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# Evolution and Functional Morphology of the Cephalic Lobes in Batoids

by

Samantha Lynn Mulvany

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
Department of Integrative Biology
College of Arts and Sciences
University of South Florida

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# DEDICATION

I would like to dedicate this to my family and friends. My family has always been very supportive of my academic endeavors, nurturing my love of biology from early childhood to this very day. My grandfather, who passed away on the day that I was accepted into the program, has been with me every step of the way on this bittersweet journey. My loving husband, who I met near the end of my graduate work, has been my own personal cheering section, encouraging me to finish up so that I can pursue a teaching career.

Most of my friends have taken on the dual role of helping me on an academic and social level. My kidneys: Laura Habegger, Jackie Guzy and Celina Bellanceau always have my back (the small of my back) and I don't know what I would do without them in my life. Susie Piacenza, Teresa Piacenza, Ilgin Hasbora, Neal Halstead, Martin Berdugo, Krissy Morrow, Maranda Holley, Nick Larghi, Kerry Bohl, Jessica Davis, David Jennings and Pat Yarnot have made my time in Tampa the most memorable and fun part of my life.

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## TABLE OF CONTENTS

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# LIST OF TABLES

المنارة للاستشارات

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# LIST OF FIGURES

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# ABSTRACT

Cephalic lobes are unique structures derived from the anterior pectoral fins, found in select myliobatid stingrays. Many benthic batoids utilize undulatory locomotion and use their pectoral fins for both locomotion and prey capture. Pelagic myliobatids that possess cephalic lobes utilize oscillatory locomotion, using their pectoral fins to locomote and their cephalic lobes for prey capture. Despite differences in habitat usage and locomotor modes, these batoids feed on very similar benthic organisms. The purpose of this study was to 1.) compare the morphology of the cephalic lobes and anterior pectoral fins in lobed and lobeless species, looking at skeletal elements, musculature and electrosensory pore distributions; 2.) compare prey capture kinematics in lobed and lobeless species and examine the role of the cephalic lobes in prey capture modulation due to elusive/non-elusive prey; 3.) analyze multiple morphological and behavioral variables to establish any correlations to the presence of cephalic lobes. Radiography, dissections and staining techniques were employed to examine the morphology of the cephalic lobes and anterior pectoral fins in six species of batoids. High speed videography was used to film prey capture behavior in five batoid species, using elusive and non-elusive prey. Continuous morphological and behavioral variables were used to determine any correlations with the presence of the cephalic lobes, taking phylogeny into account. Results indicate that the skeletal components of the pectoral fins of oscillatory species are very different from pectoral fins of undulatory species as well as the cephalic lobes. Second moment of area ($I$), showed that the cephalic lobes and pectoral fins in undulatory species had greater resistance to bending in

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multiple directions and were also more flexible. The cephalic lobes had a novel muscle layer compared to the pectoral fin musculature. Electorsensory pores were absent from the anterior pectoral fins in oscillatory batoids, but numerous on the cephalic lobes and anterior pectoral fins in undulatory batoids. The distribution of the electrosensory pores was uniform with the exception of *Rhinoptera bonasus*, which possessed higher pore numbers along the edges of the cephalic lobes. Overall, the morphology of the cephalic lobes is distinct, but more similar to the pectoral fins of undulators compared to oscillators. Kinematic data showed that species with cephalic lobes localize prey capture to the cephalic region of the body. Lobed species were faster at pouncing and tenting prey, but slower during biting. The cephalic lobes were able to move more in the vertical and horizontal plane compared to the anterior pectoral fins. All species were able to modulate prey capture behavior to some degree. Species lacking lobes spent more time handling elusive prey compared to non-elusive prey. For all species, elusive prey were farther from the mouth during biting but prey escapes were rare. Lobed species were overall faster in prey capture, but did not display more modulation or feeding success than lobeless species. Phylogenetically corrected correlations showed that most morphological variables correlated to the appearance of the cephalic lobes, while kinematics variables did not. There was also a correlation among habitat, locomotion and the cephalic lobes. The cephalic lobes may have played a key role in partitioning prey capture to the head region, maintaining dexterity in the lobes while allowing the pectoral fins to shift to oscillatory locomotion and consequently a pelagic lifestyle.

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# CHAPTER 1: GENERAL INTRODUCTION

Form and function are closely linked, and often influence an organism's ecology and consequently fitness (Bock, 1980; Barel *et al*. 1989). The evolution of unique structures can be linked to morphological, behavioral and ecological changes in organisms that often help characterize clades. Feathers allowed body temperature regulation and flight, opening up new aerial habitats and helping characterize the Aves clade, while mammary glands increased nourishment and development of offspring, ultimately paving the way for increased brain size in mammals (Wideliz et al., 2007). Novel appendages with specialized functions for locomotion, sensory abilities, or feeding have arguably contributed to the immense success of arthropods (Angelini and Kaufman, 2005). Spinnerets in spiders, derived from other appendages, allow web-building behavior to arise, opening up new niches and certainly unique methods of prey capture that help to define the clade (Pechmann et al., 2010). In centipedes, the forcipules represent the only known example of locomotor appendages evolving into venomous prey capture appendages, concurrent with a shift from open habitat to leaf-litter habitat (Dugon et al., 2012). Ballistic tongue projection in chameleons allows feeding at lower temperatures compared to other lizards, as tongue projection is temperature-independent due to the elastic recoil mechanism involved (Anderson and Deban, 2010). Novel muscle insertions, ligaments and bone elements in cyprinodontiforms resulted in a unique upper jaw protrusion mechanism that allowed a picking and scraping feeding mode to evolve (Hernandez et al., 2009). In this study, I examine such an innovation in the family Myliobatidae.

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Batoidea is a clade that includes approximately 630 species of skates and rays, which represents about half of the known chondrichthyan species (Aschliman et al., 2012). Batoids are distinguished by their dorso-ventrally depressed bodies and enlarged pectoral fins. Within the batoid clade, a derived family, Myliobatidae, possess unique appendages called cephalic lobes. These lobes have evolved from the anterior pectoral fins (Bigelow and Schroeder, 1953; Nishida, 1990; Miyake et al., 1992) and are distinct from the pectoral fins. While some rays have one continuous lobe, others have one discontinuous lobe or two completely separate lobes (McEachran et al., 1996). Skeletal components of the pectoral fins and cephalic lobes consist of cartilaginous radials that extend from the propterygium, bifurcating at the distal ends (Bigelow and Schroeder, 1953). The cephalic lobe musculature has not been studied. Electrosensory pores, used to detect prey (Kalmijn, 1971; Tricas and Sisnero, 2004), are found on the ventral surface, including the pectoral fins and cephalic lobes (Chu and Wen, 1979; Sasko et al., 2006).

The pectoral fins are used for locomotion and in most species lacking cephalic lobes, the pectoral fins are also used to form a tent around the prey, constraining and pinning prey to the substrate during feeding (Wilga et al., 2012). In species with cephalic lobes, the functions of the pectoral fins have been partitioned such that the pectoral fins are used for primarily locomotion and the cephalic lobes are used for prey capture. The lobes are used in prey detection, digging through the substrate, excavation and handling, (Smith and Merriner, 1985; Moss, 1977; Sasko et al., 2006) and in some rays, such as mobulids and mantas, the lobes help channel water and plankton into the mouth (Notarbartolo-di-Sciara and Hillyer, 1989). With the lobes taking on the function of prey capture, the pectoral fins in these species have shifted to a different locomotor mode compared to other batoids.

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Batoids can utilize undulatory locomotion (waves of bending traveling down the pectoral fin), oscillatory locomotion (flapping the pectoral fins), or intermediate locomotion, a combination of both (Rosenberger, 2001). The majority of lobeless batoids are primarily undulatory, ideal for locomotion near the substrate, with high maneuverability and lower cruising speeds. Lobed batoid species are all oscillatory, ideal for long distance, pelagic migrations and higher cruising speeds in the water column (Rosenberger, 2001). Oscillatory species have stiffer pectoral fins compared to undulatory species as a result of this swimming style and the subsequent forces acting on the fins (Schaefer and Summers, 2005). The switch from undulatory to oscillatory locomotion, along with the change from lobeless to lobed batoids, coincides with the shift from benthic to pelagic habitats.

