

Ontogeny of feeding behavior and cranial morphology in the whitespotted bambooshark *Chiloscyllium plagiosum*

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Abstract Morphological and behavioral development of the feeding apparatus over early ontogeny can profoundly affect the ability of an organism to obtain nourishment, ultimately impacting survival. The interplay between morphology and behavior over the first year of life was studied in the whitespotted bambooshark *Chiloscyllium plagiosum* (Bennett 1830) beginning in March of 2002 using high-speed videography and dissection. Externally measured variables describing cranial growth, and jaw weight, scaled at or near isometry while jaw and hyoid musculature, especially the coracohyoideus, demonstrated considerable hypertrophication. The difference between the volume of the buccal cavity when open and closed scaled with substantial positive allometry while the time to reach maximum jaw and hyoid abduction exhibited weak allometry, resulting in the capacity for more rapid and greater volumetric intake during feeding. In addition, the relative forward motion of the predator during a strike decreased over ontogeny and the feeding modality became more suction-dominated. Kinematic variables exhibited little variability and the primary aspect of food capture that

was modulated in response to food type was the forward motion of the predator. An increase in capture success was noted for live, elusive shrimp over ontogeny indicating that morphological and behavioral changes have direct consequences for prey acquisition. Conservation of head shape coupled with a narrow behavioral repertoire is hypothesized to increase prey capture success in the wild over ontogeny as individuals become more proficient in the execution of a single, low-variability, suction-dominated capture behavior.

Introduction

Nutrient acquisition is crucial to survival, dictating that an organism's feeding apparatus is under selective pressure to perform at every developmental stage in which nutrients are exogenously obtained (Galis 1990; Galis et al. 1994). Aquatically feeding organisms are also subjected to additional functional constraints because of the density and viscosity of water (Lauder 1980; Liem 1990). Inertial suction feeding involves the generation of subambient buccal pressures, and maximizing the capacity to generate this pressure has been linked to numerous behavioral and morphological specializations (Liem 1993; Sanford and Wainwright 2002; Svanback et al. 2002; Carroll et al. 2004). These functional specializations in turn may lead to a more stenophagous (Sanderson 1991; Motta et al. 1995) or catholic diet (Castro 2000) depending on the degree of versatility and performance capacity of the feeding apparatus.

Qualitatively large differences in feeding behavior and morphology among aquatically feeding vertebrate species have been widely studied (e.g. Muller and Osse 1984; Liem

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and Summers 2000; Wilga et al. 2000), as have more subtle differences among populations of a single species (Cutwa and Turingan 2000; Huskey and Turingan 2001). The interaction between feeding morphology and behavior during ontogeny has received less attention despite the fact that variation among individuals over ontogeny is a key ecological and evolutionary precept (Van Valen 1965; Bolnick et al. 2003). Early ontogeny can be a period of rapid learning and morphological development that provides an opportunity to study substantial changes in feeding behavior and morphology. If capture performance during aquatic feeding is enhanced by the generation of greater subambient buccal pressure, then directional development of behavioral and morphological aspects that enhance suction is expected over ontogeny in species that rely on inertial suction feeding. In short, individuals should become more proficient (Sanderson 1991) at suction feeding over ontogeny as their morphology and behavior are incrementally tailored to meet the requirements for the generation of greater suction forces.

Sharks are exemplary organisms within which to investigate the development of feeding over early ontogeny because they feed readily in captivity and possess a mechanically simple feeding apparatus comprising relatively few structural elements. Furthermore, unlike teleost fishes and amphibians that undergo drastic metamorphic changes in their feeding apparatus (Reilly 1995; Hunt von Herbing 2001; Hernandez et al. 2002), sharks exhibit non-metamorphic, continuous growth allowing the study of developmental trajectories without these confounding effects. Additionally, much is known about the feeding of sub-adult and adult sharks both in laboratory and natural settings (Cortes 1999; Fouts and Nelson 1999; Motta and Wilga 2001; Motta 2004), but little is known about the development of feeding morphology and behavior over early ontogeny (but see Ferry-Graham 1997, 1998a, b). To assess natural ontogenetic shifts in diet we must first understand patterns of change in feeding morphology and behavior that affect prey capture capacity.

The whitespotted bambooshark *Chiloscyllium plagiosum* (Orectolobiformes: Hemiscylliidae) was selected as the subject for this study. *Chiloscyllium plagiosum* is a common reef-dwelling, benthic species of the Indo-West Pacific and eastern coast of Southern Asia (Compagno 1984b). *Chiloscyllium plagiosum* is oviparous, with hatchlings averaging 12–20 cm TL (A. Cornish, personal communication; Tullis and Petterson 2000), and breeds readily in captivity making it possible to observe first feeding. *Chiloscyllium plagiosum* is an opportunistic generalist that feeds primarily on benthic invertebrates and occasionally fish (A. Cornish, personal communication). Wu (1993) determined that adult *C. plagiosum* primarily use inertial suction to capture prey. This species was

expected a priori to be a suction feeder throughout ontogeny based on previous studies of the feeding apparatus and behavior of orectolobiform sharks (Wu 1993, 1994; Motta and Wilga 1999; Motta et al. 2002; Robinson and Motta 2002; Matott et al. 2005). The goals of this study were: (1) to describe ontogenetic changes in the behavior of *C. plagiosum* feeding on a variety of food types and sizes; (2) to determine the contribution of behavioral differences among individuals, among food types, and among food types within an individual to overall variability in feeding behavior throughout early ontogeny; and (3) to describe ontogenetic changes in the feeding morphology of *C. plagiosum*.

