

The Early Ontogeny of Feeding in Two Shark Species:
Developmental Aspects of Morphology, Behavior, and Performance

by

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Dedication

This work is dedicated to researchers everywhere that seek knowledge for its inherent value and strive for its practical application every day.

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ABSTRACT

Early ontogeny is a time of rapid anatomical and behavioral development in most organisms. The degree of synchrony between form and function during this period, and the concomitant performance consequences, can strongly impact individual survival. Understanding the development of feeding during early ontogeny is important because nutrient acquisition universally influences organismal biology. A one-year, longitudinal feeding study was conducted for two elasmobranch species that were selected for their disparate morphology, behavior, and habitat: the whitespotted bambooshark *Chiloscyllium plagiosum* and the leopard shark *Triakis semifasciata*. To quantify changes in cranial morphology, external attributes of the feeding apparatus were measured weekly. Additionally, specimens were dissected to examine trends in the growth of select muscles and the volume of the buccal cavity. To quantify feeding behavior, individuals were observed weekly using high-speed digital cameras as they consumed various food types. Suction performance was evaluated using particle image velocimetry and direct measurements of suction pressure. The cranial morphology of *C. plagiosum* exhibited primarily isometric growth while the cranial morphology of *T. semifasciata* was dominated by allometric growth. Allometric increases were noted in

the cross-sectional area of every muscle examined in both species, though the primary hyoid depressor, the coracohyoideus, hypertrophied to a greater degree in *C. plagiosum*. Although intra-individual differences throughout ontogeny complicated comparison, modulation in response to food attributes was clearly evident in *T. semifasciata* but broadly absent in *C. plagiosum*. Over ontogeny *C. plagiosum* generated allometrically greater suction while *T. semifasciata* generated relatively less. The shape of the parcel of water ingested during feeding did not change over ontogeny in either species. The capacity to perform diverse feeding behaviors throughout ontogeny is not constrained in *T. semifasciata* but tends to be stereotyped and accompanied by enhanced performance in *C. plagiosum*. A functionally generalized feeding apparatus and repertoire may benefit *T. semifasciata* by allowing the use of diverse feeding behaviors in variable environments, such as estuaries, over ontogeny. Morphological and behavioral conservation of the feeding apparatus throughout ontogeny, however, may allow *C. plagiosum* to exploit taxonomically varied crevice-dwelling reef organisms using a single specialized behavior.

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Chapter 1: Introduction

For every organism, obtaining nutrients from resources available in the environment is paramount to survival. In gnathostomes, feeding is a crucial aspect of interaction with the external environment that has been facilitated by the novel evolution of jaws supported by a rigid endoskeleton (Kent and Carr, 2001; Benton, 2004). Modification of the anatomy, morphology, mechanical function, and utilization of the jaws over evolutionary time represents a major axis of phylogenetic and ecological diversification that has contributed to the radiation of this diverse lineage (Lauder, 1985a; 1985b; Liem, 1990; Schwenk, 2000; Wilga, et al., 2000; Ferry-Graham and Lauder, 2001).

The long-term effects of feeding within the life of an individual are numerous and diverse. Generation of tissue for growth, repair, and development can be drastically affected by nutrient availability. Not only is growth rate affected by gross nutrient uptake, but also by the type and quality of nutrients consumed. Because size, both of the entire organism and of select anatomical features, has been shown to affect mechanical capacity, diverse aspects of performance, and behavioral interactions with other organisms, the rate at which size increases (i.e. growth rate) is critical in determining the niche of an individual (Werner and Gilliam, 1984; Galis, et al., 1994; Arendt and Wilson, 1997). Reproductive state and capacity are also influenced by the availability of nutrients and nutrient reserves, and the expense of energy associated with the act of mating can be

immense. As such, the fitness of an organism is contingent upon feeding, making elucidating the roots of this behavior key to understanding the ecology and evolution of a species.

Aquatic Feeding

The interaction between the anatomy, mechanical function, and behavioral application of the feeding apparatus has been broadly investigated in aquatic gnathostomes over the last forty years (Alexander, 1967; Muller and Osse, 1984; Reilly and Lauder, 1988; Motta, et al. 1997; 2002; Ferry-Graham, 1998a; 1998b; Ferry-Graham and Lauder, 2001; Wainwright, et al., 2001a; 2001b). A prominent conclusion of this research is that physical attributes of the aquatic medium substantially influence the capacity of these organisms to capture prey (Lauder and Clark, 1984; Muller and Osse, 1984; Wainwright, et al., 2001b; Higham, et al., 2005}. The principle of continuity dictates that expansion of the buccopharyngeal cavity during feeding will cause an influx of water into the cavity as a consequence of the development of subambient pressure. During inertial suction feeding, this flow of water entrains prey or food items allowing them to be ingested by the predator. Inertial suction feeding is the dominant, and presumably ancestral, mode of prey procurement in the teleost fishes (Lauder, 1985a; 1985b) and early research on osteichthian fishes identified a highly conservative feeding sequence associated with this prey capture mode. In this sequence each bite consists of four phases: preparatory, expansive, compressive, and recovery (Liem, 1978). Subsequent studies have confirmed this feeding sequence is found among taxonomically distant species of aquatic gnathostomes, including elasmobranchs (Motta, et al., 1997;

Ferry-Graham, 1998a; 1998b; Wilga and Motta, 1998a; 1998b; Robinson and Motta, 2002), amphibians (Reilly and Lauder, 1989; Reilly, 1995; Deban, 1997), and reptiles (Lauder and Prendergast, 1992; Van Damme and Aerts, 1997; Summers, et al., 1998b), with variations from the model primarily occurring in the relative duration of each phase.

Inertial suction feeding may or may not be associated with rapid forward motion of the predator (Norton and Brainerd, 1993). Simultaneous with the generation of sub-ambient buccopharyngeal pressure the predator may overtake its prey or food and consume it completely or in discrete portions, termed ram feeding (Norton and Brainerd, 1993). A single feeding strike can be described as employing a combination of the two modalities (Norton and Brainerd, 1993). Despite the apparently strict conservation of the medium-constrained, four-stage feeding sequence among aquatic gnathostomes, considerable research has shown that prey mobility, size, hardness, and type can all elicit modulation in teleosts, turtles, and elasmobranchs (Wainwright and Lauder, 1986; Lauder and Prendergast, 1992; Nemeth, 1997; Ferry-Graham, 1998a; Ferry-Graham, et al., 2001b). Modulation is the ability of an individual organism to consistently alter the motor and kinematic patterns of prey capture in response to changes in feeding conditions, including prey size and/or type (Chu, 1989; Sanderson, 1990). Another important type of deviation within the four-stage aquatic feeding model is termed variation and deals with the intrinsic unpredictability that exists both between bites from a single organism and among the bites of conspecifics employing the same kinematic and motor patterns to feed on similar prey. Variation differs from modulation in that it is not a predictable and consistent change in kinematics but, rather, a random fluctuation about

the mean within an established kinematic pattern (Wainwright and Lauder, 1986; Sanderson, 1990; Motta, et al., 1997).

Although an organism may be capable of behaviorally modulating its feeding in response to prey size or type, anatomical specializations that enhance the capacity to perform suction feeding have been noted. Notable specializations include a deep pharyngeal region, a protrusible upper jaw, a small, laterally-enclosed gape, reduced dentition, and a wide skull for the insertion of well-developed epaxialis muscles (Muller and Osse, 1984; Liem, 1993; Carroll et al. 2004). In short, these anatomical attributes enhance the capacity of the organism to generate rapid or volumetrically large influxes of water into the buccopharyngeal cavity. It is the interplay among anatomical features of the feeding apparatus and the behaviors for which they are employed that dictate the niche occupied by an organism.

The Ontogeny of Feeding

Ontogenetic changes are variations in the ecology or morphology of an organism that occur during development. Ontogenetic changes in diet (Stoner and Livingston 1984; Lowe, et al., 1996; Brickle, et al., 2003), prey capture success (Reilly and Lauder, 1988; Coughlin, 1991), and various other behavioral and morphological parameters associated with feeding have long been noted in aquatic gnathostomes (Skulason, et al., 1989; Osse, 1990; Reilly and Lauder, 1990; Ellis and Shackley, 1995; Reilly, 1995; 1996; Richard and Wainwright, 1995). Ontogenetic changes in the mechanical feeding elements and size of teleost fishes have been shown to greatly influence feeding performance and modality (Skulason, et al., 1989; Osse, 1990; Coughlin, 1991; Richard

and Wainwright, 1995; Cook, 1996; Van Wassenbergh, et al., 2005). In lineages that metamorphose between larval and juvenile life stages, such as teleost fishes and amphibians, anatomical and behavioral changes concomitant with metamorphosis can drastically influence feeding (Coughlin, 1991; Reilly, 1995; 1996; Hernandez, et al., 2002; Krebs and Turingan, 2003). A crucial attribute of ontogenetic changes in feeding ability is that the capacity to feed must be maintained throughout development if the individual is to survive (Galis, 1990; 1993; Galis, et al., 1994). Understanding the contribution of changes in behavior and morphology to deviations in performance over the course of ontogeny, allows prediction of changes in functional capacity and ecology over development.

The ontogeny of feeding morphology and mechanisms in elasmobranchs, which do not undergo metamorphosis, has not been as thoroughly studied as in other aquatic gnathostomes. Ferry-Graham (1998b) suggested that the feeding mode of the swellshark *Cephaloscyllium ventriosum* might change between the hatchling and juvenile life stages due to absolute consequences of size and the resultant inability of small sharks to generate suction, or to the higher level of swimming activity displayed by younger sharks. Robinson and Motta (2002), by contrast, determined that feeding kinematics are isometric and conservative over ontogeny in the obligate suction feeding nurse shark *Ginglymostoma cirratum*. Based on observations of feeding behavior for one individual both with and without food, Robinson and Motta (2002) suggests that feeding behavior in *G. cirratum* is, in fact, highly stereotyped. While sharks are widely recognized as top-level predators (Cortez, 1999), the ecological role of young sharks as they undergoing

ontogenetic dietary, morphological, and behavioral changes is poorly known and requires further study to make estimations of the ecological impact of early age classes.

Goals

The ultimate goal of this study was to quantify and find causation for ontogenetic changes in the feeding morphology, kinematics, and modalities of two disparate species of shark that could be used to predict ecological change during development. The whitespotted bambooshark *Chiloscyllium plagiosum* and the leopard shark *Triakis semifasciata* were the species selected because they differ substantially in cranial morphology, feeding behavior, and habitat (Talent, 1976; Compagno, 1984a; 1984b, Ferry-Graham, 1998a; Wu, 1993; Kao, 2000). In order to elucidate ontogenetic trends at both the individual and species level, a longitudinal study design was used to follow several individuals of each species over the first year of life. Yearling *T. semifasciata* are known to employ a mixture of suction and ram-feeding components in initial capture bites based on the type of prey offered, although ram tends to dominate (Ferry-Graham 1998a). Adult *C. plagiosum* are known to primarily employ suction-dominated bites and this species belongs to a clade of bottom-associated and morphologically specialized suction feeders (Wu, 1993; 1994; Motta and Wilga, 2001; Motta, 2004).

The six questions examined in this dissertation are:

- 1) Do food capture kinematics differ between *C. plagiosum* and *T. semifasciata* and, if so, in what functionally relevant ways?
- 2) Are initial capture bites of hatchling/neonatal (<3 month old) *C. plagiosum* and *T. semifasciata* suction- or ram-dominated?

- 3) Does the feeding modality and/or kinematic pattern exhibited by these species change ontogenetically and what are the effects of these changes on feeding performance?
- 3) Do these species modulate their feeding modality and/or kinematics based on food type through ontogeny?
- 4) Is cranial growth in these species isometric or allometric? What are the effects of changes in size and/or shape on feeding modality and/or kinematics?
- 5) Does the magnitude of sub-ambient buccopharyngeal pressure and pattern of water flow into the buccopharyngeal cavity change in each species over the first year of life?
- 6) Are changes in size, morphology, behavior, and performance associated with changes in foraging strategy or prey preference as indicated by gut content analysis and existing behavioral studies?

Significance

The value of this research lies in its examination of the ontogeny of food capture in two elasmobranch species that employ different feeding strategies utilizing similar anatomical mechanisms. The longitudinal design of this study allows collection of data from the same individuals at intervals throughout early development and correlation of changes in performance with changes in morphological and behavioral parameters. The importance of examining ecomorphological questions during a variety of life stages in a single species in order to present as complete an analysis as possible has been underscored by Barel, et al. (1989), Motta and Kotrschal (1992), Galis, et al. (1994), and Liem and Summers (2000). Since the feeding kinematics of adult *C. plagiosum* and *T. semifasciata* have already been previously investigated (Wu 1993; Ferry-Graham 1998a),

the information gained from this study will supplement previous studies and expand their pertinence through ontogeny. Comparison of these findings with other studies of elasmobranch feeding will allow a more thorough understanding of the relationship between feeding structure and function both over ontogeny and through evolutionary time. Additionally, because the functional demands imposed by the aquatic medium on the feeding of elasmobranchs are also applicable to other aquatically feeding gnathostomes (Lauder, 1980; Muller and Osse, 1980; Liem, 1990), these findings will enhance understanding of the evolution of the feeding apparatus of all gnathostomes.

Chapter 2: The ontogeny of feeding behavior and cranial morphology in the leopard shark *Triakis semifasciata* (Girard 1854): a longitudinal perspective

Abstract

The relationship between form, function, and biological role in determining the niche occupied by an organism depends on diverse factors. Understanding this relationship is further complicated by considering the interplay among factors over ontogeny. While much is known about the ecological and functional morphology of feeding in lower vertebrates, studies of elasmobranch feeding morphology and behavior over ontogeny are broadly lacking. In this study, the ontogeny of feeding behavior and morphology was investigated in neonatal and young-of-the-year leopard sharks *Triakis semifasciata* using morphometric measurements of growth and high-speed videography in a longitudinal study. Five food types were used during filming sessions to facilitate differentiation of modulation and variation over ontogeny. Functional aspects of muscle and buccal volume scaling were investigated through dissection. Size was shown to influence several kinematic variables and intra- and inter-individual variability was the dominant factor contributing to variability in feeding behavior. Modulation of feeding behavior based on food size and elusivity was present for timing variables and predator motion during the strike, but not for food motion or the relative extent of buccal expansion. Allometric growth occurred in all aspects of external cranial morphology measured, resulting in a shallower head profile, anterior displacement of the mouth, and

relatively larger jaw musculature over ontogeny. While the degree to which morphology constrains or enhances behavior can not be directly quantified, variability in behavior greatly exceeds variability in morphology over early ontogeny. Maintenance of a behaviorally and morphologically versatile feeding apparatus throughout ontogeny is proposed to enhance the exploitation of resources and facilitate a diverse diet in *T. semifasciata* under variable environmental conditions.

Introduction

A central tenet of functional morphology is that a reciprocal interaction between morphology and behavior (i.e. form and function) exists and affects the capacity of an organism to exploit resources (Bock, 1980; Wainwright, 1994). Tying this capacity to the ecology and evolutionary fitness of a species requires quantification of the performance of the form-function complex (Arnold, 1983; Lauder, 1990) as well as its biological role (Bock and von Wahlert, 1965; Bock, 1980). In the context of evolutionary biology, the functional and ecological morphology of feeding is of interest because the ability to obtain nutrients directly influences individual survival, lifetime reproductive capacity, and fitness (Schwenk, 2000; Ferry-Graham, et al., 2002). Despite being subjected to diverse and variable selective forces over both ontogeny and phylogeny, maintenance of the utility of the form-function complex throughout development is necessary (Galis, et al., 1994) to ensure the competitive capacity of an individual or species.

For aquatically feeding vertebrates, a prey capture paradigm characterized by a posteriorly directed wave of sequential buccopharyngeal expansion has been developed based on studies of numerous clades (Lauder and Lanyon, 1980; Motta, et al., 1991; Lauder and Prendergast, 1992; Reilly and Lauder, 1992). Muller and Osse (1984) and Lauder (1985) noted that functional convergence in aquatic feeding behavior and morphology can largely be attributed to constraints imposed by physical properties of water, such as density and viscosity. In order to capture prey, organisms may exploit the density and viscosity of the fluid by entraining prey within a parcel of water that is drawn into the mouth (inertial suction feeding), or overtake and consume their prey (ram

feeding) (Liem, 1980b; Norton and Brainerd, 1993). Although ram-feeding and suction-feeding specialists exist, most aquatic vertebrates use a combination of these two modalities to exploit a broad taxonomic and functional diversity of prey types (Liem, 1990). Morphological specializations correlated with a predisposition towards use of a specific feeding mode include those facilitating generation of a broad, unobstructed buccal aperture, in the case of ram feeding, or a narrow buccal aperture leading into an expansible buccopharyngeal cavity, in the case of suction feeding (Muller and Osse, 1984; Liem, 1993).

Despite the overall conservation of the aquatic feeding paradigm and its associated morphology across taxa, systematic behavioral modulation in response to prey elusivity, size, hardness, and type has been described in many aquatically feeding vertebrates (Liem, 1978; Lauder and Prendergast, 1992; Anderson, 1993; Ferry-Graham, 1998a). Existing simultaneously with modulation, intrinsic variability in kinematic and motor patterns is nearly universal both intra- and inter-individually among conspecifics feeding on the same prey or food (Shaffer and Lauder, 1985a;b; Sanderson, 1990; Cook, 1996). In many cases inter-individual variability in feeding behavior is so extensive that it overwhelms inter-specific differences and complicates comparison among species (Wainwright and Lauder, 1986; Reilly and Lauder, 1989; Norton, 1991).

Though much is known about modulation and variation in prey capture behavior within the adult life stages of aquatically feeding vertebrates, less is known about the effects of these factors over the course of ontogeny (Cook, 1996). Several aspects of a predator change over ontogeny, directly affecting its interactions with prey and, consequently, its diet. Dramatic changes can occur in the size and shape of a predator

during early stages of development, especially in metamorphic species, when growth rates are often at their highest (Grossman, et al., 1980; Osse, 1990; Coughlin, 1991; Reilly, 1996). Additionally, small incremental changes in morphology and anatomy can accrue over ontogeny, enhancing the ability of the predator to exploit existing or novel prey types (Cook, 1996; Hernandez and Motta, 1997; Cutwa and Turingan, 2000;). Exposure to prey items early in development can cause behavioral preferences in a predator that lead to dietary specialization as a result of experience (Werner, et al., 1981). Additionally, when a predator repeatedly interacts with a particular prey type it can apply the results of its experience to future encounters, facilitating faster, more efficient handling (Coughlin, 1991).

Elasmobranchs are a model clade in which to study the relationship between feeding morphology and behavior over ontogeny for a number of reasons. In contrast to osteichthian fishes, the elasmobranch feeding mechanism comprises relatively few structural elements. Anatomical complexity can often be employed as a predictor of functional potential, especially when considered in a phylogenetic context (Lauder, 1981; Friel and Wainwright, 1999). Despite the comparable lack of anatomical complexity in elasmobranch feeding systems, however, prey capture behavior, diet, and ecological role differ greatly among species (Cortes, et al., 1996; Lowe, et al., 1996; Motta and Wilga, 2001). While the feeding morphology and behavior of sub-adult and adult elasmobranchs is well-studied (Wu, 1994; Motta and Wilga, 2001; Wilga, 2001; Robinson and Motta, 2002), little is known about the interplay between these factors over early ontogeny. Elasmobranchs exhibit continuous, non-metamorphic growth and while size variation can be vast within a species (Compagno, 1984a;b), shape variation of

cranial elements over ontogeny is comparably small for those species for which it has been studied (Ellis and Shackley, 1995; Ferry-Graham, 1998b; Robinson and Motta, 2002).

The objective of this study was to quantify changes in cranial morphology and feeding behavior over early ontogeny in the leopard shark *Triakis semifasciata* (Girard 1854) (Triakidae). Identifying changes in morphology and behavior is the first step to isolating the contribution of each of these factors to feeding performance and ecology. *Triakis semifasciata* was chosen because this species is documented as a dietary generalist (Russo, 1975; Talent, 1976), potentially indicating a diverse prey capture repertoire, and the feeding kinematics and modality of early stage juveniles have been previously quantified (Ferry-Graham, 1998a). A longitudinal experimental design was employed to allow discrimination among individuals and food type effects across ontogeny. The specific goals of this study were to:

- 1) assess the role of individual variability and modulation of feeding behavior in response to food type over the course of ontogeny;
- 2) assess patterns of size and shape variation in cranial morphology over early ontogeny and their relationship to feeding behavior; and
- 3) identify form-function complexes that vary through ontogeny and have the capacity to influence prey capture performance and feeding ecology in neonatal and young-of-the-year (YOY) *T. semifasciata*.

Methods and Materials

Experimental Animals

Triakis semifasciata is a common neritic, demersal species found along the Pacific coast of North America and northern Central America (Compagno, 1984b).

Triakis semifasciata is aplacental viviparous, giving birth yearly in May or June to litters of 7-36 young (Ackerman, 1971). Pups are approximately 20-26 cm at birth and grow between 2 and 4 cm/year (Compagno, 1984b; Kusher, et al., 1992). Adults can reach a maximum size of 1.98 m TL (Miller and Lea, 1972), though most do not exceed 1.60 m TL (Compagno, 1984b).

Triakis semifasciata is an opportunistic generalist that feeds on a broad diversity of prey including benthic invertebrates (Ackerman, 1971; Russo, 1975; Talent, 1976; Kao, 2000). In Elkhorn Slough, California the diet of individuals between 40 and 70 cm TL was dominated by small grapsid crabs and other mobile crustaceans, although fishes, clam siphons, fish eggs, and echiuroid worms increased in relative importance with maturation (Talent, 1976).

Filming Techniques

Four neonatal *T. semifasciata* (average TL 25.85 cm) were obtained through a commercial aquarium collector and raised for 52 weeks at Mote Marine Laboratory, Sarasota, Florida. Animals were maintained in a 2.4-meter diameter, 1400-liter semicircular communal tank at $27\pm 1^{\circ}\text{C}$ and 32 ± 2 ppt salinity. During experimental sessions, individuals were transferred into a 150-liter filming tank filled with water from

the communal holding tank. The temperature of the filming tank was maintained throughout experimental sessions at the above temperature.

Animals were fed a maintenance diet consisting of 3-4% of their body weight in frozen market squid *Loligo opalescens*, frozen Atlantic threadfin herring *Opisthonema oglinum*, and live and frozen grass shrimp *Palaemonetes pugio*, *ad libitum* three times per week. Experimental sessions began within two weeks of arrival of the sharks at Mote Marine Laboratory, when individuals were determined to be no more than three weeks old. Five food types were offered during experimental sessions: 1) cut squid scaled to one half mouth width (MW); 2) cut squid scaled to MW; 3) cut herring scaled to one half MW; 4) cut herring scaled to MW; and 5) live shrimp scaled to MW in carapace length. These food types compose a set intended to examine modulation in food capture kinematics in response to small versus large and elusive versus non-elusive items. The number of failed attempts occurring prior to food capture was recorded for each capture event throughout the study. During filming sessions, which occurred weekly over the one-year experimental period, food items were presented in a haphazardly determined order until the individual approached satiation, as evidenced by a decrease in overall activity level and an unwillingness to feed. Although a filming session often comprised as many as ten capture sequences for a given individual, only the first five were considered for analysis in order to avoid potentially confounding effects of satiation (Sass and Motta, 2002).

To obtain recordings of feeding events, a Redlake PCI 1000 high-speed digital camera (Redlake, San Diego, CA, USA) was placed perpendicular to the aquarium providing a lateral view. Recordings were made at 250 fps and illumination was

provided by two, 500-Watt quartz-halogen lights. Animals were trained to feed under illumination prior to the experiment and were allowed a 20-minute acclimation period prior to each feeding session. A Plexiglas false bottom divided the tank vertically, allowing a ventral view to be obtained by placing a mirror beneath the shark. A ruler beside the shark provided distance measure and only orthogonal views were retained for analysis. Kinematic data were obtained from recordings using Redlake MotionScope PCI software version 2.21.1 (Redlake, San Diego, CA, USA) and SigmaScan Pro version 4 (SPSS Inc.) The variables measured were selected for their availability over the course of ontogeny, functional relevance, and prior employment in other studies of elasmobranch feeding (Motta, et al., 1997; Ferry-Graham, 1998a; Wilga and Motta, 1998b). The 52-week experimental time period was broken into four equal segments to facilitate statistical analysis. A total of five capture sequences per food type (N=5) per individual (N=4) per time segment (N=4) were recorded, for a total of 400 sequences. Sequences were not always obtained weekly for each food type/individual combination due to satiation. From the onset of mandible depression (time 0 ms), the following kinematic variables were quantified, with descriptions provided for uncommon measures: 1) strike distance, from the closest point on the food to the lower jaw of the shark (cm) at time 0 ms; 2) maximum gape (cm); 3) time to maximum gape (ms); 4) maximum cranial elevation angle (degrees), measured relative to resting head position by calculating the difference in the angle formed between the dorsum of the head and the body with its vertex at the inflection point of the head and the body; 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), measured relative to resting head position at the position of the hyoid; 11) time to maximum hyoid depression (ms); 12) time to hyoid retraction (ms); 13) duration of hyoid depression (ms); 14) total strike duration (ms), from onset of lower jaw depression to jaw closure on the food; 15) duration of food movement (ms), from the field at which the food began to move until the food entered the mouth; 16) distance moved by the food (cm), over the duration of the prior variable; 17) velocity of the food (cm s^{-1}), over the course of the movement measured in the prior variable; 18) distance moved by the predator (cm), during the duration of food movement; and 19) velocity of the predator (cm s^{-1}), over the course of the movement measured in the prior variable. No measures were made of upper jaw protrusion excursion or timing because protrusion was not present in all cases.

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, and indicates the relative contribution of forward motion of the predator and motion of the prey 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 (Lauder and Clark, 1984; Muller and Osse, 1984), it is used here primarily for comparison with other studies of fish feeding kinematics and as a generalized indicator of overall feeding modality.

Morphological Measurements

To chronicle changes in individual shape over ontogeny, both the lateral and ventral camera views were used to measure a suite of external morphological variables. The definitions of these variables followed Compagno (1984b) and 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 that these measurements were accurate and not influenced by error associated with taking them from video images, individuals were removed from the tank every 7 weeks and the same measurements taken. As the measurement error associated with these variables was always less than 2.8%, measurements taken from the recorded footage were deemed valid for describing growth trends.