The majority of undulatory batoids are benthic, locomoting and feeding near the substrate (Compagno, 1977; McEachran and Carvalho, 2002). Oscillatory batoids, however, are pelagic, locomoting in the water column at higher speeds (Rosenberger, 2001 Fontanella et al., 2013). Mobulid and manta species are truly pelagic, spending all of their time swimming and filter feeding in the water column (Notarbartolo-di-Sciara and Hillyer, 1989), while other lobed species feed in the benthos (Bigelow and Schroeder, 1953; Compagno, 1977). Lobeless and lobed batoids that feed on benthic organisms typically feed on polychaetes, bivalves, shrimp, fish and other crustaceans (Smith and Merriner, 1985; Michael, 1993; Compagno, 1997; Ebert and Cowley, 2003; Ebert and Bizzarro, 2007; Collins et al., 2007; Ajemian and Powers, 2012; Jacobsen and Bennett, 2013). The only known examples of pelagic batoids without lobes are the pelagic stingray, *Pteroplatytrygon violacea*, and electric rays in the family Torpedinidae. *Pteroplatytrygon violacea* uses a combination of oscillatory and undulatory locomotion (Rosenberger, 2001) and wraps its pectoral fins around prey in the water column (Jordan, 2008).

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*Torpedo* rays utilize body-caudal-fin locomotion to swim in the water column (Roberts, 1969) and wrap their pectoral fins around prey, stunning prey with electric organs (Wilson, 1953; Lowe et al., 1994). The cephalic lobes may offer a unique evolutionary solution to the problem of the stiffness needed for oscillatory locomotion and the flexibility needed for prey capture.

The purpose of this study was to examine: the morphology of the anterior pectoral fins and cephalic lobes, the function of the cephalic lobes during prey capture, and correlations of the presence/absence of cephalic lobes with morphological, behavioral and ecological variables to better understand the role of this evolutionary novelty in shaping the Myliobatidae clade. Three lobeless batoids: *Raja eglanteria* (Bosc, 1800); yellow stingray, *Urobatis jamaicensis* (Cuvier, 1816); Atlantic stingray *Dasyatis sabina* (Lesueur, 1824) and six lobed batoids: spotted eagle ray *Aetobatus narinari* (Euphrasen, 1790); cownose ray *Rhinoptera bonasus* (Mitchill, 1815); *Mobula japonica* (Müller and Henle, 1841); *Mobula thurstoni*, (Lloyd, 1908); *Mobula munkiana* (Notarbartolo-di-Sciara, 1987), *Manta birostris* (Walbaum, 1792) were used in this study, though *Mobula* and *Manta* species were grouped together because of small sample sizes.

The morphological goal of this study was to compare the skeleton, muscle and electrosensory pores of the cephalic lobes and anterior pectoral fins. Second moment of areas for skeletal cross sections, patterns of calcification, muscular complexity and pore distributions were examined. The goal of the kinematic study was to compare prey capture kinematics and investigate modulatory ability in lobed and lobeless species with varying prey types. I hypothesized that species with cephalic lobes would: have shorter prey capture durations, be more successful in retaining captured prey, and display a greater capacity to modulate prey capture behavior with different prey types. After accounting for the phylogenetic relatedness of

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the species, I hypothesized that both morphological and kinematic variables will correlate with the presence/absence of the cephalic lobes.

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# CHAPTER 2: THE MORPHOLOGY OF THE CEPHALIC LOBES AND ANTERIOR PECTORAL FINS IN SIX SPECIES OF BATOIDS [1]

## ABSTRACT

Many benthic batoids utilize their pectoral fins for both undulatory locomotion and feeding. Certain derived, pelagic species of batoids possess cephalic lobes, which evolved from the anterior pectoral fins. These species utilize the pectoral fins for oscillatory locomotion while the cephalic lobes are used for feeding. The goal of this article was to compare the morphology of the cephalic lobes and anterior pectoral fins in species that possess and lack cephalic lobes. The skeletal elements (radials) of the cephalic lobes more closely resembled the radials in the pectoral fin of undulatory species. Second moment of area (*I*), calculated from cephalic lobe radial cross sections, and the number of joints revealed greater flexibility and resistance to bending in multiple directions as compared to pectoral fin radials of oscillatory species. The cephalic lobe musculature was more complex than the anterior pectoral fin musculature, with an additional muscle on the dorsal side, with fiber angles running obliquely to the radials. In *Rhinoptera bonasus*, a muscle presumably used to help elevate the cephalic lobes is described.

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Electrosensory pores were found on the cephalic lobes (except *Mobula japonica*) and anterior pectoral fins of undulatory swimmers, but absent from the anterior pectoral fins of oscillatory swimmers. Pore distributions were fairly uniform except in *R. bonasus*, which had higher pore numbers at the edges of the cephalic lobes. Overall, the cephalic lobes are unique in their anatomy but are more similar to the anterior pectoral fins of undulatory swimmers, having more flexibility and maneuverability compared to pectoral fins of oscillatory swimmers. The maneuverable cephalic lobes taking on the role of feeding may have allowed the switch to oscillatory locomotion and hence, a more pelagic lifestyle.

## INTRODUCTION

Evolutionary novelties are of great interest in the study of functional morphology because they form the basis for defining clades and offer unique adaptive solutions to a changing environment. Furthermore, the evolution of novel structures can provide insight into how changes in form are linked to changes in ecology (Lachaise et al., 2000; Widelitz et al., 2007; Konow et al., 2008; Hernandez et al., 2009).

Five genera of derived rays (*Aetobatus, Aetomylaeus, Rhinoptera, Mobula*, and *Manta*) possess novel structures called cephalic lobes, which are modifications of the anterior portions of the pectoral fin (Bigelow and Schroeder, 1953; Nishida, 1990; Miyake et al., 1992). Rays can exhibit one continuous lobe, one discontinuous lobe, or two distinct lobes with the most derived rays exhibiting two distinct, movable lobes (McEachran et al., 1996). The lobes extend anteriorly beyond the head and in the most derived clades are clearly separated from the pectoral fins (Fig. 2.1). The skeletal components of the cephalic lobes are similar to the pectoral fins, with series of

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cartilaginous radials extending out from the propterygeal cartilage with bifurcations of the radials at the distal ends of the fin rays (Bigelow and Schroeder, 1953).

In batoids, a series of laterally oriented cartilaginous radials compose a fin ray (Schaefer and Summers, 2005). Electrosensory canals line the ventral side of the cephalic lobes (Chu and Wen, 1979), providing a means of detecting prey (Tricas and Sisneros, 2004). The internal anatomy of the cephalic lobes, which may include novel muscles and subdivisions, as well as their function(s), has not been closely examined.

The pectoral fins of batoids are used primarily for either undulatory or oscillatory locomotion. A combination of the two modes is frequent, with certain species falling within a gradient of undulation and oscillation (Rosenberger, 2001). With the derivation of the cephalic lobes from the anterior portion of the pectoral fins, the primary function of the lobes has evolved to prey capture, prey detection, and holding/trapping prey against the substrate as well as maneuvering it toward the mouth (Smith and Merriner, 1985; Sasko et al., 2006). In the most derived batoids (*Manta* and *Mobula*), the cephalic lobes are uncurled and positioned around the mouth during feeding, presumably directing water and plankton into the mouth (Notarbartolo-di-Sciara and Hillyer, 1989).

Shifts in habitat also coincide with changes in locomotor modes and the appearance of the cephalic lobes. Basal batoids are generally bottom living, feed on benthic organisms (Compagno, 1977), lack cephalic lobes and use undulatory locomotion (Campbell, 1951; Rosenberger and Westneat, 1999; Rosenberger, 2001), which allows the body to remain close to the substrate while moving. The undulatory mode of swimming also allows high maneuverability while still maintaining close contact to the substrate, though swimming velocity is low (Rosenberger,

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2001). More derived, epi- benthic and pelagic rays utilize an oscillatory swimming mode, which is well suited for pelagic species that locomote at higher velocities and in most cases have cephalic lobes (Rosenberger, 2001). However, many epibenthic rays still feed on benthic organisms (Compagno, 1977; Jardas et al., 2004; Collins et al., 2007) and the emergence of the cephalic lobes is found in some of these epi-benthic species. Oscillatory locomotion is less maneuverable (Rosenberger, 2001), which may hinder prey capture. Kinetic cephalic lobes may facilitate prey restraint and capture for oscillatory batoids with reduced maneuverability (Sasko et al., 2006). In batoids, locomotor patterns correlate to calcification patterns in the radials of the pectoral fin (Schaefer and Summers, 2005). Undulatory batoids tend to have more catenated calcification, where chains of calcified cartilage are deposited along the radials, whereas oscillatory batoids tend to have crustal calcification, where a layer of calcified cartilage superficially coats the radials (Fig. 2.2). Oscillatory batoids also possess cross-bracings, with adjacent radials connected to one another via projections of cartilage, typically near the joints (Schaefer and Summers, 2005). Crustal calcification and cross-bracing provide more stiffness, which is presumably advantageous for oscillatory locomotion. In addition, some undulatory dasyatid rays exhibit joint staggering of the radials on the lateral margins of the pectoral fins, providing greater stiffness similar to how bricks are staggered when constructing a wall (Schaefer and Summers, 2005).