Methods and materials

Filming techniques

Five *C. plagiosum* (Bennett 1830) were hatched (average TL 15.76 cm, average weight 10.22 g) at SeaWorld, Orlando, Florida in March of 2002 and raised for 52 weeks, attaining an average TL of 43.64 cm and an average weight of 230.13 g. Sharks were maintained in a 340-l communal tank at $26 \pm 1^\circ\text{C}$ and 33 ± 2 salinity. During experimental sessions, individuals were isolated in a 100-l subsection of the holding tank to avoid influence by interactions with other individuals. Sharks were fed a maintenance diet consisting of 3–4% of their body weight in frozen krill *Euphausia superba*, frozen clam *Mercenaria mercenaria*, and live and frozen grass shrimp *Palaemonetes pugio*, ad libitum twice per week. This feeding frequency was maintained throughout the study except for feedings preceding experimental sessions, when the ration was cut to 2%. Experimental sessions began at first feeding, which occurred within 2 weeks of hatching.

To investigate directional changes of feeding behavior in response to food size, type, and elusivity (i.e. modulation), five food types were offered during experimental sessions: (1) chopped krill scaled to one half mouth width (MW); (2) chopped krill scaled to MW; (3) chopped clam (shelled) scaled to one half MW; (4) chopped clam scaled to MW; and (5) live shrimp scaled to MW in carapace length. Filming sessions occurred weekly over 1 year. During filming sessions food was presented in haphazard order until the individual approached satiation, as evidenced by decreased feeding activity. Though a filming session often comprised ten or more capture sequences for an individual, only the first five were considered for analysis to avoid satiation effects (Sass and Motta 2002).

Recordings of feeding events were obtained using a Redlake PCI 1000 high-speed digital camera (Redlake, San

Diego, CA, USA), which was placed perpendicular to the aquarium providing both a lateral view and, via a mirror beneath the shark, a ventral view. Recordings were made at 250 fps and illumination was provided by two, 500-W quartz-halogen lights. Individuals were trained to feed under illumination and were allowed a 20-min acclimation period prior to each session. For statistical purposes, the 52-week experimental time period was broken into four even segments. A total of five capture sequences per food type ($N = 5$) per individual ($N = 5$) per time segment ($N = 4$) were recorded, for a total of 500 sequences. Sequences were not obtained weekly for each food type/individual combination due to satiation. Kinematic data were obtained using Redlake MotionScope PCI software version 2.21.1 (Redlake, San Diego, CA, USA) and SigmaScan Pro version 4 (SPSS Inc.) From the onset of mandible depression (time 0 ms), the following kinematic variables were quantified: (1) strike distance (cm) at time 0 ms; (2) maximum gape (cm); (3) time to maximum gape (ms); (4) maximum cranial elevation angle (degrees); (5) time to maximum cranial elevation angle (ms); (6) time to onset of cranial elevation (ms); (7) time to offset of cranial elevation (ms); (8) duration of cranial elevation (ms); (9) time to onset of hyoid depression (ms); (10) maximum hyoid depression (cm); (11) time to maximum hyoid depression (ms); (12) time to hyoid retraction (ms); (13) duration of hyoid depression (ms); (14) total strike duration (ms); (15) duration of food movement (ms); (16) distance moved by the food (cm); (17) velocity of the food (cm s^{-1}); (18) distance moved by the predator (cm) during the duration of food movement; and (19) velocity of the predator (cm s^{-1}). No measures were made of the extent or timing of upper jaw protrusion because protrusion was often obscured by movement of the labial cartilages. Variables (16) and (18) were used to calculate the ram-suction index (RSI) (Norton and Brainerd 1993). The RSI is calculated as

$$(D_{\text{PREDATOR}} - D_{\text{PREY}})/(D_{\text{PREDATOR}} + D_{\text{PREY}})$$

where D is the distance moved by either the predator or prey. This index indicates the relative contribution of predator and prey motion to a given capture event. An RSI value of 1 indicates a purely ram-based bite and a value of -1 indicates a purely suction-based bite. Because of inherent problems with the RSI (Van Damme and Aerts 1997), including the rapidly decreasing effect of suction with increasing distance from the mouth of the predator (Muller and Osse 1984; Day et al. 2005), it is used here primarily to allow comparison with other studies of suction feeding. The number of failed attempts occurring prior to food capture was recorded for each capture event throughout the study.

