rotation is positive, and greater dorsal-to-caudal rotation is associated with greater depression. Feeding attempts with only negative (dorsal margin rotating rostrally) long-axis rotation showed lower depression, but still sufficient for a successful strike. It is worth noting that ventral translation did not vary significantly among individuals including trials those trials without positive long-axis

rotation, suggesting that during those food captures there was an alternative successful mechanism for lowering the distal end of the ceratohyal beyond just ceratohyal depression while rotating the dorsal margin caudally.

Long-axis rotation may reorient the distal end of the ceratohyal relative to the basihyal. As the basihyal descends and moves caudally it may reorient so that the rostral end depresses relative to the caudal end, or it may stay level relative to the cranium. If the basihyal stays level during depression and retraction the distal end of the ceratohyal must slide rostrally along the basihyoceratohyal joint because the proximal end of the ceratohyal is protruding rostrally with the distal end of the hyomandibula. Based on the CT scans of each individual the distal end of the ceratohyal is roughly triangular in shape. The three points of the triangle are rostroventral, caudoventral and caudodorsal. The caudodorsal point rests behind and underneath the basihyal. Rostral translation of the distal end of the ceratohyal along the basihyoceratohyal joint is not possible while caudodorsal tip of the ceratohyal stays level with the basihyal. If this was the case, the caudodorsal point of the ceratohyal would be forced laterally along the basihyal, rotating the rostral margin of the distal end of the ceratohyal into the basihyal or disarticulating the caudodorsal end of the ceratohyal from the joint.

Positive long-axis rotation would rotate the caudodorsal point of the distal end of the ceratohyal caudal and ventral to the basihyal, as the basihyal is retracted, allowing the distal end of the ceratohyal to maintain contact with the basihyal during hyoid arch expansion. This scenario requires that positive long-axis rotation of the ceratohyal should coincide with retraction and depression of the ceratohyal, as was

observed here. If the rostral end of the basihyal is depressing then the ceratohyal should have negative long axis rotation as the caudodorsal point of the distal end follows the articulation of with the basihyal rostrally. Negative long-axis rotation was observed prior to maximum gape in all trials and continued through ceratohyal depression and retraction in three trials. Both orientations of the basihyal during hyoid expansion are therefore likely to be possible, and both mechanics successfully captured food through suction. Rather than being integral to the suction mechanism itself, long-axis rotation of the hyomandibula and ceratohyal may instead allow for variation in the timing and extent of ventral expansion and lateral compression of the hyoid arch during feeding.

Depression of hyoid elements may be closely linked to long-axis rotation in hyomandibula and the ceratohyal, but the amount of protrusion/retraction appears to vary independent of depression or long-axis rotation (Table 4). The rostro-caudal motions of the joints of the hyoid arch are timed closely with the depression of the hyoid elements, but the magnitudes of the motions appear to be independent of depression or long-axis rotation, such that how much the hyomandibula depresses does not affect the amount that it protrudes rostrally (Table 4). Decoupling of the magnitudes of protrusion and depression in the expansion mechanism may account for the high variability in suction pressures that characterizes bamboo sharks as a functional generalist (Wilga *et al.* 2012). Minor changes in head shape are estimated to produce noticeable changes in pressures (Van Wassenbergh *et al.* 2006), so increased protrusion/retraction of hyoid elements may lengthen the buccal cavity and alter pressure profiles during strikes, increasing or decreasing pressure, or shifting the

timing of peak pressure according to possible prey items. Variation in the kinematic variables driving buccal expansion and suction mechanics in bamboo sharks may be one of the main characteristics that allow to adjustments their highly specialized feeding strategy (Wilga et al. 2007; Wilga et al. 2012) for a wide variety of prey (Compagno 1984). Further research focusing on the suction performance of elasmobranchs should incorporate three dimensional kinematic reconstructions of the hyoid elements with changes in oral volume and in-vivo measurements of pressure (Camp *et al.* 2015)

Long-axis rotation may also have broader implications for estimates of loading and force distribution across and between elements during suction capture events. Long-axis rotation may transmit compressive or tensile forces from the elements to the ligamentous connections that support and connect the feeding apparatus of bamboo sharks and other suction feeding shark species (Ramsay 2012; Motta and Wilga 1999), reducing the load during suction events. A redistribution of forces could have particular significance for the hyomandibula as it is exceptionally stiff compared to other shark species and is expected to be under exceptional load (Balaban *et al.* 2014).

Individuals varied considerably in amount of rotation of the hyomandibula and ceratohyal. Variation in motions of these elements may also explain the variation in pressure from other bamboo shark feeding studies (Wilga and Sanford 2008; Wilga *et al.* 2012). This variation was less apparent in translation of the distal ends of the same elements, so it is possible that differences in motions between individuals are adjustable depending on position or type of prey, but the overall suction mechanics

constrain the changes in dimensions of the oral cavity to ensure sufficient flow for capturing and keeping prey.

CONCLUSION

Bamboo sharks are dietary generalists, despite capturing prey almost entirely through suction. The general diet of bamboo sharks has been attributed to their ability to vary suction performance. Suction is generated by movement of the hyoid arch. This study describes additional degrees of motion beyond those previously attributed to suction. These motions vary considerably in magnitude and may explain the high variation in suction performance.

Figures

Figure 1: Proposed motions of the hyoid arch, including the hyomandibula (dark grey) and ceratohyal (light grey) of a white-spotted bamboo shark during a feeding event (Wu, 1994), and (Wilga, 2008). A) Cartilaginous elements of the head, in left lateral view. B) Cartilaginous elements at maximum gape and hyoid depression. The distal end of the ceratohyal moves ventrally and caudally (thick black arrows 1) as the proximal end moves rostrally and medially (black arrow 2). C) Anterior view of the cranium and hyoid elements in resting position and D) depressed position, with the arch expanding ventrally (black arrows 1) and constricting medially (black arrows 2). **By**, Basihyal; **Cr**, Cranium; **Cy**, Ceratohyal; **Hy**, Hyomandibula; **HC**, Hyomandibuloceratohyal joint; **MC**, Meckel's Cartilage; **MK**, Knob on Meckel's cartilage that articulates with hyomandibula at the hyomandibulomandibular joint; **NC**, Nasal Capsule; **Or**, Orbit; **PQ**, Palatoquadrate.

Figure 2. Positions of implanted markers in Lateral (left) and Dorsal (right) views. Anterior is to the left of each image. Marker colors differ by cartilage element. Positions shown here are for Bamboo 1, but markers were placed in approximately the same position other individuals as well. Scale bar is 10 mm. **Cr**, Cranium; **Cy**, Ceratohyal; **Hy**, Hyomandibula; **MC**, Meckel's Cartilage; **PQ**, Palatoquadrate.

Figure 3: Orientations of the joint coordinate systems (JCSs) for the hyomandibula and ceratohyal. A) JCS for measuring motion of the hyomandibula relative to the chondrocranium. B) JCS for measuring motion of the ceratohyal relative to the chondrocranium. For both JCSs, negative rotation about the Z-axis is depression and positive is elevation (right-hand rule); rotation about Y-axis is protraction (positive) and retraction (negative); rotation about X-axis is long-axis rotation.