Because the role of the anterior pectoral fins shifts from locomotion to prey detection and capture with the advent of cephalic lobes, the predominant movements and thus stresses on the cephalic lobes will differ, resulting in structural and biomechanical changes in the supporting cartilaginous radials, as compared to the radials of the pectoral fins. As the cephalic lobes are used to manipulate prey, the radials may have less calcification to allow increased flexibility for

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grasping behavior. However, the spotted eagle ray, *Aetobatus narinari* utilizes its single cephalic lobe to dig benthic prey out of the substrate, thus the skeletal structures will most likely be reinforced in ways that facilitate digging behavior. Furthermore, because the cephalic lobes house the electroreceptive ampullae of Lorenzini (Chu and Wen, 1979) they can serve for prey detection (Tricas and Sisneros, 2004). Analysis of the electroreceptor pore distributions and total pore counts on the cephalic lobes has yet to be done.

The purpose of this study was to examine the functional anatomy of cephalic lobes and compare their morphology to their evolutionary precursors, the anterior pectoral fins in closely related batoid species, exploring the evolutionary responses of form to changes in function and ecology. Six phylogenetically representative batoid groups were chosen to represent the diversity of cephalic lobe structure. Our goal was to compare the skeletal elements, musculature, and electrosensory pores of the cephalic lobes and pectoral fins to determine any phylogenetic patterns. The distribution of material (second moment of area) of the radials, calcification patterns and presence of cross-bracings in the skeletal elements were examined, along with complexity of muscular elements and the distribution and density of electrosensory pores across the cephalic lobes and pectoral fins.

## MATERIALS AND METHODS

### *Specimens*

Specimens of nine batoid species were collected from local fishermen and acquired through museum loans (Table 2.1). Because of low availability and similar anatomy, the *Mobula* and *Manta* data were combined, resulting in six groups representing a phylogenetic series of different

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head shapes in batoids (Fig. 2.1) Museum specimens were preserved with formalin and held in 70% ethanol, while specimens collected from local fishermen were kept frozen until dissected. Both male and female specimens were used. The specimens included predominantly mature animals, but all mobulid and manta specimens were neonates as mature animals were not available (Table 2.1).

### *Musculature and Skeleton*

A minimum of four specimens per species were used to investigate the musculature and skeletal components, with the exception of the mobulid and manta species (N = 1 each) which were combined (N = 4). The anterior portion of the pectoral fins and cephalic lobes were skinned and dissected to reveal the origin and insertion of each muscle, as well as the orientation and number of muscle layers. The cartilage was exposed to examine the number of joints and orientation of the radials. Radiographs and digital photographs of the muscle dissections and cartilage were taken with a PXS10–16W Kevex digital X-ray machine and a Canon PowerShot A710IS camera, and the photographs used to create illustrations of the skeletal elements and musculature using Adobe Illustrator CS2 version 12.0.1 (Adobe Systems, San Jose, CA).

The cephalic lobe and anterior pectoral fin ray cartilage from 2–3 specimens of each species with the exception of the mobulids was then dissected from the body and stained using a modified protocol from Deban (1997). Per museum restrictions, the fin ray cartilage from one cephalic lobe in one *M. munkiana* was allowed to be used to represent the mobulids. From all of the above specimens, a subsample of every fifth fin ray (from the most anterior radial) was detached from the propterygium and soaked in 95% ethanol for 12–24 h, then in an alcian blue

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solution (700 ml 100% ethanol, 300 ml glacial acetic acid, and 700 mg alcian blue) for 4–24 h to stain the cartilage. The cartilage was then rinsed with distilled water and transferred to a dilute alizarin red solution (100 ml of distilled water with 10 drops of alizarin red S-saturated distilled water) for 1–3 days to further stain the calcified cartilage. The fin rays were then transversely cut every centimeter, starting from the medial margin to the lateral edge. In this manner, a total of approximately 3–5 fin rays were examined from each species and each fin ray yielded 3–5 cross sectional areas (CSAs). The sectioned radial was examined under a Wild stereozoom M3 microscope and digitally photographed with a Canon PowerShot A710IS camera at 10–30x magnification. CSAs and diameters were calculated from the digital images using SigmaScan Pro v4.01.003 (Systat Software, San Jose, CA). The thickness of the crustal calcification was measured as well as the radius and distance from the lateral and dorso-ventral axes for the catenated calcification.

Calcification of the cartilaginous elements can vary by species, age and region of the body in elasmobranchs (Summers et al., 2004; Macesic and Summers, 2012). While calcification undoubtedly increases stiffness (Currey, 2002), the material properties of the radials are unknown. The majority of the radial is comprised of uncalcified cartilage and it is likely that this composite material comprised of both calcified and uncalcified regions contribute significantly to the stiffness of the radials (Seki et al., 2006; Chen et al., 2008; Meyers et al., 2013). Therefore, the second moment of area ($I$), was calculated for the radial cross sections as a whole and also for only the calcified portions of the radial cross sections. Initially ignoring the calcification, the equation for the second moment of area $I$ of an elliptical CSA was used to determine $I$ for each radial cross section in both the dorso-ventral and lateral plane: $I_{\mathrm{Lateral}} = \pi/4$ x $ab^3$, where $a$ is the radius along the lateral axis and $b$ is the radius along the dorso-ventral axis; $I_{\mathrm{dorso\text{-}ventral}} = \pi/4$ x

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$a_1^3b_1$ (Fig. 2.3A). A ratio of $I_{\text{Lateral}}/I_{\text{dorso-ventral}}$ ($I_{\text{Lat}}/I_{\text{DV}}$) was then taken for each cross section to determine the ability of the radial, at that region, to resist bending forces in the dorso-ventral and lateral plane. A ratio of one indicates equal resistance (a circular shape), a ratio greater than one indicates a higher resistance to bending in the lateral plane, and a ratio less than one indicates a higher resistance to bending in the dorso-ventral plane. Similar methods were used to determine $I$ for only the calcified portions of each radial. The equation for a hollow ellipse was used for the crustal calcification, where $I_{\text{hollow Lat}} = \pi/4(a_1b_1^3 - a_2b_2^3)$ and $I_{\text{hollow DV}} = \pi/4(a_1^3b_1 - a_2^3b_2)$ (Fig. 2.3B). The parallel axis theorem was used to determine $I$ for the catenated calcification, where each circular calcification was measured with the equation: $x[\pi r^4/4 + \pi r^2 d]$, where $r$ = radius of the calcified circular areas, $d$ = distance from the neutral axis, with $d = 0$ for areas that lie on the neutral axis, and $x$ is the number of circular calcifications within each cross section (Fig. 2.3C). For cross sections with multiple circular calcifications, the calcified $I$s were then summed to obtain the total calcified $I$ for each radial CSA. A ratio of $I_{\text{Lat}}/I_{\text{DV}}$ was then taken for each cross section to compare resistance patterns. A Kruskal–Wallis nonparametric test was run using SigmaStat 3.1 (Systat Software, San Jose, CA) to determine any significant difference in $I_{\text{Lat}}/I_{\text{DV}}$ among species and among the anterior pectoral fins of oscillatory swimmers, undulatory swimmers and the cephalic lobes as well as differences in $I_{\text{Lat}}/I_{\text{DV}}$ within species (if $I_{\text{Lat}}/I_{\text{DV}}$ changes among and along the different radials sectioned), followed by a Dunn's post hoc test.

*Electrosensory Pores*

Electrosensory pore distributions across the ventral anterior regions of the pectoral fins and the entire ventral side of the cephalic lobes were calculated for *R. eglanteria* (n = 4), *D. sabina* (n

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= 6), *U. jamaicensis* (n = 7), *A. narinari* (n = 5), *R. bonasus* (n = 7), and combined *Mobula* and *Manta* specimens, including *Mobula thurstoni* (n = 1), *M. japonica* (n = 2), *M. munkiana* (n = 1), and *Manta birostris* (n = 1). The propterygium was used as the medial border for all species. Because the anterior region of the pectoral fin is continuous with the rest of the pectoral fin, the first fin ray attached to the propterygium anterior to the mouth was used as the posterior border for species lacking cephalic lobes. Pores not plainly visible were dyed black by applying India ink to the skin surface and wiping away excess ink. The targeted area of the batoid was placed on an HP Scanjet 3570c digital scanner and scanned at 300–600 dpi. Electrosensory pores on the left and right side were counted and total pore counts were then averaged for each species. A Kruskal–Wallis nonparametric test was run in SigmaPlot 11.0 to determine if pore counts varied among the species or between left and right sides, with Dunn's post hoc tests to determine which species differed. A pore map was constructed from the scans showing the pore distribution across the ventral side of the anterior pectoral fins and cephalic lobes for each species.