To obtain measurements of muscle mass, muscle cross-sectional area, and buccal volume that could not be obtained from live individuals, fresh-dead specimens (N=8) spanning the entire range of total lengths of live specimens used in this study were obtained from commercial fishers. The previously described morphological variables measured on live sharks were also measured for dead specimens. Wet muscle mass and cross-sectional area were obtained by excising key muscles whose role in feeding is known (Motta, et al., 1991; Wu, 1994; Motta and Wilga, 1995; 1999). The coracomandibularis, coracohyoideus, and coracoarcualis were selected due to their role as jaw abductors and hyoid depressors, and the quadratomandibularis was selected for its role in jaw adduction. The quadratomandibularis was separated into its constituent divisions (anterior, posterior, superficial, and ventral) for measurement because it is a

complex muscle, and Huber and Motta (2004) found that treating its divisions separately produced the most accurate estimate of bite force. The cross-sectional area from the four divisions was then added together to obtain the morphological cross-sectional area of the muscle as a whole. SigmaScan Pro version 4 (SPSS Inc.) was used to determine anatomical cross-sectional area from digital photographs taken with a Nikon Coolpix 4300. The palatoquadrate and Meckel's cartilages were also excised and their combined weight recorded because they are the skeletal elements upon which the jaw-abducting and -adducting musculature acts and their allometric growth could influence the velocity and force of feeding motions.

Measures of buccal volume were obtained by injecting silicone sealant into the mouth of each dead specimen (N=8) and allowing it to cure for 36 hours before removal and weighing. Using the mass-density of silicone reported by Cook (1996) (0.06 g ml^{-1}), this weight was converted into a 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 was termed the buccal reserve volume and used to indicate the maximum potential change in the volume of the buccal cavity during a capture event at discrete points over ontogeny.

Statistical Analyses

All kinematic data were log-transformed and checked for normality and homogeneity of variance, employing Kolmogorov-Smirnov and Levene Median tests, respectively, using SigmaStat Pro version 3.1 (SPSS Inc.). A Spearman Rank Order Correlation test was performed, as several variables exhibited skewed distributions, to

establish the relationship between each variable and total length. Several variables were found to be highly correlated ($P < 0.01$) with total length. To assess the nature of these relationships, Model II linear regressions were performed using the average value for each individual for which kinematic data were available at a given total length (Richard and Wainwright, 1995). Model II regressions were appropriate because both of the variables used in these comparisons contained measurement error. Regressions were performed for all individuals combined and then for each individual separately to compare regression coefficients among individuals. Differences among individuals within a given kinematic variable were assessed using a modified Student's t-test (Zar, 1999).

To standardize the statistical handling of all data for further analysis, all variables that did not show a relationship to total length were 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 correlation matrix-based Principal Components Analysis (PCA) that reduced the expansive kinematic data set to a few, orthogonally oriented composite variables whose contribution to overall patterns of variation in the data could be elucidated (Quinn and Keough, 2002). An Equamax rotation was used because it produced higher loadings than any other rotation, enhancing data interpretation. Variables that loaded above an absolute value of 0.5 were identified as contributing heavily to the variability within the respective principal component (PC). Principal components with an eigenvalue greater than 1.0 were retained for further analysis. The factor loading scores for each capture sequence on each principal component were then used in a two-way, mixed-model repeated

measures MANOVA to identify differences between clusters of capture sequences 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 because it is robust to multivariate deviance from normality (Zar, 1999). To further investigate differences identified in multivariate space 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 among differences detected by the RM ANOVA was evaluated using Fisher's LSD with an α level of 0.05. To determine the order of the best-fit equation describing trends over the repeated measure time, single degree of freedom polynomial contrasts were utilized. Bonferroni corrections were not applied to the α level of any tests due to their tendency to increase the rate of type II errors (Cabin and Mitchell, 2000; Moran, 2003). The PCA, MANOVA, and RM ANOVA tests were performed using Systat 11 (SPSS Inc.).

Morphological variables measured on living specimens were log-transformed and regressed against the logarithm of total length using Model II linear regressions in order to investigate changes in the relative dimensions of these variables over time. If the pattern of growth was isometric for a given variable, a slope of one was predicted for these regressions. Significant deviations from a slope of one were tested using Student's *t* (Zar, 1999) and indicated a relative retardation or acceleration in growth rate 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 that were measured on both live and dead specimens, the data taken from dead specimens

were compared against the confidence intervals for the live individuals to determine if the data fell within this range, indicating that it could have been drawn from the same population. Comparing live and dead scaling data via this method allowed application of data collected exclusively on dead specimens to live specimens within the appropriate size range. Morphological variables that were measured exclusively on dead specimens were log-transformed and regressed against the logarithm of total length using Model II linear regressions. The slope of the regression equation for each variable was tested against the expected slope using Student's *t*. The expected slope of these regressions depended on the dimensionality of the variable being considered, with a slope of one expected for linear variables, a slope of two expected for planar variables (e.g. muscle cross section), and a slope of three expected for cubic variables (e.g. muscle mass, buccal volume).

Results

Individuals generally swam slowly around the tank until a piece of food was introduced. The shark would then orient to the food within ~2-5 seconds and approach rapidly. Food was typically engulfed on the first pass, except in the case of live shrimp (see 'Kinematic Trends' below). Food capture kinematics began with either cranial elevation or lower jaw depression and continued in a posteriorly directed fashion (Fig. 1). The temporal sequence of kinematic events was conserved over the period of ontogeny studied, with the exception of maximum gape and maximum cranial elevation, which were occasionally transposed. When upper jaw protrusion was apparent it occurred after maximum gape and reached its maximum during jaw closure. The absolute distance

from the lower jaw to the food at time zero (strike distance) did not change over ontogeny ($P=0.261$), regardless of individual ($P=0.588$) or food type ($P=0.264$), and averaged 0.94 ± 0.46 cm. The relative distance to the food as a percent of head length averaged $11.0\pm 5.8\%$ and did not exhibit a trend over ontogeny ($P=0.230$).

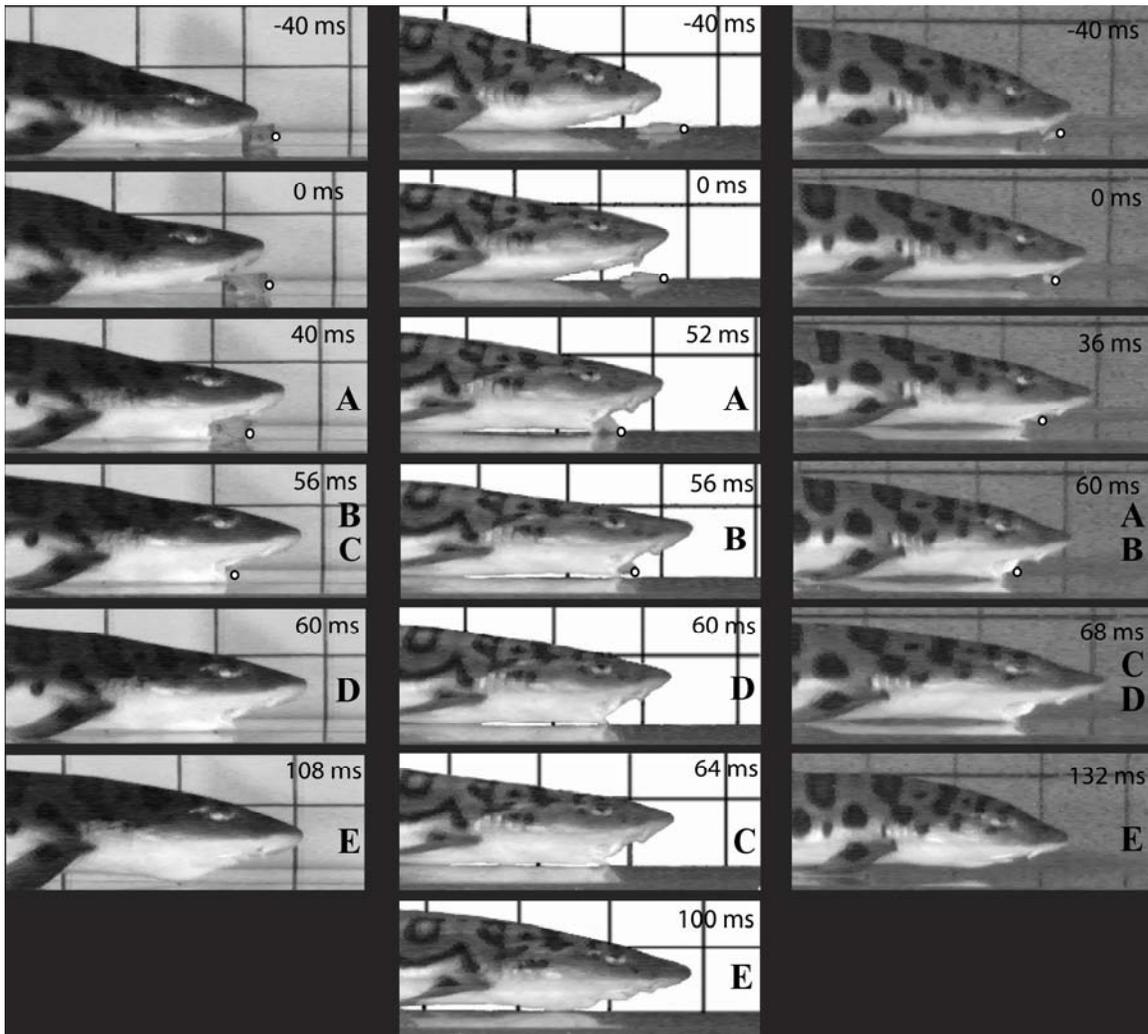


Figure 1: Composite photographic series of representative prey capture sequences for three of the five food types utilized. The subset of food types selected illustrates maximum differences in capture kinematics. The left column depicts capture of a large piece of fish, the center column depicts capture of a small piece of squid, and the right column depicts capture of a live shrimp. 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. Time is shown in the upper right corner of each field. Time 0 ms corresponds to the beginning of rapid jaw opening. A indicates maximum gape; B indicates maximum cranial elevation; C indicates maximum hyoid depression; D indicates food capture; E indicates jaw closure on the food. Note that the sequence of kinematic events is largely conserved but that absolute timing of events varies among food types.

Scaling of Kinematics

Approximately half of the kinematic variables measured exhibited a relationship with total length when individuals were combined, with displacement variables tending toward smaller values, timing and velocity variables tending toward longer and slower values, and the RSI tending toward ram dominance over ontogeny (Table 1). Within a given variable, however, significant differences among individual scaling coefficients existed over ontogeny for nearly three-quarters of the variables (Table 1). For variables quantifying time to and extent of maximum linear and angular displacements, relationships with total length were identified for measures describing motion of the jaws and cranium but not hyoid depression.

Table 1: Regression parameters for kinematic variables versus total length (cm) for *Triakis semifasciata*. Results shown are for all individuals combined (N=4). 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.

Variable	Individuals Combined				Individuals Separated	
	Slope	y-int	StErr	P	Range of Slopes	
Max Gape	-0.56	1.01	0.15	<0.001*	-1.25	-0.59
Time to Max Gape	0.35	1.18	0.23	0.021*	0.17*	0.54*
Max Cranial Elev Angle	-2.08	3.54	0.60	<0.001*	-3.82**	0.70**
Time to Max Cranial Elev Angle	--	--	--	0.269	0.10**	1.10**
Time to Onset of Cranial Elev	--	--	--	0.641	-0.88**	0.64**
Time to Offset of Cranial Elev	-0.42	2.60	0.21	0.044*	-0.49*	0.22*
Duration of Cranial Elev	-1.26	3.78	0.48	0.010*	-2.29**	-0.35**
Time to Onset of Hyoid Depress	--	--	--	0.077	-0.02*	1.01*
Max Hyoid Depress	--	--	--	0.853	-0.30	0.80
Time to Max Hyoid Depress	--	--	--	0.497	-0.71	0.10
Time to Hyoid Retraction	--	--	--	0.314	-1.27**	0.24**
Duration of Hyoid Depress	--	--	--	0.190	-1.45**	0.13**
Total Strike Duration	--	--	--	0.130	-0.49*	0.62*
Distance Moved by Food	-0.95	1.46	0.29	0.002*	-1.87**	-0.26**
Duration of Food Movement	0.56	0.88	0.17	<0.001*	0.20*	0.99*
Velocity of Food	-1.51	3.58	0.33	<0.001*	-2.55**	-0.45**
Distance Moved by Predator	--	--	--	0.141	-0.21**	1.75**
Velocity of Predator	--	--	--	0.305	-0.89	0.66
RSI	0.46	-0.69	0.14	<0.001*	0.32	1.00
Strike Distance	--	--	--	0.143	-1.25	1.01

Kinematic Trends

Principal components analysis reduced the kinematic data into six components that accounted for 78.11% of the overall variability in the data set (Table 2). Timing and duration variables loaded heavily on principal components (PC) 1, 2, and 4. Variables describing cranial elevation and depression loaded heavily on PC2, variables describing hyoid retraction loaded heavily on PC4, and the remaining timing variables loaded heavily on PC1. Though not a timing variable, maximum cranial elevation angle also loaded on PC2. Together the three timing components accounted for 46.20% of the overall variability in the data set (Table 2). PC3 and PC5 were characterized by variables quantifying forward motion of the food and predator, respectively, over the course of the strike. RSI loaded on both PC3 and PC5, but in opposite directions as would be expected from the nature of this index. Lastly, PC6 was characterized by measures of maximum hyoid and gape displacement distance, or buccal excursion. Strike distance did not load heavily on any component.

The RM MANOVA conducted simultaneously on all six principal components indicated that differences existed over ontogeny (Pillai Trace $df=3$; $F=5.37$; $P=0.001$), among individuals over ontogeny ($df=9$; $F=4.45$; $P<0.001$), among food types over ontogeny ($df=12$; $F=1.83$; $P=0.044$), and among food types for a given individual over ontogeny ($df=36$; $F=1.61$; $P=0.020$). Despite a high degree of overlap, when each PC was examined with a separate RM ANOVA for individual differences irrespective of time and food type, differences were found on all components (Table 3, Fig. 2). Food type differences, independent of time and individual, were present on all components except PC3 (food motion) and PC6 (buccal excursion) (Table 3). Bites on large fish

Table 2: Principal component loadings of kinematic variables associated with capture sequences in *Triakis semifasciata*. Bold face values indicate variables determined to load heavily on the respective component (loading scores >|0.5|) (N=4). Together the six components explain 78.11% of the overall variability in the data set. For clarity, all loadings <|0.25| are replaced by 0. Max=maximum; Depress=depression; Elev=elevation.

Variable	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6
Time to Max Gape	0.852	0	0	0	0	0
Duration of Food Movement	0.838	0	0	0	0	0
Time to Max Hyoid Depress	0.828	0	0	0	0	0
Total Strike Duration	0.823	0	0	0	0	0
Time to Onset of Hyoid Depress	0.718	0	0	0	0	0
Time to Max Cranial Elev Angle	0.508	0	0	0	0	0.446
Duration of Cranial Elev	0	0.945	0	0	0	0
Max Cranial Elev Angle	0	0.869	0	0	0	0
Time to Offset of Cranial Elev	0.458	0.608	0	0	0	0.39
Time to Onset of Cranial Elev	0	-0.604	0	0.364	0	0.344
Distance Moved by Food	0	0	0.954	0	0	0
Velocity of Food	-0.332	0	0.920	0	0	0
RSI	0	0	-0.699	0	0.668	0
Duration of Hyoid Depress	0	0	0	0.937	0	0
Time to Hyoid Retraction	0	0	0	0.926	0	0
Velocity of Predator	-0.278	0	0	0	0.921	0
Distance Moved by Predator	0.389	0	0	0	0.878	0
Max Hyoid Depress	0	0	0	0	0	0.789
Max Gape	0	0	0	0.487	0	0.502
Strike Distance	0	0.328	0.306	-0.318	0	0.381
Eigenvalue	4.282	2.605	2.467	2.353	2.198	1.715
Percent Variance Explained	21.408	13.027	12.336	11.766	10.990	8.575

tended to have the largest values on all components, followed by bites on small fish. This trend was reversed on PC1 (general timing variables), with bites on large squid having high values and bites on both sizes of fish having small values. Bites on live shrimp tended to have the lowest values on PCs 1, 4, and 5 (general and hyoid timing variables and predator motion), indicating that these strikes were characterized by brief, fast cephalic motions, and a smaller ram component (Fig. 3). Differences among sharks for a given food (i.e. effect interactions) were only found on PC6 (buccal excursion), with the values for one individual being consistently high and the values for two individuals

fluctuating greatly based on food type. In summary, while a high degree of intra- and inter-individual variability was present in all aspects of food capture over ontogeny, individuals modulated timing and modality aspects of food capture, but not the extent of buccal excursion, in a consistent manner in response to food type.

Table 3: Results of RM ANOVAs performed separately on each principal component from a PCA of prey capture kinematics in *Triakis semifasciata* (N=4). df=Degrees of Freedom; TS=Time Segment. For all between subjects comparisons the error degrees of freedom are 80, while for all within subjects comparisons the error degrees of freedom are 240.

		PC1 - General Timing			PC2 - Cranial Timing, Displacement		
		df	F	P	df	F	P
Between Subjects:	Shark	3	15.278	<0.001*	3	22.147	<0.001*
	Food	4	7.700	0.004*	4	7.316	0.005*
	Shark*Food	12	0.625	0.815	12	1.517	0.135
Within Subjects:	Time Segment	3	5.452	0.004*	3	0.623	0.671
	TS*Shark	9	0.791	0.625	9	2.936	0.003*
	TS*Food	12	3.767	<0.001*	12	3.281	<0.001*
	TS*Shark*Food	36	0.94	0.571	36	1.338	0.105
		PC3 - Food Motion			PC4 - Hyoid Timing		
		df	F	P	df	F	P
Between Subjects:	Shark	3	14.544	<0.001*	3	34.266	<0.001*
	Food	4	1.278	0.132	4	61.700	<0.001*
	Shark*Food	12	0.819	0.630	12	1.400	0.183
Within Subjects:	Time Segment	3	2.077	0.136	3	19.964	<0.001*
	TS*Shark	9	1.991	0.041*	9	3.219	0.001*
	TS*Food	12	0.435	0.948	12	3.026	0.001*
	TS*Shark*Food	36	0.991	0.489	36	1.543	0.031*
		PC5 - Predator Motion			PC6 – Buccal Excursion		
		df	F	P	df	F	P
Between Subjects:	Shark	3	10.398	<0.001*	3	11.948	<0.001*
	Food	4	8.533	0.002*	4	2.85	0.075
	Shark*Food	12	1.494	0.144	12	2.214	0.018*
Within Subjects:	Time Segment	3	1.394	0.256	3	6.202	0.001*
	TS*Shark	9	3.626	<0.001*	9	3.254	0.001*
	TS*Food	12	1.985	0.026*	12	1.417	0.158
	TS*Shark*Food	36	1.761	0.007*	36	1.549	0.030*

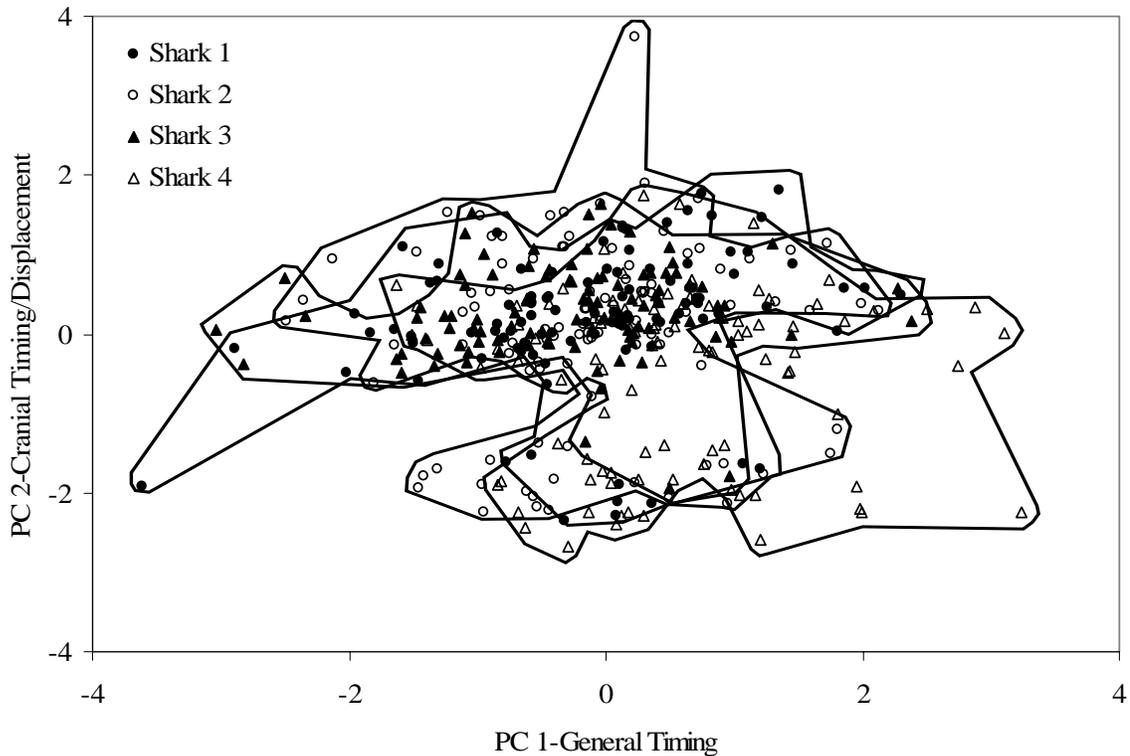


Figure 2: Comparison of principal component 1 (PC1) (general timing) and PC2 (cranial timing/displacement) scores among individuals irrespective of time for *Triakis semifasciata*. Together the variability explained by these two components is 34.4% of the overall variability in the kinematic data set. Polygons delimit the region of the plot occupied by bites from each individual. Note the high degree of intra-individual variability and the large degree of overlap among individuals, which is also a prevailing trend on the remaining PCs. On both components Shark 4 is significantly different ($P=0.004$ and 0.005 , respectively) from all other individuals, and on PC2 Shark 3 is significantly different ($P=0.041$) from all other individuals.

Differences over ontogeny regardless of individual or food type occurred on PCs 1, 4, and 6 (Table 3), indicating significant changes in timing and excursion aspects of food capture with growth. The trend along each of these components was a gradual linear decrease in factor loading scores over the first 6-9 months. This was followed by a quadratic increase in values over the following 3-6 months for general timing variables (PC 1) and buccal excursion variables (PC 6). Directional trends were not found over ontogeny within cranial timing variables (PC2) or the motion of the food or predator (PCs 3 and 5) (Table 3). Differences in individual trajectories over ontogeny, however, were

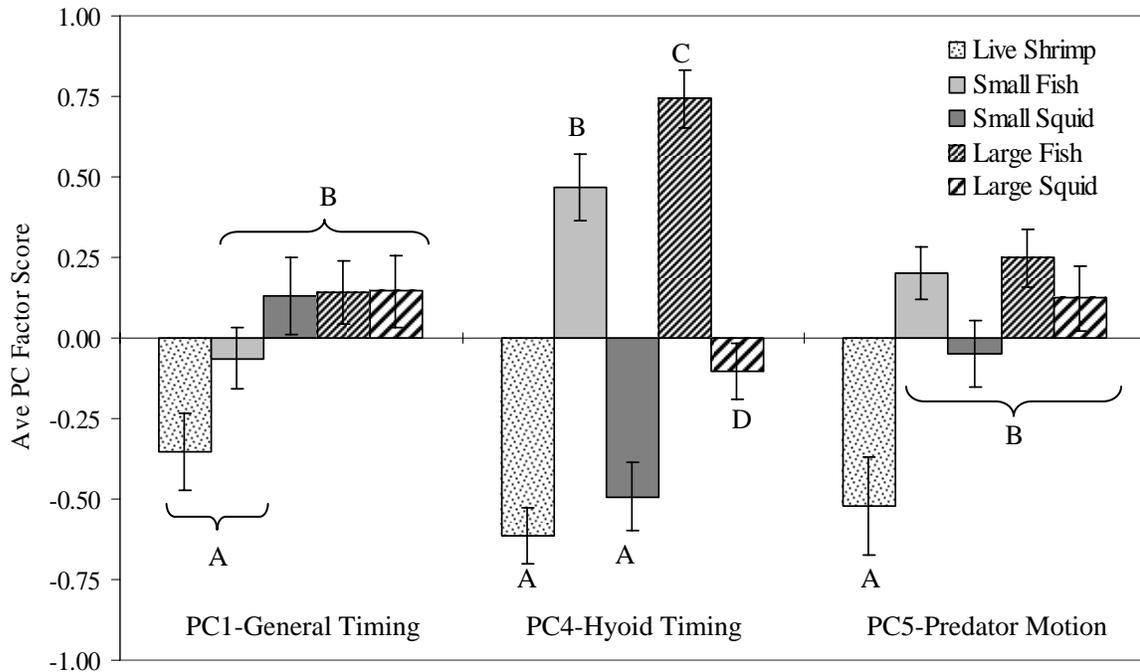


Figure 3: Comparison of average principal component (PC) scores for food types irrespective of individual and time for the components upon which overall differences between foods were found. Error bars indicate one standard error. Bars labeled with different letters differ at the $\alpha=0.05$ level. Note that bites on live shrimp are characterized by rapid, brief cranial movements and slow, short predator movement relative to other food types.

identified on all PCs except PC1 (general timing variables). Thus, within the general trends described over ontogeny there were distinct inter-individual differences among cranial and hyoid timing, food and predator motion, and excursion variables.

The trajectory over ontogeny differed by food type on PCs 1, 2, 4, and 5 (Table 3). General timing variables (PC1) decreased linearly over ontogeny for large fish and live shrimp, but increased linearly for both sizes of squid and small fish (Fig. 4).

Differences in cranial timing variables (PC2) were attributable to food type rather than size, with bites on fish, squid, and shrimp exhibiting different patterns over time. Bites on fish showed an initial increase in cranial timing variable values before decreasing during the last 6 months. Bites on squid exhibited the opposite trend, while cranial

timing variables from bites on shrimp decreased nearly linearly over time. These differences due to food type may be obfuscated by size effects because fish pieces tended to be thicker than squid pieces in their smallest dimension and may have required greater cranial elevation to accommodate their girth. Differences in hyoid timing variables (PC4) were attributable to food size rather than type, with large food of both types eliciting more brief hyoid motions over time and small food eliciting prolonged hyoid motion during the first 3 months followed by more brief motions during the last 9 months (Fig. 5). Live shrimp, which were comparable in size to large food, exhibited the same pattern of deviation over time as large food. The pattern of change over time was not significantly different for food types with regard to buccal excursion variables (PC6), indicating that similar relative buccal expansion occurred regardless of trends in the duration of hyoid motion. The trend over time for predator motion (PC5) differed between all types of dead food and live shrimp. For dead food, predator motion either increased slightly or remained constant over the first 9 months and then increased or remained constant during the last 3-month segment (Fig. 6). For live shrimp, predator motion decreased over the first 3 months before increasing markedly and then declining slightly. The number of attempts it took individuals to capture live shrimp remained constant at ~2.5 during the first 9 months and only began to decline when predator aspects of modality reached a peak (Fig. 7). As predator motion declined past this point so did the number of attempts needed to capture shrimp, eventually reaching an average of 1.5. On PCs 4-6 interactions between shark and food type over time exist (Table 3), indicating that the pattern of deviation observed is influenced partially by non-additive effects of individual and food type. While this weakens the ability to generalize about the

trends described above for these factors in isolation, the majority of additive effects include small squid, the food type characterized by the greatest variability in food capture kinematics, and various individuals.