Figure 4. XROMM animation of a typical suction capture event. Feeding sequence starts in resting position (-100 ms), to peak gape (0 ms), peak ceratohyal depression (56 ms), and recovery phase (288 ms). Left side elements only are animated, and hyomandibula (orange) and ceratohyal (purple) are highlighted. Sequence is shown from A) left lateral view, B) ventral view, and C) anterior view with upper and lower jaws removed. Time from peak gape is shown on the left beside each row.

Figure 5. Rotations of the hyomandibula and ceratohyal relative to the cranium for the same suction capture event as Figure 4. A) Hyomandibula. B) Ceratohyal. Colors on the graphs correspond to rotation around the colored axes shown in the inset JCS images. Red is the x-axis, showing long axis rotation. Green is the y axis, showing protraction (positive) and retraction (negative), and blue is the z-axis, showing depression (negative). Light grey box shows mouth opening, dark grey shows mouth closing and peak gape occurs at time 0.

Figure 6. Translation of the distal ends of the hyomandibula and ceratohyal relative to a cranial anatomical coordinate system (ACS). Insets show the ACS and the yellow and black circles show the points tracked. A) Translation of point attached to the distal end of the hyomandibula. B) Translation of point attached to the distal end of the ceratohyal. The ACS is oriented such that translation along the red (X-axis) is medial (positive) and lateral (negative), green (Y-axis) is rostral (positive) and caudal (negative), and blue (Z-axis) is dorsal (positive) and ventral (negative).

Figure 7. Diagrams of long-axis rotation of the hyomandibula (dark grey) and the ceratohyal (light grey) following peak gape. A) Lateral view. B) Anterior view. Black arrows show the motions of the hyomandibula and the ceratohyal. White arrows show direction of long-axis rotation of the hyomandibula and ceratohyal. Here the ceratohyal is fully depressed, corresponding to the second last row in Fig. 4.

Table 1. Table of pooled means, variances, ranges and single factor ANOVA p-values for timing and magnitude of rotations of the hyomandibula and ceratohyal. L.A.R. long-axis rotation, Levene's test of homogeneity and Shapiro-Wilke test of normality. P-values below $\alpha=0.05$ are highlighted in blue.

Hyomandibula Timing	Mean	Variance	Min.	Max.	S.E.M. (±)	p-values	Levene's Test	Shapiro- Wilke
L.A.R. (X-axis Negative)	54.3	18.53	48.5	60.6	1.24	0.404	0.556	0.239
Protrusion (Y-axis Positive)	50.8	87.20	30.3	60.6	2.69	0.850	0.352	0.132
Depression (Z-axis Negative)	59.6	369.96	45.5	115.2	5.55	0.112	0.156	0.001
Hyomandibula Magnitude								
L.A.R. (X-axis Negative)	-34.4	125.02	-50.6	-11.3	3.23	0.129	0.129	0.754
Protrusion (Y-axis Positive)	29.8	44.306	17.4	40.3	1.92	0.002	0.241	0.907
Depression (Z-axis Negative)	-28.2	157.36	-43.6	-3.7	3.62	0.035	0.101	0.573
Ceratohyal Timing								
L.A.R. (X-axis Negative)	-6.7	349.66	-30.3	24.2	5.40	0.003	0.574	0.303
L.A.R. (X-axis Positive)	-47.2	28738	-	451.5	63.6	48.94	0.321	0.233
Caudal (Y-axis Negative)	47.5	75.99	30.3	57.6	2.52	0.006	0.145	0.088
Depression (Z-axis negative)	50.8	21.63	45.5	57.6	1.34	0.407	0.061	0.144
Ceratohyal Magnitude								
L.A.R. (X-axis Negative)	-12.2	45.63	-21.7	-2.8	1.95	0.001	0.246	0.237
L.A.R. (X-axis Positive)	21.2	298.99	0.086	41.1	4.99	0.016	0.122	0.032
Caudal (Y-axis Negative)	-43.4	45.75	-52.8	-29.3	1.95	0.047	0.519	0.794
Depression (Z-axis negative)	-47.0	234.69	-65.9	-20.8	4.42	0.074	0.305	0.309

Table 2. Table of pooled means, variances, ranges and single factor ANOVA p-values for timing and magnitude of translations of the distal ends of the hyomandibula and ceratohyal. L.A.R. long-axis rotation, Levene's test of homogeneity and Shapiro-Wilke test of normality. P-values below $\alpha=0.05$ are highlighted in blue.

Hyomandibula Timing (ms)	Mean	Variance	Min.	Max.	S.E.M. ($\pm$)	p-values	Levene's Test	Shapiro-Wilke
Rostral (X-axis Positive)	49.0	260.47	12.5	65.6	4.66	0.998	0.3284	0.030
Ventral (Y-axis Negative)	53.7	141.94	25	72.7	3.44	0.103	0.382	0.279
Medial (Z-axis Positive)	47.9	163.37	18.8	63.6	3.69	0.584	0.444	0.023
Hyomandibula Magnitude (mm)								
Rostral (X-axis Positive)	7.25	2.63	3.8	9.61	0.47	0.087	0.093	0.761
Ventral (Y-axis Negative)	-6.2	5.27	-8.7	-1.2	0.66	0.120	0.087	0.173
Medial (Z-axis Negative)	6.84	3.58	2.9	9.2	0.55	0.411	0.546	0.431
Ceratohyal Timing (ms)								
Caudal (X-axis Negative)	48.3	58.64	30.3	57.6	2.21	0.043	0.316	0.168
Ventral (Y-axis Negative)	69.0	447.87	39.4	100	6.11	0.124	0.355	0.351
Ceratohyal Magnitude (mm)								
Caudal (X-axis Negative)	-13.3	13.91	-19.0	-6.7	1.08	0.068	0.519	0.888
Ventral (Y-axis Negative)	-12.8	6.66	-16.1	-6.0	0.74	0.744	0.153	0.027

Table 3. P-values for tukey adjusted multiple comparisons of significant ANOVAs from Table 1. Individual sharks (Bamboo_1, Bamboo_2, Bamboo_3) that differ significantly ($\alpha=0.05$) from each other for timing or magnitude of a given parameter are highlighted. Individuals grouped either in A (dark grey) or B (white) are significantly different. Individuals grouped as both A and B (light grey) are not significantly different from either other individual. Means across four trials for each individual are given. Ceratohyal caudal translation has individual differences according to ANOVA (p-value<0.05), but individual variation was not significant according to a Tukey multiple comparisons test.

	Individual		
Timing (ms)	Bamboo 1	Bamboo 2	Bamboo 3
Ceratohyal - LAR	-21.2	-13.3	14.4
Ceratohyal Retraction	37.9	50.8	53.8
Ceratohyal Caudal Translation	40.9	51.6	52.3
Magnitude			
Hy protrusion (°)	-24.8	-27.1	-37.4
Hy Depression (°)	-19.7	-40.2	-24.7
Ceratohyal – LAR (°)	-7.6	-8.7	-20.3
Ceratohyal + LAR (°)	21.2	36.9	5.4
Ceratohyal Retraction (°)	-37.2	-45.1	-48.0

Table 4. Table of regression values show effect of Long-axis rotation on rotation and translation of depression of the hyomandibula and ceratohyal, and protrusion and retraction of those elements. Significant p-values (<0.05) are highlighted in blue. For ceratohyal regression analyses only 8 trials exhibiting positive long axis rotation were used.