Morphological measurements

To chronicle ontogenetic changes in morphology, frames from digital recordings of feeding were used to measure several external morphological variables. The definitions of these variables follow Compagno (1984b) and they consisted of: (1) total length; (2) mouth width; (3) mouth length; (4) preoral length; (5) preorbital length; (6) prebranchial length; (7) branchial length; (8) prepectoral length; (9) anterior pectoral fin base to lower jaw; (10) head length; (11) head width; and (12) head depth at the position of the hyoid. To verify measurement accuracy, individuals were removed from the tank every 4 weeks and the same measurements taken. Measurement error was always less than 2.3%.

To acquire measurements of muscle mass, muscle cross-sectional area, and buccal volume that could not be obtained from live individuals, fresh-dead specimens ($N = 9$) spanning the size range of live specimens used in this study were dissected. The morphological variables measured on live sharks were also measured for dead specimens. Wet muscle mass and cross-sectional area were obtained by excising muscles involved in lower jaw and hyoid depression, specifically the coracomandibularis, coracohyoideus, and coracoarcualis (Motta et al. 1991; Wu 1994; Motta and Wilga 1999). SigmaScan Pro version 4 (SPSS Inc.) was used to measure anatomical cross-sectional area from digital photographs of bisected muscles taken with a Nikon Coolpix 4300. The palatoquadrate and Meckel's cartilage were also excised and their combined weight recorded because their growth could influence the velocity and force of feeding motions. The combined weight was used because these elements are tightly anatomically linked and protruded together as a unit (Wu 1993, 1994; Matott et al. 2005).

Buccal volume was obtained by injecting silicone into the buccal cavity of each dead specimen and allowing it to cure for 36 h before removal and weighing. Using the mass-density of silicone reported by Cook (1996) (0.06 g ml^{-1}), weight was converted to volume. Casts were made of the buccal cavity in a resting position and in a maximally expanded position based on kinematic footage. The difference between these two volumes, the buccal reserve volume, indicates the maximum possible change in buccal volume during feeding.

Statistical analyses

All kinematic data were checked for normality and homogeneity of variance using SigmaStat Pro version 3.1 (SPSS Inc.). As several variables exhibited skewed distributions, a Spearman Rank Order Correlation test was

performed to establish the relationship between each variable and total length. Several variables were highly correlated ($P < 0.01$) with total length, so data were log-transformed to meet the assumptions of normality and Model II linear regressions were performed to examine these relationships. Regressions were performed for individuals combined to obtain an overall scaling coefficient. Regressions were then performed for each individual separately, allowing comparison of coefficients among individuals. Differences among individuals were assessed using a modified Student's t test (Zar 1999).

To standardize statistical handling of all data for further analysis, variables that lacked a relationship with total length were log-transformed and regressed against total length using Model II linear regressions and the studentized residuals obtained (Quinn and Keough 2002). Size-corrected, studentized residuals for all kinematic variables were then used in a principal components analysis (PCA) that reduced the expansive kinematic data set to a few, orthogonally oriented composite variables. An Equamax rotation was used because it produced higher loadings than other rotations, enhancing data interpretation. Variables loading above $|0.5|$ were considered to contribute heavily to variability within the respective principal component. Principal components with an eigenvalue greater than 1.0 were retained for further analysis. Factor loading scores for each capture sequence on each principal component were then used in a two-way, mixed-model, repeated measures (RM) MANOVA to identify differences in multivariate space over the repeated measure time. Individual was a random factor and food type was a fixed factor tested over the interaction term. Significance was assessed using Pillai's trace. To further investigate differences identified by the RM MANOVA, a two-way, mixed-model RM ANOVA was performed for each principal component separately using individual as a random factor and food type as a fixed factor tested over the interaction term. Statistical significance was evaluated using Fisher's LSD. To determine the order of the best-fit polynomial describing trends over time, single degree of freedom polynomial contrasts were utilized. The PCA, MANOVA, and RM ANOVA tests were performed using Systat 11 (SPSS Inc.).

To investigate changes over time in the relative dimensions of morphological variables measured on living specimens, data were log-transformed and regressed against the logarithm of total length using Model II linear regressions. A slope of one for these regressions indicated isometric growth. Significant deviations from a slope of one were tested using Student's t (Zar 1999) and indicated allometry for the given feature. Regressions were performed for each individual separately and then 95% confidence intervals were determined for all live individuals combined. For variables measured on both live and dead specimens, data

from dead specimens were compared against the confidence intervals for live individuals to determine if they could have been drawn from the same population. Comparing live and dead scaling data via this method allowed data collected exclusively on dead specimens to be applied to live specimens in the appropriate size range. Morphological variables measured exclusively on dead specimens were log-transformed and regressed against the logarithm of total length using Model II linear regressions. The expected slope of these regressions depended on the dimensionality of the variable being considered.