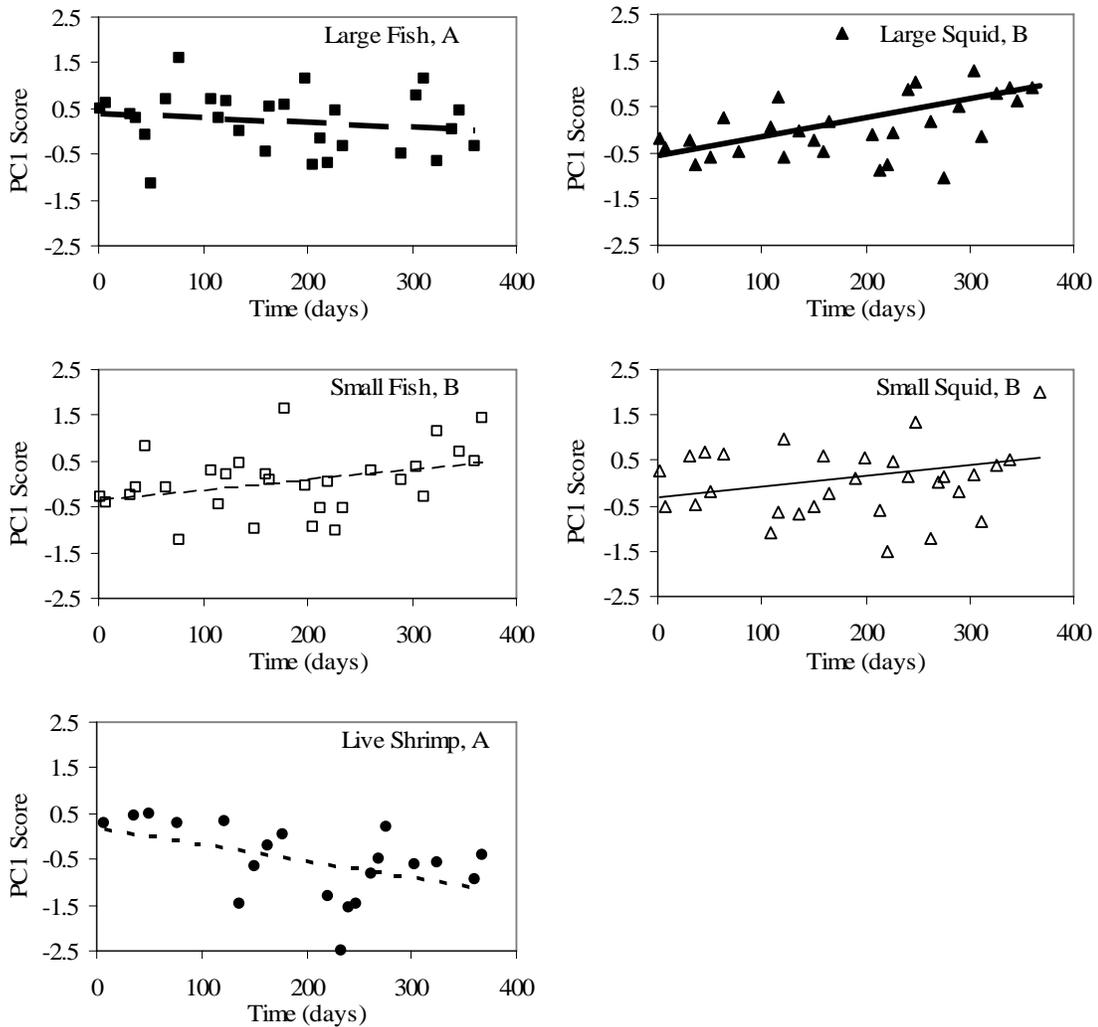


Figure 4: Trends in general timing variables (PC1) over time by food type in *Triakis semifasciata*. Model II regressions are based on all bites from all individuals but, for clarity, only daily averages are shown. Graphs labeled with different letters differ in the trend displayed over time at the $\alpha=0.05$ level. Over time bites on small food and large squid get longer and involve slower movements, while bites on large fish and live shrimp become more brief and faster. The regression equations for each food type are: Large fish $y=-0.001x+0.383$; Large squid $y=0.004x-0.485$; Small fish $y=0.002x-0.407$; Small squid $y=0.002x-0.203$; Live Shrimp $y=-0.003x+0.318$.

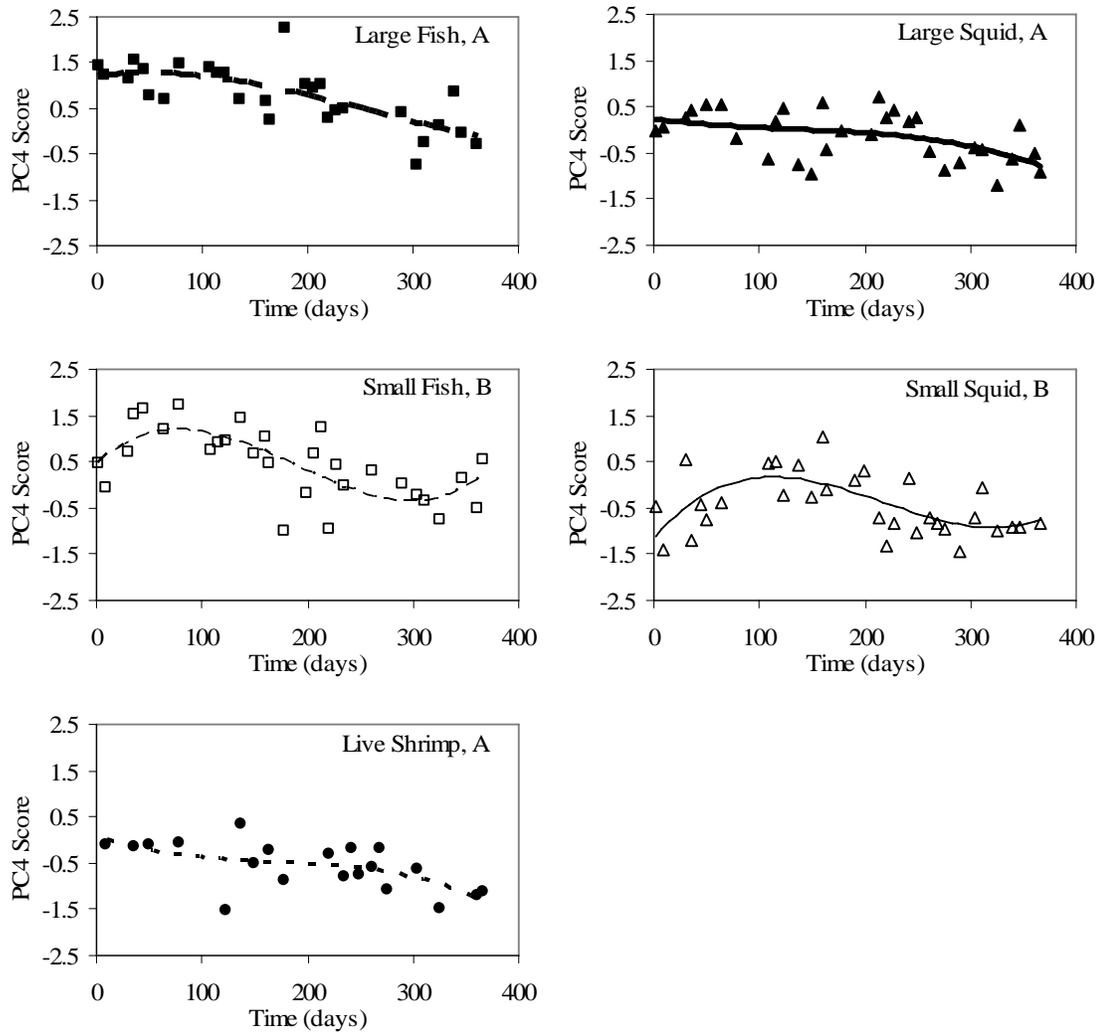


Figure 5: Trends in hyoid timing variables (PC4) over time by food type for *Triakis semifasciata*. Best fit polynomial trend lines are based on all bites from all individuals but, for clarity, only daily averages are shown. Graphs labeled with different letters differ in the trend displayed over time at the $\alpha=0.05$ level. Over time bites on both types of small food showed a similar pattern of initial increase in duration before exhibiting a decrease. Bites on both types of large food and live shrimp generally exhibited a decrease in hyoid timing variables over time. Changes in hyoid timing variables over time are primarily attributable to food size as live shrimp were selected to be ~ 1 mouth width in carapace length throughout the study.

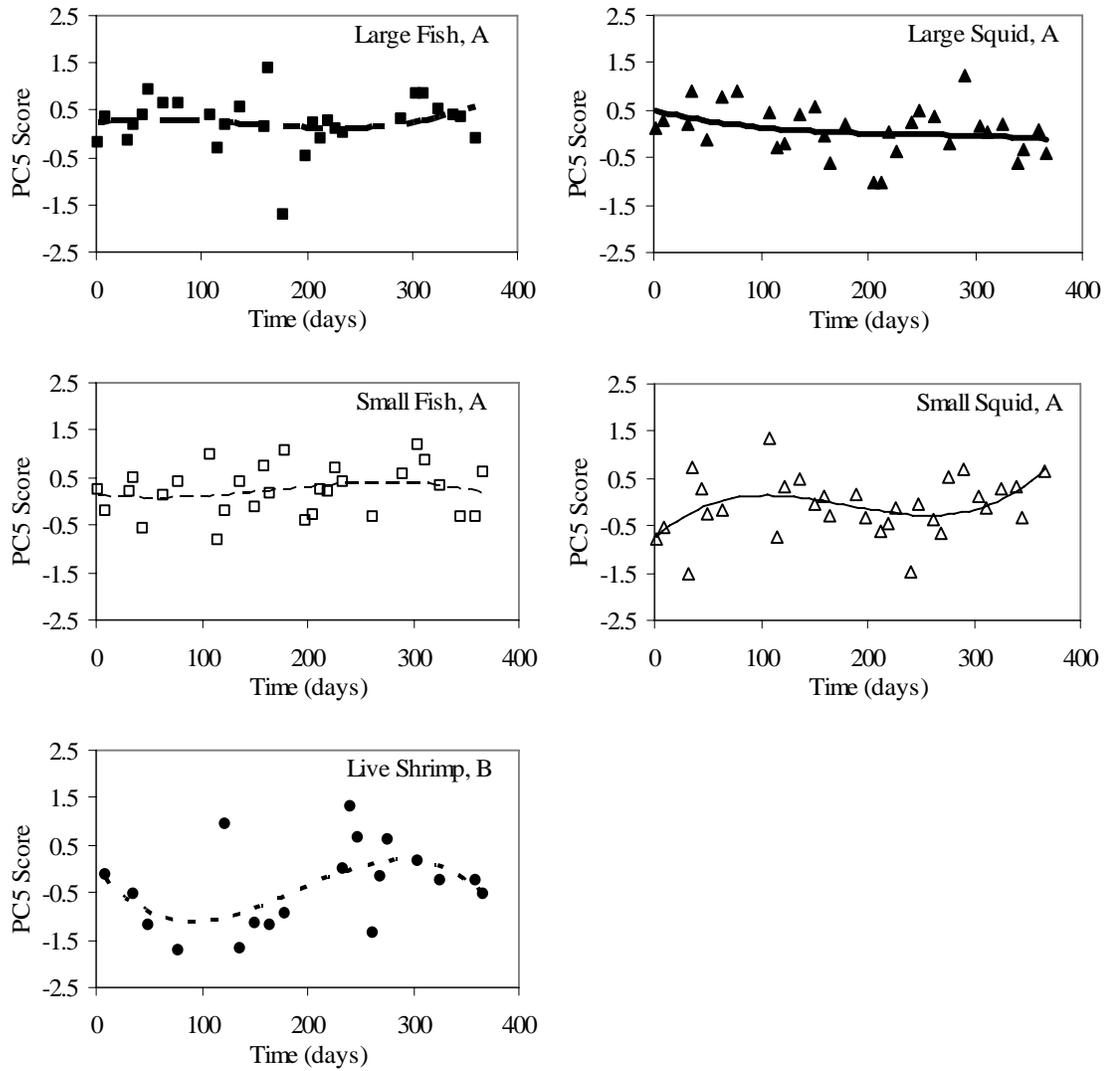


Figure 6: Trends in predator motion (PC5) over time by food type for *Triakis semifasciata*. Best fit polynomial trend lines are based on all bites from all individuals but, for clarity, only daily averages are shown. Graphs labeled with different letters differ in the trend displayed over time at the $\alpha=0.05$ level. The pattern of size-corrected forward motion of the predator over time is similar for all dead food types. For live shrimp, bites during the first six months involve short, slow movement of the predator, whereas during the last six months the motion of the predator more closely resembled that observed for bites on dead food.

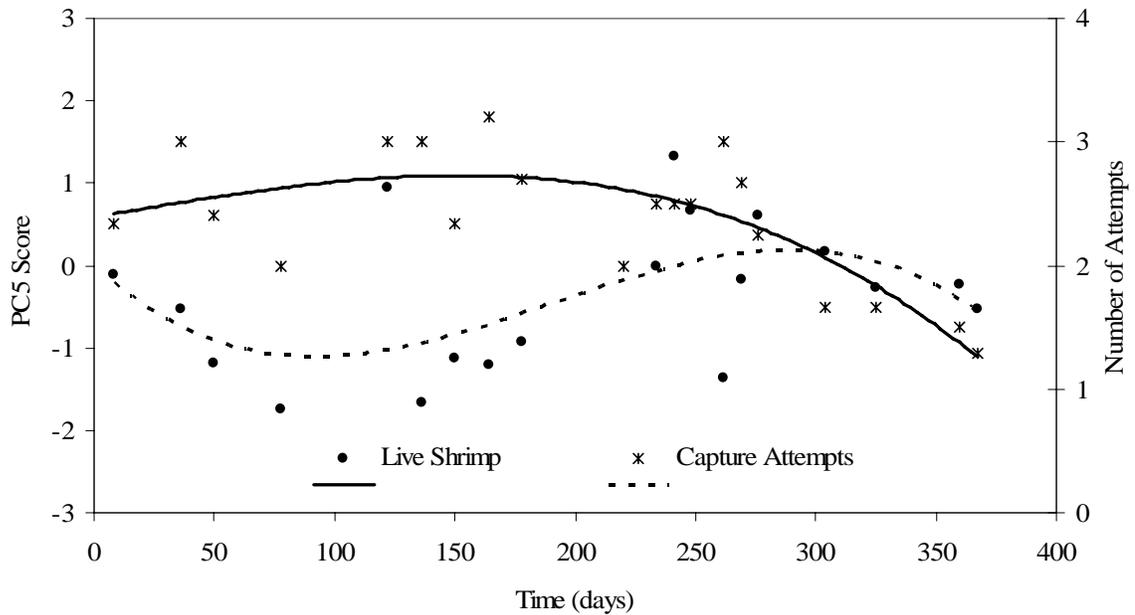


Figure 7: The trend in predator motion (PC5) over time compared to the number of attempts required to capture live shrimp in *Triakis semifasciata*. Best fit polynomial trend lines are based on all bites on live shrimp from all individuals but, for clarity, only daily averages are shown. Note that an increase in capture success coincides with an increase in the forward velocity and distance covered by the predator during a strike.

Scaling of Morphology

The majority of external morphological variables scaled with negative allometry relative to total length, however pre-branchial length and pectoral fin base to lower jaw length scaled positively (Fig. 8, Table 4), creating changes in overall head shape through ontogeny. Closed and open buccal volume scaled with negative allometry but the coefficient for open volume was larger, producing isometric growth in buccal reserve volume (Fig. 9, Table 5). All measures of muscle mass and cross-sectional area scaled with positive allometry (Table 5), with the mandibular abductor, the coracomandibularis, exhibiting the greatest scaling coefficient. Combined jaw weight, however, scaled isometrically.

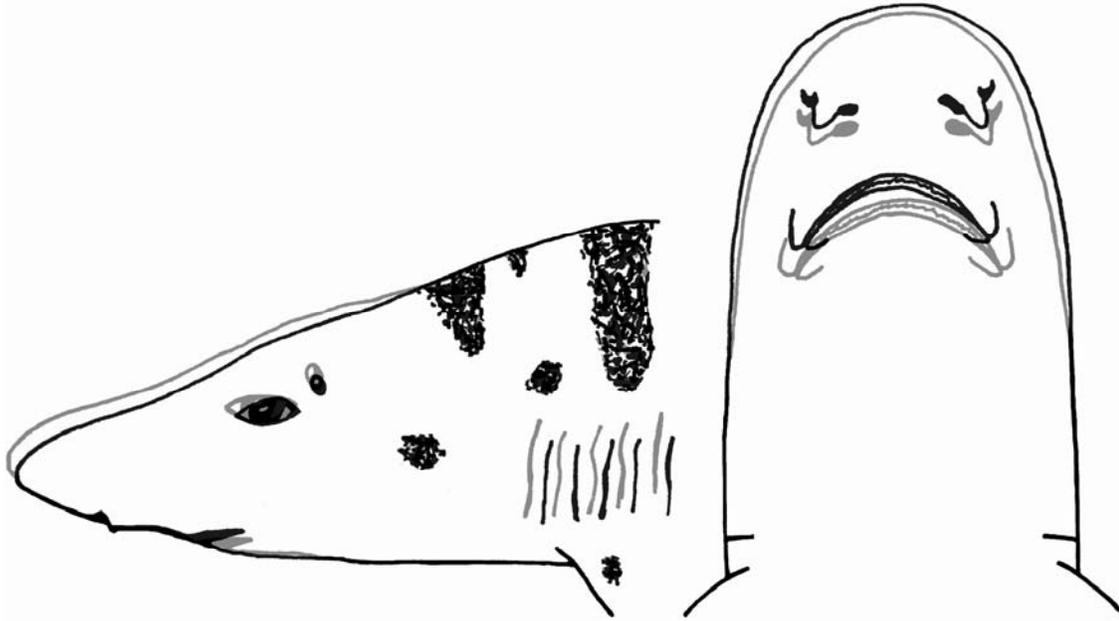


Figure 8: Scaled drawing of external morphology of *Triakis semifasciata* at age 1 day (light gray lines) and age 365 days (black lines). Scaling is based upon the Model II regression line for all live individuals (N=4) and is indicative of growth trends exhibited by all individuals. Note that older individuals tend to have a relatively narrower, more anteriorly placed mouth and a shallower, broader head profile from the rostrum to the position of the hyoid.

Table 4: Parameters for log-log regressions against total length (cm) of external morphological variable data measured on live specimens of *Triakis semifasciata*. Results shown are averages for individuals (N=4). The expected slope for isometry is one in all cases. $t_{0.05(1),33}=1.692$.

Variable	Slope	y-intercept	r^2	St Error	t	P
Mouth Width	0.790	-0.86	0.99	2.8E-4	-875.39	<0.001*
Mouth length	0.879	-1.53	0.99	4.1E-4	-299.27	<0.001*
Pre-Oral Length	0.772	-0.87	0.88	7.9E-4	-293.61	<0.001*
Pre-Orbital Length	0.935	-1.02	0.94	8.4E-4	-107.32	<0.001*
Pre-Branchial Length	1.090	-0.77	0.96	9.2E-4	29.11	<0.001*
Pre-Pectoral Length	0.981	-0.70	0.99	5.4E-4	-61.63	<0.001*
Pectoral Fin Base to LJ	1.153	-1.14	0.99	5.8E-4	257.98	<0.001*
Head Length	0.982	-0.63	0.97	7.7E-4	-120.61	<0.001*
Branchial Length	1.011	-1.37	0.99	5.2E-4	6.63	<0.001*
Head Depth at Hyoid	0.830	-0.87	0.97	6.6E-4	-364.21	<0.001*
Head Width	0.983	-0.91	0.91	7.4E-4	-71.85	<0.001*

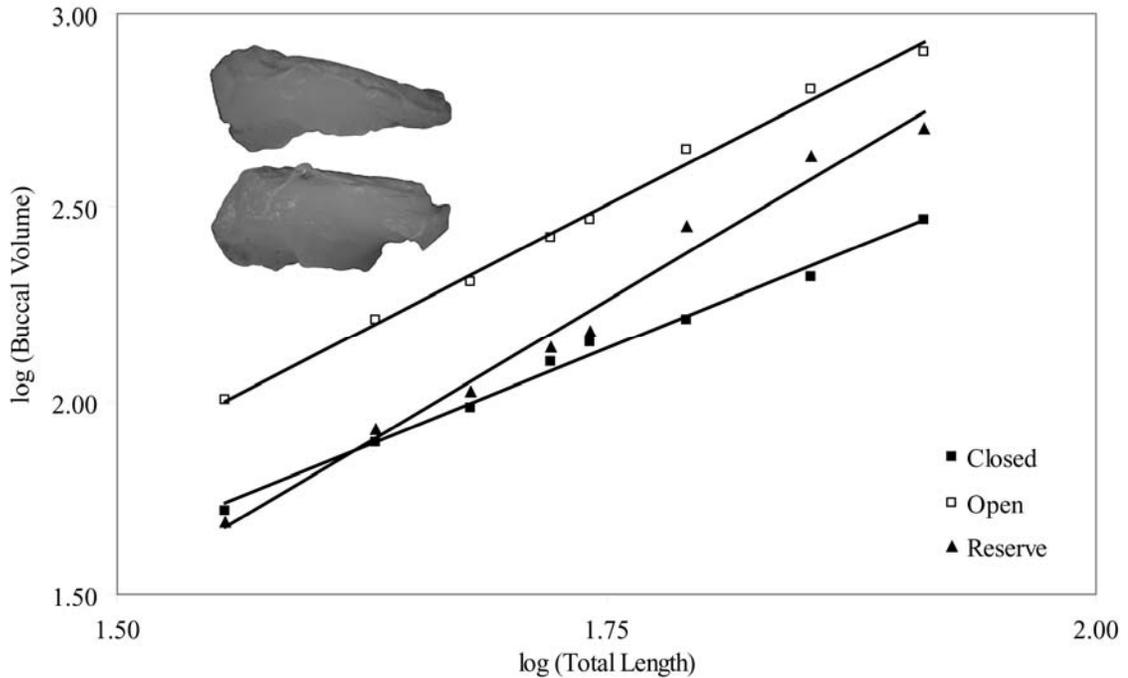


Figure 9: Scaling of buccal volume relative to total length in *Triakis semifasciata*. Measurements calculated from the weight of silicone casts obtained from dead specimens placed in resting and maximally expanded positions based on kinematic footage. Reserve volume is the difference between the open and closed position volume for a specimen. Inset shows lateral view of closed (top) and open (bottom) silicone casts from a representative individual. The expected slope for isometry is 3 in all cases and only Buccal Reserve Volume scaled isometrically. Model II regression equations are: Buccal Volume Closed $y=2.050x-1.453$; Buccal Volume Open $y=2.600x-2.043$; Buccal Reserve Volume $y=3.007x-3.003$.

Table 5: Parameters for log-log regressions against total length (cm) of morphological variable data taken from dead specimens only of *Triakis semifasciata*. The expected slope for isometry is given in the column labeled Sl_{EXP} . $t_{0.05(1), 6}=1.943$.

Variable	Sl_{EXP}	Slope	y-intercept	r^2	St Error	P
Buccal Volume Closed (ml)	3	2.050	-1.45	0.99	4.6E-3	<0.001*
Buccal Volume Open (ml)	3	2.600	-2.04	0.99	5.0E-3	<0.001*
Buccal Reserve Volume (ml)	3	3.007	-3.00	0.98	1.0E-2	0.265
Combined Jaw Weight (g)	3	3.020	-4.68	0.96	1.5E-2	0.130
Quadratmandibularis Area (mm ²)	2	2.108	-3.72	0.97	9.4E-3	<0.001*
Coracohyoideus Area (mm ²)	2	2.356	-4.83	0.96	1.0E-2	<0.001*
Coracomandibularis Area (mm ²)	2	2.535	-5.61	0.95	1.5E-2	<0.001*
Coracoarcualis Area (mm ²)	2	2.109	-3.95	0.98	7.6E-3	<0.001*
Quadratmandibularis Weight (g)	3	3.340	-8.90	0.98	1.2E-2	<0.001*
Coracohyoideus Weight (g)	3	3.069	-8.52	0.99	7.5E-3	<0.001*
Coracomandibularis Weight (g)	3	4.273	-11.84	1.00	6.5E-3	<0.001*
Coracoarcualis Weight (g)	3	3.310	-8.94	0.98	9.9E-3	<0.001*

Discussion

Differences in the feeding behavior of *T. semifasciata* exist irrespective of as well as over the course of ontogeny that can be attributed to absolute consequences of size, individual variability, and modulation in response to food attributes. These differences occur concomitant with allometric changes in cranial morphology that have the potential to influence feeding performance by affecting the generation of sub-ambient buccal pressure and bite force during feeding.

Variability in feeding kinematics among individuals was greater than modulation among food types, except in hyoid timing variables. Individual variability was, in fact, the only factor that contributed to overall differences in food motion and buccal excursion variables. The predominance of individual variability in feeding behavior is consistent with other studies of feeding in aquatic vertebrates including sharks (Shaffer and Lauder, 1985a; Summers, et al., 1998b; Wilga and Motta, 1998b). Among individuals, predator motion (i.e. the ram component of the strike) exhibited the least variability and may reflect the similar degree of experience with particular food types possessed by individuals at a given age. The return of the hyoid to its resting position (hyoid retraction), however, exhibited the greatest variability. Successful entrainment of prey, especially elusive prey, via suction feeding requires temporally coordinated, low-variability expansion of the buccal cavity and depression of the hyoid (Muller and Osse, 1984; Wainwright, et al., 2001a; Svanback, et al., 2002). Once the food is secured within the buccal cavity, however, the recovery of the hyoid to its resting position does not appear to have a functionally relevant temporal component and occurs slowly with high variability.