This study was approved by the University of South Florida Institutional Animal Care and Use Committee under protocol # T 3566 and T 2957.

## RESULTS

### *Skeleton*

The anterior pectoral fins are supported by the propterygium (protopterygium) and fin rays (series of radials) that extend distally from the propterygium in all species. For species that lack cephalic lobes, the orientation of the pectoral fin rays gradually shifts from a cranial orientation at the anterior portion of the pectoral fin to a lateral orientation at the middle portion of the

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pectoral fin and a caudal orientation at the posterior portion of the fin (Fig. 2.4). For *A. narinari, R. bonasus*, and the mobulid and manta species, the pectoral fin rays only extend laterally. The fin rays of the cephalic lobes extend anteriorly for all species that possess lobes. The pectoral fin and cephalic lobe fin rays of all species bifurcate at their distal end at least once.

The base of each fin ray in the cephalic lobe in *R. bonasus* is semispherical and lies in socketsalong the propterygium, attached via connective tissue. Each fin ray in the *A. narinari* cephalic lobe, as well as that of the pectoral fins for all species examined, has a flat base and is attached to the surface of the propterygium by connective tissue. Clearing and staining show crustal calcification patterns for the pectoral fin and cephalic lobe radials of *A. narinari, R. bonasus*, and *M. munkiana*, and catenated calcification patterns for the pectoral fin radials in *R. eglanteria, U. jamaicensis,* and *D. sabina* (Fig. 2.2). Occasional cartilaginous cross bracings, connections between adjacent radials, are found in the pectoral fin radials of *D. sabina*, while the pectoral fin radials of *A. narinari* and *R. bonasus* are heavily cross-braced to the point that separating an individual radial is almost impossible.

The inter-radial joints of the pectoral fins are not staggered in any of the species with the exception of *D. sabina*. In *D. sabina*, only the bifurcated distal radials display joint staggering (Fig. 2.4B), as noted by Schaefer and Summers (2005). Cephalic lobe radials do not display joint staggering. The average number of joints per cm varied significantly among species (P-value: $<0.001$). Significant differences (P-value: $\leq 0.023$) were found among all groups except *R. bonasus* cephalic lobe and *U. jamaicensis* pectoral fin radials (the two groups with the highest number of joints per cm), *R. bonasus* and *A. narinari* pectoral fin radials (the two groups with the lowest number of joints per cm), and *D. sabina* and *R. eglanteria* pectoral fin radials (Fig. 2.5).

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Measurements of radial CSA revealed inter and intraradial shape differences. For *R. eglanteria, U. jamaicensis, D. sabina* and the cephalic lobe radials of *A. narinari, R. bonasus*, and *M. munkiana*, the cross sectional shapes are oval and circular. For the pectoral fins of *A. narinari* and *R. bonasus*, the shapes also include irregular ovals and more rectangular cross sections (Fig. 2.2). In almost all cases, the radials for all species are dorso-ventrally compressed to some degree. Calcification patterns also occur, though patterns vary by species. For *A. narinari, R. bonasus*, and *M. munkiana*, crustal calcification occurs within the pectoral fin and cephalic lobe radials, while *R. eglanteria*, *U. jamaicensis,* and *D. sabina* have catenated calcification, with 1–3 calcified struts running through the dorsal and ventral edges of the radials. At the distal tips of the fin rays, any calcified struts run through the center of the radials.

The average $I_{\mathrm{Lat}}/I_{\mathrm{DV}}$ of the radial CSAs for all species ranges from 2.18 in *A. narinari* cephalic lobe to 8.02 in *R. bonasus* anterior pectoral fin, indicating that all the radials offer greater resistance to lateral bending than dorso-ventral bending (Fig. 2.6A). The average $I_{\mathrm{Lat}}/I_{\mathrm{DV}}$ for just the calcified regions of the radials ranges from 0.29 in *U. jamaicensis* to 5.53 in *R. bonasus* pectoral fin, indicating that the calcified regions in *U. jamaicensis* resist dorso-ventral bending while *R. bonasus* radials, as well as all the other species, resist lateral bending (Fig. 2.6B). No significant differences in $I_{\mathrm{Lat}}/I_{\mathrm{DV}}$ were found among the radials (anterior to posterior, proximal to distal) within each species. (P-value: >0.05), therefore the data were combined for interspecific comparison. There was a significant difference in $I_{\mathrm{Lat}}/I_{\mathrm{DV}}$ for the radials among the species (P-value: <0.001). Post hoc tests showed that the $I_{\mathrm{Lat}}/I_{\mathrm{DV}}$ for the radial CSAs of *A. narinari* and *Mobula* cephalic lobe are significantly lower from all the other species (P- value: <0.05), indicating that the cephalic lobe radials of *Mobula* and *A. narinari* are more circular and withstand resistance from all directions, whereas the other groups withstand bending more in the

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lateral plane (Fig. 2.6A).

The $I_{Lat}/I_{DV}$ ratios for the calcified regions of the radial are significantly different among the species (P-value: <0.001; Fig. 2.6B). Post hoc tests revealed that the groups with the highest $I_{Lat}/I_{DV}$, *R. bonasus* and *A. narinari* pectoral fin radials and cephalic lobe radials, along with *Mobula* cephalic lobes do not differ significantly from each other. *Raja eglanteria* pectoral fins, *D. sabina* pectoral fins, *A. narinari* cephalic lobes and *Mobula* cephalic lobes do not significantly differ from each other forming a second group. The third group with the lowest $I_{Lat}/I_{DV}$, U. *jamaicensis, R. eglanteria*, and *D. sabina* pectoral radials, do not significantly differ from each other.

When all pectoral fin radials were grouped by swimming mode and examined along with all the cephalic lobe radials, the $I_{Lat}/I_{DV}$ were significantly different (P-value: <0.001). Post hoc tests showed that $I_{Lat}/I_{DV}$ in the anterior pectoral fins of oscillatory species were significantly different from the other groups (P-value: <0.05). There was no significant difference between anterior pectoral fins of undulatory swimmers and the cephalic lobes (P-value: >0.05). This indicates that the cross-sectional shape of the cephalic lobe radials is more similar to that of pectoral fin radials of undulatory swimmers. That is, the radial cross sections of the cephalic lobes and the pectoral fins of undulatory swimmers are rounder compared to the cross sections of radials in oscillatory swimmers. However, when examining the calcified regions of the radials alone, there were significant differences (P-value: <0.001) with all three groups showing distinct differences. The undulatory swimmers had the lowest $I_{Lat}/I_{DV}$ while the oscillatory swimmers had the highest $I_{Lat}/I_{DV}$, indicating the calcified regions of the radials in undulatory swimmers are suited to resist bending forces in all directions, as compared to the oscillatory swimmers which have calcified regions that best resist bending in the lateral plane (Table 2.2).

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*Musculature*

The anterior pectoral fin musculature is highly conserved across species. The dorsal surface of the anterior pectoral fin is comprised of two muscle layers, the abductor superficialis and the abductor profundus, separated by a tendinous sheath (Fig. 2.7). The abductor superficialis originates on the propterygium and inserts on the tendinous sheath of the abductor profundus, with the muscle fibers running dorsal to ventral proceeding proximal to distal. The abductor profundus originates on the propterygium and inserts on the radials, with muscle fibers running ventro-dorsally and proximo-distally on the fin. The ventral surface of the anterior pectoral fins is similarly comprised of an adductor profundus and superficialis, originating on the propterygium and inserting into the radials or the tendinous sheath of the deeper muscle, respectively. The fibers of the adductor profundus run dorsoventrally and proximo-distally while those of adductor superficialis run ventro-dorsally and proximo-distally (Fig. 2.7).

Differences in the distal point of insertion into the tendinous sheath of abductor and adductor superficialis were found. In *D. sabina* and *R. eglanteria*, the abductor superficialis extended three fifths the length of the fin rays while the adductor superficialis extended half the length of the fin rays. In *U. jamaicensis* and *M. thurstoni*, the abductor superficialis extended four fifths down the length of the fin rays while the adductor superficialis extended three fifths the length of the fin rays. For *R. bonasus* and *A. narinari*, the superficialis muscles extended down the length of the entire pectoral fin to the most distal radial.