Cy L.A.R. Regression	Cy Depression/Cy L.A.R.	Cy Retraction/ Cy L.A.R.	Cy Depression/ Cy Retraction
R ²	0.9208	0.0584	0.1044
Standard Error	0.079	0.120	0.678
p-Value	<0.0001	0.4494	0.3056
Regression Coefficient	-0.8502	-0.0945	0.7319
Hy L.A.R. Regression	Hy Depression/ Hy L.A.R.	Hy Protrusion/ L.A.R	Hy Depression/Hy L.A.R.
R ²	0.930	0.001	0.0005
Standard Error	3.4696	0.188	0.5958
p-Value	<0.0001	0.9215	0.948
Regression Coefficient	-1.08	0.019	0.04

List of Symbols and Abbreviations

ACS, anatomical coordinate system

By, Basihyal

Cr, Cranium

Cy, ceratohyal

HC, Hyomandibuloceratohyal joint

Hy, hyomandibula

JCS, joint coordinate system

MC, Meckel's cartilage

MK, Knob on Meckel's cartilage

NC, Nasal Capsule

Or, Orbit

PQ, palatoquadrate

XROMM, X-ray reconstruction of moving morphology

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Competing Interests

The authors have no competing interests with the contents of this study.

Author Contributions

Bradley Scott is responsible for the introduction and discussion of the study and analysis of the trials, with input and commentary from the other authors. Implantation, anaesthesia, feeding, and contributions to the written components of the study came from Dr. Wilga. Dr. Brainerd ran the X-ray equipment, and contributed the experimental protocol used to record, track and analyze motions of bamboo sharks, as well as the written contributions to description of methods and results of the study.

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CHAPTER 2

Suction Capture Kinematics of the Mandibular Arch of the White-Spotted

Bamboo Shark, *Chiloscyllium plagiosum*

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Summary Statement

A precise three-dimensional reconstruction of jaw motions in a suction feeding shark.

Abstract:

The white-spotted bamboo shark is a generalist suction-feeding elasmobranch, capable of generating exceptional subambient pressures within its mouth. The main mechanism used to generate this drop in pressure is an expansion of the hyoid arch; however, the motions of the upper and lower jaws are still an important part of prey capture. The timing and extent of depression and protrusion of the lower and upper jaws is well documented, but hypotheses of the precise three-dimensional motions of the jaws have remained untested. Supination, or medial rotation of the ventral margin, of the lower jaw has been hypothesized to erect teeth during prey capture by tensing the dental ligament, and medial translation of the jaw joint has been hypothesized to enhance protrusion of the jaws. Long-axis rotations of the upper and lower jaws of an elasmobranch are measured for the first time, along with in-vivo measurements of upper jaw protrusion and medial translation of the jaw joint during feeding. The lower jaw supinates as the mouth opens and pronates past resting position as the jaws close. Medial translation of the jaw joint occurs following maximum gape and after prey capture, and too late to enhance the protrusion of the upper jaw. Pronation of the lower jaw is likely too low in magnitude to change buccal volume or contribute to suction. Instead pronation may be a result of extreme stress from activation of the hypertrophied jaw adductors of bamboo sharks as the jaws are closed.

Introduction:

Aquatic vertebrates use a numerous feeding strategies to obtain prey. Methods range from ram, filter and biting to suction feeding (Motta and Wilga 2001). The mechanisms underlying different feeding strategies vary based on the underlying morphology and the range of motion. The presence of feeding mechanisms can be tested by comparison with kinematic data for various taxa. In elasmobranchs (sharks, skates, and rays) the feeding strategies, and underlying mechanisms, are varied, but share constraints on the number of elements in the feeding apparatus (Motta and Wilga 2001; Wilga *et al.* 2007). Much of the flexibility in feeding strategy may come from a decoupling of the motions of the mandibular and hyoid arches (Motta and Wilga 1999; Wilga *et al.* 2007). The jaws open and close the mouth, biting or processing prey, while the hyoid either protrudes the jaws or generates negative pressure inside the mouth (Motta *et al.* 1997; Motta *et al.* 2002; Wilga 2008; Wilga and Sanford 2008). Suction feeding elasmobranchs use powerful hyoid motions to expand the oral cavity ventrally and generate powerful suction forces in front of the mouth. While motions of the hyoid arch have been studied due its importance for generating suction forces (Wilga 2008; Wilga and Sanford 2008; Wilga *et al.* 2012; Wu 1994; Chapter 1), three-dimensional motions of the mandibular arch in suction feeding sharks are less well-understood (Wu 1994; Ramsay and Wilga 2007; Ramsay 2012). White-spotted bamboo sharks, *Chiloscyllium plagiosum*, are specialized for suction capture, yet have a generalized diet (Wilga *et al.* 2012). Additional degrees of motion of the jaws may allow a broader range of mechanisms for different prey. While timing and magnitude of protrusion and depression of the jaws has been measured in other studies (e.g.

Motta *et al.* 2002; Motta *et al.* 2008; Wilga and Sanford 2008), medial translation of the jaw joint, a possible contributor to jaw protrusion, and long-axis rotation, a possible mechanism for grasping prey, have not been examined. Here X-ray Reconstruction of Moving Morphology (XROMM) is used to measure additional degrees of motion including medial translation of the jaw joint (Wu 1994) and long-axis rotation of the upper (Frazzetta and Prange 1987) and lower jaws (Ramsay and Wilga 2007).

The mandibular arch of elasmobranchs is suspended from the cranium as a palatoquadrate and Meckel's cartilage, supported by the hyomandibula and connected to the hyoid arch at the hyomandibular joint (Fig. 1A; Motta and Wilga 1999; Ramsay 2012). In elasmobranchs suction force is primarily generated by ventral expansion of the hyoid arch (Wilga 2008; Wilga and Sanford 2008; Motta *et al.* 2002; Motta *et al.* 2008). This contrasts with teleosts, where the hyoid elements are closely connected to the upper jaws (Westneat 2006), and protrusion of the upper jaws contributes considerably to suction force at the mouth (Holzman *et al.* 2007). Among elasmobranchs, feeding initiates when the lower jaw depresses, then the jaws protruded to close rapidly (Fig. 1B, C; Motta *et al.* 2008; Wilga *et al.* 2007), but jaw protrusion contributes little to suction forces (Motta *et al.* 1997; Motta *et al.* 2008). Instead protrusion of the jaws reduces the time required to close the mouth following prey capture (Motta *et al.* 2008).