Individual differences in the developmental trajectory of feeding behavior over the course of early ontogeny have not previously been described for elasmobranchs, a fact attributable to the lack of studies examining feeding during the neonatal/hatchling stage (but see Ferry-Graham 1997; 1998a; 1998b). Subtle differences among individuals in morphology, growth trajectories, and experience can directly impact the development and execution of behavior (Bryan and Larkin, 1972; Morse, 1980), as well as establish a base of variation upon which natural selection can act. That individuals within a population utilize different resources and are not necessarily ecological equivalent, especially during different life stages, has long been recognized (Wainwright, 1994; Bolnick, et al., 2003;). In *T. semifasciata* developmental differences in aspects of cranial and hyoid timing, predator and food motion, and buccal expansion exist among individuals that may translate to differences in prey capture performance resulting in differences in diet. The magnitude of behavioral differences over ontogeny is likely to be amplified in natural settings due to greater variability in predator experience and heterogeneity of available resources (Morse, 1980). An ontogenetic dietary study that distinguishes differences in individual exploitation of prey is needed before a definitive conclusion can be reached regarding the long-term ecological implications of individual dietary specialization in *T. semifasciata*.

The effect of growth on the timing, velocity, and subsequent performance of animal motion has been reported for diverse behaviors (Ferry-Graham, 1998b; Verwaijen, et al., 2002; Elsworth, et al., 2003). The scaling model of feeding kinematics proposed by Richard and Wainwright (1995), based on largemouth bass *Micropterus salmoides*, predicts an isometric increase in linear displacement and maximum linear

velocity over ontogeny. This model also predicts a scaling coefficient of 0.3 for angular displacement, angular velocity, and time to reach peak displacements based on decreases in per sarcomere contraction velocity over ontogeny (Richard and Wainwright, 1995). While this model and others that examine relationships between organism size and behavioral response typically assume isometric growth (Hill, 1950; O'Reilly, et al., 1993), allometric growth occurred in every quantified attribute of *T. semifasciata* cranial morphology (Table 4). Most notable were an anteriorly directed shift of the mouth, accompanied by a slight narrowing, and the development of a shallower head profile at the level of the hyoid. Despite this allometry, the scaling coefficient of the time to reach maximum gape for *M. salmoides* (0.31) (Richard and Wainwright, 1995), the nurse shark *Ginglymostoma cirratum* (0.33) (Robinson and Motta, 2002), and *T. semifasciata* (0.35) are very similar, suggesting that a physiological limitation to sarcomere contraction rate may generally exist in fishes even under conditions of allometric growth. This relationship may be ecologically important because growth, and associated changes in nutritional needs, can drive variation in resource use (Olson, 1996; Lima-Junior and Goitein, 2003). As the ability to generate sub-ambient buccal pressures is partially dependent on the rate of buccal expansion (Muller and Osse, 1984; Svanback, et al., 2002), prey capture efficiency may be reduced by an increase in the time needed to open the mouth, which could provoke ontogenetic dietary shifts.

If prey capture performance is negatively impacted by a decrease in mouth opening speed, it is possible that behavioral modulation can compensate by affecting factors other than mouth opening speed that determine the magnitude of buccal pressure, such as acceleration, impulse, and magnitude of the volume entrained (Muller and Osse,

1984; Carroll, et al., 2004). One notable departure from the Richard and Wainwright (1995) model in *T. semifasciata* is the scaling coefficient of maximum gape distance over ontogeny (-0.555). While the absolute size of the mouth increased over time, the size of the oral aperture during feeding was behaviorally regulated to a relatively smaller size. Additionally, when feeding on live, elusive shrimp all timing variables decreased over the course of ontogeny and the forward motion of sharks during a strike tended to increase. Thus, while the time needed to open the mouth to a given gape size increased, presumably limiting suction generation capacity, individuals were able to behaviorally compensate by employing a smaller oral aperture and expanding the buccal cavity more rapidly, presumably increasing suction. The end product of learning to regulate feeding behavior in this way was an increase in the capture efficiency of live, elusive shrimp over ontogeny (Fig. 7). In addition to behavioral modulation, the positively allometric relationships between jaw musculature size, in concert with the isometric relationship for jaw weight, indicate the potential for relatively greater forces to act on relatively smaller skeletal elements over the course of ontogeny. Provided that changes in jaw opening and closing lever mechanics are not occurring (Barel, 1983; Westneat, 1994), this pattern of growth would produce the capacity for more rapid jaw opening and more forceful jaw closing, thereby increasing suction feeding ability as well as maximum bite force (Huber and Motta, 2004). Both suction and biting are employed by *T. semifasciata* in the wild (Russo, 1975; Talent, 1976) and the enhancement of both behaviors may be facilitated by allometric growth of cranial components.

The only behavioral factors not modulated in response to differing food types were food motion and the extent of buccal expansion. The lack of differences in food

motion among types could represent a hydrodynamic constraint acting on the generation of effective suction that was not overcome by behavior (Ferry-Graham, et al., 2001a), especially considering buccal reserve volume scaled with isometry and the relative size of food items was kept constant. Both absolute and relative strike distance were conservative throughout ontogeny, perhaps representing the maximum distance that *T. semifasciata* can displace food using suction under these experimental conditions. The force generated by a flow of water that is capable of imparting momentum to a food particle decays as the cube of distance from its source (Muller, et al., 1982; Muller and Osse, 1984; Svanback, et al., 2002). Provided that the entire buccal reserve volume is being employed, which it appears to be based upon the constant relative degree of buccal expansion, a cubic increase in buccal reserve volume would generate constant relative food displacement. The lack of apparent change in absolute food velocity and displacement could be a result of this exponential relationship, which requires that relatively large differences in buccal pressure be generated to produce differences in water velocity that would create a noticeable effect on food (Wainwright, et al., 2001a). In order to determine how much force is being imparted to the food item by the flow generated during suction feeding it is necessary to describe the pattern of flow directly in front of and into the oral aperture. While this pattern has been described for various osteichthian fishes with terminal mouths (Lauder and Clark, 1984; Muller and Osse, 1984; Ferry-Graham, et al., 2003), the contribution of a subterminal mouth and a prominent rostrum to flow pattern remains undescribed and could vary significantly over ontogeny as a result of behavioral and morphological changes such as those detected in *T. semifasciata*.

The detection of modulation in response to food size and elusivity in *T. semifasciata* in this study is in contrast to the findings of Ferry-Graham (1998a), who described juvenile *T. semifasciata* as typically employing a feeding modality intermediate between ram and suction that was unaffected by food type. The reason suggested by Ferry-Graham (1998a) for the apparent lack of modulation was that the offered live food, mud shrimp, did not behave elusively. Russo (1975) and Talent (1976) proposed that *T. semifasciata* was capable of performing suction-dominated bites based on the presence of whole clams and burrowing worms in the gut of several individuals. The capacity to modulate feeding behavior and employ a greater ram component when feeding on elusive prey may play an important role in the early development of *T. semifasciata* by facilitating exploitation of a broader prey base. While ontogenetic dietary shifts have been noted for *T. semifasciata* larger than those used in this study (Russo, 1975; Talent, 1976; Kao, 2000), dietary data for neonates and YOY are lacking. Based on the versatile feeding repertoire demonstrated in this study, it is predicted that neonates and YOY, while exhibiting individual dietary specialization and preferential usage of select resources, will behave as opportunistic generalists and a shift toward more elusive prey will occur with increases in predator experience and size.

In conclusion, to elucidate changes in feeding performance, diet, and ecological niche through ontogeny, longitudinal studies must begin by understanding variability and modulation in the form-function complex of the feeding apparatus. Over early ontogeny the feeding behavior and cranial morphology of *T. semifasciata* change considerably. Despite extensive inter-individual variability in capture kinematics, behavioral modulation occurs in response to food attributes, most notably elusivity and size, and

consists of changes in the timings of cranial movements as well as the extent of overall predator motion during the strike. Live shrimp were captured using rapid buccal expansion and forward motion of the predator, while non-elusive food items were captured using slower motions. Though buccal reserve volume increases isometrically across ontogeny, movement of the food via inertial suction appears to be limited by hydrodynamic constraints, as evidenced by a lack of difference in food movement during the strike over ontogeny. Growth of cranial elements produces a morphology tailored for both rapid buccal expansion, through positively allometric growth of jaw-abducting musculature and a relative narrowing of the mouth, and forceful biting, via positively allometric growth of the jaw-adducting musculature. Utilization of a broad behavioral repertoire enhanced by morphological development is hypothesized to permit exploitation of a broad prey base in *T. semifasciata* over early ontogeny.

Chapter 3: The ontogeny of feeding behavior and cranial morphology in the whitespotted bambooshark *Chiloscyllium plagiosum* (Bennett 1830): a longitudinal perspective

Abstract

The 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* 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 positive allometry while the time to reach maximum jaw and hyoid abduction showed no trend with size, indicating 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

A 'specialist' is, in its most inclusive sense, an organism that exhibits limited breadth in one or more aspects of its niche. Specializations may include behavioral, physiological, anatomical, and morphological aspects of organisms and have the capacity to both greatly restrict and expand the realized niche of a species (Kiltie, 1982; Herrel, et al., 1999; Bohn and Amundsen, 2001; Sibbing and Nagelkerke, 2001). On the individual level, specialization can lead to variation in performance, the ability to perform a task, which can lead to differential survival and fitness among members of a population (Liem, 1980a; Ferry-Graham, et al., 2002). By virtue of their impact on fitness, performance differences are an underlying force driving adaptation over phylogenetic time (Lauder, 1981; Arnold, 1983; Norton, et al., 1995).

Obtaining nutrients is crucial to survival, dictating that the feeding apparatus must be under selective pressure to perform at every stage of development in which nutrients are obtained from external sources (Galis, 1990; Galis, et al., 1994). However, aquatically feeding animals are subjected to additional functional constraints because of the density and viscosity of water (Lauder, 1980; Liem, 1990; 1993; Lauder and Shaffer, 1993). If feeding on unattached prey, aquatic organisms may either use the medium to entrain the prey (suction feed) or overcome the medium and engulf the prey (ram feed) (Liem, 1980b). Inertial suction feeding involves the generation of sub-ambient buccal pressures, and maximizing the capacity to generate this pressure has been linked to numerous behavioral and morphological specializations (Liem, 1993; Wainwright, et al., 2001a; 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., 1995a; Hernandez and Motta, 1997) or catholic diet (Castro, 2000), the difference in part being attributable to the degree of versatility and performance capacity of the feeding apparatus. Employing a specialized function (e.g. generating sub-ambient buccal pressure) to perform versatile biological roles (e.g. capturing elusive prey in the water column and benthic prey in crevices) may lead to broader exploitation of the available food base than if the function were employed only for a single biological role.

Qualitatively large differences in feeding behavior and morphology among aquatically feeding vertebrate species are easily recognized and have been widely studied (Muller and Osse, 1984; Motta, et al., 1995b; Liem 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, but variation among individuals over ontogeny is a key ecological and evolutionary precept (Van Valen, 1965; Bolnick, et al., 2003). Early ontogeny is often a period of rapid learning and morphological development and provides an opportunity to study rapid changes in feeding behavior and morphology. If capture performance of the feeding apparatus is enhanced by the generation of greater sub-ambient buccal pressure, then directional development of the functionally relevant behavioral and morphological aspects that enhance suction is expected over ontogeny in species that primarily employ this mode of prey capture. 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 composed of comparatively 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; Huber and Motta, 2004; Motta, 2004), but little is known about the development of feeding morphology and behavior over early ontogeny (but see Ferry-Graham, 1997; 1998). To assess ontogenetic shifts in feeding and how they affect performance, we must first describe and understand the patterns of change in feeding morphology and behavior.

The whitespotted bambooshark *Chiloscyllium plagiosum* (Hemiscylliidae) was selected as the subject for this study because specimens are readily available from captive breeders, making it possible to study individuals from first feeding through the first year of life. This species was expected *a priori* to be an obligate suction feeder based on studies of the feeding apparatus and behavior of orectolobid 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 feeding morphology and behavior of *Chiloscyllium plagiosum* feeding on a variety of food types and sizes; 2) to determine the contribution of behavioral differences among individuals, among food types (modulation), and among food types within an individual, throughout

ontogeny; 3) to compare the ontogeny of prey capture behavior and morphology to that of a behavioral and morphological generalist, the leopard shark *Triakis semifasciata* (Chapter 2); and 4) to identify form-function complexes that change over early ontogeny and may influence feeding performance among sharks with respect to the generation of subambient buccal pressure.

Methods and Materials

Experimental Animals

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, hatching at 12-20 cm TL (A. Cornish, pers. comm.; Tullis and Peterson, 2000). Adult females attain a maximum size of ~1 m TL, while males typically reach ~0.7 m. *Chiloscyllium plagiosum* is an opportunistic generalist that feeds primarily on benthic invertebrates and occasionally fish (A. Cornish, pers. comm.). Wu (1993) determined that adult *C. plagiosum* primarily use suction to capture prey.

Filming Techniques

Five *C. plagiosum* (average TL 15.76 cm at hatching) were hatched at SeaWorld, Orlando, Florida and raised for 52 weeks, attaining an average TL of 43.64 cm. Animals were maintained in a 340-liter communal tank at $26\pm 1^{\circ}\text{C}$ and 33 ± 2 ppt salinity. During experimental sessions, individuals were isolated within a 100-liter subsection of the holding tank so their behavior would not be influenced by interactions with other individuals. Animals 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 but for feedings immediately preceding experimental sessions the ration was cut to 2% to encourage active feeding during filming. Experimental sessions began within two weeks of hatching and included first-feeding in three of the five individuals.

To investigate directional changes of feeding behavior in response to food size, type, and elusivity (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 scaled to one half MW; 4) chopped clam (shelled) scaled to MW; and 5) live shrimp scaled to MW in carapace length. Filming sessions occurred weekly over the entire one-year experimental period. During filming sessions food items were presented in a haphazard order until the individual approached satiation, as evidenced by a decrease in feeding activity. Though a filming session often comprised as many as ten capture sequences for a given individual, only the first five were considered for analysis to avoid confounding effects of satiation (Sass and Motta, 2002).

The methods for obtaining kinematic and morphological data are described in detail elsewhere (Chapter 2). In brief, a Redlake PCI 1000 high-speed digital camera (Redlake, San Diego, CA, USA) was placed perpendicular to the aquarium providing both a lateral view and, via a mirror beneath the shark, a ventral view. Only orthogonal views were retained for analysis. Recordings were made at 250 fps and illumination was provided by two, 500-Watt quartz-halogen lights. Animals were trained to feed under illumination and were allowed a 20-minute acclimation period prior to each feeding

session. 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.) The variables measured parallel those of Chapter 2. 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); 3) maximum cranial elevation angle (degrees); 4) time to maximum cranial elevation angle (ms); 5) time to onset of cranial elevation (ms); 6) time to offset of cranial elevation (ms); 7) duration of cranial elevation (ms); 8) time to onset of hyoid depression (ms); 9) maximum hyoid depression (cm); 10) time to maximum hyoid depression (ms); 11) time to hyoid retraction (ms); 12) duration of hyoid depression (ms); 13) total strike duration (ms); 14) duration of food movement (ms); 15) distance moved by the food (cm); 16) velocity of the food (cm s^{-1}); 17) distance moved by the predator (cm) during the duration of food movement; and 18) 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 (15) and (17) 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, and indicates the relative contribution of forward motion of the predator and motion of the prey 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. 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 the digital recordings were used to measure several external morphological variables. The definitions of these variables follow Compagno (1984b) and 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 location of the hyoid. To verify that these measurements were accurate, 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 total length 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 feeding, specifically the coracomandibularis, coracohyoideus, coracoarcualis, and quadratomandibularis (Motta, et al., 1991; Wu, 1994; Motta and Wilga, 1995; 1999). SigmaScan Pro version 4 (SPSS Inc.) was used to determine anatomical cross-sectional area from digital photographs taken with a Nikon Coolpix 4300. The palatoquadrate (upper jaw) and Meckel's cartilage (lower jaw) were also excised and their combined

weight recorded. These skeletal elements were considered because their growth could influence the velocity and force of feeding motions.

Measures of buccal volume were obtained by injecting silicone into the buccal cavity of each dead specimen and allowing it to cure for 36 hours before removal and weighing. Using the mass-density of silicone reported by Cook (1996) (0.06 g ml^{-1}), this weight was converted into a 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, was used to indicate the maximum potential change in the volume of the buccal cavity during a capture event.

Statistical Analyses

All kinematic data were log-transformed and checked for normality and homogeneity of variance via the Kolmogorov-Smirnov and Levene Median tests, respectively, 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 Model II linear regressions were performed to describe the nature of these relationships. Model II regressions were appropriate because both variables used in these comparisons contained error (McGowan, 1988). Regressions were performed for all individuals combined then for each individual separately to compare regression 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, all variables that lacked a relationship to total length were 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 correlation matrix-based 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 any other rotation, enhancing data interpretation. Variables loading above an absolute value of 0.5 were considered to contribute heavily to the variability within the respective principal component (PC). 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 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 with an α level of 0.05. To determine the order of the best-fit equation describing trends over the repeated measure time, single degree of freedom polynomial contrasts were utilized. Bonferroni corrections were not applied to any tests due to their tendency to increase the rate of type II errors (Cabin and Mitchell, 2000;

Moran, 2003). The PCA, MANOVA, and RM ANOVA tests were performed using Systat 11 (SPSS Inc.).

Morphological variables measured on living specimens were log-transformed and regressed against the logarithm of total length using Model II linear regressions to investigate changes in the relative dimensions of these variables over time. 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 taken from dead specimens was compared against the confidence intervals for the live individuals to determine if it 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 within 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, with a slope of one expected for linear variables, a slope of two expected for planar variables (e.g. muscle cross section), and a slope of three expected for cubic variables (e.g. muscle mass, buccal volume).

Results

Individuals generally rested on the bottom or swam slowly around the filming chamber until food was introduced. The shark would then initiate searching behavior, which was characterized by rapid movement along the bottom in a sweeping pattern that covered most of the filming chamber. 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. 10). The temporal sequence of kinematic events was conservative across ontogeny and among food types. Capture success was generally high with all dead food types, averaging 1.1 ± 0.3 attempt per capture and changing little over ontogeny, however the number of attempts needed to capture live shrimp declined steadily from 2.5 ± 0.8 during the first time segment to 1.6 ± 0.5 during the last.

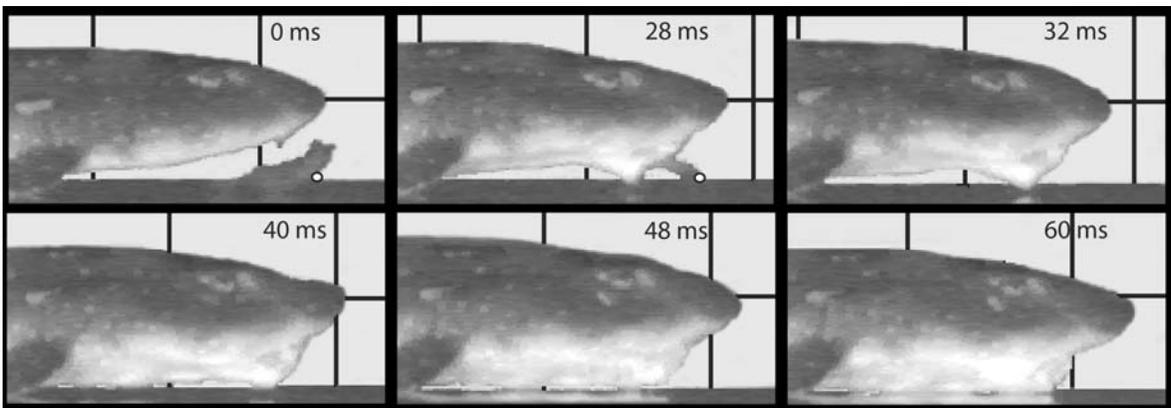


Figure 10: Composite photographic series of a representative food capture sequence for *Chiloscyllium plagiosum*. The point on the food that is farthest from the shark's mouth is indicated by a white dot. This was the point used in calculating the RSI. Times for this capture are shown in the upper right 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.

Scaling of Kinematics

When individuals were combined, the majority of kinematic variables scaled with positive coefficients over ontogeny indicating that slower, more extensive motions accompanied growth (Table 6). The only two variables that decreased over ontogeny were the time to onset of hyoid depression and RSI, indicating that as sharks grew they began depressing their hyoid earlier in the strike and their overall feeding modality became more suction-dominated. The distance moved by the predator and the velocity of the predator during the strike did not change with size, while the distance moved by the food increased slightly (Table 6). Taken together, these relationships indicate that the decrease in RSI 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 6), indicating that while substantial inter-individual variability exists in the scaling of kinematics over ontogeny the general trends presented above are representative.

Kinematic Trends

The 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 PCs (Table 7). Three of these related to cranial elevation during the period from onset to maximum, indicating that the timing and extent of cranial elevation was characterized by relatively 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 over the course of the strike loaded on PC2 while variables describing the motion of the food loaded on PC5. The RSI, even though it is an index based on motion of both the predator and food, only loaded heavily on PC2. This indicates that variability in aspects of predator motion was greater than for aspects of food motion. Lastly, variables describing the extent of buccal expansion, as indicated by maximum gape and maximum hyoid depression, loaded on PC6 (Table 7).

Table 6: Regression parameters for kinematic variables versus total length (cm) for *Chiloscyllium plagiosum*. 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.

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**

Table 7: Principal component loadings of kinematic variables associated with capture sequences in *Chiloscyllium plagiosum*. 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.

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

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 ($df=48$; $F=1.05$; $P=0.338$). Despite a high degree of overlap, when each PC was examined with a separate RM ANOVA for differences among individuals they were detected regardless of ontogeny on all components (Fig. 11) and over ontogeny on all components except PC2 (predator motion) (Table 8), indicating the presence of

substantial inter-individual differences in kinematics. 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. 12). 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) (Fig. 13) and 5 (prey motion), indicating that modulation in response to food attributes did not occur in the timing or extent of cranial motion over ontogeny. Effect interactions among sharks within food types over ontogeny existed only on PCs 5 (prey motion) and 6 (buccal expansion) indicating complex interactions over time on these two components that are due to the combined influence of individual and food type.

Scaling of Morphology

Morphometric variables tended to scale with isometry or very slight allometry (Table 9). The greatest allometric scaling coefficient was quantified for mouth width (1.05) and suggests that the width of the mouth at an age of 1 year is ~12% greater than would be predicted assuming isometry. The scaling coefficient exhibited by other morphological attributes typically produced an estimate varying by less than 3% from that predicted by isometry. The apparently high level of statistical significance associated with these deviations is attributable to the low variability generated by taking measurements on a weekly basis. Measurements taken on dead specimens fell within the 95% confidence intervals of those taken from live specimens and were dominated by isometric or very slightly allometric patterns of growth.

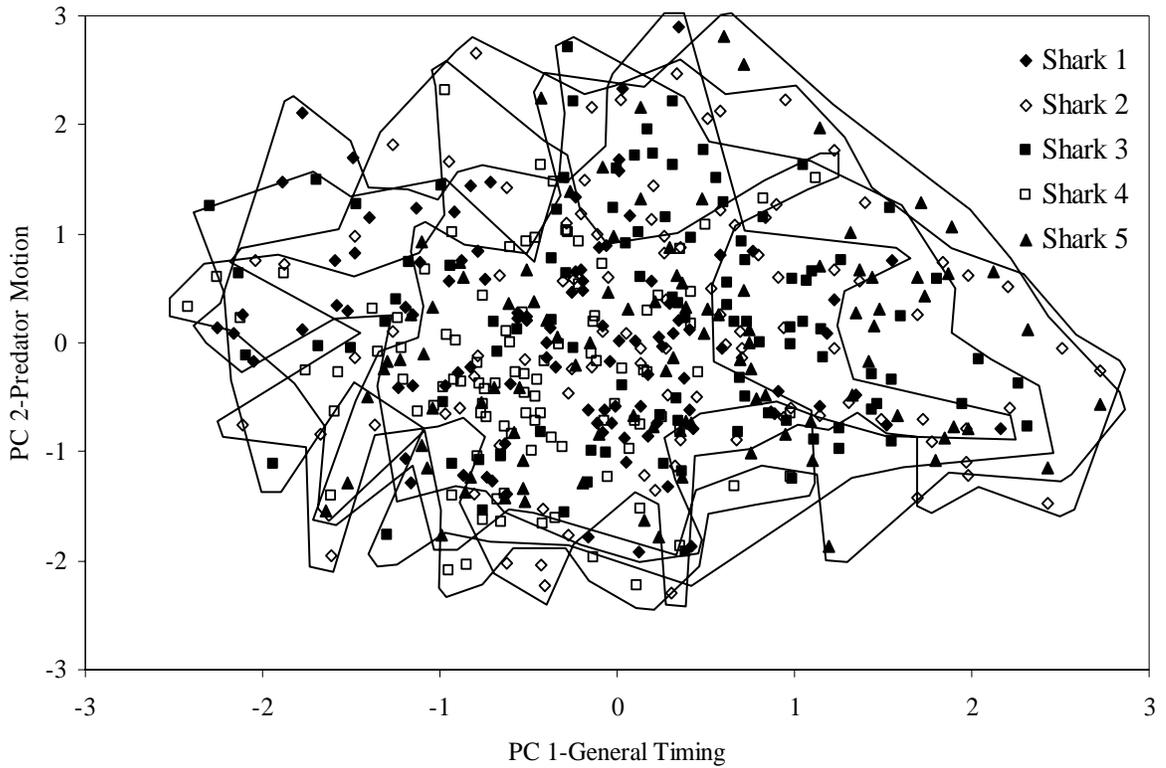


Figure 11: Comparison of principal component 1 (PC1) (general timing) and PC2 (predator motion) scores among individuals irrespective of time for *Chiloscyllium plagiosum*. Together the variability explained by these two components is 33.99% of the overall variability in the kinematic data set. Polygons delimit the region of the plot occupied by bites from each individual. Note the high degree of intra-individual variability and the large degree of overlap among individuals, which is also a prevailing trend on the remaining PCs. On PC1 sharks 1 and 4 differ from the remaining sharks ($\alpha=0.05$) and on PC2 sharks 4 and 5 differ ($\alpha=0.05$).

Table 8: Results of RM ANOVAs performed separately on each principal component from a PCA of prey capture kinematics in *Chiloscyllium plagiosum* (N=5). df=Degrees of Freedom; TS=Time Segment. 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.