Results

Individuals generally rested on the bottom or swam slowly around the filming chamber until food was introduced. The shark would then rapidly search along the bottom in a circular sweeping pattern. Food capture kinematics began with lower jaw depression or cranial elevation and progressed in a posteriorly directed fashion, with the hyoid beginning to depress shortly after the onset of jaw opening and reaching its maximum excursion well after the time of maximum gape (Fig. 1). The temporal sequence of kinematic events was conservative across ontogeny and among food types. Capture success was high with all dead food types, averaging 1.1 ± 0.3 attempts per capture and changing little over ontogeny. The number of attempts needed to capture live shrimp, however, declined steadily from 2.5 ± 0.8 during the first time segment to 1.6 ± 0.5 during the last.

Scaling of kinematics

When individuals were combined, most kinematic variables scaled with positive coefficients over ontogeny demonstrating that slower, more extensive motions accompanied growth (Table 1). The only two variables that decreased over ontogeny were the time to onset of hyoid depression and RSI, indicating that as sharks grew they depressed their hyoid earlier and their feeding modality became more suction-dominated. The distance moved by and velocity of the predator during the strike did not change with size, while the distance moved by the food increased (Table 1). Taken together, these relationships indicate that the RSI decrease over ontogeny was caused exclusively by greater motion of the food due to suction.

When individuals were analyzed separately, differences were detected in the extent of the scaling coefficient for many variables but rarely the sign (Table 1). This demonstrates that while substantial inter-individual variability exists in kinematic scaling over ontogeny the general trends presented above are representative.

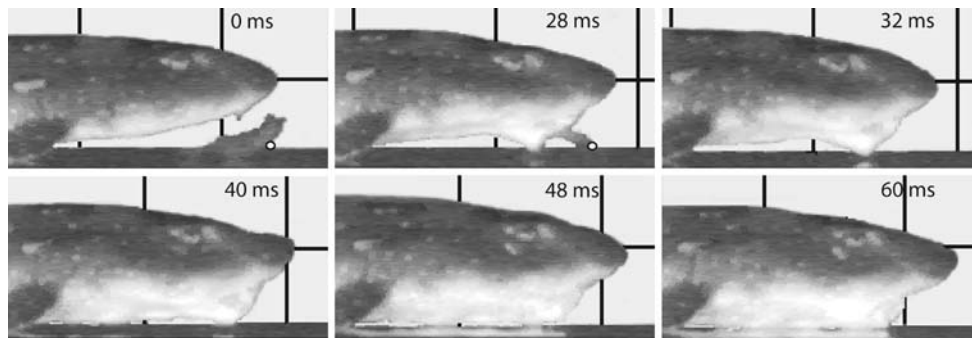


Fig. 1 *Chiloscyllium plagiosum*. Composite photographic series of a representative food capture sequences. The point on the food that is farthest from the shark's mouth is indicated by a white dot. This was the point used in calculating the RSI. Times for this capture are shown

in the upper right corner of each field. In chronological order the events shown are onset of lower jaw depression, maximum gape, food capture, maximum cranial elevation, maximum hyoid depression, and jaw closure on the food

Table 1 *Chiloscyllium plagiosum*. Regression parameters for kinematic variables versus total length (cm)

Variable	Individuals combined				Individuals separated	
	Slope	y-int	StErr	P	Range of slopes	
Max gape	0.90	-1.40	0.04	<0.001*	0.79*	1.11*
Time to max gape	0.21	1.12	0.07	0.005*	0.02**	0.37**
Max cranial elev angle	–	–	–	0.334	-0.93**	0.97**
Time to max cranial elev angle	0.29	1.17	0.07	<0.001*	0.03*	0.48*
Time to onset of cranial elev	–	–	–	0.345	-0.40	0.03
Time to offset of cranial elev	0.75	0.79	0.08	<0.001*	0.43**	1.15**
Duration of cranial elev	0.92	0.47	0.10	<0.001*	0.58*	1.50*
Time to onset of hyoid depress	-0.32	1.64	0.15	0.032*	-0.54	-0.31
Max hyoid depress	0.97	-1.77	0.08	<0.001*	0.54**	1.30**
Tim to max hyoid depress	0.37	1.10	0.06	<0.001*	0.13*	0.62*
Time to hyoid retraction	0.26	1.65	0.05	<0.001*	0.08	0.47
Duration of hyoid depress	0.38	1.42	0.06	<0.001*	0.19*	0.55*
Duration of food movement	0.30	0.96	0.09	<0.001*	0.03*	0.49*
Total strike duration	–	–	–	0.118	-0.48**	0.11**
Distance moved by food	0.32	-0.58	0.08	<0.001*	0.13	0.41
Velocity of food	–	–	–	0.797	-0.12*	0.47*
Distance moved by predator	–	–	–	0.878	-0.27**	0.23**
Velocity of predator	–	–	–	0.078	-0.53**	-0.09**
RSI	-0.22	0.07	0.11	0.039*	-0.51*	-0.07*
Strike distance	0.87	-1.65	0.13	<0.001*	0.55**	1.55**