The white-spotted bamboo shark is a suction-feeding generalist (Wilga *et al.* 2012) that eats a wide variety of prey items such as small fish and crustaceans (Compagno, 1984). Capable of strong suction forces for capturing prey, but also

highly variable in the timing and magnitude of pressures and kinematics during feeding (Wilga *et al.* 2007; Wilga and Sanford 2008; Wilga *et al.* 2012; Chapter 1), Bamboo sharks may use this flexibility in feeding to adjust for different prey or feeding strategies, such as using substrate to enhance suction forces (Nauwelerts *et al.* 2007) and adjusting the orientation of their teeth during prey capture or processing (Ramsay and Wilga 2007). To accommodate different prey items, the three-dimensional motions of the upper and lower jaws of bamboo sharks may be more complex than depression and protrusion. Erection of the teeth during prey capture is hypothesized to activate through tension of the dental ligament (Ramsay and Wilga 2007). The dental ligament can be tensed by supination of the lower jaw (Ramsay and Wilga 2007). As the ventral margin of the jaws rotate medially, the labial margin rotates laterally, here this is termed supination, after the motion of turning the hands palm up or forward. The opposite rotation around the same axis is pronation. Lateral rotation of the labial margin of the lower jaw pulls the dental ligament by increasing the labial area of the lower jaw, resulting in tension (Ramsay and Wilga 2007). Long-axis rotation of mandibular elements has not been reported for any elasmobranch species, but bite-feeding sharks may utilize a similar mechanism for the upper jaw during protrusion (Frazzetta and Prange 1987). Long-axis rotation can have considerable effects on maneuverability and orientation of elements in actions as disparate as terrestrial locomotion (Kambic *et al.* 2014) and the hyoid elements of bamboo sharks (Chapter 1).

Other orectolobiform shark species medially constrict their jaw joints during protrusion, a mechanism proposed to enhance the magnitude of jaw protrusion during

prey capture (Wu 1994). Bamboo sharks may use a similar mechanism during feeding (Wu 1994; Ramsay 2012). The jaws are connected at the symphysis (distal end) by flexible cartilage rather than fused or interdigitated (Ramsay 2012). This allows the jaws to rotate. As the jaw joint moves medially, the angle between the jaws is reduced and the jaws are thought to protrude rostrally as well as ventrally (Fig. 1D, E; Wu 1994). If the jaws are protruded as the jaw joints move medially, then the distal end of the upper jaw should move further forward than the jaw joint at the same time.

The purpose of this study is to describe the three-dimensional motions of the upper and lower jaws during food capture in the White-spotted bamboo shark. In particular to record the long-axis rotation of the jaws and the medial translation of the jaw joint. As predicted, the upper and lower jaws compress medially at the jaw joint, and exhibit long-axis rotation during feeding. As the lower jaw depresses the ventral margin rotates medially, supinating. As the jaws close and protrude the ventral margin of the lower jaw rotates laterally, pronating. Medial translation occurs as the jaws are protruded (Wu, 1994, Ramsay 2012) following peak gape, but does not appear to contribute to additional rostral protrusion of the jaws (contrary to Wu, 1994).

Methods and Materials:

Implantation

Four conical tungsten carbide markers (Kambic *et al.* 2014) were implanted in the chondrocranium, and three markers in each of palatoquadrate, Meckel's Cartilage, hyomandibula, ceratohyal from the left side (Fig. 2) in three individual white-spotted bamboo sharks (*Chiloscyllium plagiosum* Bennett) of similar size, with body lengths

of 78.6 cm 79.2 cm 85 cm, and cranium lengths of 6.47 cm, 6.35 cm, 6.58 cm at the University of Rhode Island Graduate School of Oceanography. General anaesthesia was applied using 0.033 gL⁻¹ of MS-222 Tricaine methanesulfonate in a recirculating fish anaesthesia system (Wilga and Sanford 2008) for implantation and CT scan procedures. All sharks recovered and fed normally following implantation. Individuals were fed in a separate transparent tank to acclimate them for experimental trials. Food was withheld for one week prior to trials to encourage feeding.

CT Scan

Individuals were scanned following the first day of trials using a FIDEX CT scanner (Animage). Scans were taken at 448 X 448 pixels (416 X 416 pixels for Bamboo 3) resolution and a slice thickness of 0.185 mm. Images were compiled and reconstructed in Osirix (Pixmeo, Geneva, Switzerland). Three-dimensional surface meshes of the cranium, Meckel's, palatoquadrate, hyomandibula, and ceratohyal cartilages and as well as the markers were exported as .obj files to Maya (2015; Autodesk). The centroid for the vertices of each marker was calculated using the vertAvG tool in the XROMM_tools shelf for Maya (2015; Autodesk) available at xromm.org. Centroids of markers were exported from Maya as CT coordinates using the CTeX tool.

XROMM

Feeding trials were recorded at the Keck XROMM Laboratory, Brown University. Each individual was recorded feeding on a piece of squid or herring for each trial. Feeding events were recorded on a pair of Phantom v.10 high speed video cameras. Each camera had a fluoroscopic image intensifier and a corresponding X-ray emitter (Imaging Systems and Service; Brainerd *et al.* 2010). Food items were placed in front

of the shark and recording began when subject moved towards the food; it ended when the subject was finished processing or after ten seconds, whichever occurred first. The motion of implanted markers were recorded at 320 Hz or 330 Hz, with X-ray energies of 110–120kV and 100mA.

CT coordinates of each marker were imported into XMALab (open-sourced software authored by Ben Knörlein; bitbucket.org/xromm/xmalab). Marker positions in both videos from each trial were tracked using XMALab and the three-dimensional translations of markers in each element were used to calculate translations and rotations of rigid bodies (Brainerd *et al.* 2010). Rigid body transformations were imported into Maya and applied to 3D meshes of each element imported from the CT scans.

Joint Coordinate System Analysis

The rotations of each element in each trial were measured in Maya using a Joint Coordinate System (JCS, Brainerd *et al.* 2010; Camp and Brainerd 2015 Kambic *et al.* 2014; jointAxes tool, XROMM_tool_shelf for Maya, xromm.org). Two sets of three (x, y, z) axes were aligned to the elements. The X-axis was aligned with the long-axis of the element pointing distally, the Z-axis was aligned horizontal to the cranium and pointed medially (Fig. 3). The Y-axis is aligned perpendicular to the other two axes, pointing approximately dorsally (Fig. 3). Here axes were centered at the quadratomandibular joint. One set of axes tracked the motion of the cranium, the other tracked the motion of the distal element, the Meckel's cartilage (Fig. 3A) or the palatoquadrate (Fig. 3B), and the relative difference in motion between the sets of axes was recorded. The calculated difference in motion between the two axes gave the

motion of lower jaw or upper jaw relative to the cranium (Fig. 3). Timing and magnitude of the peak rotation along each of the three axes was recorded. Timings were recorded relative to peak gape. Magnitudes were recorded as relative to resting position. Resting position was recorded at a time prior feeding, where the ceratohyal was neither elevating nor depressing, or the initial frame of the trial if feeding began shortly after the start of filming. A total of 12 trials were analyzed for three individuals; four trials per individual.

Anatomical Coordinate System Analysis

Translation data were obtained using an Anatomical Coordinate System (ACS, Brainerd *et al.* 2010; Gidmark *et al.* 2012) in Maya, applied to the same trials as the JCS. A single set of axes was aligned with the cranium. As in Chapter 1 the Y-axis pointed rostral, the Z-axis pointed dorsally, and the X-axis pointed medially (relative to the left side cranial elements) at right angles to each other. Locators were placed on the jaw joint and caudal angle of the Meckel's cartilage; and the distal end of the palatoquadrate. On the palatoquadrate rostral and ventral translation was measured for the distal locator. Medial, rostral and ventral translation were recorded for the locator on the jaw joint of the Meckel's cartilage (Table 2). The medial translation of the caudal angle of the Meckel's cartilage was also measured (Table 2). All translation was relative to the cranium.