The cephalic lobe musculature in *A. narinari, R. bonasus*, and mobulid species (*M. japonica, M. thurstoni*, and *M. munkiana*) is comprised of three muscle layers on the dorsal side

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of the radials. The most superficial layer, here termed the dorsal oblique, has muscle fibers that run obliquely to the radials (Fig. 2.8). In *A. narinari*, the dorsal oblique is very thick and is interspersed with white connective tissue, possibly collagen. In *R. bonasus*, the dorsal oblique is divided into superficial and deep divisions. The deep division is darker in color than the superficial layer. In mobulid species and *A. narinari*, the dorsal oblique is undivided. In *A. narinari, R. bonasus*, and *Mobula*, the dorsal oblique originates on the propterygium and inserts onto the muscle layer deep to it, the abductor superficialis. The dorsal oblique muscle in *A. narinari* was noticeably thicker than in other species.

Similar to the pectoral fin musculature, the abductor superficialis and abductor profundus muscles in the cephalic lobes of *A. narinari, R. bonasus,* and *Mobula* run in the same direction as the radials, originate on the propterygium, and insert onto the profundus and along the radials, respectively. The superficialis muscle fibers run dorsoventrally and proximo-distally while the profundus muscle runs ventro-dorsally and proximo-distally. Unlike the pectoral fin musculature, both superficialis muscles extend down the length of the lobe while the profundus muscles taper off at two fifths the length of the fin rays. However, the profundus muscles have multiple tendons that extend down the entire length of the fin rays, inserting distally on the cephalic lobe. On the ventral side of the cephalic lobes in *A. narinari, R. bonasus,* and *Mobula*, the adductor superficialis and adductor profundus originate on the propterygium and insert onto the adductor profundus and radials, respectively. Similar to the dorsal side of the cephalic lobes, the superficialis runs down the length of the radials while the profundus tapers to a muscular insertion two fifths down the length of the radials and tendons that extend to the distal edge of the cephalic lobes.

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In *R. bonasus*, there is a muscle located near the medio-dorsal side of the cephalic lobes, which originates from the neurocranium and inserts onto the most medial fin ray of the cephalic lobes (Fig. 2.9). This muscle, here termed the cephalic lobe levator, appears to elevate the antero-medial portion of the cephalic lobes. Interestingly, the depressor rostri muscle in *M. thurstoni* inserts onto the ventral base of the cephalic lobes via an aponeurosis, contrary to Gonzalez-Isais (2003) who states that the depressor rostri inserts onto the lateral part of the nasal capsules via an aponeurosis.

### *Electrosensory Pores*

The qualitative distribution of the electrosensory pores on the surface of the pectoral fins and cephalic lobes is similar for all species with the exception of *R. bonasus* (Fig. 2.10). The electrosensory pores of the cephalic lobes of *R. bonasus* appear to increase in density around the edges of the cephalic lobes.

The number of electrosensory pores does not significantly differ from left side to right side on any species (P-value: >0.1). The total number of pores among species differs (P-value: <0.001), with the cephalic lobes of *R. bonasus* and *A. narinari* having the most pores and the manta/mobulid species having no visible pores on the cephalic lobes, and *R. bonasus, A. narinari*, and the manta/mobulid species having no visible electrosensory pores on the anterior pectoral fins (Fig. 2.11). Tukey's post hoc test revealed three groups that differed in pore count (P-value: ≤0.001). *Aetobatus narinari* and *R. bonasus* cephalic lobes had the greatest number of pores. The second group formed *U. jamaicensis, D. sabina,* and *R. eglanteria* pectoral fins had fewer pores and the remaining species did not have any pores.

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## DISCUSSION

Cephalic lobes are anterior extensions of the pectoral fins found in some derived, oscillatory myliobatid rays (Bigelow and Schroeder, 1953; Nishida, 1990; Miyake et al., 1992). Having undergone a suite of morphological modifications to skeletal, muscular, and electrosensory components, the cephalic lobes have shifted from a once primarily locomotor function to the detection, capture, and manipulation of prey (Smith and Merriner, 1985; Sasko et al., 2006). In a similar manner, diversification of appendages and thus shifts in function and the emergence of novel locomotory, feeding, and reproductive behaviors have played key roles in the evolution of other taxa (Angelini and Kaufman, 2005; Pechmann et al., 2010). The separation of the cephalic lobes and pectoral fins may have aided myliobatids in the expansion into a pelagic niche via oscillatory locomotion while maintaining the ability to capture prey. Whereas a few other pelagic batoids exist without cephalic lobes, they use different forms of locomotion and feeding strategies. Torpedo electric rays, including *Torpedo californica*, utilize body-caudal fin propulsion (Roberts, 1969), leaving the pectoral fins free to wrap around and electrically stun prey prior to capture (Wilson, 1953; Belbenoit and Bauer, 1972; Michaelson et al., 1979; Lowe et al., 1994). The pelagic stingray *Pteroplatytrygon violacea* utilizes an intermediate locomotor mode between undulation and oscillation (Rosenberger, 2001) wrapping its pectoral fin around prey to capture it (Jordan et al., 2009). However, the majority of pelagic batoids employ oscillatory locomotion and possess cephalic lobes, which are used in feeding.

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### *Skeleton*

The skeletal anatomy of cephalic lobes is distinct from the anterior pectoral fins of basal batoids, and differs markedly from the pectoral fins of oscillatory species that possess cephalic lobes. While the cross sectional shapes of the pectoral fin radials in oscillatory species are quite diverse, encompassing rectangular, oval, diamond, and irregular shapes, the cross sectional shapes of the pectoral fin radials in undulatory species and the cephalic lobe radials of all examined species are more oval and circular. The second moment of area ratio for the radials disregarding calcification ($I_{\mathrm{Lat}}/I_{\mathrm{DV}}$) differed among species, with *A. narinari* and *Mobula* cephalic lobes having the most circular radial CSAs and thus radials that resist bending equally in all directions (Fig. 2.6A). This pattern is biomechanically advantageous for structures that move in multiple planes and experience forces from multiple directions (Wainwright et al., 1980). The cephalic lobes encounter multidirectional forces, as the cephalic lobes are laterally extended and depressed during prey capture and oscillated dorso-ventrally during prey excavation (Sasko et al., 2006).

Nearly all of the radials of most species (with the exception of the cephalic lobe radials of *A. narinari, M. munkiana,* and *R. bonasus*) were dorso-ventrally flattened to some degree (Fig. 2.2), meaning that the radials would actually be more biomechanically suited to resisting forces in the lateral plane than the dorso-ventral plane (as the cartilage is placed further away from the longitudinal neutral axis than the dorso-ventral neutral axis). This shape confers greater flexibility in the dorso-ventral axis which would be suitable for locomotory movement. This could also be the result of having a dorso-ventrally depressed body plan, requiring depression of the radials to maintain a flatter overall body shape and pectoral appendage. Dorso-ventrally depressed radials may also increase the area of pectoral fin and cephalic lobe muscle attachment,

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as the dorsal and ventral musculature attach to the dorsal and ventral surfaces of the radials. For the oscillatory species in particular, heavy cross-bracing, in effect, transforms each individual fin ray of the pectoral fin into one collective structure, transferring force among all the fin rays. In a similar manner, abutting molariform teeth transfer forces laterally during compressive biting of hard prey (Nobiling, 1977). As oscillatory swimming requires a collective depression of all the fin rays during a downstroke (and similarly a collective elevation of the fin rays during an upstroke), it is advantageous to possess heavy cross bracings that mechanically link each radial to the adjacent radials. Thus, examining each individual radial may not be representative of how they are biomechanically utilized and how the forces act on them.

The lack of cross bracing and joint staggering (with the exception of *D. sabina*), along with increased number of joints, in the anterior pectoral fin of undulatory species and the cephalic lobes of oscillatory species may reflect the need for greater flexibility and maneuverability. Undulatory locomotion requires more independent fin rays, as multiple waves per fin length travel down the body (Rosenberger, 2001). At any given time, one fin ray along the body will be depressed while another fin ray at a different point along the body will be elevated. These undulatory species also capture prey with their pectoral fins, pinning prey against the substrate, forming a tent over the prey and maneuvering prey toward the mouth (Wilga et al., 2012), which requires flexibility. The proximal radials of *R. bonasus* cephalic lobes are spherical at their base and lie in sockets on the propterygium, no doubt providing a greater range of motion compared to the proximal radials of other species, which lie flat against the propterygium. This, coupled with the fact that the cephalic lobes of *R. bonasus* have the highest number of joints per cm, with *A. narinari* cephalic lobes being comparable to undulatory species (Fig. 2.5), suggests that the skeletal components of the cephalic lobes of *R. bonasus, M. munkiana,* and *A. narinari* are

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highly flexible and maneuverable. All of these attributes make the cephalic lobes ideal for grasping and manipulation of prey (Sasko et al., 2006) and even digging through the substrate, as the eagle ray is known to do (Gudger, 1914).