Results shown are for all individuals combined ($N = 5$). Where differences among individuals were detected using a modified Student's t test, the magnitude of these differences is indicated

max maximum; depress depression; elev elevation

Kinematic trends

PCA reduced the set of kinematic variables into six principal components that accounted for 72.80% of the overall variability in the data set. Five kinematic variables did not load heavily on any of the principal components (PCs) (Table 2). Three of these related to cranial elevation during the period from onset to maximum, indicating that the

timing and extent of cranial elevation exhibited little variation. Timing variables loaded on PCs 1, 3, and 4, with general timing variables loading on PC1, variables describing depression of the cranium from maximum excursion loading on PC3, and variables describing the recovery of the hyoid to its resting position loading on PC4. Variables associated with forward motion of the predator loaded on PC2 while variables describing the motion of the

Table 2 *C. plagiosum*. Principal component loadings of kinematic variables associated with food capture sequences

Variable	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6
Duration of prey movement	0.844	0	0	0	0	0
Total strike duration	0.626	0.309	0	0	0	0
Time to max gape	0.623	0	0	0	0	0
Velocity of food	-0.528	0	0	0	-0.770	0
Time to max hyoid depress	0.523	0	0	0.260	0	0
Distance moved by predator	0	-0.966	0	0	0	0
RSI	0	-0.914	0	0	0.382	0
Velocity of predator	-0.368	-0.870	0	0	0	0
Duration of cranial elev	0	0	0.949	0	0	0
Time to offset of cranial elev	0	0	0.910	0	0	0
Duration of hyoid depress	0	0	0	0.971	0	0
Time to hyoid retraction	0	0	0	0.953	0	0
Distance moved by food	0	0	0	0	-0.949	0
Max gape	0	0	0	0	0	0.754
Max hyoid depress	-0.488	0	0	0	0	0.561
Time to onset of cranial elev	0	0	0	0	0	0
Time to onset of hyoid depress	0	0	0	0	0	0
Time to max cranial elev angle	0.356	0	0.491	0	0	0
Max cranial elev angle	-0.298	0	0.400	0	0	0.376
Strike distance	0	-0.303	0	0	-0.306	0.465
Eigenvalue	4.72	2.65	2.27	1.89	1.63	1.08
Percent variance explained	19.99	14.00	11.56	10.55	10.15	6.56

Bold face values indicate variables determined to load heavily on the respective component (loading scores >|0.5|) ($N = 5$). Together the six components explain 72.80% of the overall variability in the data set. For clarity, all loadings <|0.25| are replaced by 0

max maximum; *depress* depression; *elev* elevation

food loaded on PC5. The RSI, though it is an index based on motion of both the predator and food, only loaded heavily on PC2. This indicates that variability in predator motion was greater than variability in food motion. Lastly, variables describing the extent of buccal expansion, as indicated by maximum gape and maximum hyoid depression, loaded on PC6 (Table 2).

The RM MANOVA conducted simultaneously on all six principal components indicated that differences existed over ontogeny (Pillai Trace $df = 3$; $F = 5.99$; $P = 0.001$) and among individuals over ontogeny ($df = 12$; $F = 3.61$; $P < 0.001$), but not among food types over ontogeny ($df = 12$; $F = 1.35$; $P = 0.190$) or among food types for a given individual over ontogeny ($df = 48$; $F = 1.05$; $P = 0.338$). When each PC was examined with a separate RM ANOVA for differences among individuals they were detected on all components regardless of ontogeny and on all components except PC2 (predator motion) over ontogeny (Table 3). Thus, despite a high degree of overlap among individuals, substantial inter-individual differences in kinematics did exist. Differences among captures on various food types regardless of ontogeny existed only on PCs 1 (general timing) and 2 (predator motion), with

strikes on live, small, and large prey separating on both PCs (Fig. 2). Interactions between the factors shark and food type were only found on PCs 1 (general timing) and 4 (hyoid timing). Differences over ontogeny among captures of various food types were only detected on PCs 2 (predator motion) and 5 (food motion), indicating that modulation in response to food attributes did not occur in the timing or extent of cranial motions over ontogeny. Effect interactions among sharks within food types over ontogeny existed only on PCs 5 (food motion) and 6 (buccal expansion), indicating complex interactions attributable to the combined influence of individual and food type.