Statistics

Single factor ANOVAs (Table 1; $\alpha=0.05$) tested for individual variation in the timing and magnitude of each rotation and translation. Single factor ANOVAs were calculated for positive and negative long-axis (x-axis) rotation, positive and negative medial (y-

axis) rotation, and depression (negative z-axis rotation) of the Meckel's cartilage at the jaw joint. ANOVAs for the rotations of the palatoquadrate were calculated for positive long-axis rotation, positive medial rotation, and depression. ANOVAs were also calculated for the timing and magnitude of all measured translations: distal palatoquadrate, palatoquadrate jaw joint, Meckel's jaw joint, Meckel's caudal angle (Tables 2, 3). Standard errors are based on Student's t-distribution as the sample size was low ($n=12$, <30). For timings or magnitudes with significant ANOVAs a Tukey adjusted multiple comparisons test was used to determine which individuals differed (Table 4). Levene's test and Wilkes-Sharro test were used to determine validity of assumptions of homogeneity of variance and normality respectively. All statistical analyses were performed with SAS 9.4 software (SAS Institute, Cary NC).

To measure the difference in rostral protrusion possibly resulting from medial translation of the jaw joint, rostral translation of the upper jaw at the jaw joint was subtracted from rostral translation of the distal end of the upper jaw for all trials. If medial translation of the jaw joint enhances rostral protrusion, then distal rostral translation should be greater than proximal rostral translation of the upper jaw at the time of peak medial translation of the jaw joint. Timing of peak medial translation of the jaw joint of the palatoquadrate was compared against the timing of peak positive difference in rostral translation between the distal and jaw joint landmarks of the palatoquadrate using a student's t-test adjusted for unequal variability between samples (Welch 1947).

RESULTS

Feeding Sequence

Food was captured by inertial suction and transported to the oral cavity in a single motion for all trials analyzed except one. For the single exception the food item was drawn to the edge of the mouth, grasped, and then transported in a later motion. While the magnitude of hyoid motions of that trial were much lower than other trials (Chapter 1) no obvious impact to the mandibular arch motions was observed. Cranial elevation was present prior to feeding in some trials to position the head in front of the food; however, little or no cranial elevation was observed during feeding strikes. Opening of the mouth appears to begin earlier in these trials compared to other studies of kinematics in bamboo sharks (Wilga 2008; Wilga *et al.* 2008). This is due to a difference in definition. Initiation of the expansive phase typically begins when gape is 10% of maximum (Wilga and Sanford 2008); however, some motions, such as supination of the lower jaw, begin as the jaws start to open and prior to 10% of maximum gape, so here the initial jaw depression is defined as the last jaw closure before maximum gape.

Feeding was initiated with depression of the lower jaw and opening of the gape (Fig. 4, at -139 ms). The lower jaw supinated as it depressed (most easily seen in Fig. 4B, C at -18 ms). The ventral margin of the Meckel's cartilage moved medially. Near peak gape the jaw joint began to protrude, moving rostrally, as well as ventrally and medially (Fig. 4A at -18 ms). The upper jaw also began to pronate, the ventral margin rotating laterally (Fig. 4 0 ms). Protrusion of the jaw joint coincided with the beginning of depression and retraction of the ceratohyal and adduction and protrusion of the hyomandibula (Fig. 4A, -18 – 0 ms, and Chapter 1 fig. 4). Peak

depression of the Meckel's cartilage did not always coincide with peak gape, as depression of the palatoquadrate began at varied times among trials. At or slightly before peak gape pronation of the palatoquadrate peaked and the distal end of the palatoquadrate began to translate rostrally and ventrally as the upper jaw depressed (Fig. 4, 0 ms). As the jaws closed the distal end of the hyomandibula fully protruded rostrad, depressed and adducted, while the ceratohyal fully retracted and depressed (Fig. 4A, 52 ms; Chapter 1). At this time the lower jaw fully pronated as the jaw joint maximally protruded and constricted medially (Fig. 4, 52 ms). During recovery the Meckel's cartilage and the palatoquadrate retracted and elevated towards their original position, but often began processing prior to full recovery.

Rotations from joint coordinate systems (JCSs)

Long-axis rotation of the upper and lower jaws can be seen as slight medial and lateral displacements of the ventral margins relative to the jaw joint in ventral and anterior views (Fig. 4B, C), but the motions are best quantified using JCSs (Fig. 5). Supination of the lower jaw is smaller than pronation, with means ($\pm$ s.e.m) of -8.0 ± 1.1 deg and 11.2 ± 1.5 deg respectively (Fig. 5A and Table 1). Pronation of the upper jaw (12.3 ± 0.9 deg) is slightly greater than pronation of the lower jaw (Table 1). The depression of the lower jaw is the largest rotation of the jaw joints at -22.7 ± 0.6 deg., but depression of the upper jaw is also quite high, -15.7 ± 1.8 deg (Fig. 5, Table 1).

Motions of the mandibular arch are much more sequential compared to the hyoid arch, where majority of the motion happens at the same time (Chapter 1). Peak supination of the lower jaw at -19 ± 7 ms is followed by peak pronation of the upper jaw 0 ± 1.6 ms, then depression of the lower jaw (6 ± 2 ms), depression of the

palatoquadrate (55 ± 5 ms) and finally pronation of the lower jaw at 87 ± 6 ms (Fig. 5, Table 1).

Translations of points in an anatomical coordinate system (ACS)

Rotations of the mandibular arch elements resulted in translation of the jaw joint, the caudal angle of the lower jaw, and the distal end of the upper jaw (Fig. 6 and Table 1). Medial/lateral translation of the caudal angle did not correspond with the supination and pronation of the lower jaw. Instead it moved laterally a mean -1.3 ± 0.4 mm then medially 1.6 ± 0.15 mm before and after peak gape respectively. Translation of the caudal angle relative to the cranium was primarily dependent on the timing of supination and pronation with the medial translation of the jaw joint (5.3 ± 0.5 mm). Depression of the upper jaw combined with ventral translation of the jaw joint of -7.2 ± 0.4 mm, resulted in a ventral translation of the distal end of the palatoquadrate of 13.4 mm. Rostral protrusion of the distal end of the upper jaw nearly the same as mean rostral translation of the jaw joint. 5.1 ± 0.3 mm and 4.6 ± 0.4 mm respectively. When rostral translation of the distal end of the palatoquadrate was compared with rostral translation of the joint of the palatoquadrate (nearly identical to the translation of the jaw joint of the Meckel's cartilage shown in Fig. 6A) the greatest difference is generally quite low (1 ± 0.7 mm) and the timing is considerably earlier than the medial translation of the jaw joint, 7 ± 0.2 ms after peak gape compared with 54 ± 4 ms after peak gape.