		PC1 - General Timing			PC2 - Predator Motion		
		df	F	P	df	F	P
Between Subjects:	Shark	4	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		
		df	F	P	df	F	P
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		
		df	F	P	df	F	P
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*

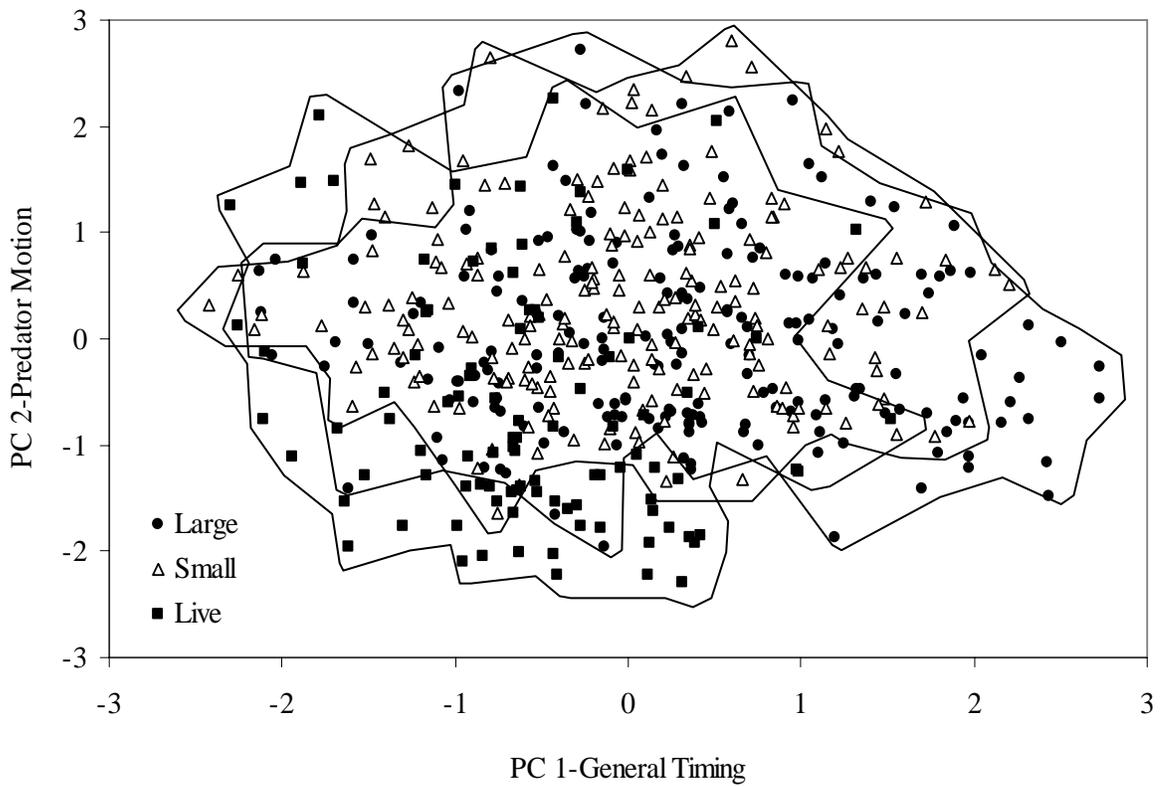


Figure 12: Comparison of principal component 1 (PC1) (general timing) and PC2 (predator motion) scores for large, small, and live food irrespective of time for *Chiloscyllium plagiosum*. Together the variability explained by these two components is 33.99% of the overall variability in the kinematic data 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.

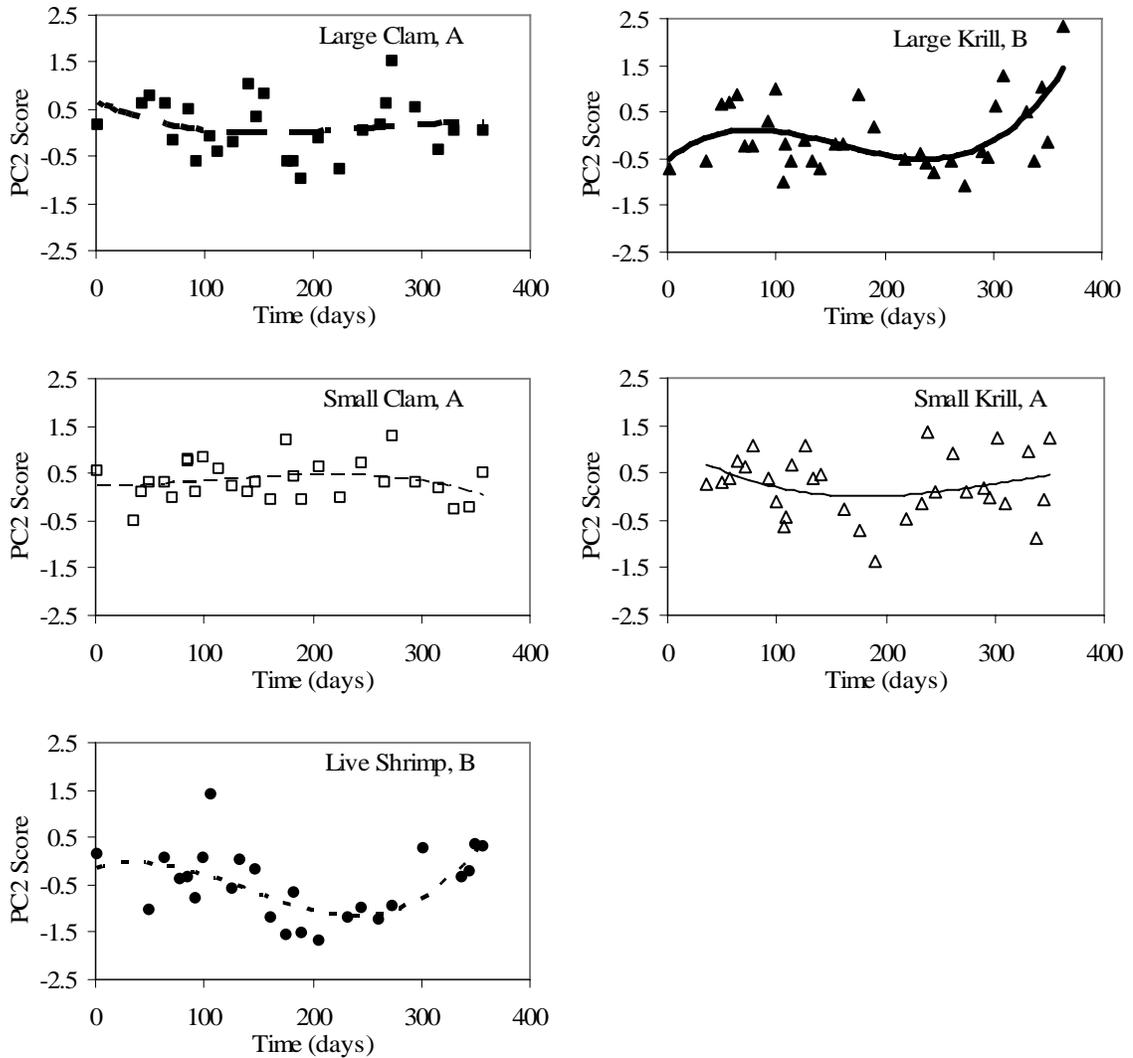


Figure 13: Trends in predator motion (PC2) over time by food type in *Chiloscylidium plagiosum*. Model II regressions are based on all bites from all individuals but, for clarity, only daily averages are shown. Graphs labeled with different letters differ in the trend displayed over time at the $\alpha=0.05$ level. Over time bites on small (one half mouth width) food and large (mouth width) clam exhibit a slight trend toward greater predator motion, while bites on large (mouth width) krill and live shrimp diverge from this trend at ~250 days and are characterized by relatively less predator motion. Regression lines labeled with different letters differ at the $\alpha=0.05$ level.

Table 9: Parameters for log-log regressions against total length (cm) of external morphological variable data measured on live specimens of *Chiloscyllium plagiosum*. Results shown are averages for individuals (N=5). The expected slope for isometry is one in all cases. $t_{0.05(1), 40}=1.684$.

Variable	Slope	y-intercept	r ²	St Error	P
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*

The growth trajectories calculated for the masses and cross-sectional areas of muscles, as well as buccal volume, were dominated by substantial allometry (Table 10). The cross-sectional area of all of the jaw abducting and adducting muscles scaled with positive allometry. The scaling 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. Paradoxically, the weight of both the coracohyoideus and the coracomandibularis scaled with negative allometry (Table 10), which may be a factor of unquantified changes in other aspects of muscle size (e.g. length) that occur 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 the muscles acting on these elements. 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 scaling coefficient for the buccal reserve volume indicating that as *C. plagiosum* grows its relative buccal reserve volume substantially increases (Fig. 14).

Table 10: Parameters for log-log regressions against total length (cm) of morphological variable data taken from dead specimens only of *Chiloscyllium plagiosum*. The expected slope for isometry is given in the column labeled Sl_{EXP} . $t_{0.05(1), 8}=1.860$.

Variable	Sl_{EXP}	Slope	y-intercept	r^2	St Error	P
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*
Quadratmandibularis Area (mm ²)	2	2.44	-4.17	0.98	1.5E-2	<0.001*
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*
Quadratmandibularis Weight (g)	3	3.13	-5.21	0.98	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*

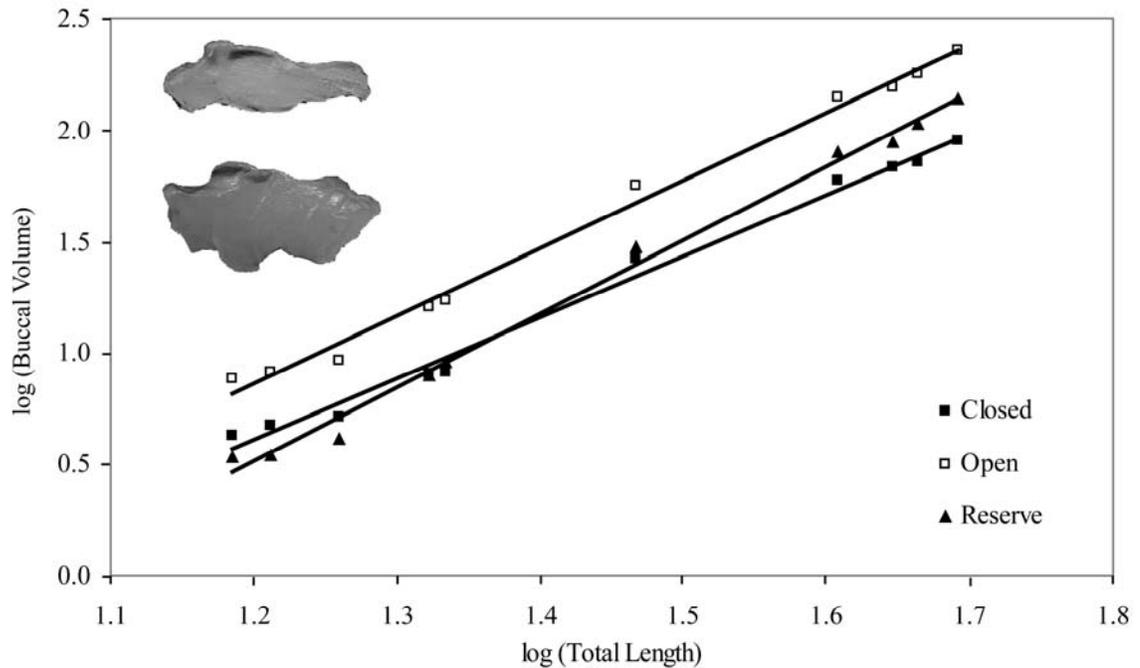


Figure 14: Scaling of buccal volume relative to total length in *Chiloscyllium plagiosum*. Measurements calculated from the weight of silicone casts obtained from dead specimens placed in resting and maximally expanded positions based on kinematic footage. Reserve volume is the difference between the open and closed position volume for a specimen. Inset shows lateral view of closed (top) and open (bottom) silicone casts from a representative individual. The expected slope for isometry is 3 in all cases. Model II regression equations are: Buccal Volume Closed $y=2.75x-2.69$; Buccal Volume Open $y=3.04x-2.79$; Buccal Reserve Volume $y=3.31x-3.46$.

Discussion

Changes in morphology and behavior associated with the feeding apparatus of *Chiloscyllium plagiosum* over early ontogeny appear to enhance the capacity of this species to generate subambient pressures in the buccal cavity during feeding, facilitating inertial suction feeding. Though most morphological aspects of the head scale with isometry, the musculature associated with jaw and hyoid abduction, and jaw adduction, hypertrophies with growth. Additionally, buccal reserve volume grows allometrically at a rate greater than the positive allometric increase in the time to maximum gape and maximum hyoid depression. Considered together 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 in morphology and kinematics between hatchling and juvenile swellsharks *Cephaloscyllium ventriosum* and found isometric growth but differences in RSI that were attributed 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* suggested 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 over ontogeny 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, as quantified by the RSI (Cook, 1996). To verify whether the apparent 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 with size, 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 in large part due to divergence in the velocity and duration of food movement. Because these variables are determined by the interaction between the food and the water flow generated by the shark, which is partially dictated by physical attributes of the food, the apparent modulation should be interpreted cautiously. If the force exerted by the entraining flow of water generated by the shark is the same among bites on different food types, the response of different food types to this flow could vary in a consistent manner that would be statistically detected as modulation. Assessment of the response of various food types to controlled suction forces is necessary to firmly assert that *C. plagiosum* is modulating kinematic timings in response to food type. When food presentation is via tongs, restricting food motion, the nurse shark *Ginglymostoma cirratum* does not modulate its feeding 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 strike kinematics but modulation of overall predator motion in response to food type (Table 8).

Despite the conservative kinematic patterns described above, differences were detected among individuals both irrespective of, and over the course of, ontogeny. The occurrence of considerable variability in feeding kinematics among and between individual fish during discrete life stages is nearly universal (Wainwright and Lauder,

1986; Sanderson, 1990; Cook, 1996; Ferry-Graham, 1998a), even in species like *G. cirratum* that are obligate suction feeders and exhibit considerable stereotypy (Motta, et al., 2002). The general lack of longitudinal studies that follow target individuals across ontogeny has led to a paucity of information regarding intra-specific variability in developmental trajectories with regard to feeding morphology and behavior (but see Chapter 2). 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 compromised if inter-individual variability is not considered. The magnitude of developmental differences over ontogeny are likely to be amplified in natural settings due to greater variability in predator experience and heterogeneity of available resources (Morse, 1980), compounding the potential 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 variations in the environment over phylogeny (Liem, 1980a).

The pattern of morphological and behavioral development described here for *C. plagiosum* is in stark contrast to that reported in Chapter 2 for the leopard shark *Triakis semifasciata*. The growth of the head of *T. semifasciata* is highly variable and strongly allometric, producing marked changes in head shape over ontogeny. This is accompanied by hypertrophy of the jaw and hyoid abducting musculature, but not to the extent seen here for *C. plagiosum*, especially in the case of the coracohyoideus. 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 (Ferry-Graham, 1998a) and modulation of timing and modality variables in response to prey size and elusivity. *Triakis semifasciata* apparently utilizes a diverse, 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 here. 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), apparently using a single, highly stereotyped feeding behavior (Motta, et al., 2002), has been demonstrated in the orectolobid shark *G. cirratum*, and is hypothesized to be prevalent in bottom-dwelling, suction-feeding elasmobranchs (Motta and Wilga, 2001; Motta, 2004). The generation of suction forces adequate to entrain prey is contingent upon temporally coordinated buccal expansion and is severely limited by physical attributes of the aquatic medium (Muller and Osse, 1980; Wainwright, et al., 2001b), suggesting that suction feeding species should exhibit little variation or modulation in strike kinematics. For both *C. plagiosum* and *T. semifasciata* 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.

Chapter 4: The comparative ontogeny of feeding performance in two sharks: the leopard shark *Triakis semifasciata* and the whitespotted bambooshark *Chiloscyllium plagiosum*

Abstract

Development of the capacity and ability to exploit prey is crucial to the survival of predatory organisms in the wild. Ontogenetic trends in food capture performance were quantified using direct measures of suction pressure and flow in front of the mouth for two elasmobranchs, the ram-feeding generalist leopard shark *Triakis semifasciata* and the suction-feeding specialist whitespotted bambooshark *Chiloscyllium plagiosum*, at four times during the first year of life. At any given total length, *C. plagiosum* tended to produce greater maximum subambient pressure and ingest more rounded, high-aspect ratio parcels of water than *T. semifasciata*. Growth, and feeding kinematics that have been shown to scale with growth in these species, primarily accounted for variation in performance aspects over time. Maximum subambient pressure scaled with negative allometry in *T. semifasciata* and was mediated by an allometric increase in the time to reach maximum gape. Despite similar allometric increases in the timing of buccal expansion, maximum subambient pressure in *C. plagiosum* scaled with isometry, presumably as a consequence of earlier onset of hyoid depression over ontogeny and an allometrically increasing buccal reserve volume. The length of the ingested parcel of water relative to mouth width and the aspect ratio of the parcel did not change with growth in either species, demonstrating physical constraints imposed by water on the use

of suction in feeding. Though total length was the primary factor responsible for developmental trends in both species, behavioral changes irrespective of size also contributed to performance. The contribution of these behavioral changes was minor relative to the effect of growth, and size-independent behavioral variability is hypothesized to contribute little to overall performance differences over the course of early ontogeny.

Introduction

The performance of the feeding apparatus of aquatic vertebrates is influenced both by the structure and spatial organization of its elements and the temporal and spatial pattern of motion of those elements during a feeding event (Reilly and Lauder, 1988; Ferry-Graham, et al., 2001b; Motta and Wilga, 2001; Carroll, et al., 2004). Changes in the mechanical properties of structural elements (Summers, et al., 1998b; Summers, 2000), allometry of musculoskeletal elements (Richard and Wainwright, 1995; Robinson and Motta, 2002; Herrel, et al., 2005), mechanical advantage of muscular lever systems (Hernandez and Motta, 1997; Wainwright, et al., 2000; Adriaens, et al., 2001; Westneat, 2003), and morphology of the feeding apparatus (Carroll, et al., 2004; Dean and Motta, 2004) have all been shown to affect aspects of feeding performance. While structural changes within an individual take time to develop, even during metamorphosis when change occurs rapidly (Reilly and Lauder, 1990; Galis, 1993; Reilly, 1996), variation in the behavioral repertoire at discrete points during ontogeny is nearly universally in aquatically feeding vertebrates (Wainwright and Lauder, 1986; Sanderson, 1990). Additionally, distinct modulation of food capture behavior by aquatic vertebrates is thoroughly documented (Deban, 1997; Ferry-Graham, et al., 2001b; Motta and Wilga, 2001) and has been shown to influence several aspects of performance (Norton, 1991; Wainwright, et al., 2001a; Day, et al., 2005; Higham, et al., 2005).

Inertial suction feeding is the dominant prey capture mechanism employed by aquatically feeding vertebrates (Lauder, 1985b) and several morphological and behavioral correlates for enhancing suction performance have been identified (Liem, 1993; Carroll, et al., 2004; Gibb and Ferry-Graham, 2005; Van Wassenbergh, et al.,

2005). As suction feeding requires imparting force to a food item via displacement of the water surrounding it, traits that increase the force available to be transferred are characteristic of suction feeders. Morphological attributes that enhance suction include a smaller buccal aperture that is often laterally enclosed, larger jaw and hyoid abductor muscles, reduced oral dentition, a deep head profile, hypertrophied epaxial musculature and an expansible buccopharyngeal cavity (Muller and Osse, 1984; Liem, 1993; Carroll, et al., 2004). Behavioral traits that are correlated with increased suction performance include rapid, extensive hyoid depression, buccopharyngeal expansion, and cranial rotation (Svanback, et al., 2002; Carroll, et al., 2004; Gibb and Ferry-Graham, 2005). Over ontogeny gradual changes may occur in these morphological and behavioral traits, producing the potential for substantial developmental changes in performance (Motta and Kotrschal, 1992; Galis, et al., 1994; Koehl, 1996).

While several aspects of the anatomy and food capture behavior of teleost fishes have been studied over ontogeny (Coughlin, 1991; Cook, 1996; Hernandez, 2000; Krebs and Turingan, 2003; Van Wassenbergh, et al., 2005), few studies have focused on the development of these aspects in elasmobranchs (but see Ferry-Graham, 1997; 1998a; 1998b; Chapters 2 and 3). Adult elasmobranchs are known to possess morphologically and functionally varied feeding mechanisms (Shirai and Nakaya, 1992; Wu, 1994; Motta, et al., 1997; Wilga and Motta, 1998a; Wilga and Motta, 2000) that are employed to exploit diverse prey (Cortes, 1999; Sims, 2000; Motta and Wilga, 2001). Ontogenetic dietary changes are well documented in elasmobranchs (Lowe, et al., 1996; Cortes, 1999; Kao, 2000; Ebert, 2002) and have been primarily attributed to changes in habitat and growth. It is likely, however, that developmental changes in morphology and behavior

affect performance in ways that could enable either the exploitation of novel prey, facilitating ontogenetic dietary changes, or a change in how prey are captured. The use of disparate prey capture behaviors to occupy diverse feeding niches despite the anatomical simplicity of their feeding apparatus (Motta and Wilga, 2001; Motta, 2004), as well as the absence of metamorphic changes in feeding morphology, makes elasmobranchs a worthwhile clade in which to study the development of feeding morphology and behavior.

The elasmobranch species selected for study were the leopard shark *Triakis semifasciata* (Triakidae) and the whitespotted bambooshark *Chiloscyllium plagiosum* (Hemiscylliidae). *Triakis semifasciata* is a common demersal species found along the Pacific coast of North America and northern Central America where it frequents estuaries, lagoons, and shallow bays (Compagno, 1984a). This species is aplacental viviparous, giving birth to pups that are approximately 20-26 cm and grow between 2 and 4 cm yr⁻¹ (Compagno, 1984a; Kusher, et al., 1992). *Triakis semifasciata* is an opportunistic generalist that feeds on a broad taxonomic and functional diversity of prey including benthic invertebrates and fishes throughout ontogeny (Talent, 1976; Kao, 2000). Despite possessing several morphological attributes typical of suction feeders, previous study has shown that *T. semifasciata* typically employs ram-dominated food capture (Ferry-Graham, 1998a). This species is, however, capable of modulating capture kinematics in response to food size and elusivity to utilize greater suction (Chapter 2). The whitespotted bambooshark *Chiloscyllium plagiosum* is a common shallow water, reef-dwelling, epibenthic species of the Indo-West Pacific and eastern coast of Southern Asia (Compagno, 1984b). *Chiloscyllium plagiosum* is oviparous, hatching at 12-20 cm

TL (A. Cornish, pers. comm.; Tullis and Peterson, 2000). This species is an opportunistic generalist that feeds primarily on benthic invertebrates and, with age, occasionally fish (A. Cornish, pers. comm.). Previous study has shown that *C. plagiosum* displays characteristic suction-feeding morphology and employs a more behaviorally conserved, suction-dominated food capture behavior (Wu, 1993; Chapter 3).

The goals of this study were: 1) to quantify developmental changes in food capture performance for two elasmobranch species, a ram-feeding generalist and a suction-feeding specialist, from birth/hatching through the first year of life. The study was limited to the first year of life because juvenile mortality is high in many elasmobranchs but declines with age (Manire and Gruber, 1993; Heupel and Simpfendorfer, 2002), making this a critical developmental period.; 2) to determine the contribution of organism size and behavioral development to performance changes over ontogeny in both species; and 3) to integrate knowledge of morphology, behavior, and performance to propose general developmental trends in the feeding behavior of young-of-the-year sharks that might impact individual prey capture capacity and survival.

Methods and Materials

Experimental Animals

Twelve neonatal *T. semifasciata* were obtained from Mote Marine Laboratory (MML), Sarasota, Florida and six hatchling *C. plagiosum* were obtained from SeaWorld, Orlando, Florida. *Triakis semifasciata* were approximately 1 month old upon arrival at MML, as determined by their size (Kusher, et al., 1992), and had been previously fed commercial aquarium feed, while *C. plagiosum* were reared at SeaWorld and obtained

prior to first feeding. *Triakis semifasciata* were maintained in a 2.4-meter diameter, 1400-liter semicircular communal holding tank at $27 \pm 1^\circ\text{C}$ and 32 ± 2 ppt salinity while *C. plagiosum* were maintained in a 340-liter communal holding tank at $26 \pm 1^\circ\text{C}$ and 33 ± 2 ppt salinity. During experimental sessions, individuals of each species were isolated into a 90cm×45cm×30cm filming tank containing 55 liters of water from their respective holding tank.

Animals were maintained on a diet consisting of 3-4% of their body weight in various live and dead food types of various sizes *ad libitum* twice per week. This feeding frequency was maintained throughout the study but for feedings immediately preceding experimental sessions the ration was cut to 2% to encourage active feeding during filming (see below). During experimental sessions, only pieces of Atlantic threadfin herring *Opisthomena oglinum* (for *T. semifasciata*) or the clam *Mercenaria mercenaria* (for *C. plagiosum*) that were scaled to the mouth width of the animal were fed. These food types were selected from a subset of food types for which the behavioral response of these two species is known (Chapters 2 and 3) in order to maximize suction effort and performance (Carroll, et al., 2004). Though an experimental session often consisted of as many as ten food capture events per individual, only the first five were considered for analysis in order to avoid the effects of satiation (Sass and Motta, 2002).

Experimental Techniques

The experimental period for this study was 52 weeks and was broken into four even segments for analysis, beginning with birth/hatching of the individuals. Experimental sessions only occurred during the middle five weeks of each 13-week

segment, providing a sample of feeding performance at four discrete times during the young-of-the-year period for both species. Feeding performance was measured during each time segment using two independent techniques (suction pressure and particle motion), both of which were accompanied by high-speed recordings from which kinematic data were obtained. Recordings of capture events were made with a Redlake PCI 1000 high-speed digital camera (Redlake, San Diego, CA, USA) that was placed perpendicular to the aquarium to provide a lateral view. Recordings were made at 250 fps with a shutter speed of 1/1000 ms and illumination was provided by two, 500-Watt quartz-halogen lights. Animals were trained to feed under illumination prior to the experiment and were allowed a 20-minute acclimation period prior to each feeding session. A ruler beside the shark provided distance measure and only orthogonal views were retained for analysis. Kinematic data were measured from recordings using Redlake MotionScope PCI software version 2.21.1 and SigmaScan Pro version 4. The variables measured were selected for their functional relevance and prior employment in other studies of elasmobranch feeding (Motta, et al., 1997; Ferry-Graham, 1998a; Wilga and Motta, 1998b). From the onset of lower jaw depression (time 0 ms), the following kinematic variables were quantified: 1) strike distance, from the closest point on the food to the lower jaw of the shark (cm); 2) maximum gape (cm); 3) time to maximum gape (ms); 4) time to close, from time maximum gape was first reached to jaw closure (ms); 5) maximum cranial elevation (degrees); 6) time to maximum cranial elevation (ms); 7) time to onset of cranial elevation (ms); 8) time to offset of cranial elevation (ms); 9) duration of cranial elevation (ms); 10) time to onset of hyoid depression (ms); 11) maximum hyoid depression (cm); 12) time to maximum hyoid depression (ms); 13) time to hyoid

retraction (ms); 14) duration of hyoid depression (ms); 15) time gills start to open (ms); 16) total strike duration from onset lower jaw depression to jaw closure (ms); 17) duration of food movement during the strike (ms); 18) distance the food moves during the strike (cm); 19) velocity of the food over the course of the strike (cm s^{-1}); 20) distance the predator moves during the strike (cm); and 21) velocity of the predator over the course of the strike (cm s^{-1}). Variables (18) and (20) were used to calculate the ram-suction index (RSI) (Norton and Brainerd, 1993). The RSI is calculated as $(D_{\text{PREDATOR}} - D_{\text{PREY}}) / (D_{\text{PREDATOR}} + D_{\text{PREY}})$ and indicates the relative contribution of forward motion of the predator and motion of the prey to a given capture event. Both the distance moved by and velocity of the food were used as indicators of performance in subsequent analyses (see below). No measures were made of upper jaw protrusion excursion or timing because protrusion was not present in all cases.