Though the cephalic lobe radial ultrastructure is more similar to the pectoral fin radials in undulatory species, in terms of potential flexibility and maneuverability, the calcification patterns appeared to be phylogenetically, not functionally related. The pectoral radials of *R. eglanteria, U. jamaicensis,* and *D. sabina* displayed catenated calcification, with chains of calcified cartilage running along the dorsal and ventral sides of the radials. The cephalic lobes and pectoral fin radials of *A. narinari* and *R. bonasus* displayed crustal calcification, with calcified cartilage coating the entire radial. Crustal calcification is linked primarily to oscillatory swimmers, while catenated calcification is linked primarily to undulatory swimmers, with crustal calcification being the basal condition that was secondarily derived in Myliobatidae (Schaefer and Summers, 2005). With catenated calcification being less energetically costly to produce and maintain, it is assumed that crustal calcification confers some benefit, presumably increased stiffness, to oscillatory swimmers (Schaefer and Summers, 2005). This suggests that secondarily derived crustal calcification evolved with a shift to oscillatory locomotion. Since the cephalic lobes display crustal calcification, this suggests that the cephalic lobes evolved after the shift to crustal calcification and oscillatory locomotion.

When comparing the second moment of area ratio ($I_{\mathrm{Lat}}/I_{\mathrm{DV}}$) of the total radial cartilage to the calcified cartilage of the undulatory swimmers with catenated calcification (*R. eglanteria, U. jamaicensis,* and *D. sabina*), the calcified $I_{\mathrm{Lat}}/I_{\mathrm{DV}}$ values are closer to or less than one (Fig. 2.6B). A ratio of one indicates bending resistance in all planes, and a ratio less than one indicates greater resistance to bending in the dorso-ventral plane. Though the radials of these basal batoids

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are dorso-ventrally flattened, the calcification patterns are well placed to resist bending in both lateral and dorso-ventral planes compared to the crustal calcification found in the more derived species (*A. narinari, R. bonasus,* and *Mobula*) which are well placed to resist bending in the lateral plane. Because no physical testing of resistance to bending was performed on the actual cephalic lobes or pectoral fins and the mineral content is unknown, the extent of influence that calcification has on the bending resistance of the structures cannot be determined. Because the radials are composed of a calcified outer region with a cartilaginous and more flexible inner region they can be considered composite materials (Vogel, 2003). Without knowing the material properties of the two regions, as well as that of the overlying skin and muscle, we can assume that the actual $I_{\mathrm{Lat}}/I_{\mathrm{DV}}$ values of the lobes and pectoral fins are some combination of Figures 2.6A and 2.6B. Regardless, it appears that the cephalic lobe radial architecture is best suited for manipulation and flexibility.

### *Musculature*

Although the cephalic lobes are derived from the anterior pectoral fins, there are marked anatomical differences in the muscle architecture and complexity. The origins and insertions of the muscles remain consistent, with the propterygium anchoring the muscles as they attach along the radials. However, the adductor and abductor profundus muscles of the cephalic lobes have tendons that extend to the distal ends of the lobes, similar to the flexor and extensor digitorum profundus muscles in humans that control the flexible distal digits (Gray, 1977). *Rhinobatus bonasus, A. narinari*, and the manta/mobula species examined have a novel dorsal muscle in the cephalic lobes, the dorsal oblique, which is markedly different in orientation from the other

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musculature. Because the dorsal oblique is oriented at an angle to the radials, this muscle may provide the cephalic lobes with increased dexterity and a wider range of movement compared to the pectoral fins, including adduction and abduction of the fin rays.

*Aetobatus narinari* is known to use its cephalic lobe to dig through the substrate during prey excavation (Gudger, 1914). The dorsal oblique muscle may aid in this behavior, helping to fan out the fin rays and elevate the lobe, much like a shovel. The thickness of the dorsal oblique suggests that in *A. narinari*, it is quite powerful, whereas in *R. bonasus* and manta/mobula species it is much thinner. The divisions of the dorsal oblique found in *R. bonasus* may facilitate multibehavioral usage. *Rhinoptera bonasus* is known to repeatedly depress and elevate the cephalic lobes during prey excavation and feeding events to fluidize the sediment, whereas during swimming the lobes remain elevated (Sasko et al., 2006). The cephalic lobes are also depressed close to the sediment when searching for prey, presumably to detect the weak electric fields of the prey with the ampullae of Lorenzini (Sasko et al., 2006) and/or to detect tactile stimulation from prey (Maruska and Tricas, 1998; Maruska and Tricas, 2004). The difference in muscle coloration may indicate that this muscle is fatigue-resistant red myotomal muscle (Bone, 1978) and reflect the need to repeatedly move the lobes during searching and feeding. In mobulid species, the dorsal oblique was very thin, although all specimens examined were neonates. During swimming, mobulids curl the cephalic lobes such that they face anteriorly (Notarbartolo-di-Sciara and Hillyer, 1989). The dorsal oblique muscle may assist in this behavior.

The cephalic lobe levator muscle, found only in *R. bonasus*, most likely aids in elevating the antero-medial edges of the cephalic lobes. *Rhinoptera bonasus* possesses two separate cephalic lobes that meet at the anterior margin of the head. When swimming, the cephalic lobe

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levator muscle may be important in retracting the medial portion of the cephalic lobes, making the head more hydrodynamic and therefore reducing drag during locomotion.

### *Electrosensory Pores*

Ampullae of Lorenzini are electrosensory receptors that can detect voltage gradients below 1 nV $cm^{-1}$ (Kajiura and Holland, 2002; Kajiura, 2003; Jordan et al., 2009), with studies showing responses to simulated prey from over 25 cm away (Jordan et al., 2009; McGowan and Kajiura, 2009). Ampullae in batoids not only surround the mouth but are also found on both the dorsal and ventral surfaces of the head and on the pectoral fins and cephalic lobes (Chu and Wen, 1979). Electroreception is used in prey detection, predator detection, conspecific communication and geonavigation in batoids (Tricas and Sisneros, 2004).

As batoids have a dorso-ventrally compressed body plan, the ventral surface of the body is extremely flat. Basal batoids typically locomote with bodies parallel to the substrate, such that all areas of the ventral surface are approximately equidistant from the substrate (personal observation). Concurrently, the electrosensory pore distribution of the anterior pectoral fins in the basal batoids, *R. eglanteria, U. jamaicensis,* and *D. sabina*, is very uniform. *Rhinoptera bonasus* and *A. narinari* lack electrosensory pores on the anterior pectoral fins, suggesting that the role of prey electrosensory detection is more isolated to the cephalic lobes rather than the pectoral fins.

The electrosensory pore distribution of the cephalic lobe in *A. narinari* is very uniform (Fig. 2.10). When *A. narinari* feeds on benthic prey and the cephalic lobes are depressed, the body typically is pitched downward such that the cephalic lobes and mouth are approximately parallel to and close to the substrate (Fig. 2.12A). The electrosensory pore distribution on the

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cephalic lobes of *R. bonasus* is concentrated on the distal edges of the lobes. Because *R. bonasus* possesses two distinct lobes, the lobes are more laterally positioned compared to the single lobe of *A. narinari*. This makes it impossible to have both cephalic lobes entirely parallel to the substrate when depressed (as the ray would have to pitch forward and simultaneously to the left and right). Instead, the body of *R. bonasus* remains parallel to the substrate while depressing the cephalic lobes (Sasko et al., 2006; Fig. 2.12B). The distal edges of the cephalic lobes are closest to the substrate, while the proximal portion of the lobes is furthest away, as they articulate with the propterygium. Consequently, the ventral surface of the cephalic lobes is not evenly distributed across the substrate. Thus when searching for prey items, the area of the lobes closest to the substrate has the highest density of electrosensory pores, increasing the spatial resolution (Raschi, 1978) and distance of the field of detection from the body.

All mobulid/manta specimens examined lacked electrosensory pores on the anterior pectoral fins, as well as the cephalic lobes. *Manta birostris* is known to lack electrosensory pores on the cephalic lobes (Chu and Wen, 1979), with claims that all manta species have ampullary organs only in the hyoid region (Albert and Crampton, 2006). Because mantas and mobulids ram filter-feed on plankton, the need to utilize the cephalic lobes for prey detection via electrosensory systems is most likely reduced. Their prey is not buried underneath the substrate, but rather in the water column where, at times, high densities of plankton form distinct visible patches or layers that mobulas and mantas will repeatedly swim through (Notarbartolo-di-Sciara and Hillyer, 1989). Though paddlefish, a filter-feeding fish with up to 75,000 electrosensory pores on its rostrum (Nachtrieb, 1910), utilize electroreception to capture plankton, the rivers they inhabit are turbid and have very low visibility (Wilkens et al., 1997). In contrast, mantas frequently inhabit near-shore waters and reefs (Michael, 1993) that are less turbid and may not need to rely on

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electrosensory receptors to detect prey. The megamouth shark and basking shark, are also filter feeders and possess relatively few electrosensory receptors, a total of 225 and 301 pores, respectively, for the entire head, (Kempster and Collin, 2011a; Kempster and Collin, 2011b), which undoubtedly indicates very low pore densities for such massive fishes.