Scaling of morphology

Morphometric variables scaled with isometry or very slight, though statistically significant, allometry (Table 4). The greatest allometric coefficient was quantified for mouth width (1.05), producing a deviation of 12% from isometry at the age of 1 year. Scaling coefficients exhibited by other morphological attributes produced estimates varying by less than 3% from predictions of isometry after 1 year. Measurements from dead specimens fell within the

Table 3 *C. plagiosum*. Results of RM ANOVAs performed separately on each principal component from a PCA of prey capture kinematics ($N = 5$)

	PC1: General timing			PC2: Predator motion		
	<i>Df</i>	<i>F</i>	<i>P</i>	<i>Df</i>	<i>F</i>	<i>P</i>
Between subjects						
Shark	04	20.562	<0.001*	4	3.842	0.006*
Food	4	6.203	0.004*	4	13.952	<0.001*
Shark × Food	16	3.161	<0.001*	16	1.143	0.327
Within subjects						
Time segment	3	8.421	<0.001*	3	5.409	0.003*
TS × Shark	12	4.705	<0.001*	12	1.385	0.172
TS × Food	12	1.649	0.072	12	2.151	0.014*
TS × Shark × Food	48	1.153	0.238	48	1.363	0.065
	PC3: Cranial timing			PC4: Hyoid timing		
	<i>Df</i>	<i>F</i>	<i>P</i>	<i>Df</i>	<i>F</i>	<i>P</i>
Between subjects						
Shark	4	3.771	0.007*	4	4.974	0.001*
Food	4	2.457	0.089	4	1.222	0.386
Shark × Food	16	1.024	0.438	16	2.173	0.010*
Within subjects						
Time segment	3	1.836	0.155	3	2.536	0.076
TS × Shark	12	3.184	<0.001*	12	1.966	0.027*
TS × Food	12	1.362	0.193	12	0.663	0.786
TS × Shark × Food	48	1.359	0.073	48	1.884	0.147
	PC5: Food motion			PC6: Buccal excursion		
	<i>Df</i>	<i>F</i>	<i>P</i>	<i>Df</i>	<i>F</i>	<i>P</i>
Between subjects						
Shark	4	10.431	<0.001*	4	10.740	<0.001*
Food	4	2.530	0.082	4	2.881	0.061
Shark × Food	16	1.643	0.071	16	2.881	0.061
Within subjects						
Time segment	3	2.462	0.070	3	0.299	0.887
TS × Shark	12	4.161	<0.001*	12	3.994	<0.001*
TS × Food	12	3.803	<0.001*	12	1.522	0.115
TS × Shark × Food	48	1.581	0.012*	48	2.257	<0.001*

For all between subjects, comparisons the error degrees of freedom are 100, while for all within subjects comparisons the error degrees of freedom are 300

Df degrees of freedom; *TS* time segment

95% confidence intervals of those from live specimens and were dominated by isometric or very slightly allometric growth patterns.

Growth trajectories calculated for masses and cross-sectional areas of muscles, in addition to buccal volume, were dominated by substantial allometry (Table 5). The cross-sectional area of all jaw abducting muscles scaled with positive allometry. The coefficient for the cross-sectional area of the coracohyoideus was especially

remarkable (3.04), reflecting a difference of ~51% from the area predicted by isometry. Despite their cross-sectional areas scaling with positive allometry, the weight of the coracohyoideus and coracomandibularis scaled with negative allometry (Table 5). This paradox is likely attributable to unquantified changes in muscle length over ontogeny. The combined weight of the palatoquadrate and Meckel's cartilage scaled with positive allometry, but to a lesser extent than the cross-sectional area of muscles

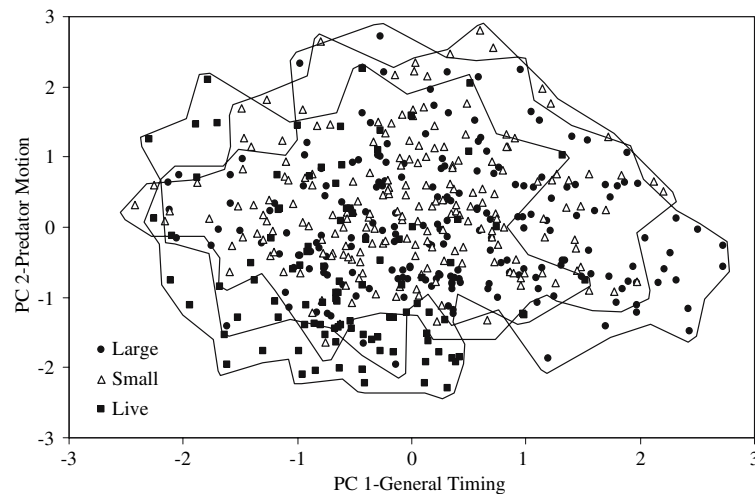


Fig. 2 *C. plagiosum*. Comparison of principal component 1 (*PC1*) (general timing) and predator motion (*PC2*) scores for large, small, and live food irrespective of time. Together the variability explained by these two components is 33.99% of the overall variability in the

kinematic data set. Polygons delimit the region of the plot occupied by bites for each food type and indicate significant difference at the $\alpha = 0.05$ level. Note the high degree of overlap among food types, which is also a prevailing trend on the remaining PCs

Table 4 *C. plagiosum*. Parameters for log–log regressions against total length (cm) of external morphological variable data measured on live specimens