Individual Variation

Timing and magnitude of showed considerably variation among the individuals, particularly the medial translation of the jaw joint and long-axis rotation

of the upper and lower jaws (Table 1). The timing of supination (p-value=0.032) pronation (p-value=0.023) medial translation of the jaw joint (p-value=0.048) and the caudal angle (p-value=0.019) of the Meckel's cartilage varied among individuals. Magnitude of supination (p-value=0.047) and medial rotation (p-value=0.029) of the lower jaw, and medial translation (p-value=0.048) of the jaw joint also varied among individuals. Magnitude of medial translation of the joint of the palatoquadrate (p-value=0.022), and depression (p-value=0.005) and ventral translation (p=0.022) of the distal end of the palatoquadrate all varied between individuals as well.

Individual Bamboo 1, achieved peak supination earlier than the other two individuals, and peak pronation later than Bamboo 2 (Table 4). Bamboo 1 also had later medial translation of the lower jaw joint and depression of the upper jaw compared to Bamboo 2, but not significantly later than Bamboo 3, who was typically in between the other individuals in terms of timing (Table 4). Magnitude of medial translation and rotation of the lower jaw, as well as depression and ventral translation of the distal end of the upper jaw was less extreme in Bamboo 1 than the other individuals (Table 4).

The relationship between medial translation of the jaw joint and rostral protrusion was also tested. If the medial translation of the jaw joint increased rostral protrusion of the jaws, then differences in rostral translation between the distal end of the upper jaw and the jaw joint should have occurred at the same time as medial translation. Peak medial translation occurred as the jaws closed, while the peak difference in rostral protrusion occurred at maximum gape (p-value<0.001).

DISCUSSION

The elements of the mandibular arch have highly complex three-dimensional motions during feeding. In addition to opening and closing the jaws, and ventral depression of the upper jaw, white-spotted bamboo sharks, *Chiloscyllium plagiosum*, use long-axis rotation of the jaws to assist in prey capture. Long-axis rotation of the upper and lower jaws times consistently with prey capture. Medial translation of the jaw joint is present; however, it occurs after prey capture and does not appear to contribute to rostral protrusion of the jaws. Despite specializing in suction feeding, bamboo sharks appear to have additional mechanisms for capturing prey increasing the range of prey types that they can consume.

Supination peaks prior to peak gape, consistent with the timing predicted for aiding prey capture by tensing the dental ligament and erecting the teeth (Ramsay and Wilga 2007). Following peak gape the lower jaw pronates, swinging the caudal angle of the meckel's cartilage medial to lateral relative to the jaw joint. While pronation of the lower jaw could improve suction performance by expanding the buccal cavity, as the caudal angle moves laterally, pronation and lateral translation of the caudal angle have extensive variation in timing as well as magnitude (Fig. 6A, Tables 1 and 2). In addition the caudal angle moves laterally during supination, while during pronation the caudal angle moves medially (Fig. 6A), the opposite motion that would be expected if pronation expanded the buccal cavity. Medial translation of the caudal angle coincides with medial translation of the jaw joint, but is of lower magnitude. As the jaw joint moves medially pronation of the lower jaw may act to reduce the compression of buccal cavity during feeding, rather than increase total volume. Instead

of increasing volume pronation of the Meckel's cartilage may be a byproduct of the force from the hypertrophied jaw adductors of *C. plagiosum* (Ramsay and Wilga 2012; Wilga *et al.* 2007). The muscles insert along the caudal and ventral margins of the Meckel's cartilage and activate during compression to close the mouth (Motta and Wilga 1999; Motta *et al.* 2008; Wilga *et al.* 2007; Ramsay 2012). With sufficient force they may pull the ventral margin laterally relative to the labial margin of the lower jaw.

The pronation of the upper jaw at peak gape may have a similar function to lower jaw supination. As the upper jaw pronates the labial margins move apart from each other, potentially tensing the dental ligament and erecting upper teeth as well (Frazzetta and Prange 1987). This would occur shortly after supination in the lower jaw, as the palatoquadrate depressed, where it could come into contact with larger prey items. Other sharks may use a similar mechanism for erecting teeth despite broad ecological differences. Pronation during jaw protrusion has been hypothesized for bite-feeding Carcharinid shark species for the same role (Frazzetta and Prange 1987) despite distinctly different ecologies compared with to bamboo sharks (Compagno 1984). While the timing of long-axis rotation of the upper and lower jaws is consistent with grasping prey (Ramsay and Wilga 2007), erection of teeth during supination was not examined, and food was grasped during prey capture in only one trial, while long-axis rotation was found in all trials. As with direction and sequence of kinematics for other cranial elements during feeding (Motta *et al.* 2002; Motta *et al.* 2008; Wilga and Sanford 2008), the sequence of long-axis rotation may be highly consistent during

feeding events, but the duration and magnitude may be highly variable even within the same species (Wilga and Sanford 2008; Wilga et al. 2012; Chapter 1)

Assessing significance of supination and long-axis rotation of elements of the mandibular arch for the feeding mechanics and ecomorphology of suction feeding shark species requires further comparison with other elasmobranchs. Morphological correlates of supination and pronation may include a loosely connected symphysis as well as a distinct angle between the distal ends of the jaws that allows the ventral margins of the lower jaw to rotate medially without abutting (Ramsay and Wilga 2007; Wu, 1994, attributes the symphyseal angle to accommodation of medial compression of the jaws in *Orectolobiformes*). Elasmobranchs with flexible symphyses (eg. dogfish *Squalus acanthias*, smoothhounds *Mustelus canis*, Gerry *et al.* 2008), could all be capable of long-axis rotation, but if supination erects teeth for capturing prey (Ramsay and Wilga 2007), then it would be expected to occur in other elasmobranchs engaging in suction or grasping behaviours, particularly those with tooth morphologies similar to bamboo sharks, such as nurse sharks (*Ginglymostomatidae*) and blind sharks (*Brachaeluridae*) (Ramsay and Wilga 2007). Pronation of the upper jaw may be more widespread as it can erect teeth for gouging prey during biting by ventral protrusion of the upper jaw. (Frazzetta and Prange 1987; Motta and Wilga 2001)

Food items were kept small during trials to maximize number of feeding events before satiation, but if long-axis rotation improves prey capture by increasing the ability of teeth to grasp (Ramsay and Wilga 2007), then larger food items may induce a greater, or more stereotypical response. Younger bamboo sharks also use less

suction and more locomotion when feeding (Lowry and Motta 2007). As bamboo sharks grow in size suction becomes the dominant component of feeding (Lowry and Motta 2007). Grasping prey may be a more significant component of the feeding strategy of young bamboo sharks until suction performance alone is sufficient to capture prey items, and as such long-axis rotation of the jaws may have greater significance for success of juvenile bamboo sharks than for adults. For this study body size varied little among the individuals, allowing differences in feeding kinematics to be associated for factors other than ontogeny, but, if long axis rotation, particularly supination, is of greater importance earlier in ontogeny then magnitude of long-axis rotation would be expected to scale negatively with body size. Pronation of the lower jaw, if related to suction performance may increase in magnitude later in ontogeny, as bamboo sharks transition to a more suction dominated feeding strategy (Lowry and Motta 2007). Magnitude of pronation of the lower jaw may be small, but it starts at approximately the same time as peak buccal pressure, and peak rate of increase in hyoid area (Wilga and Sanford 2008). Estimation of change in buccal volume of *C. plagiosum* during feeding could allow a more accurate assessment of the impact of pronation on changes in overall buccal volume and suction forces.