## CONCLUSIONS

The cephalic lobes are a novel structure, both anatomically and functionally, that aid batoids in prey detection, excavation and manipulation. Cephalic lobes are found exclusively in certain oscillatory swimmers, possibly aiding in the switch to oscillatory locomotion by taking on the role of feeding. While oscillatory locomotion involves more rigid skeletal elements and cross-bracing of radials, the demands of prey capture and feeding are quite opposite. The cephalic lobes maintain the flexibility and maneuverability needed to capture prey via increased joints, rounded radials and increased muscle complexity.

Although batoids that lack cephalic lobes*, R. eglanteria, U. jamaicensis,* and *D. sabina*, utilize their pectoral fins to locomote and capture prey, species with cephalic lobes, *R. bonasus, A. narinari,* and manta/mobula species have localized locomotion to the pectoral fins and prey capture to the cephalic lobes. The absence of electrosensory pores on the pectoral fins of all the oscillatory swimmers is further evidence of this separation of function. The cephalic lobes may have played a key role in the transition from benthic to pelagic habitats. Oscillatory locomotion allows for lift during locomotion and more efficient cruising, resulting in larger home ranges, more pelagic habitats and potential exploitation of expanded niches and resources. The trade-off of this locomotor mode is less maneuverability. The cephalic lobes offer a unique solution to this

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problem such that maneuverability can be retained in the lobes while the pectoral fins can retain an efficient oscillatory locomotor mode.

## ACKNOWLEDGMENTS

The authors thank the following people and institutions for specimen loans and collections: Jeff Williams and Sandra Raredon of the Smithsonian National Museum of Natural History, H.J. Walker of the Scripps Institute of Oceanography, George Burgess and Robert Robins of the Florida Museum of Natural History, Richard Spieler of Nova Southeastern University, Carl Luer and Jack Morris of Mote Marine Laboratory and Captain Roy Herndon.

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## TABLES AND FIGURES

Table 2.1. Materials examined.

| Species | # of specimens | Type | Size range (DW) | Museum specimen catalog number | Sex # of F/M/? |
|---|---|---|---|---|---|
| *Raja eglanteria* | 4 | frozen | 25-42cm | | 1/1/2 |
| *Urobatis jamaicensis* | 7 | preserved or frozen | 15-21cm | FLMNH77997 | 3/4/0 |
| *Dasyatis sabina* | 6 | frozen | 21-29cm | | 2/4/0 |
| *Rhinoptera bonasus* | 7 | frozen | 50-78cm | | 5/2/0 |
| *Aetobatus narinari* | 8 | preserved or frozen | 50-110cm | US28348, USNM204769, US205415, USNM52823, US17510, FLMNH32679 | 2/1/5 |
| *Manta birostris* | 1 | preserved | 113cm | US163933 | 0/1/0 |
| *Mobula japonica* | 2 | preserved | 73-85cm | SIO 82-9 | 1/1/0 |
| *Mobula munkiana* | 1 | preserved | 87cm | SIO 85-35 | 0/1/0 |
| *Mobula thurstoni* | 1 | preserved | 57cm | SIO 85-36 | 0/1/0 |

US and USNM = National Museum of Natural History, Smithsonian Institution, Washington D.C., SIO = Scripps Institute of Oceanography, La Jolla, California, FLMNH = Florida Museum of Natural History, Gainesville, Florida.

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Table 2.2. Average $I_{Lat}/I_{DV}$ of the radials and calcified portions of the radials in oscillatory swimmers, undulatory swimmers and the cephalic lobes. * and ** indicate significant differences among the groups.

| $I_{Lat}/I_{DV}$ of cephalic lobes and locomotor modes | | |
|---|---|---|
| | Radial $I_{Lat}/I_{DV}$ | Calcified Radial $I_{Lat}/I_{DV}$ |
| Oscillatory swimmers | 5.4** | 5.4* |
| Undulatory swimmers | 3.3 | 1.0* |
| Cephalic lobes | 3.3 | 2.9* |

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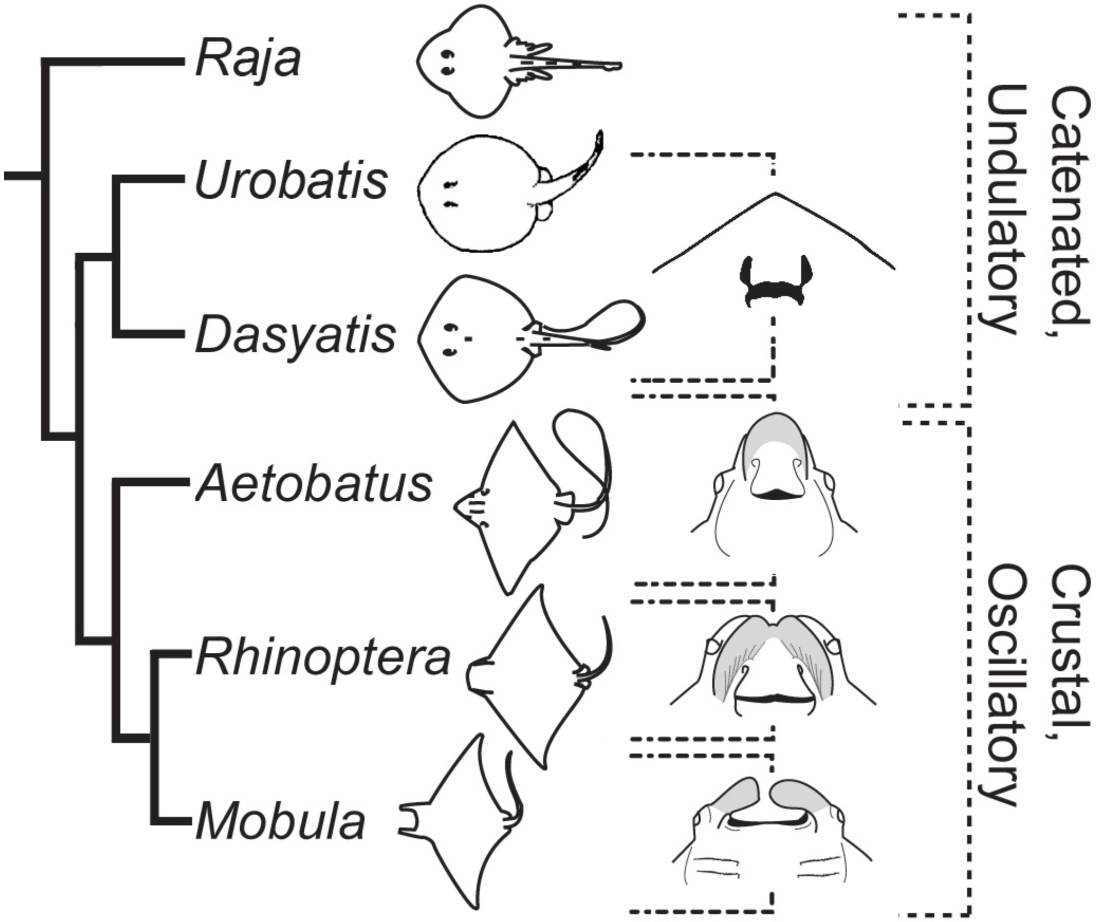

Figure 2.1. The phylogeny of select batoids based on phylogenetic trees from Nishida (1990), Dunn et al. (2003), and Aschliman et al. (2012). Head shape is shown with cephalic lobes shaded in gray. The primary locomotor mode, undulatory/oscillatory, and type of calcification pattern of the radials, catenated/crustal, are also defined for each representative batoid. Modified from Sasko et al. (2006).