Variable	Slope	y-intercept	r^2	St Error	<i>P</i>
Mouth width	1.05	−1.48	1.00	6.1E−4	<0.001*
Mouth length	1.02	−2.46	0.95	2.1E−3	<0.001*
Pre-oral length	0.99	−1.35	0.99	7.8E−4	<0.001*
Pre-orbital length	1.00	−1.13	0.99	9.3E−4	0.350
Pre-branchial length	1.01	−0.86	0.99	1.1E−3	<0.001*
Pre-pectoral length	0.99	−0.78	0.99	1.0E−3	<0.001*
Pectoral fin base to LJ	1.00	−0.95	0.99	8.0E−4	0.309
Head length	1.01	−0.73	0.99	9.4E−4	<0.001*
Branchial Length	1.00	−1.33	0.97	1.6E−3	<0.001*
Head depth at hyoid	0.99	−1.14	0.99	9.8E−4	<0.001*
Head width	0.98	−0.94	0.99	9.3E−4	<0.001*

Results shown are averages for individuals ($N = 5$). The expected slope for isometry is one in all cases.
 $t_{0.05(1), 40} = 1.684$

Table 5 *C. plagiosum*. Parameters for log–log regressions against total length (cm) of morphological variable data taken from dead specimens only

Variable	SI_{EXP}	Slope	y-intercept	r^2	St Error	<i>P</i>
Buccal volume closed (ml)	3	2.75	−2.69	0.99	1.2E−2	<0.001*
Buccal volume open (ml)	3	3.04	−2.79	0.99	1.2E−2	0.003*
Buccal reserve volume (ml)	3	3.31	−3.46	0.99	1.2E−2	<0.001*
Combined jaw weight (g)	3	3.08	−4.54	0.96	2.9E−2	0.018*
Coracohyoideus area (mm ²)	2	3.04	−5.36	0.99	1.7E−2	<0.001*
Coracomandibularis area (mm ²)	2	2.36	−4.52	0.99	1.3E−2	<0.001*
Coracoarcualis area (mm ²)	2	2.39	−4.13	0.97	2.0E−2	<0.001*
Coracohyoideus weight (g)	3	2.55	−4.51	0.99	9.0E−3	<0.001*
Coracomandibularis weight (g)	3	2.53	−4.41	0.97	2.0E−2	<0.001*
Coracoarcualis weight (g)	3	3.13	−5.16	0.97	2.4E−2	<0.001*

The expected slope for isometry is given in the column labeled SI_{EXP} . $t_{0.05(1), 8} = 1.860$

acting on them. The volume of the buccal cavity when closed scaled with negative allometry while the volume of the buccal cavity when open scaled with slight positive allometry. This discrepancy lead to a substantial

positive allometric coefficient for the buccal reserve volume indicating that as *C. plagiosum* grows its relative capacity for buccal expansion substantially increases (Fig. 3).

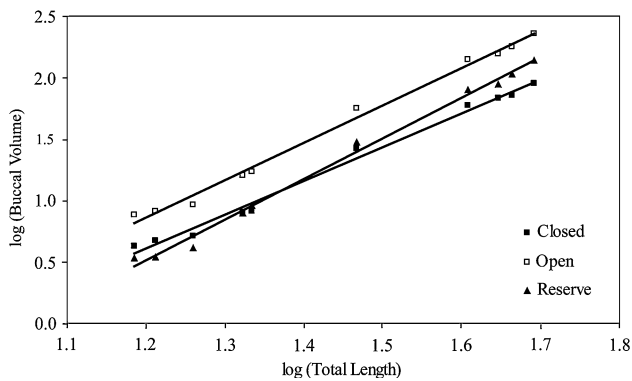


Fig. 3 *C. plagiosum*. Scaling of buccal volume relative to total length. Measurements calculated from the weight of silicone casts obtained from dead specimens placed in resting and maximally expanded positions based on kinematic footage. Reserve volume is the difference between the open and closed position volume for a specimen. The expected slope for isometry is three in all cases. Model II regression equations are: buccal volume closed $y = 2.75x - 2.69$, $r^2 = 0.99$; buccal volume open $y = 3.04x - 2.79$, $r^2 = 0.99$; buccal reserve volume $y = 3.31x - 3.46$, $r^2 = 0.99$

Discussion

Changes in feeding morphology and kinematics in *Chiloscyllium plagiosum* over early ontogeny coincide with theoretically predicted changes that enhance the generation of subambient buccal pressure during feeding, presumably increasing feeding performance. Though most morphological aspects of the head scale with isometry or slight allometry, the musculature associated with jaw and hyoid abduction hypertrophies over ontogeny. Similar hypertrophication of the feeding musculature has also been shown in the suction-feeding African catfish *Clarias gariepinus* (Herrel et al. 2005). Buccal reserve volume grows allometrically at a rate greater than the positive allometric increase in the time to maximum gape and maximum hyoid depression. Coincident with a decrease in overall predator motion, the combined effect of these relationships is a positively allometric increase in the relative contribution of suction through ontogeny in *C. plagiosum*.