Medial translation of the jaw joint compresses with the hyoid cavity (Wilga 2008, Wilga and Sanford 2008), during and following peak gape, when food is typically already captured (Motta *et al.* 2002) and peak buccal pressure has passed (Wilga and Sanford 2008). Medial translation of the jaw joint also comes after the peak increase in rostral translation of the distal end of the upper jaw relative to the jaw joint (Fig. 6C). The distal end of the upper jaw protrudes rostrally more than the jaw

joint at peak gape in all but one trial, but the medial translation of the jaw joint does not peak until well after peak gape, delayed by approximately 50 ms (Table 2). Because of this difference in timing it is unlikely that medial translation of the jaw joint protrudes the jaws rostrally. Rather, given the similarity in timing to the pronation of the lower jaw, medial translation of the jaw joint may be the result of pronation, through medial rotation of the labial margin of the lower jaw as the jaws close. The presence of medial translation of the jaw joint in other suction feeding orectolobiform sharks (Wu 1994) indicates that the long-axis rotations of the lower jaw observed here may be widespread within the Orectolobiformes, a group that includes numerous suction feeding specialists and that feeds on a variety of prey in complex environments (Compagno 1984; Motta *et al.* 2002; Wu 1994). Rostral translation of the jaws also occurs too late to contribute to grasping of prey or suction performance. Depression of the upper jaw reduces the time required to close the mouth, closing gape before the lower jaw is fully elevated (Fig. 5A, the blue depression trace is still negative at end gape, i.e. not returned to resting position) as in other elasmobranchs (Motta *et al.* 2002; Motta *et al.* 2008). Rostral protrusion of the jaws may also extend the buccal cavity to prevent escape of prey once captured.

Variation in mandibular arch motions was greater between Bamboo 1 and 2 than 3, strikingly different from the motions of the hyoid arch, where differences in long-axis rotation of the ceratohyal resulted in greatest variation being between Bamboo 3 and the other two individuals. Differing trends in variation between hyoid and mandibular arches may result from their independent depression mechanisms. The independence of depression of the mandibular and hyoid arches of bamboo sharks

allows for greater variety in the timing and magnitude of strike motions, while a consistent sequence of motions is retained (Wilga *et al.* 2012). This may allow individuals to adjust for various environmental effects including substrate (Nauwelearts *et al.* 2007) and a wide variety of prey items (Compagno 1984) that they may encounter.

CONCLUSIONS

This study shows that the elements of the mandibular arch undergo complex three-dimensional motions during feeding, including long-axis rotation of the upper and lower jaws. In addition to varying suction performance, bamboo sharks may use long-axis rotation of the upper and lower jaws as additional mechanisms for obtaining a variety of prey. By rotating the jaws around their long-axes the teeth may be erected to aid in grasping of soft bodied prey. Alternatively, long-axis rotation may improve suction performance by making the inside of the mouth more conical. Medial motion of the jaw joint does not contribute to prey capture and may be the result of long-axis rotations of the jaws during feeding.

FIGURES

Figure 1. Cartilaginous elements and motions of the oral jaws in the white-spotted bamboo shark, *Chiloscyllium plagiosum*. A) Cartilaginous elements in left lateral view. B) Depression of Meckel's cartilage opens the gape. C) Depression of the palatoquadrate and rostro-ventral protrusion of the jaws as they close. D) Mandibular arch in resting position in ventral view. E) During jaw closing, the jaw joints move medially, protruding the distal ends of the jaws rostrally as proposed in Wu (1994). **Ca**, caudal angle of lower jaw; **Cr**, Cranium; **Cy**, Ceratohyal; **Hy**, Hyomandibula; **HC**, Hyomandibuloceratohyal joint; **MC**, Meckel's Cartilage; **MK**, Knob on Meckel's cartilage that articulates with hyomandibula at the hyomandibulomandibular joint; **NC**, Nasal Capsule; **Or**, Orbit; **PQ**, Palatoquadrate.

Figure 2. Position of implanted markers in Lateral (left) and Dorsal (right) views. Anterior is to the left of each image. Marker colors differ by element. Positions are based on Bamboo 1 however, markers are placed in approximately the same position other individuals as well. Scale bar is 10 mm. **Cr**, Cranium; **Cy**, Ceratohyal; **Hy**, Hyomandibula; **MC**, Meckel's Cartilage; **PQ**, Palatoquadrate.

Figure 3. Orientations of the joint coordinate systems (JCSs). A) JCS for measuring motion of the Meckel's cartilage relative to the chondrocranium. B) JCS for measuring motion of the palatoquadrate relative to the chondrocranium. For both JCSs, negative rotation about the Z-axis is depression and positive is elevation (right-hand rule); rotation about Y-axis is medial (positive) and lateral (negative); rotation about X-axis is long-axis rotation.

Figure 4. XROMM animation of a typical suction capture event. Left side elements only are animated; palatoquadrate is light blue, Meckel's cartilage is dark blue. Sequence from start of jaw opening (-139 ms) to peak ceratohyal depression (52 ms). The same sequence in three different views: lateral (A), anterior (B), and ventral (C). Time zero is peak gape.

Figure 5. Rotations of Meckel's cartilage and palatoquadrate relative to the cranium for the same suction capture event as Figure 4. A) Meckel's cartilage. B) Palatoquadrate. Colors on the graphs correspond to rotation around the colored axes shown in the inset JCS images. Red is the X-axis, showing long-axis rotation. Green is the Y-axis, showing protraction (positive) and retraction (negative), and blue is the z-axis, showing depression (negative). Light grey box shows mouth opening, dark grey shows mouth closing and peak gape occurs at time 0.

Figure 6. Translations of points on the jaws relative to a cranial anatomical coordinate system (ACS). Insets show the ACS and the yellow and black circles show the points tracked. A) Translation of point attached to the jaw joint on the Meckel's cartilage: red (X-axis) is medial (positive) and lateral (negative), green (Y-axis) is rostral (positive) and caudal (negative), and blue (Z-axis) is dorsal (positive) and ventral (negative) and in yellow, translation of a point attached to the caudal angle of the Meckel's cartilage, medial (positive) and lateral (negative). B) Translation of point attached to the distal end of the palatoquadrate. The ACS is oriented such that translation along the red (X-axis) is medial (positive) and lateral (negative), green (Y-axis) is rostral (positive) and caudal (negative), and blue (Z-axis) is dorsal (positive) and ventral (negative).

Table 1. Table of pooled means, variances, ranges and single factor ANOVA p-values for timing and magnitude of rotations of the Meckel's and palatoquadrate. L.A.R. long-axis rotation, Levene's test of homogeneity and Shapiro-Wilke test of normality. P-values below $\alpha=0.05$ are highlighted in blue.