Simultaneous with the recordings described above, food capture performance was measured by quantifying the subambient suction pressure at the position of the food. To obtain these data, a 5-French Millar MPC-500 Mikro-tip® catheter (Millar Instruments, Inc., Houston, TX, USA) was inserted through an aperture in a Plexiglas false bottom that divided the filming tank vertically. Food items were then notched with a knife such that, when wrapped around the catheter, the tip of the pressure transducer was exposed directly to the flow generated by the shark during capture. The catheter was placed at the level of the food rather than within the buccopharyngeal cavity of the shark because the suction pressure available to elicit food displacement was the performance variable of interest. Additionally, the long-term effects of cannula implantation or permanent catheterization on elasmobranch growth are unknown and frequently repeated implantation was not

feasible on such small animals. The catheter was connected to a Millar TCB-500 transducer control unit that was calibrated and zeroed at the beginning of each experimental session to record pressure differential at depth. The output from the control unit was recorded with a Yokogawa DL 716 digital oscilloscope and exported to a computer for subsequent analysis using SigmaScan Pro version 4 (SPSS Inc.). Recordings from the camera and oscilloscope were synchronized via a cyclically repeating electronic pulse. The performance variables measured from the synchronized recordings were: 1) the duration of the subambient pressure pulse (pulse duration) (ms); 2) the area under the curve of the sub-ambient pressure pulse (pulse area) (kPa ms); 3) the maximum subambient pressure (kPa); 4) the time to reach maximum subambient pressure from onset of lower jaw depression (ms); 5) the time to reach maximum subambient pressure from onset of hyoid depression (ms); and 6) the rate of pressure decrease from the onset of subambient pressure until maximum subambient pressure is reached (kPa ms^{-1}). Twenty suction pulses with synchronized video were recorded from each time segment for each species, resulting in the analysis of a total of 160 captures. No more than three captures per individual during a given time segment were included in the analysis to avoid bias due to individual differences.

In addition to measuring subambient pressure generation, performance was quantified during independent experimental sessions within each time segment by describing the pattern of particle motion into the buccopharyngeal cavity during food capture. To accomplish this, 4.1 g of *Artemia* spp. cysts were soaked for 1-3 hours in seawater until neutrally buoyant and then added to the filming tank, producing a final seeding density of $\sim 3 \text{ g l}^{-1}$ (Lauder and Clark, 1984). *Artemia* spp. cysts were selected in

lieu of polystyrene or hollow-ground silver spheres (Muller and Osse, 1984; Ferry-Graham, et al., 2003; Day, et al., 2005; Higham, et al., 2005), because they are biodegradable and repeated exposure over ontogeny is not detrimental to experimental animals. The filming tank was then enclosed within a photo-opaque, non-reflective box that admitted light only through two, 3-mm wide slits on the top and one side of the box producing a sheet of quartz halogen illumination perpendicular to the camera that ran through the midsagittal plane of the shark. Illuminating the filming tank in this way allowed discrimination of particles directly in-line with the shark and allowed the displacement of these particles to be manually tracked during food captures. Particles that entered the oral aperture during the strike were tracked backward field-by-field from the time they were ingested to their initial resting position at the onset of lower jaw depression. Connecting the initial positions of the distal-most particles in each direction allowed description of the parcel of water ingested by the shark during food capture from the lateral aspect (Fig. 15) (Day, et al., 2005; Higham, et al., 2005). A minimum of 20 particle trajectories were described per capture event and additional particles were tracked until subsequent trajectories did not alter the shape determined for the ingested parcel. The following attributes of the ingested parcel were then quantified as indicators of performance using SigmaScan Pro version 4: 1) maximum parcel length at any position (cm and relative to mouth width [MW]); 2) maximum parcel height at any position (cm and MW); and 3) parcel area (cm²). Parcel height was then divided by parcel length to obtain an aspect ration that was used to describe the relative shape of the ingested parcel (Day, et al., 2005; Higham, et al., 2005). Ten flow patterns from independent capture events were recorded for each time segment for each species,

resulting in the analysis of a total of 80 captures. No more than two captures per individual during a given time segment were included in the analysis to avoid bias due to individual differences.

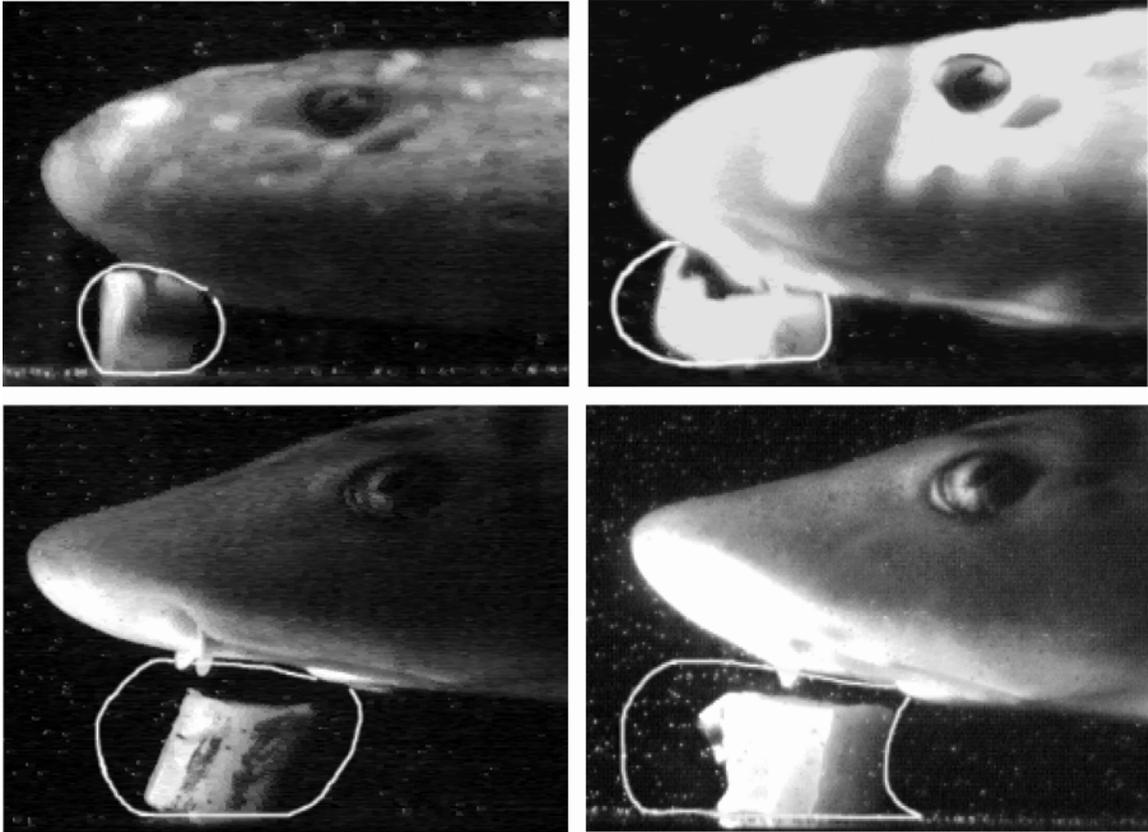


Figure 15: Illustrative examples of the range of relative size and shape of the parcel of water ingested by *Chiloscyllium plagiosum* (top) and *Triakis semifasciata* (bottom) during capture events. Images have been scaled such that head length is equal in all cases. The parcels are truncated ventrally by the Plexiglas bottom and dorsally by the rostrum of the shark. Frames to the left depict relatively rounded, high aspect ratio parcels while those to the right depict more elongated, low aspect ratio parcels relative to head length. Overall, high aspect ratio parcels (average= 0.80 ± 0.21) are typical of *C. plagiosum* (upper left) while low aspect ratio parcels (0.57 ± 0.11) are typical of *T. semifasciata* (lower right).

Statistical Analyses

To ascertain whether the use of the catheter or particles influenced food capture behavior, kinematic data were compared against data for the same species feeding on the same food type when neither method to measure performance was employed (Chapters 2 and 3). All kinematic data were log-transformed and checked for normality and

homogeneity of variance using the Kolmogorov-Smirnov and Levene Median tests, respectively. Model II linear regressions were performed against total length for each kinematic variable and the studentized residuals obtained (Quinn and Keough, 2002). Size-corrected, studentized residuals for all kinematic variables were then used in a correlation matrix-based Principal Components Analysis (PCA) (Quinn and Keough, 2002). An Equamax rotation was used because it produced higher loadings than any other rotation, enhancing data interpretation, and variables that loaded above an absolute value of 0.5 were identified as contributing heavily to the variability within the respective principal component (PC). Principal components with an eigenvalue greater than 1.0 were retained for further analysis. The factor loading scores for each capture event on each principal component were then used in a series of one-way MANOVAs to identify differences between clusters of capture sequences in multivariate space. Capture sequences from each time segment using each technique to quantify performance were independently compared against sequences during the same time segment in which neither technique was employed (Chapters 2 and 3). Significance was assessed using Pillai's trace because it is robust to multivariate deviation from normality (Zar, 1999). Regressions were performed using SigmaStat Pro version 3.1 (SPSS Inc.) while the PCA and MANOVA tests were performed using Systat 11 (SPSS Inc.).

Once the effects of the presence of the catheter and particles had been assessed, log-transformed performance data for each variable were regressed against log-transformed total length data using Model II linear regressions to describe changes in performance over ontogeny. Model II linear regressions were appropriate because error existed in measurements of both the dependent and independent variables (Quinn and

Keough, 2002). Significance was assessed via ANOVA. In addition to testing for the presence of trends in performance variables over ontogeny, the nature of these relationships was assessed to determine whether performance scaled isometrically or allometrically. The predicted isometric scaling coefficient for each performance variable was determined by the dimensionality of the variable and existing kinematic and anatomical scaling data for these two species (Chapters 2 and 3). Linear variables, such as maximum parcel height and velocity of the food, were predicted to scale with a coefficient of one, whereas planar variables, such as parcel area and pulse area, were predicted to scale with a coefficient of two. Maximum parcel height and length relative to mouth width (i.e. with size accounted for), as well as parcel aspect ratio, were predicted to scale with a coefficient of zero. As strike duration does not scale with size in either species (Chapters 2 and 3), the duration of the pressure pulse and the time of its onset relative to the onset of lower jaw depression were predicted to scale with coefficients of zero. The onset of hyoid depression does not scale with size in *T. semifasciata* (Chapter 2), but scales with a coefficient of -0.3 in *C. plagiosum* (Chapter 3), thus the time of maximum subambient pressure relative to the onset of hyoid depression was expected to scale with coefficients of zero and -0.3, respectively. Maximum subambient pressure, though influenced by behavioral deviation in the rate and duration of buccal expansion (Muller and Osse, 1984; Carroll, et al., 2004), has been shown to correlate strongly with volume change in the buccal cavity during feeding (Svanback, et al., 2002; Carroll, et al., 2004). The difference in the volume of the buccal cavity (buccal reserve volume) has been shown to scale with a coefficient of 3.0 in *T. semifasciata* (Chapter 2) and 3.3 in *C. plagiosum* (Chapter 3), so maximum subambient

pressure was predicted to scale similarly. Deviation from an isometric coefficient was tested for each variable using a modified Student's t-test (Zar, 1999). Differences between regressions at the species level for a given variable were also assessed using a modified Student's t-test (Zar, 1999).

To determine whether changes in performance variables occurred over ontogeny irrespective of size, studentized residuals were obtained from the regressions of each variable against total length and these values were regressed against log-transformed age data (in days). Deviation from a coefficient of zero was tested using ANOVA and indicated differences in that performance variable over ontogeny that could not be accounted for simply by an increase in TL. For each performance variable that displayed a trend over ontogeny that could not be accounted for by TL alone, a backward stepwise multiple regression was used to elucidate the contribution of specific kinematic variables to this relationship (F-to-Enter=2.0, F-to-Remove=1.9, 20 steps) (Quinn and Keough, 2002). Only variables directly related to the timing, duration, and extent of motion of cranial features of the shark were retained for this analysis (i.e. strike distance, duration of food movement, and RSI were not included). Because significant correlations among variables included in multiple regressions can lead to problems of multicollinearity that substantially weaken the conclusions of the test (Quinn and Keough, 2002), a Spearman rank order correlation analysis was performed to determine if kinematic variables were correlated. Several significant correlations ($P < 0.05$) were detected, so a correlation matrix-based PCA was performed to consolidate related variables into several uncorrelated PCs. Variables that loaded above an absolute value of 0.5 were identified as contributing heavily to the variability within the respective PC. Principal components

with an eigenvalue greater than 1.0 were retained for further analysis. The PCs were then used as independent variables in a second set of backward stepwise multiple regressions with the operational criteria provided above for each performance variable that exhibited a trend over ontogeny that could not be accounted for by TL alone. All regressions, including the backward stepwise multiple regressions, were performed with SigmaStat Pro version 3.1.

Results

Sharks of both species generally swam slowly around the filming tank or rested on the bottom until food was introduced. On average, strikes were typically faster in *C. plagiosum* (average total strike duration of 69 ± 17 ms) than in *T. semifasciata* (96 ± 16 ms), involved relatively less excursion of structural elements, and were characterized by substantially less variability. For a detailed description of feeding kinematics in both species over ontogeny see Chapters 2 and 3. Differences were not detected in multidimensional space during any time segment between capture events with and without catheterized food, or between capture events with and without particles present (Table 11). At any given total length, the maximum subambient pressure generated by *C. plagiosum* tended to be greater than that generated by *T. semifasciata*, though considerable overlap was apparent (Fig. 16). Additionally, at any given total length the aspect ratio of the parcel ingested tended to be higher (i.e. more rounded) in *C. plagiosum*, but lower (i.e. more elliptical) in *T. semifasciata* (Fig. 17).

Table 11: Comparison between capture events with food catheterized and without, and with particles present and absent, during each time segment for both species via MANOVA based on principal component loading scores. Values shown are for Pillai's trace. df=degrees of freedom. Note that effects were not detected during any time segment for either technique used to quantify performance.

		<i>Triakis semifasciata</i>			<i>Chiloscyllium plagiosum</i>		
		df	F	P	df	F	P
With catheter vs. without:	Segment 1	5, 34	1.255	0.306	5, 39	0.752	0.590
	Segment 2	5, 34	0.768	0.579	5, 39	2.077	0.096
	Segment 3	5, 34	0.796	0.560	5, 39	0.756	0.587
	Segment 4	5, 34	1.525	0.208	5, 39	2.118	0.084
With particles vs. without:	Segment 1	6, 23	0.983	0.459	5, 29	0.299	0.909
	Segment 2	6, 23	2.193	0.081	5, 29	1.468	0.231
	Segment 3	6, 23	1.464	0.209	5, 29	0.504	0.771
	Segment 4	6, 23	1.763	0.152	5, 29	1.049	0.408

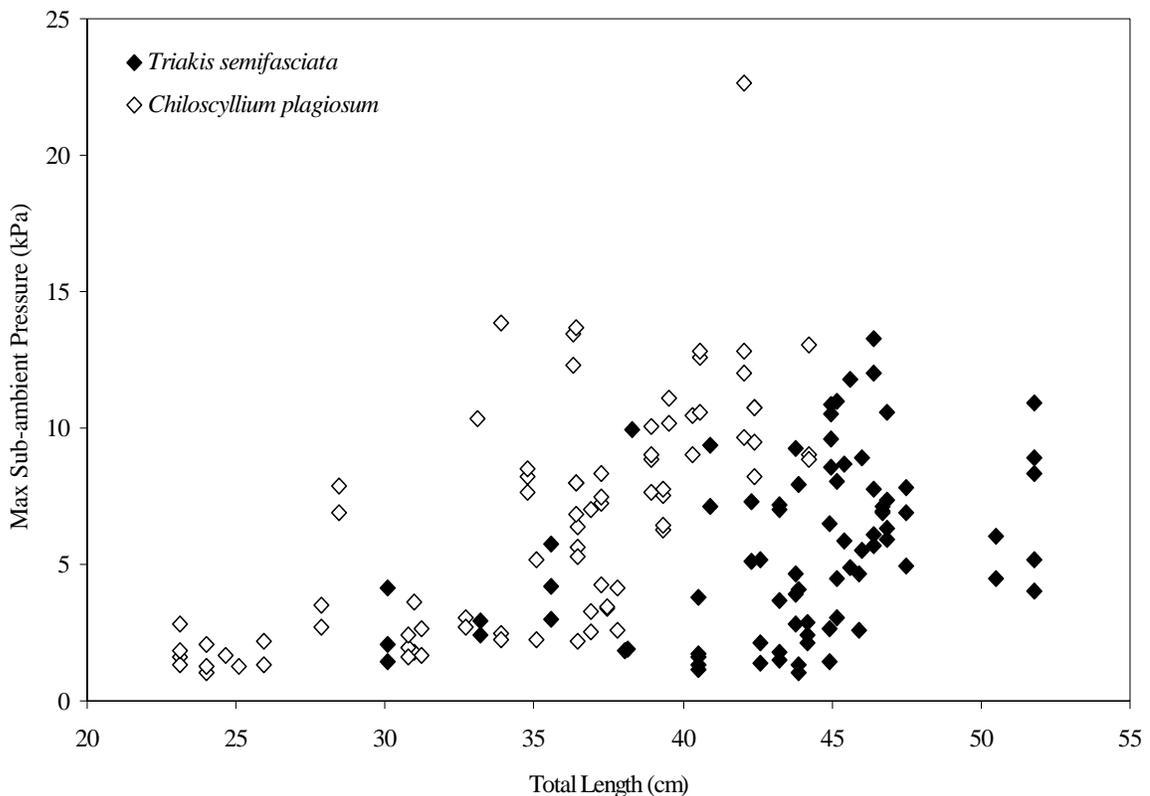


Figure 16: Values of maximum subambient pressures generated during food capture events in *Chiloscyllium plagiosum* and *Triakis semifasciata* relative to total length (TL). Note that at any given TL values tend to be higher (i.e. greater subambient pressure drop) in *C. plagiosum* than in *T. semifasciata*.

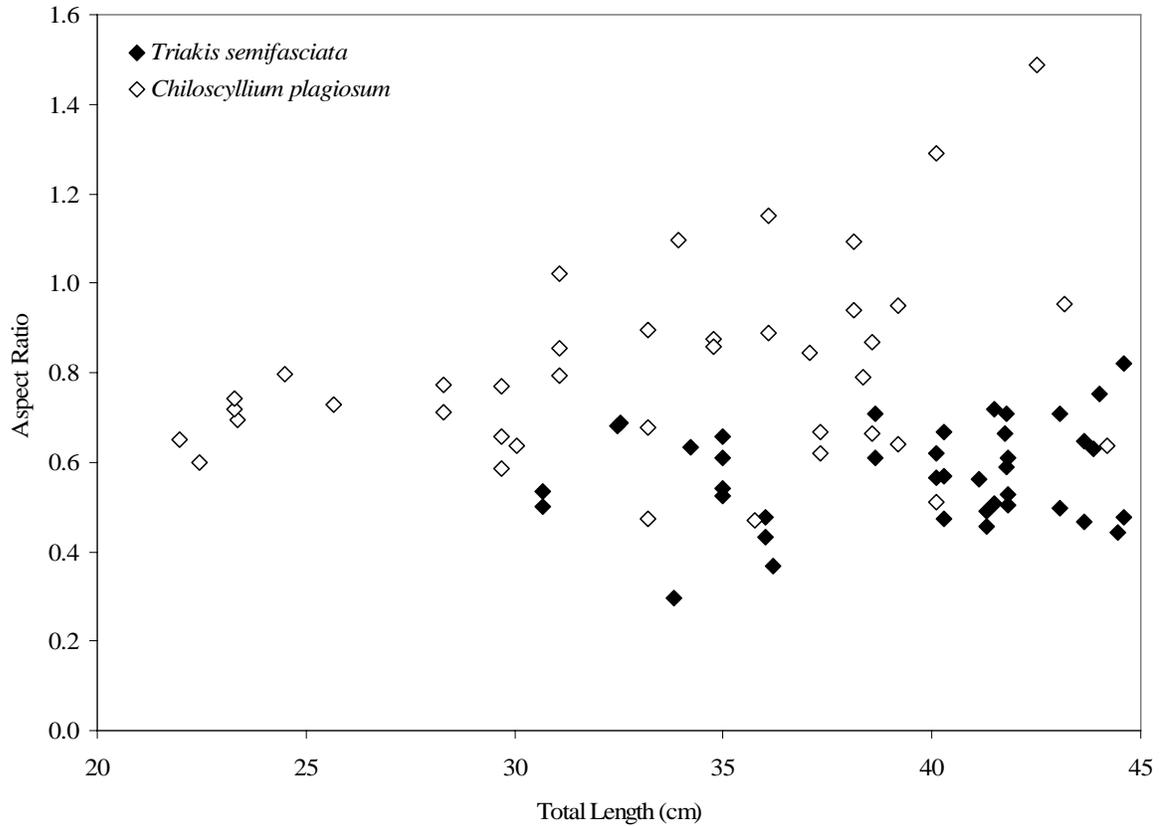


Figure 17: Aspect ratio values for parcels of water ingested during food capture events in *Chiloscylidium plagiosum* and *Triakis semifasciata* relative to total length (TL). Note that at any given TL values tend to be higher (i.e. more rounded) in *C. plagiosum* than in *T. semifasciata*.

Scaling of Performance

When compared to a slope of zero, most performance variables exhibited some trend relative to growth, with the value of all variables for which trends were present in *T. semifasciata* displaying positive coefficients (Table 12, Slope and P columns). The same was not true for *C. plagiosum*, with pulse duration and the time to reach maximum subambient pressure relative to the onset of both lower jaw and hyoid depression decreasing with growth (Table 12). Though the rate of pressure decrease did not change with growth in *T. semifasciata*, it tended to increase substantially (slope=4.42) ($P < 0.001$) in *C. plagiosum* (Table 12). Although maximum parcel height relative to mouth width tended to increase slightly in *T. semifasciata*, it exhibited no trend with growth in *C.*

plagiosum. Velocity of the food during the strike, maximum parcel length relative to mouth width, and parcel aspect ratio notably did not change with growth in either species.

Table 12: Regression parameters for performance variables versus total length and tests of isometry for both species. The observed slope (Slope) and expected slope (Sl_{EXP}) are given. Standard error and P values are from ANOVA tests for relationships between each variable and total length. The expected slope for isometry is given in the column labeled Sl_{EXP} . Where the expected slope was not zero, a modified Student's t-test was used to assess isometry. Where the expected slope was zero (n/a) the results of the ANOVA were defaulted to. For variables quantified with catheterized food, $t_{0.05(1), 78}=1.665$. For variables quantified with particles present $t_{0.05(1), 38}=1.686$.

Variable	Slope	y-int	r ²	StErr	P	Sl _{EXP}	t
<i>Triakis semifasciata</i>							
Pulse Duration	1.08	-0.06	0.10	0.37	0.005*	0.0	n/a
Pulse Area	3.93	-4.86	0.24	0.79	<0.001*	2.0	79.85*
Maximum Subambient Pressure	2.45	-3.35	0.18	0.59	<0.001*	3.0	-30.26*
Time of Maximum Pressure Relative:							
To Onset of Lower Jaw Depression	--	--	--	--	0.481	0.0	n/a
To Onset of Hyoid Depression	--	--	--	--	0.812	0.0	n/a
Rate of Pressure Decrease	--	--	--	--	0.134	1.0	-2.70*
Distance Food Moves	1.45	-2.15	0.25	0.41	0.001*	1.0	37.57*
Velocity of the Food	--	--	--	--	0.115	1.0	-3.77*
Maximum Parcel Length (cm)	1.05	-1.21	0.34	0.24	<0.001*	1.0	7.67*
Maximum Parcel Length (MW)	--	--	--	--	0.292	0.0	n/a
Maximum Parcel Height (cm)	1.40	-2.00	0.39	0.28	<0.001*	1.0	47.69*
Maximum Parcel Height (MW)	0.60	-1.13	0.10	0.28	0.042*	0.0	n/a
Parcel Area	2.74	-3.81	0.56	0.40	<0.001*	2.0	63.72*
Parcel Aspect Ratio	--	--	--	--	0.262	0.0	n/a
<i>Chiloscyllium plagiosum</i>							
Pulse Duration	-1.38	3.49	0.28	0.25	<0.001*	0.0	n/a
Pulse Area	6.55	-8.21	0.62	0.58	<0.001*	2.0	154.91*
Maximum Subambient Pressure	3.26	-4.31	0.59	0.31	<0.001*	3.3	-1.63
Time of Maximum Pressure Relative:							
To Onset of Lower Jaw Depression	-1.11	3.12	0.26	0.21	<0.001*	0.0	n/a
To Onset of Hyoid Depression	-1.53	3.61	0.15	0.42	<0.001*	-0.3	-57.90*
Rate of Pressure Decrease	4.42	-7.20	0.58	0.42	<0.001*	1.0	160.16*
Distance Food Moves	0.66	-1.03	0.13	0.28	0.026*	1.0	-21.82*
Velocity of the Food	--	--	--	--	0.876	1.0	-52.26*
Maximum Parcel Length (cm)	0.73	-1.03	0.37	0.16	<0.001*	1.0	-31.35*
Maximum Parcel Length (MW)	--	--	--	--	0.065	0.0	n/a
Maximum Parcel Height (cm)	1.12	-1.72	0.53	0.17	<0.001*	1.0	12.40*
Maximum Parcel Height (MW)	--	--	--	--	0.622	0.0	n/a
Parcel Area	1.16	-1.80	0.37	0.25	<0.001*	2.0	-61.95*
Parcel Aspect Ratio	--	--	--	--	0.067	0.0	n/a

Behavioral Effects on Performance

Though growth accounted for most of the variability in performance over ontogeny, when the studentized residuals from the regressions of performance variables against total length were regressed against time, a number of trends remained (Table 13). For *T. semifasciata* pulse duration, pulse area, distance moved by the food, maximum parcel height, and parcel area exhibited weakly increasing trends over time (Table 13). Backward stepwise multiple regressions indicated that numerous kinematic variables partially accounted for these trends (Table 14). The relative extent of maximum gape was largely accountable for trends in all of these performance variables except pulse area, which was accounted for primarily by total strike duration (Table 14). In addition to maximum gape, trends in the remaining four performance variables were accounted for by a variety of kinematic variables, with no clear tendency for any single variable to dictate performance (Table 14). Two notable exceptions to this generalization were the time to hyoid retraction and the duration of hyoid depression, which exhibited strong relationships with distance the food moves during a strike (Table 14). PCA consolidated the numerous initial kinematic variables into six PCs (Table 15) and eliminated problems of multicollinearity due to correlation among variables. Backward stepwise multiple regressions for *T. semifasciata* based on these PCs determined that trends in pulse duration and pulse area could be accounted for primarily by PC4 (timing variables describing cranial elevation, maximum gape, and initial hyoid displacement), with PC6 (the duration and extent of cranial elevation) also contributing to pulse duration (Table 16). Trends in the distance that the food moved and parcel area were accounted for primarily by PC5 (maximum gape and the time the gills open). The trend in maximum

parcel height was also explained by PC5, but PC1 (duration of hyoid and buccal displacement) also contributed (Table 16).