Ferry-Graham (1998b) described differences between hatchling and juvenile swellsharks *Cephaloscyllium ventriosum* with respect to cranial morphology and feeding kinematics. She noted isometric growth but differences in RSI attributable to increased predator motion masking the suction component of the strike in hatchlings. A mathematical model of buccal volume changes during feeding in *C. ventriosum* indicated isometric growth of the buccal cavity, leading to an isometric prediction for suction generation in this species (Ferry-Graham 1998b). Isometric growth of the buccal cavity was also noted in the cottid fish *Clinocottus analis*, but differential motion of the body

relative to the premaxilla during strikes lead to an increase in the apparent degree of suction over ontogeny (Cook 1996). To establish whether the increase in suction over ontogeny in *C. plagiosum* is mediated by an allometric increase in suction generation or simply due to a decrease in predator motion direct measurements of suction pressure are underway.

Within the broader trend of directional development toward increasing utilization of suction, limited modulation in response to food type was observed in *C. plagiosum*. Differences in general timing variables (PC1) detected among small, large, and live food bites regardless of ontogeny are largely due to divergence in the velocity and duration of food movement. Because these variables are affected by interactions between food and the flow generated by the shark, which are partially dictated by physical attributes of the food, the modulation should be interpreted cautiously. If the force exerted by the entraining flow of water is the same among bites on different food types, the response of food types to this flow could vary in a consistent manner that would be statistically detected as modulation. Assessment of the response of various foods to controlled suction forces is necessary to assert that *C. plagiosum* is modulating kinematic timings in response to food type. When food presentation is via tongs, restricting food motion, the obligate suction-feeding nurse shark *Ginglymostoma cirratum* does not modulate its kinematics in response to food type (Matott et al. 2005). The lack of differences in the pattern of response over ontogeny in *C. plagiosum* argues for stereotypy of feeding kinematics but modulation of overall predator motion in response to food type (Table 3).

Despite the conservative kinematic patterns described above, differences were detected among individuals both irrespective of and over the course of ontogeny. The incidence of notable variability in feeding kinematics among and within individuals is nearly universal (Wainwright and Lauder 1986; Cook 1996; Ferry-Graham 1998a), even in species like *G. cirratum* that exhibit marked stereotypy (Motta et al. 2002). A deficiency of studies chronicling feeding behavior and morphology of individuals across ontogeny has led to a lack of information regarding intra-specific variability in developmental trajectories. As disparities among individuals can influence performance, niche utilization, and potentially survival (Van Valen 1965; Bolnick et al. 2003), the application of laboratory studies of feeding to natural settings is limited if inter-individual variability is not considered. The magnitude of developmental differences over ontogeny is likely to increase in natural settings due to variability in predator experience and heterogeneity of available resources (Morse 1980), compounding the impact of intra-individual effects on niche diversification. Intra-individual developmental

differences could serve as a fertile source of variability, especially in species that exhibit conservative growth and narrow behavioral repertoires, permitting response to environmental variations over phylogeny (Liem 1980).

The pattern of morphological and behavioral development described for *C. plagiosum* is in stark contrast to that reported in a similar study of the leopard shark *Triakis semifasciata* (Lowry et al. 2007). The growth of the head of *T. semifasciata* is strongly allometric, producing marked changes in head shape over ontogeny. This is accompanied by hypertrophication of the jaw and hyoid abducting musculature, but not to the extent measured for *C. plagiosum*. Additionally, BRV increases isometrically in *T. semifasciata*, which is both a dietary and behavioral generalist (Talent 1976; Compagno 1984a; Kao 2000). The feeding kinematics of *T. semifasciata* are characterized by extensive variability and modulation of timing and modality variables in response to prey size and elusivity (Ferry-Graham 1998a; Lowry et al. 2007). *Triakis semifasciata* apparently utilizes a variable, ram-dominated feeding repertoire over early ontogeny to capture functionally and taxonomically diverse prey in the wild. By contrast, *C. plagiosum* employs a narrow range of behavior characterized by limited variability and modulation over the range of food types tested. Dietary information for young *C. plagiosum* is lacking, but the ability to capture elusive shrimp is clearly demonstrated here. Capture of diverse prey items (Castro 2000) using a single, highly stereotyped feeding behavior (Motta et al. 2002) has been demonstrated in the orectolobiform shark *G. cirratum*. This narrow behavioral range is hypothesized to predominate in bottom-dwelling, suction-feeding elasmobranchs (Motta and Wilga 2001; Motta 2004). For both *C. plagiosum* and *T. semifasciata* (Lowry et al. 2007) an increase in capture success when feeding on live shrimp was noted, demonstrating that with experience young sharks are able to capture more elusive food regardless of whether their behavioral repertoire consists of a single, specialized behavior or numerous, highly variable behaviors. Thus, for young-of-the-year sharks, at least two developmental strategies exist that both have the potential to facilitate exploitation of a taxonomically diverse food base.

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