Meckel's					S.E.M.			
Timing	Mean	Variance	Min.	Max.	($\pm$)	p-value	Levene's Test	Shapiro-Wilke
L.A.R. (X-axis Negative)	-18.7	540.2	-81.82	0	6.7	0.0319	0.1012	0.0032
L.A.R. (X-axis Positive)	86.6	477.2	68.75	136.4	6.3	0.0234	0.2001	0.0208
Lateral (Y-axis Negative)	-6.9	43.5	-18.18	3.0	1.9	0.4173	0.0024	0.3041
Medial (Y-axis Positive)	74.9	1001	45.45	154.6	9.1	0.0645	0.0602	0.0048
Depression (Z-axis Negative)	6.1	65.3	-6.06	21.2	2.3	0.5308	0.1461	0.8173
Meckel's Magnitude								
LAR (X-axis Negative)	-8.0	14.4	-14.31	-1.1	1.1	0.0470	0.4987	0.6843
LAR (X-axis Positive)	11.2	27.2	0.27	19.5	1.5	0.0642	0.0033	0.1608
Lateral (Y-axis Negative)	-8.7	2.3	-11.07	-6.7	0.4	0.2296	0.9038	0.3087
Medial (Y-axis Positive)	7.8	18.2	0.23	12.7	1.2	0.0292	0.1867	0.0569
Depression (Z-axis Negative)	-22.7	3.7	-26.86	-19.9	0.6	0.6362	0.7016	0.6090
Palatoquadrate Timing								
L.A.R. (X-axis Positive)	-0.1	29.4	-9.38	6.1	1.6	0.0211	0.3798	0.1334
Medial (Y-axis Positive)	51.8	61.2	36.36	63.6	2.3	0.1066	0.0185	0.8868
Depression (Z-axis Negative)	55.2	272.4	31.25	87.9	4.8	0.0047	0.1204	0.7037
Palatoquadrate Magnitude								
LAR (X-axis Positive)	12.3	9.3	7.36	18.1	0.9	0.1498	0.3611	0.9891
Medial (Y-axis Positive)	7.1	6.2	1.22	10.6	0.7	0.0881	0.7376	0.1535
Depression (Z-axis Negative)	-15.7	40.5	-21.62	-2.2	1.8	0.0048	0.0456	0.0050

Table 2. Descriptive statistics of the timing of translation of locators in the mandibular arch, relative to the cranium. Including the jaw joint and caudal angle of the Meckel's cartilage. Values for mean, variance, Minimum (Min.), maximum (Max.), standard error, ANOVA p-value, Levene's test of equality of variance, and the Shapiro-Wilke test of normality are given. *A trial where the distal end of the palatoquadrate did not translate rostrally more than the jaw joint was excluded.

Meckel's Joint Timing (ms)	Mean	Variance	Min.	Max.	S.E.M. (±)	p-value	Levene's Test	Shapiro-Wilke
Rostral (Y-axis Positive)	10.5	5638.9	-225.0	56.3	21.7	0.420	0.157	<0.001
Ventral (Z-axis Negative)	47.2	86.87	21.2	54.6	2.7	0.178	0.142	0.003
Medial (X-axis Positive)	54.2	186.30	18.8	69.7	3.9	0.048	0.319	0.040
Meckel's Caudal Timing (ms)								
Medial (X-axis Positive)	-22.8	18371	-303.0	57.6	57.8	0.019	0.038	<0.001
Lateral (X-axis Negative)	175.9	40148	-15.2	N/A	39.1	0.061	0.055	0.036
PQ Distal Timing (ms)								
Rostral (Y-axis Positive)	28.0	280.0	12.5	69.7	4.8	0.203	0.025	0.023
Ventral (Z-axis Negative)	50.9	287.5	30.3	90.9	4.9	0.187	0.101	0.255
Distal-Proximal Rostral Translation Timing (ms)								
Positive*	6.7	49.1	-3.0	24.2	2.1	0.416	0.097	<0.001
Negative	56.4	7537.5	-181.8	142.4	25.1	0.440	0.160	0.009

Table 3. Descriptive statistics of the Magnitude of translation of locators in the mandibular arch, relative to the cranium. Including the jaw joint and caudal angle of the Meckel's cartilage. Values for mean, variance, Minimum (Min.), maximum (Max.), standard error, ANOVA p-value, Levene's test of equality of variance, and the Shapiro-Wilke test of normality are given.

Meckel's Joint					S.E. M.		Levene's	Shapiro
Magnitude (mm)	Mean	Variance	Min.	Max.	($\pm$)	p-value	Test	-Wilke
Rostral (y-axis)								
Positive	4.6	2.21	0.5	6.2	0.4	0.147	0.222	0.007
Depression (Z-axis) Negative	-7.2	2.36	-9.3	-3.6	0.4	0.083	0.818	0.188
Medial (X-axis)								
Positive	5.3	3.14	1.5	7.8	0.5	0.049	0.222	0.367
Meckel's Caudal Magnitude (mm)								
Medial (X-axis)								
Positive	1.6	2.21	0.1	5.8	0.2	0.105	0.219	0.006
Lateral (X-axis)								
Negative	-1.3	0.29	-2.4	-0.7	0.4	0.214	0.210	0.256
PQ Distal Magnitude (mm)								
Rostral (Y-axis)								
Positive	5.1	1.07	2.9	6.5	0.3	0.913	0.352	0.575
Depression (Z-axis) Negative	-13.5	16.34	-16.9	-4.7	1.2	0.022	0.026	0.002
Difference in Rostral Translation Magnitude (mm)								
Greatest	1.1	0.45	0.2	2.4	0.2	0.669	0.242	0.032
Lowest	-1.5	3.18	-6.5	0.5	0.5	0.918	0.178	0.003

Table 4. P-values for tukey adjusted multiple comparisons of significant ANOVAs from Table 1. Individual sharks (Bamboo_1, Bamboo_2, Bamboo_3) that differ significantly ($\alpha=0.05$) from each other for timing or magnitude of a given parameter are highlighted. Individuals grouped either in A (dark grey) or B (white) are significantly different. Individuals grouped as both A and B (light grey) are not significantly different from either other individual. Means across four trials for each individual are given.

	Individual		
	Bamboo 1	Bamboo 2	Bamboo 3
Timing (ms)			
Meckel's - LAR	-40.9	-2.3	-12.9
Meckel's + LAR	109.1	71.9	81.8
MC Joint Medial	64.4	42.2	56.1
MC Angle Medial	-162.9	42.2	52.3
PQ + LAR	1.5	-5.5	3.8
PQ Depression	70.455	38.3	56.8
Magnitude			
Meckel's - LAR (°)	-6.03	-6.28	-11.57
Meckel's Medial (°)	3.69	1.79	8.97
MC Joint Medial (mm)	3.74	5.7	6.58

List of Symbols and Abbreviations

ACS, anatomical coordinate system

By, Basihyal

Cr, Cranium

Cy, ceratohyal

HC, Hyomandibuloceratohyal joint

Hy, hyomandibula

JCS, joint coordinate system

MC, Meckel's cartilage

MK, Knob on Meckel's cartilage

NC, Nasal Capsule

Or, Orbit

PQ, palatoquadrate

XROMM, X-ray reconstruction of moving morphology

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Competing Interests

The authors have no competing interests with the contents of this study.

Author Contributions

Bradley Scott is responsible for the introduction and discussion of the study and analysis of the trials, with input and commentary from the other authors. Implantation, anaesthesia, feeding, and contributions to the written components of the study came from Dr. Wilga. Dr. Brainerd ran the X-ray equipment, and contributed the experimental protocol used to record, track and analyze motions of bamboo sharks, as well as the written contributions to description of methods and results of the study.

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