Table 13: Regression parameters for performance variables against age in days for both species irrespective of size. For variables quantified in the presence of the catheter, $t_{0.05(1), 19}=1.729$. For variables quantified with particles present, $t_{0.05(1), 9}=1.833$.

Variable	Slope	y-int	r ²	StErr	P
<i>Triakis semifasciata</i>					
Pulse Duration	0.13	-0.27	0.08	0.05	0.013*
Pulse Area	0.26	-0.54	0.07	0.11	0.020*
Maximum Sub-ambient Pressure	--	--	--	--	0.080
Time of Maximum Pressure Relative to:					
Onset of Lower Jaw Depression	--	--	--	--	0.286
Onset of Hyoid Depression	--	--	--	--	0.638
Rate of Pressure Decrease	--	--	--	--	0.650
Distance Food Moves	0.13	-0.28	0.13	0.05	0.022*
Velocity of the Food	--	--	--	--	0.119
Maximum Parcel Length (cm)	--	--	--	--	0.300
Maximum Parcel Height (cm)	0.09	-0.20	0.14	0.04	0.016*
Parcel Area	0.13	-0.27	0.13	0.05	0.023*
Parcel Aspect Ratio	--	--	--	--	0.166
<i>Chiloscyllium plagiosum</i>					
Pulse Duration	0.43	-0.92	0.00	0.13	0.001*
Pulse Area	--	--	--	--	0.808
Maximum Sub-ambient Pressure	0.17	-0.35	0.07	0.07	0.021*
Time of Maximum Pressure Relative to:					
Onset of Lower Jaw Depression	--	--	--	--	0.832
Onset of Hyoid Depression	--	--	--	--	0.497
Rate of Pressure Decrease	--	--	--	--	0.120
Distance Food Moves	--	--	--	--	0.597
Velocity of the Food	--	--	--	--	0.508
Maximum Parcel Length (cm)	--	--	--	--	0.701
Maximum Parcel Height (cm)	--	--	--	--	0.918
Parcel Area	--	--	--	--	0.725
Parcel Aspect Ratio	--	--	--	--	0.834

Table 14: Kinematic variables contributing to performance differences over time irrespective of size in *Triakis semifasciata*. Only variables included in the final step of the backward stepwise multiple regressions are shown (e.g. those that contribute significantly). The total percent variance explained is a measure of how well the multiple regression comprising the final kinematic variables describes the trend in the respective performance variable. Max=maximum; Ret=retraction; Depress=depression; Pred=predator; Var=variance.

Variable	Pulse Duration			Pulse Area			Distance Food Moves			Max Parcel Height			Parcel Area		
	Coeff	StErr	P	Coeff	StErr	P	Coeff	StErr	P	Coeff	StErr	P	Coeff	StErr	P
Constant	0.00	0.02	--	0.00	0.04	--	0.00	0.02	--	0.00	0.01	--	0.00	0.01	--
Max Gape	0.80	0.25	0.002	--	--	--	1.11	0.29	<0.001	0.59	0.20	0.006	1.11	0.25	<0.001
Time to Max Gape	0.58	0.26	0.031	--	--	--	--	--	--	-0.58	0.21	0.009	--	--	--
Time to Close	0.47	0.13	<0.001	--	--	--	--	--	--	--	--	--	--	--	--
Time to Onset Hyoid Depress	--	--	--	--	--	--	0.46	0.21	0.035	--	--	--	--	--	--
Max Hyoid Depress	--	--	--	--	--	--	--	--	--	0.30	0.13	0.031	--	--	--
Time to Max Hyoid Depress	-0.78	0.31	0.015	--	--	--	--	--	--	0.42	0.20	0.048	--	--	--
Time to Hyoid Retraction	--	--	--	--	--	--	-3.18	1.26	0.016	--	--	--	--	--	--
Duration of Hyoid Depress	--	--	--	--	--	--	2.50	1.02	0.019	--	--	--	--	--	--
Time Gills Start to Open	--	--	--	--	--	--	--	--	--	0.09	0.03	0.011	0.15	0.04	<0.001
Total Strike Duration	--	--	--	1.48	0.55	0.008	--	--	--	--	--	--	-0.45	0.18	0.015
Distance Predator Moves	--	--	--	--	--	--	--	--	--	--	--	--	0.19	0.08	0.028
Total % Var Explained	31.7			14.6			40.0			43.7			57.9		

Table 15: Principal component loadings after Equamax rotation of kinematic variables exclusively associated with motion of the shark during capture events in *Triakis semifasciata*. Bold face values indicate variables determined to load heavily on the respective component (loading scores > |0.5|) (N=12). Together the six components explain 83.42% of the overall variability in the data set. For clarity, all loadings < |0.25| are replaced by 0. Depress=depression; Max=maximum; Elev=elevation; Var=variance.

Variable	PC1	PC2	PC3	PC4	PC5	PC6
Duration of Hyoid Depress	0.922	0	0	0	0	0
Time to Hyoid Retraction	0.887	0	0	0.281	0	0
Time to Close	0.716	0	0.406	0	0	0.261
Total Strike Duration	0.667	0	0	0.538	0	0.295
Max Hyoid Depress	0	-0.969	0	0	0	0
Time to Max Hyoid Depress	0	0.933	0	0	0	0
Distance Predator Moves	0	0	0.955	0	0	0
Velocity of the Predator	-0.265	0	0.877	0	0	0
Onset of Cranial Elev	0	0	0	0.787	0	-0.321
Time to Max Cranial Elev	0	0.251	0	0.749	0	0.370
Time to Max Gape	0.444	0	0	0.738	0	0
Time to Onset of Hyoid Depress	0	0	0	0.706	0	0.302
Offset of Cranial Elev	0	0	0	0.592	0	0.619
Max Gape	0	0	0	0	0.800	0
Time Gills Start to Open	0	0	0	0	0.646	0
Duration of Cranial Elev	0	0	0	0	0	0.853
Max Cranial Elev	0	0.284	0	0	0	0.768
Eigenvalue	3.10	2.09	2.13	3.17	1.37	2.32
Percent Var Explained	18.25	12.27	12.55	18.64	8.08	13.64

Table 16: Partial regression coefficients from the backward stepwise multiple regressions of principal components (PC) that contribute to performance differences over time irrespective of size in *Triakis semifasciata*. Only PCs included in the final step of the backward stepwise multiple regressions are shown (e.g. those that contribute significantly). The total percent variance explained is a measure of how well the multiple regression comprising the final PCs describes the trend in the respective performance variable. Var=variance; Max=maximum.

Variable	Pulse Duration			Pulse Area			Distance Food Moves			Max Parcel Height			Parcel Area		
	Coeff	StErr	P	Coeff	StErr	P	Coeff	StErr	P	Coeff	StErr	P	Coeff	StErr	P
Constant	0.00	0.02	--	0.00	0.04	--	0.00	0.02	--	0.00	0.01	--	0.00	0.01	--
PC1	--	--	--	--	--	--	--	--	--	-0.02	0.01	0.040	--	--	--
PC4	0.06	0.02	<0.001	0.08	0.04	0.048	--	--	--	--	--	--	--	--	--
PC5	--	--	--	--	--	--	0.07	0.02	<0.001	0.04	0.01	<0.001	0.08	0.01	<0.001
PC6	-0.04	0.02	0.041	--	--	--	--	--	--	--	--	--	--	--	--
Total % Var Explained	18.2			4.9			29.5			32.3			44.6		

For *C. plagiosum*, only pulse duration and maximum subambient pressure displayed increasing trends over time irrespective of growth (Table 13). Backward stepwise multiple regression revealed that these trends could be accounted for primarily by variables associated with the time taken to reach and extent of maximum cranial elevation, for pulse duration, or total strike duration, for maximum subambient pressure (Table 17). The clarity and significance of these relationships was not enhanced by PCA so results of this analysis are not presented here.

Table 17: Kinematic variables contributing to performance differences over time irrespective of size in *Chiloscyllium plagiosum*. Only variables included in the final step of the backward stepwise multiple regressions are shown. The total percent variance explained is a measure of how well the multiple regression comprising the final kinematic variables describes the trend in the respective performance variable. Max=maximum; Var=variance.

Variable	Pulse Duration			Max Subambient Pressure		
	Coeff	StErr	P	Coeff	StErr	P
Constant	-0.02	0.39	--	1.59	0.42	--
Max Cranial Elevation	-0.17	0.08	0.045	--	--	--
Time of Max Cranial Elevation	0.60	0.20	0.005	--	--	--
Onset of Cranial Elevation	-0.10	0.05	0.037	--	--	--
Total Strike Duration	--	--	--	-0.42	0.28	<0.001
Total % Var Explained	16.6			17.5		

Discussion

Trends exist in several measures of food capture performance for both *T. semifasciata* and *C. plagiosum* over ontogeny that can be accounted for by size increase and, to a lesser degree, behavioral changes. Size effects are prevalent in most studies of biomechanical performance (Losos, 1990; Elsworth, et al., 2003; Herrel, et al., 2005) and have led to the generation of numerous models intended to predict scaling coefficients of kinematic measures (Hill, 1950; O'Reilly, et al., 1993; Richard and Wainwright, 1995). Explanatory mechanisms for trends with size typically assume isometric growth and

range from changes in muscle contraction rate to the disparity between the scaling coefficient of muscular force production (slope=2) and the mass of skeletal elements (slope=3). The model used here to predict performance was based on knowledge of the scaling of kinematics in *T. semifasciata* and *C. plagiosum*, which primarily conform to the model of Richard and Wainwright (1995) but deviate because of allometric growth in several morphological and anatomical features (Chapters 2 and 3).

The generation of greater subambient suction pressure during feeding in teleost fishes is correlated with increased cross-sectional area of muscles that abduct and expand the buccopharyngeal cavity, faster buccopharyngeal and hyoid expansion, and increased cranial elevation (Muller and Osse, 1984; Norton and Brainerd, 1993; Ferry-Graham, et al., 2001b; Sanford and Wainwright, 2002; Carroll, et al., 2004). In both *T. semifasciata* and *C. plagiosum* the absolute subambient suction pressure increases with size, but to a greater extent in *C. plagiosum*. In *T. semifasciata* the time to reach maximum hyoid depression and maximum cranial elevation do not change with size but the time to open the mouth (time to maximum gape) increases (slope=0.35), despite being accompanied by positively allometric growth in the weight and cross-sectional area of jaw abducting muscles and isometric growth in buccal reserve volume (Chapter 2). This suggests that in *T. semifasciata* maximum subambient pressure should scale negatively allometrically as the rate of oral expansion decreases and results show that this is indeed the case. In *C. plagiosum* the time to open the mouth, maximum hyoid depression, and maximum cranial elevation all increase (slopes between 0.2 and 0.4) despite positively allometric growth in abducting musculature (Chapter 3), suggesting that maximum subambient pressure should also scale with negative allometry. Maximum subambient pressure, however,

scaled with isometry in *C. plagiosum*. This discrepancy may be due to the relationship between the time of onset hyoid depression and size. In *T. semifasciata* the onset of hyoid depression exhibits no relationship with growth (Chapter 2), but in *C. plagiosum* the hyoid begins to be depressed relatively earlier in the strike with growth (slope=-0.32) (Chapter 3). Functionally this means that flow would be generated through a slowly expanding, smaller oral aperture earlier in the strike, which should increase the velocity of flow into the buccopharyngeal cavity and, therefore, maximum subambient pressure (Muller and Osse, 1984; Svanback, et al., 2002). Indeed, in *C. plagiosum* the time of maximum subambient pressure relative to both the onset of lower jaw and hyoid depression scales with negative allometry, while the rate of pressure decrease scales with positive allometry (slope=4.42) (Table 12), resulting in isometric scaling of maximum subambient pressure. The timing of hyoid depression as the primary determinant of maximum subambient pressure has been hypothesized for orectolobid sharks (Motta, 2004), and changes in the relative timing of hyoid depression appear to enhance pressure generation over ontogeny in *C. plagiosum*.

Both species used in this study have been shown to utilize inertial suction during food capture (Chapters 2 and 3; Ferry-Graham, 1998a; Wu, 1993; 1994), although the relative contribution of suction tends to be greater in *C. plagiosum*. The viscosity and density of the aquatic medium dictate that suction is a relatively near-field phenomenon, as the effects of flow degrade as the cube of distance from the oral aperture (Muller and Osse, 1984; Svanback, et al., 2002; Ferry-Graham, et al., 2003). The greatest parcel length of water sucked into the mouth by *T. semifasciata* was 4.45 cm (1.73 MW) and 2.15 cm (1.38 MW) for *C. plagiosum*. Absolute maximum parcel length (cm) scaled with

slight positive allometry in *T. semifasciata* but with negative allometry in *C. plagiosum*, while maximum parcel length relative to mouth width did not exhibit any trend with size in either species. These values correspond well with theoretical and empirical measures of suction distance (Alexander, 1967; Lauder and Clark, 1984; Muller and Osse, 1984; Wainwright, et al., 2001a) and demonstrate the severe constraint imposed by the fluid medium on the effective range of suction feeding. The ecological consequence of this relationship is that suction feeders must approach their prey closely before striking, potentially necessitating a stalking or ambushing predatory behavior (Wainwright, et al., 2001b; Motta, 2004).

Both empirical (Muller and Osse, 1984; Higham, et al., 2005) and theoretical work (Weihs, 1980; Muller, et al., 1982) has shown that one mechanism of overcoming constraints on suction distance imposed by the aquatic medium is to increase predator velocity, generating a more focused, elongated region of effective suction. Though the aspect ratio of the ingested parcel did not exhibit a trend with growth in either species, it tended to be higher (i.e. more rounded) in *C. plagiosum* than in *T. semifasciata* at any given size. In the current study the strike velocity of *T. semifasciata* averaged $19.10 \pm 6.75 \text{ cm s}^{-1}$ while for *C. plagiosum* it was only $9.37 \pm 6.06 \text{ cm s}^{-1}$. Additionally, the feeding modality of *T. semifasciata* is typically ram-dominated (Ferry-Graham, 1998a; Chapter 2), while the feeding modality of *C. plagiosum* is strongly suction-dominated (Chapter 3). This corroborates the tendency for increased forward motion of the predator during the strike to influence the shape and length of the parcel of water ingested. A second mechanism to overcome the limited effective distance of suction generation in the aquatic medium involves the use of the substrate to truncate and focus flow (P.

Wainwright, pers. comm.). Food was presented on the bottom to both species in the current study and flow was clearly ventrally truncated by the substrate (Figure 15). Additionally, the rostrum of both species appeared to limit the dorsal extent of the flow to some degree, an effect not noted in previous studies of species with terminal mouths (Lauder and Clark, 1984; Muller and Osse, 1984; Day, et al., 2005; Gibb and Ferry-Graham, 2005). The additive effects of truncating flow into the mouth by feeding near the substrate or other aquatic structures and having a subterminal mouth may enhance suction capacity by focusing suction force over a smaller area, overcoming limitations imposed by the aquatic medium. This could have consequences for prey capture performance, especially in species that live or hunt in close association with the substrate or within crevices.

The small degree of variability in suction performance that was not accounted for by growth was often accounted for by relatively straightforward physical relationships described by kinematic variables. In *T. semifasciata* variation in pulse duration and area were accounted for by variables describing the timing of cranial elevation and the duration that the oral aperture was open. In *C. plagiosum* variation in pulse duration was accounted for only by variables describing the timing and extent of cranial elevation. The importance of cranial elevation in determining suction feeding performance has been underscored for teleost fishes by Carroll et al. (2004). Variability in the distance moved by the food, maximum parcel height, and parcel area in *T. semifasciata* were accounted for by the extent of maximum gape and the time the gills began to open. Subtle variation in maximum gape can drastically affect the rate of flow at the oral aperture (Ferry-Graham and Lauder, 2001; Day, et al., 2005), affecting both the force available to impart

motion to the food and the volume of fluid ingested. This relationship, however, assumes that strike duration does not change over ontogeny, which it does not in *T. semifasciata* (Chapter 2). With growth accounted for, maximum subambient pressure increased as strike duration decreased in *C. plagiosum*. The low partial regression coefficients of kinematic variables after growth had been accounted for, however, suggest that size-independent behavioral variability contributes relatively little to overall performance over the course of ontogeny in either species.

Ecological and Evolutionary Implications

Substantial changes in suction feeding performance occur over ontogeny in both *T. semifasciata* and *C. plagiosum* that are primarily attributable to growth and trends in kinematic variables with growth. In *T. semifasciata* these developmental trajectories generate an organism that is functionally adept at suction feeding, but less so than *C. plagiosum*. Despite physical constraints imposed by the aquatic medium, the propensity to ram feed throughout ontogeny and produce greater absolute pressure with growth likely facilitate the known ontogenetic dietary shift in *T. semifasciata* toward more functionally diverse and elusive prey items (Talent, 1976; Kao, 2000). Ram feeding has been shown in several species of bony fish to facilitate consumption of more elusive prey (Norton, 1991; Ferry-Graham and Lauder, 2001; Wainwright et al., 2001b) and is typified by the ingestion of a more oval parcel of water (Higham, et al., 2005). As increasingly diverse benthic prey types are taken over ontogeny it is also possible that what suction *T. semifasciata* is able to generate is made more effective by feeding in close association with the substrate, which substantially modifies the focus and anterior extent of flow into

the buccopharyngeal cavity. Shifts in habitat use over ontogeny may also influence dietary composition as leopard sharks are known to decreasingly utilize shallow, homogenous mud flats and increasingly utilize more complex, sandy- or rocky-bottomed habitats as they age (Barry and Cailliet, 1981; Compagno, 1984a).

As *C. plagiosum* becomes functionally, anatomically, and behaviorally canalized to feed via inertial suction over ontogeny, it generates absolutely greater suction but maintains a high aspect ratio, more rounded parcel of ingested water. These developmental aspects stringently constrain the distance from which *C. plagiosum* can effectively entrain prey, but provide for a more rapid, brief, and forceful suction capture over ontogeny. Knowledge of the biological role for which this feeding mechanism is employed is crucial to making assertions about diet over ontogeny in this species. Orectolobiform sharks including *C. plagiosum* (C. Wilga, pers. obs.), *G. cirratum* (Compagno, 1984b; P. Motta, pers. obs.), and the epaulette shark *Hemiscyllium ocellatum* (Heupel and Bennett, 1998) are known to feed by thrusting their heads into soft sediment or crevices in rocky substrate. The capacity to capture prey via suction feeding may be augmented over ontogeny by the development of ambushing, stalking, or excavating behaviors that make available crevice-dwelling or burrowing prey by positioning them close to the mouth. If such changes in foraging behavior do occur over ontogeny, *C. plagiosum* is anticipated to increasingly utilize benthic prey over ontogeny as opposed to exploiting prey in the water column. Knowledge of ontogenetic shifts in microhabitat utilization by *C. plagiosum* could augment this prediction, but this information is unavailable on the scale needed to make accurate dietary predictions.

Despite substantial differences in the degree of anatomical complexity of the feeding apparatus of teleost fishes, aquatically feeding tetrapods, and elasmobranchs, commonalities exist in the behavioral mechanism and effect of subambient buccal pressure generation that are dictated by physical attributes of the aquatic medium (Lauder and Shaffer, 1993; Wainwright, et al., 2001b; Dean, 2003; Motta, 2004; Gibb and Ferry-Graham, 2005). Regardless of the number of musculoskeletal components present or the temporal relationships governing the interactions of these components during a strike, the generation of subambient pressure is enhanced by specific morphological and behavioral attributes (Wainwright, et al., 2001b; Svanback, et al., 2002; Carroll, et al., 2004; Gibb and Ferry-Graham, 2005; Higham, et al., 2005). Over ontogeny and phylogeny this means that even large morphological changes may produce relatively small suction performance changes (Gibb and Ferry-Graham, 2005), dictating that expansion of the dietary niche of an individual or a species may be best accomplished by the learning of novel pre-strike hunting behaviors or the occupation of novel habitats once physical limits of performance imposed by the medium are reached.

References

- Ackerman, L.T., 1971. Contributions to the biology of the leopard shark, *Triakis semifasciata* (Girard) in Elkhorn Slough, Monterey Bay, California. Sacramento State College, Sacramento, pp. 54.
- Adriaens, D., Aerts, P., Verraes, W., 2001. Ontogenetic shift in mouth opening mechanisms in a catfish (Clariidae, Siluriformes): A response to increasing functional demands. *Journal of Morphology* 247, 197-216.
- Alexander, R.M., 1967. The functions and mechanisms of the protrusible upper jaws of some acanthopterygian fish. *Zoological Journal of the Linnaean Society* 151, 43-64.
- Anderson, C.W., 1993. The modulation of feeding behavior in response to prey type in the frog *Rana pipiens*. *Journal of Experimental Biology* 179, 1-12.
- Arendt, J.D., Wilson, D.S., 1997. Optimistic growth: Competition and an ontogenetic niche-shift select for rapid growth in pumpkinseed sunfish (*Lepomis gibbosus*). *Evolution* 51, 1946-1954.
- Arnold, S.J., 1983. Morphology, performance and fitness. *American Zoologist* 23, 347-361.
- Barel, C.D.N., 1983. Towards a constructional morphology of cichlid fishes (Teleostei, Perciformes). *Netherlands Journal of Zoology* 33, 357-424.
- Barel, C.D., Anker, G.C., Witte, F., Hoogerhoud, R.J., Goldschmidt, T., 1989. Constructional constraint and its ecomorphological implications. *Acta Morphologica Neerlandica Scandinavia* 27, 83-109.
- Barry, J.P., Cailliet, G.M., 1981. The utilization of shallow marsh habitats by commercially important fishes in Elkhorn Slough, California. *Cal-Neva Wildlife Transactions* 1981, 38-47.
- Benton, M.J., 2004. *Vertebrate Paleontology*, 3rd Ed. Blackwell Publishers, 455 pp.
- Bock, W.J., 1980. The definition and recognition of biological adaptation. *American Zoologist* 20, 217-227.
- Bock, W.J., von Wahlert, G., 1965. Adaptation and the form-function complex. *Evolution* 19, 269-299.
- Bohn, T., Amundsen, P., 2001. The competitive edge of an invading specialist. *Ecology* 82, 2150-2163.
- Bolnick, D.I., Svanback, R., Fordyce, R.E., Yang, L.H., Davis, J.M., Hulsey, C.D., Forister, M.L., 2003. The ecology of individuals: Incidence and implications of individual specialization. *The American Naturalist* 161, 1-28.
- Brickle, P., Laptikhovskiy, V., Pompert, J., Bishop, A., 2003. Ontogenetic changes in the feeding habits and dietary overlap between three abundant rajid species on the Falkland Islands shelf. *Journal of the Marine Biological Association of the UK* 83, 1119-1125.

- Bryan, J.E., Larkin, P.A., 1972. Food specialization by individual trout. *Journal of the Fisheries Research Board of Canada* 29, 1615-1624.
- Cabin, R.J., Mitchell, R.J., 2000. To Bonferroni or not to Bonferroni: When and how are the questions. *Bulletin of the Ecological Society of America* 81, 246-248.
- Carroll, A.M., Wainwright, P.C., Huskey, S.H., Collar, D.C., Turingan, R.G., 2004. Morphology predicts suction feeding performance in centrarchid fishes. *Journal of Experimental Biology* 207, 3873-3881.
- Castro, J.I., 2000. The biology of the nurse shark, *Ginglymostoma cirratum*, off the Florida east coast and the Bahama Islands. *Environmental Biology of Fishes* 58, 1-22.
- Chu, C.T., 1989. Functional design and prey capture dynamics in an ecologically generalized surfperch (Embiotocidae). *Journal of Zoology (London)* 217, 417-440.
- Compagno, L.J.V., 1984a. FAO Species Catalogue. Sharks of the World. An Annotated and Illustrated Catalogue of Shark Species Known to Date. Part 2. Carcharhiniformes. FAO Fisheries Synopsis 125 Vol. 4.
- Compagno, L.J.V., 1984b. FAO Species Catalogue. Sharks of the World. An Annotated and Illustrated Catalogue of Shark Species Known to Date. Part 1. Hexanchiformes to Lamniformes, FAO Fisheries Synopsis 125.
- Cook, A., 1996. Ontogeny of feeding morphology and kinematics in juvenile fishes: a case study of the cottid fish *Clinocottus analis*. *Journal of Experimental Biology* 199, 1961-1971.
- Cortes, E., 1999. Standardized diet compositions and trophic levels of sharks. *ICES Journal of Marine Science* 56, 707-717.
- Cortes, E., Manire, C.A., Hueter, R.E., 1996. Diet, feeding habits, and diel feeding chronology of the bonnethead shark, *Sphyrna tiburo*, in southwest Florida.
- Coughlin, D.J., 1991. The ontogeny of feeding behavior of first feeding Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Science* 48, 1896-1904.
- Cutwa, M.M., Turingan, R.G., 2000. Intralocality variation in feeding biomechanics and prey use in *Archosargus probatocephalus* (Teleostei, Sparidae), with implications for the ecomorphology of fishes. *Environmental Biology of Fishes* 59, 191-198.
- Day, S.W., Higham, T.E., Cheer, A.Y., Wainwright, P.C., 2005. Spatial and temporal patterns of water flow generated by suction-feeding bluegill sunfish *Lepomis macrochirus* resolved by Particle Image Velocimetry. *Journal of Experimental Biology* 208, 2661-2671.
- Dean, M.N., 2003. Suction feeding in the pipid frog, *Hymenochirus bettgeri*: Kinematic and behavioral considerations. *Copeia* 2003, 879-886.
- Dean, M.N., Motta, P.J., 2004. Feeding behavior and kinematics of the lesser electric ray, *Narcine brasiliensis* (Elasmobranchii: Batoidea). *Zoology* 107, 171-189.
- Deban, S.M., 1997. Modulation of prey-capture behavior in the plethodontid salamander *Ensatina eschscholtzii*. *Journal of Experimental Biology* 200, 1951-1964.
- Ebert, D.A., 2002. Ontogenetic changes in the diet of the sevengill shark (*Notorynchus cepedianus*). *Marine and Freshwater Research* 53, 517-523.
- Ellis, J.R., Shackley, S.E., 1995. Ontogenetic changes and sexual dimorphism in the head, mouth, and teeth of the lesser spotted dogfish. *Journal of Fish Biology* 47, 155-164.

- Elsworth, P.G., Seebacher, F., Franklin, C.E., 2003. Sustained swimming performance in crocodiles (*Crocodylus porosus*): Effects of body size and temperature. *Journal of Herpetology* 37, 363-368.
- Ferry-Graham, L.A., 1998a. Effects of prey size and motility on prey-capture kinematics in leopard sharks *Triakis semifasciata*. *Journal of Experimental Biology* 201, 2433-2444.
- Ferry-Graham, L.A., 1998b. Feeding kinematics of hatchling swellsharks, *Cephaloscyllium ventriosum* (Scyliorhinidae): the importance of predator size. *Marine Biology* 131, 703-718.
- Ferry-Graham, L.A., Lauder, G.V., 2001. Aquatic prey capture in ray-finned fishes: A century of progress and new directions. *Journal of Morphology* 248, 99-119.
- Ferry-Graham, L.A., Bolnick, D.I., Wainwright, P.C., 2002. Using functional morphology to examine the ecology and evolution of specialization. *Integrative and Comparative Biology* 42, 265-277.
- Ferry-Graham, L.A., Wainwright, P.C., Bellwood, D.R., 2001a. Prey capture in long-jawed butterflyfishes (Chaetodontidae): the functional basis of novel feeding habits. *Journal of Experimental Marine Biology and Ecology* 256, 167-184.
- Ferry-Graham, L.A., Wainwright, P.C., Lauder, G.V., 2003. Quantification of flow during suction feeding in bluegill sunfishes. *Zoology* 106, 159-168.
- Ferry-Graham, L.A., Wainwright, P.C., Westneat, M.W., Bellwood, D.R., 2001b. Modulation of prey capture kinematics in the cheeklined wrasse *Oxycheilinus digrammus* (Teleostei : Labridae). *Journal of Experimental Zoology* 290, 88-100.
- Fouts, W.R., Nelson, D.R., 1999. Prey capture by the Pacific angel shark, *Squatina californica*: visually mediated strikes and ambush-site characteristics. *Copeia*, 304-312.
- Friel, J.P., Wainwright, P.C., 1999. Evolution of complexity in motor patterns and jaw musculature of tetraodontiform fishes. *Journal of Experimental Biology* 202, 867-880.
- Galis, F., 1990. Ecological and morphological aspects of changes in food uptake through the ontogeny of *Haplochromis piceatus*. In: Hughes, R.N. (Ed.), *Behavioural Mechanisms of Food Selection*. Springer-Verlag, Berlin, pp. 281-302.
- Galis, F., 1993. Interactions between the pharyngeal jaw apparatus, feeding behaviour, and ontogeny in the cichlid fish, *Haplochromis piceatus*: A study of morphological constraints in evolutionary ecology. *Journal of Experimental Zoology* 267, 137-154.
- Galis, F., Terlouw, A., Osse, J.W.M., 1994. The relation between morphology and behavior during ontogenetic and evolutionary changes. *Journal of Fish Biology* 45, 13-26.
- Gibb, A.C., Ferry-Graham, L., 2005. Cranial movements during suction feeding in teleost fishes: Are they modified to enhance suction production? *Zoology* 108, 141-153.
- Grossman, G.D., Coffin, R., Moyle, P.B., 1980. Feeding ecology of the Bay Goby (Pisces: Gobiidae). Effects of behavioral, ontogenetic, and temporal variation on diet. *Journal of Experimental Marine Biology and Ecology* 44, 47-59.
- Hernandez, L.P., 2000. Intraspecific scaling of feeding mechanics in an ontogenetic series of zebrafish, *Danio rerio*. *Journal of Experimental Biology* 203, 3033-3043.

- Hernandez, L.P., Motta, P.J., 1997. Trophic consequences of differential performance: Ontogeny of oral jaw-crushing performance in the sheepshead, *Archosargus probatocephalus* (Teleostei, Sparidae). *Journal of Zoology* (London) 243, 737-756.
- Hernandez, L.P., Barresi, M.J.F., Devoto, S.H., 2002. Functional morphology and developmental biology of zebrafish: Reciprocal illumination from an unlikely couple 1. *Integrative and Comparative Biology* 42, 222-231.
- Herrel, A., Aerts, P., Fret, J., DeVree, F., 1999. Morphology of the feeding system in agamid lizards: Ecological correlates. *Anatomical Record* 254, 496-507.
- Herrel, A., van Wassenbergh, S., Wouters, S., Adriaens, D., Aerts, P., 2005. A functional morphological approach to the scaling of the feeding system in the African catfish, *Clarias gariepinus*. *Journal of Experimental Biology* 208, 2091-2102.
- Heupel, M.R., Bennett, M.B., 1998. Observations on the diet and feeding habits of the epaulette shark, *Hemiscyllium ocellatum*, on Heron Island Reef, Great Barrier Reef, Australia. *Marine and Freshwater Research* 49, 753-756.
- Heupel, M.R., Simpfendorfer, C.A., 2002. Estimation of mortality of juvenile blacktip sharks, *Carcharhinus limbatus*, within a nursery area using telemetry data. *Canadian Journal of Fisheries and Aquatic Science* 59, 624-632.
- Higham, T.E., Day, S.W., Wainwright, P.C., 2005. Sucking while swimming: evaluating the effects of ram speed on suction generation in bluegill sunfish *Lepomis macrochirus* using digital particle image velocimetry. *Journal of Experimental Biology* 208, 2653-2660.
- Hill, A.V., 1950. The dimensions of animals and their muscular dynamics. *Science Progress* (London) 38, 209-230.
- Huber, D.R., Motta, P.J., 2004. Comparative analysis of methods for determining bite force in the spiny dogfish *Squalus acanthias*. *Journal of Experimental Zoology* 301A, 26-37.
- Hunt Von Herbing, I., 2001. Development of feeding structures in larval fish with different life histories: winter flounder and Atlantic cod. *Journal of Fish Biology* 59: 767-782.
- Huskey, S.H., Turingan, R.G., 2001. Variation in prey-resource utilization and oral jaw gape between two populations of largemouth bass, *Micropterus salmoides*. *Environmental Biology of Fishes* 61, 185-194.
- Kao, J.S., 2000. Diet, daily ration and gastric evacuation of the leopard shark (*Triakis semifasciata*). California State University, Hayward.
- Kent, G.C., Carr, R.K., 2001. *Comparative Anatomy of the Vertebrates*, 9th Ed. McGraw-Hill, Inc., New York, 544 pp.
- Kiltie, R.A., 1982. Bite force as a basis for niche diversification between rain forest peccaries (*Tayassu tajacu* and *T. pecari*). *Biotropica* 14, 188-195.
- Koehl, M.A.R., 1996. When does morphology matter? *Annual Review of Ecology and Systematics* 27, 501-542.
- Krebs, J.R., Turingan, R.G., 2003. Intraspecific variation in gape-prey size relationships and feeding success during early ontogeny in red drum, *Sciaenops ocellatus*. *Environmental Biology of Fishes* 66, 75-84.

- Kusher, D.I., Smith, S.E., Cailliet, G.M., 1992. Validated age and growth of the leopard shark, *Triakis semifasciata*, with comments on reproduction. *Environmental Biology of Fishes* 35, 187-203.
- Lauder, G.V., 1980. Hydrodynamics of prey capture by teleost fish. *Biofluid Mechanics* 2, 161-181.
- Lauder, G.V., 1981. Form and function: structural analysis in evolutionary morphology. *Paleobiology* 7, 430-442.
- Lauder, G.V., 1985a. Aquatic feeding in lower vertebrates. In: Hildebrand, M., Bramble, D.M., Liem, K.F., Wake, D.B. (Eds.), *Functional Vertebrate Morphology*. Belknap Press, Cambridge, pp. 210-229.
- Lauder, G.V., 1985b. Functional morphology of the feeding mechanism in lower vertebrates. *Fortschritte Der Zoologie* 30, 179-188.
- Lauder, G.V., 1990. Functional morphology and systematics: Studying functional patterns in an historical context. *Annual Review of Ecology and Systematics* 21, 317-340.
- Lauder, G.V., Clark, B.D., 1984. Water flow patterns during prey capture by teleost fishes. *Journal of Experimental Biology* 113, 143-150.
- Lauder, G.V.J., Lanyon, L.E., 1980. Functional anatomy of feeding in the bluegill sunfish, *Lepomis macrochirus*: *in vivo* measurements of bone strain. *Journal of Experimental Biology* 84, 33-55.
- Lauder, G.V., Prendergast, T., 1992. Kinematics of aquatic prey capture in the snapping turtle *Chelydra serpentina*. *Journal of Experimental Biology* 164, 55-78.
- Lauder, G.V., Shaffer, H.B., 1993. Aquatic vertebrate skull design. In: Hanken, J., Hall, B.K. (Eds.), *The skull*. The University of Chicago Press, Chicago, pp. 113-149.
- Liem, K.F., 1978. Modulatory multiplicity in the functional repertoire of the feeding mechanism in cichlid fishes. *Journal of Morphology* 158, 323-360.
- Liem, K.F., 1980a. Adaptive significance of intra- and interspecific differences in the feeding repertoires of cichlid fishes. *American Zoologist* 20, 295-314.
- Liem, K.F., 1980b. Acquisition of energy by teleosts: adaptive mechanisms and evolutionary patterns. In: Ali, M.A. (Ed.), *Environmental Physiology of Fishes*. Plenum Publishing Corp., New York, pp. 299-334.
- Liem, K.F., 1990. Aquatic versus terrestrial feeding modes: possible impacts on the trophic ecology of vertebrates. *American Zoologist* 30, 209-221.
- Liem, K.F., 1993. Ecomorphology of the teleost skull. In: Hanken, J., Hall, B.K. (Eds.), *The Skull. Functional and evolutionary mechanisms*. The University of Chicago Press, Chicago, pp. 422-452.
- Liem, K.F., Summers, A.P., 2000. Integration of versatile functional design, population ecology, ontogeny and phylogeny. *Netherlands Journal of Zoology* 50, 245-259.
- Lima-Junior, S.E., Goitein, R., 2003. Ontogenetic diet shifts of a Neotropical catfish, *Pimelodus maculatus* (Siluriformes, Pimelodidae): An ecomorphological approach. *Environmental Biology of Fishes* 68, 73-79.
- Losos, J.B., 1990. Ecomorphology, performance capability, and scaling of West Indian *Anolis* lizards: An evolutionary analysis. *Ecological Monographs* 60, 369-388.
- Lowe, C.G., Wetherbee, B.M., Crow, G.L., Tester, A.L., 1996. Ontogenetic dietary shifts and feeding behaviors of the tiger shark, *Galeocerdo cuvier*, in Hawaiian waters. *Environmental Biology of Fishes* 47, 203-211.

- Manire, C.A., Gruber, S.H., 1993. A preliminary report on the rate of natural mortality of juvenile lemon sharks, *Negaprion brevirostris*. NOAA, pp. 65-71.
- Matott, M.P., Motta, P.J., Hueter, R.E., 2005. Modulation in feeding kinematics and motor pattern of the nurse shark *Ginglymostoma cirratum*. *Environmental Biology of Fishes* 74, 163-174.
- McGowan, C., 1988. Differential development of the rostrum and mandible of the swordfish (*Xiphias gladius*) during ontogeny and its possible functional significance. *Canadian Journal of Zoology* 66, 496-503.
- Miller, D.J., Lea, R.N., 1972. Guide to the coastal marine fishes of California. California Department of Fish and Game, Fishery Bulletin 157, 1-235.
- Moran, M.D., 2003. Arguments for rejecting the sequential Bonferroni in ecological studies. *Oikos* 100, 403-405.
- Morse, D.H., 1980. Behavioral Mechanisms in Ecology. Harvard University Press, Cambridge, MA, 383 pp.
- Motta, P.J., 2004. Prey capture behavior and feeding mechanics of elasmobranchs. In: Carrier, J.C., Musick, J.A., Heithaus, M.R. (Eds.), *Biology of Sharks and Their Relatives*. CRC Press, Boca Raton, FL, pp. 596.
- Motta, P.J., Kotschal, K.M., 1992. Correlative, experimental, and comparative evolutionary approaches in ecomorphology. *Netherlands Journal of Zoology* 42, 400-415.
- Motta, P.J., Wilga, C.A.D., 1995. Anatomy of the feeding apparatus of the lemon shark, *Negaprion brevirostris*. *Journal of Morphology* 226, 309-329.
- Motta, P.J., Wilga, C.D., 1999. Anatomy of the feeding apparatus of the nurse shark, *Ginglymostoma cirratum*. *Journal of Morphology* 241, 1-29.
- Motta, P.J., Wilga, C.D., 2001. Advances in the study of feeding behaviors, mechanisms, and mechanics of sharks. *Environmental Biology of Fishes* 60, 131-156.
- Motta, P.J., Hueter, R.E., Tricas, T.C., 1991. An electromyographic analysis of the biting mechanism of the lemon shark, *Negaprion brevirostris*: functional and evolutionary implications. *Journal of Morphology* 201, 55-69.
- Motta, P.J., Clifton, K.B., Hernandez, P., Eggold, B.T., 1995a. Ecomorphological correlates in ten species of subtropical seagrass fishes: diet and microhabitat utilization. *Environmental Biology of Fishes* 44, 37-60.
- Motta, P.J., Hueter, R.E., Tricas, T.C., Summers, A.P., 2002. Kinematic analysis of suction feeding in the nurse shark, *Ginglymostoma cirratum* (Orectolobiformes, Ginglymatidae). *Copeia* 1, 24-38.
- Motta, P.J., Tricas, T.C., Hueter, R.E., Summers, A.P., 1997. Feeding mechanism and functional morphology of the jaws of the lemon shark *Negaprion brevirostris* (Chondrichthyes, Carcharhinidae). *Journal of Experimental Biology* 200, 2765-2780.
- Motta, P.J., Clifton, K.B., Hernandez, P., Eggold, B.T., Giordano, S.D., Wilcox, R., 1995b. Feeding relationships among nine species of seagrass fishes of Tampa Bay, Florida. *Bulletin of Marine Science* 56, 185-200.
- Muller, M., Osse, J.W.M., 1984. Hydrodynamics of suction feeding in fish. *Transactions of the Zoological Society of London* 37, 51-135.
- Muller, M., Osse, J.W.M., Verhagen, J.H.G., 1982. A quantitative hydrodynamical model of suction feeding in fish. *Journal of Theoretical Biology* 95, 49-79.

- Nemeth, D.H., 1997. Modulation of attack behavior and its effect on feeding performance in a trophic generalist fish, *Hexagrammos decagrammus*. *Journal of Experimental Biology* 200, 2155-2164.
- Norton, S.F., 1991. Capture success and diet of cottid fishes: the role of predator morphology and attack kinematics. *Ecology* 72, 1807-1819.
- Norton, S.F., Brainerd, E.L., 1993. Convergence in the feeding mechanics of ecomorphologically similar species in the Centrarchidae and Cichlidae. *Journal of Experimental Biology* 176, 11-29.
- Norton, S.F., Luczkovich, J.J., Motta, P.J., 1995. The role of ecomorphological studies in the comparative biology of fishes. *Environmental Biology of Fishes* 44, 287-304.
- Olson, M.H., 1996. Ontogenetic niche shifts in largemouth bass: variability and consequences for first-year growth. *Ecology* 77, 179-190.
- O'Reilly, J.C., Linstedt, S.L., Nishikawa, K.C., 1993. The scaling of feeding kinematics in toads (Anura: Bufonidae). *American Zoologist* 33, 147A.
- Osse, J.W.M., 1990. Form changes in fish larvae in relation to changing demands of function. *Netherlands Journal of Zoology* 40, 362-385.
- Quinn, G.P., Keough, M.J., 2002. *Experimental Design and Data Analysis for Biologists*. Cambridge University Press, Cambridge, United Kingdom, 537 pp.
- Reilly, S.M., 1995. The ontogeny of aquatic feeding behavior in *Salamandra salamandra*: Stereotypy and isometry in feeding kinematics. *Journal of Experimental Biology* 198, 701-708.
- Reilly, S.M., 1996. The metamorphosis of feeding kinematics in *Salamandra salamandra* and the evolution of terrestrial feeding behavior. *Journal of Experimental Biology* 199, 1219-1227.
- Reilly, S.M., Lauder, G.V., 1988. Ontogeny of aquatic feeding performance in the Eastern newt, *Notophthalmus viridescens* (Salamandridae). *Copeia* 1988, 87-91.
- Reilly, S.M., Lauder, G.V., 1989. Physiological bases of feeding behavior in salamanders: do motor patterns vary with prey type? *Journal of Experimental Biology* 141, 343-358.
- Reilly, S.M., Lauder, G.V., 1990. Metamorphosis of cranial design in tiger salamanders (*Ambystoma tigrinum*): a morphometric analysis of ontogenetic change. *Journal of Morphology* 204, 121-137.
- Reilly, S.M., Lauder, G.V., 1992. Morphology, behavior, and evolution: Comparative kinematics of aquatic feeding in salamanders. *Brain, Behavior, and Evolution* 40, 182-196.
- Richard, B.A., Wainwright, P.C., 1995. Scaling the feeding mechanism of largemouth bass (*Micropterus salmoides*): kinematics of prey capture. *Journal of Experimental Biology* 198, 419-433.
- Robinson, M.P., Motta, P.J., 2002. Patterns of growth and the effects of scale on the feeding kinematics of the nurse shark (*Ginglymostoma cirratum*). *Journal of Zoology* 256, 449-462.
- Russo, R.A., 1975. Observations on the food habits of leopard sharks (*Triakis semifasciata*) and brown smoothhounds (*Mustelus henlei*). *California Fish and Game* 61, 95-103.
- Sanderson, S.L., 1990. Versatility and specialization in labrid fishes: ecomorphological implications. *Oecologia* 84, 272-279.

- Sanderson, S.L., 1991. Functional stereotypy and feeding performance correlated in a trophic specialist. *Functional Ecology* 5, 795-803.
- Sanford, C.P., Wainwright, P.C., 2002. Use of sonomicrometry demonstrates the link between prey capture kinematics and suction pressure in largemouth bass. *Journal of Experimental Biology* 205, 3445-3457.
- Sass, G.G., Motta, P.J., 2002. The effects of satiation on prey capture kinematics in the largemouth bass, *Micropterus salmoides*. *Environmental Biology of Fishes* 65, 441-454.
- Schwenk, K., 2000. Tetrapod feeding in the context of vertebrate morphology. In: Schwenk, K. (Ed.), *Feeding: Form, function, and evolution in tetrapod vertebrates*. Academic Press, San Diego, pp. 3-20.
- Shaffer, H.B., Lauder, G.V., 1985a. Aquatic prey capture in Ambystomatid salamanders: patterns of variation in muscle activity. *Journal of Morphology* 183, 273-284.
- Shaffer, H.B., Lauder, G.V., 1985b. Patterns of variation in aquatic Ambystomatid salamanders: kinematics of the feeding mechanism. *Evolution* 39, 83-92.
- Shirai, S., Nakaya, K., 1992. Functional morphology of feeding apparatus of the cookie-cutter shark, *Isistius brasiliensis* (Elasmobranchii, Dalatiinae). *Zoological Science* 9, 811-821.
- Sibbing, F.A., Nagelkerke, L.A.J., 2001. Resource partitioning by Lake Tana barbs predicted from fish morphometrics and prey characteristics. *Reviews in Fish Biology and Fisheries* 10, 393-437.
- Sims, D.W., 2000. Filter-feeding and cruising swimming speeds of basking sharks compared with optimal models: they filter-feed slower than predicted for their size. *Journal of Experimental Marine Biology and Ecology* 249, 65-76.
- Skulason, S., Noakes, D.L.G., Snorrason, S.S., 1989. Ontogeny of trophic morphology in four sympatric morphs of Arctic charr *Salvelinus alpinus* in Thingvallavatn, Iceland. *Biological Journal of the Linnean Society* 38, 281-301.
- Stoner, A.W., Livingston, R.J., 1984. Ontogenetic patterns in diet and feeding morphology in sympatric sparid fishes from seagrass meadows. *Copeia* 1984, 174-187.
- Summers, A.P., 2000. Stiffening the stingray skeleton: An investigation of durophagy in myliobatid stingrays (Chondrichthyes, Batoidea, Myliobatidae). *Journal of Morphology* 243, 113-126.
- Summers, A.P., Koob, T.J., Brainerd, E.L., 1998a. Stingray jaws strut their stuff. *Nature* 395, 450-451.
- Summers, A.P., Darouian, K.F., Richmond, A.M., Brainerd, E.L., 1998b. Kinematics of aquatic and terrestrial prey capture in *Terrapene carolina*, with implications for the evolution of feeding in cryptodire turtles. *Journal of Experimental Zoology* 281, 280-287.
- Svanback, R., Wainwright, P.C., Ferry-Graham, L.A., 2002. Linking cranial kinematics, buccal pressure, and suction feeding performance in largemouth bass. *Physiological and Biochemical Zoology* 75, 532-543.
- Talent, L.G., 1976. Food habits of the leopard shark, *Triakis semifasciata*, in Elkhorn Slough, Monterey Bay, California. *California Fish and Game* 62, 286-298.

- Van Damme, J., Aerts, P., 1997. Kinematics and functional morphology of aquatic feeding in Australian snake-necked turtles (Pleurodira: Chelodina). *Journal of Morphology* 233, 113-125.
- Van Valen, L., 1965. Morphological variation and width of ecological niche. *American Naturalist* 99, 377-390.
- Van Wassenbergh, S., Aerts, P., Herrel, A., 2005. Scaling of suction-feeding kinematics and dynamics in the African catfish, *Clarias gariepinus*. *Journal of Experimental Biology* 208, 2103-2114.
- Verwajen, D., Van Damme, R., Herrel, A., 2002. Relationships between head size, bite force, prey handling efficiency and diet in two sympatric lacertid lizards. *Functional Ecology* 16, 842-850.
- Wainwright, P.C., 1994. Functional morphology as a tool in ecological research. In: Wainwright, P.C., Reilly, S.M. (Eds.), *Ecological Morphology: Integrative Organismal Biology*. The University of Chicago Press, Chicago, pp. 42-59.
- Wainwright, P.C., Lauder, G.V., 1986. Feeding biology of sunfishes: patterns of variation in the feeding mechanism. *Zoological Journal of the Linnean Society* 88, 217-228.
- Wainwright, P.C., Westneat, M.W., Bellwood, D.R., 2000. Linking feeding behavior and jaw mechanics in fishes. In: Domenici, P., Blake, R.W. (Eds.), *Biomechanics in Animal Behavior*. BIOS Scientific Publishers Ltd., Oxford, pp. 207-221.
- Wainwright, P.C., Ferry-Graham, L.A., Waltzek, T.B., Hulsey, C.D., Carroll, A.M., Svanback, R., 2001a. Evaluating suction feeding performance in fishes. *American Zoologist* 41, 1617-1617.
- Wainwright, P.C., Ferry-Graham, L.A., Waltzek, T.B., Carroll, A.M., Hulsey, C.D., Grubich, J.R., 2001b. Evaluating the use of ram and suction during prey capture by cichlid fishes. *Journal of Experimental Biology* 204, 3039-3051.
- Weih, D., 1980. Hydrodynamics of suction feeding of fish in motion. *Journal of Fish Biology* 16, 425-433.
- Werner, E.E., Gilliam, J.F., 1984. The ontogenetic niche and species interactions in size-structured populations. *Annual Review of Ecology and Systematics* 15, 393-425.
- Werner, E.E., Mittelbach, G.G., Hall, D.J., 1981. The role of foraging profitability and experience in habitat use by the bluegill sunfish. *Ecology* 62, 116-125.
- Westneat, M.W., 1994. Transmission of force and velocity in the feeding mechanisms of labrid fishes (Teleostei, Perciformes). *Zoomorphology* 114, 103-118.
- Westneat, M., 2003. A biomechanical model for analysis of muscle force, power output and lower jaw motion in fishes. *Journal of Theoretical Biology*, 269-281.
- Wilga, C.A.D., Motta, P.J., 1998a. Feeding mechanism of the Atlantic guitarfish *Rhinobatos lentiginosus*: Modulation of kinematic and motor activity. *Journal of Experimental Biology* 201, 3167-3184.
- Wilga, C.D., Motta, P.J., 1998b. Conservation and variation in the feeding mechanism of the spiny dogfish *Squalus acanthias*. *Journal of Experimental Biology* 201, 1345-1358.
- Wilga, C.D., Motta, P.J., 2000. Durophagy in sharks: feeding mechanics of the hammerhead *Sphyrna tiburo*. *Journal of Experimental Biology* 203, 2781-2796.
- Wilga, C.A.D., Wainwright, P.C., Motta, P.J., 2000. Evolution of jaw depression mechanics in aquatic vertebrates: insights from Chondrichthyes. *Biological Journal of the Linnean Society* 71, 165-185.

- Wilga, C.D., Hueter, R.E., Wainwright, P.C., and Motta, P.J., 2001. Evolution of upper jaw protrusion mechanisms in elasmobranchs. *American Zoologist* 41, 1248-1257.
- Wu, E.H., 1993. The functional morphology of feeding mechanisms in orectolobiform sharks. Harvard University, Cambridge, Massachusetts, pp. 177.
- Wu, E.H., 1994. Kinematic analysis of jaw protrusion in orectolobiform sharks: a new mechanism for jaw protrusion in elasmobranchs. *Journal of Morphology* 222, 175-190.
- Zar, J.H., 1999. *Biostatistical Analysis*. Prentice Hall, Upper Saddle River, New Jersey, 919 pp.

About the Author

David Lowry was born in St. Charles, Missouri and moved to Boise, Idaho at age five. Encouraged by his parents' love of nature, David read ravenously about diverse natural phenomena and spent time outdoors whenever possible. The mysterious ocean intrigued David from a young age and his search for knowledge soon turned toward uncovering the secrets of the animals that dwelt therein. After graduating from Borah High School in 1996, David attended Hawai'i Pacific University in Honolulu, Hawai'i where he obtained a Bachelors Degree in Marine Biology in 1999. It was during a winter visit home to Boise that David met his future wife Sonia. Immediately after graduation David and Sonia moved to Tampa, Florida to attend the University of South Florida, where David earned his Ph.D. in Biology in 2005. After completing his degree, David moved to Olympia, Washington to pursue a career in applied biological science.