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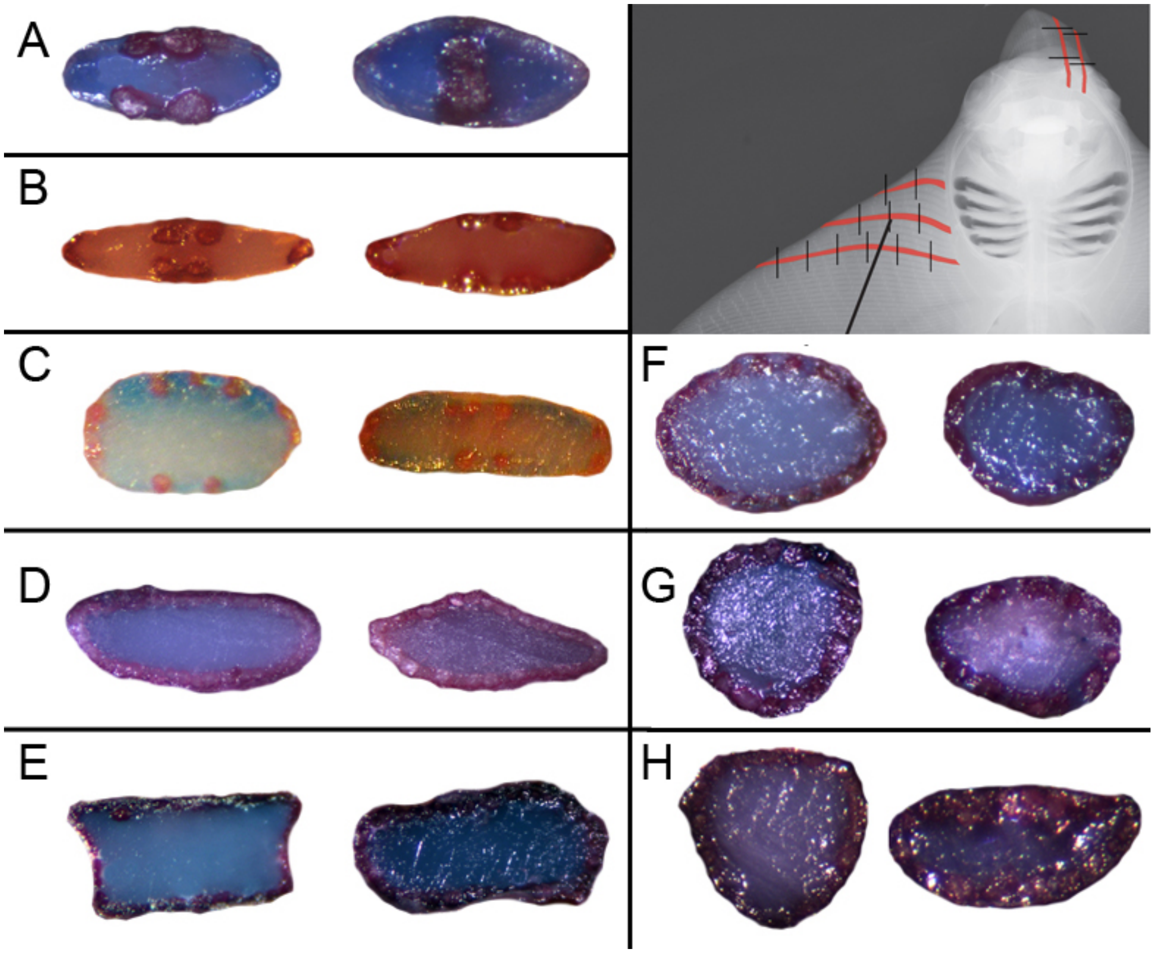

Figure 2.2. Stained cross sectional areas of select radials. The picture in the upper right has representative fin rays highlighted (fin rays #5, 10, and 15 on the pectoral fin and fin rays #5 and 10 on the cephalic lobe) with black lines representing the 1 cm sections where the CSAs were sampled. (A–H) show transverse sections of select radials from the anterior pectoral fins and cephalic lobes. For all pictures, the top of the picture is the dorsal surface. (A–E) are pectoral fin cross sections from left to right of (A) fin ray #10, 1 cm and fin ray #25, 4 cm in *R. eglanteria*; (B) fin ray #10, 1 cm and fin ray #10, 2 cm of *U. jamaicensis*; (C) fin ray #5, 1 cm and fin ray #25, 4 cm of *D. sabina*; (D) fin ray #5, 1 cm and fin ray #5, 4 cm of *A. narinari*; (E) fin ray #5, 1 cm and fin ray #15, 7 cm of *R. bonasus*. (F–H) are cephalic lobe cross sections from left to right of (F) fin ray #10, 2 cm and fin ray #10, 4 cm of *A. narinari*; (G) fin ray #10, 1 cm and fin ray #10, 4 cm of *R. bonasus*; (H) fin ray #10, 1 cm and fin ray #15, 6 cm of *M. munkiana*. The most well-stained and representative cross sections were chosen for each species.

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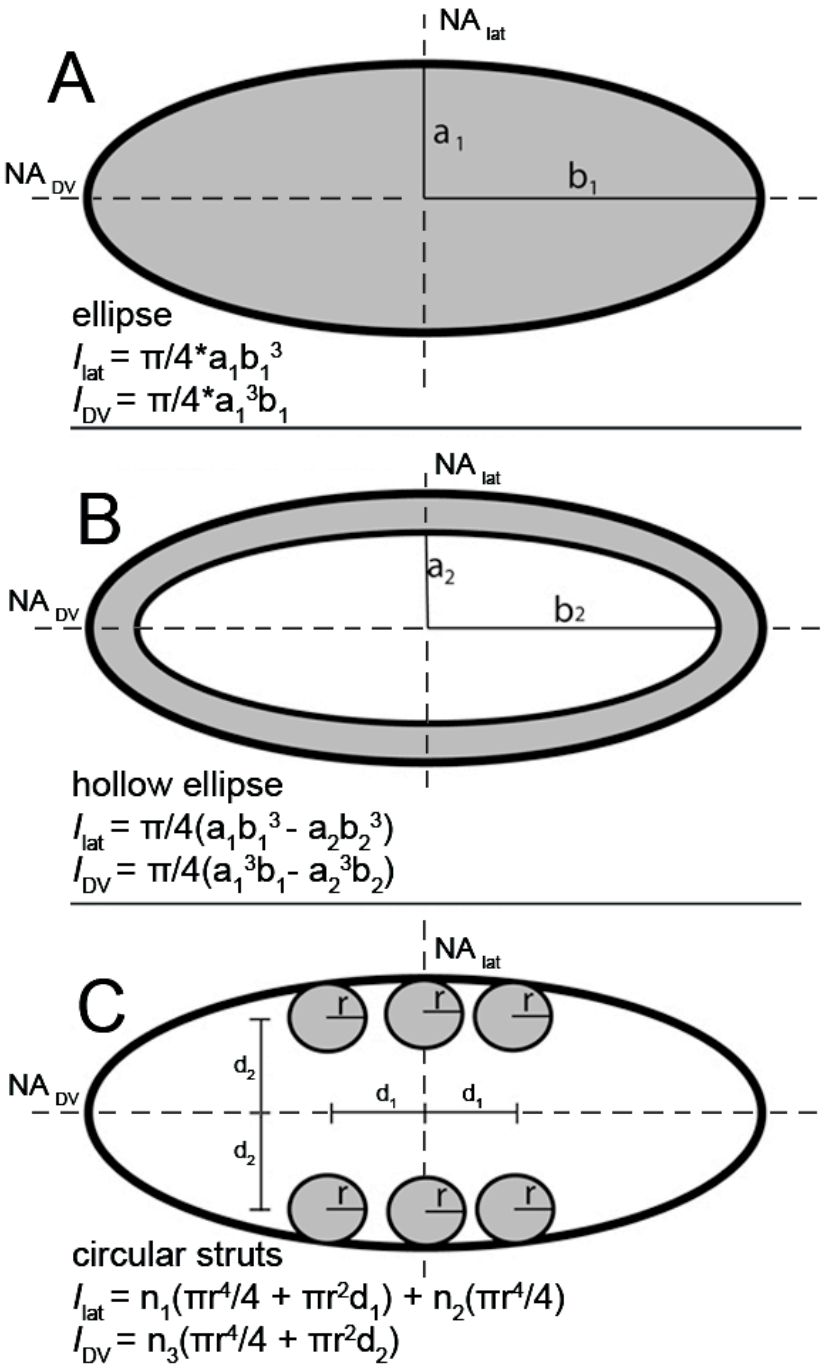

Figure 2.3. Calculations for *I*. The three large ovals represent three example radial CSAs. I is calculated for bending in the lateral plane ($I_{lat}$) and dorso-ventral plane ($I_{DV}$). (A) calculates I for all of the cartilage ignoring calcification; (B) calculates I for only the crustal calcification areas; (C) calculates I for the catenated calcification areas. Note that catenated calcification equations change based on the number and location of calcified circles. In this example, $n_1 = 4$, $n_2 = 2$, $n_3 = 6$, $n_4 = 0$. NA = neutral axis.

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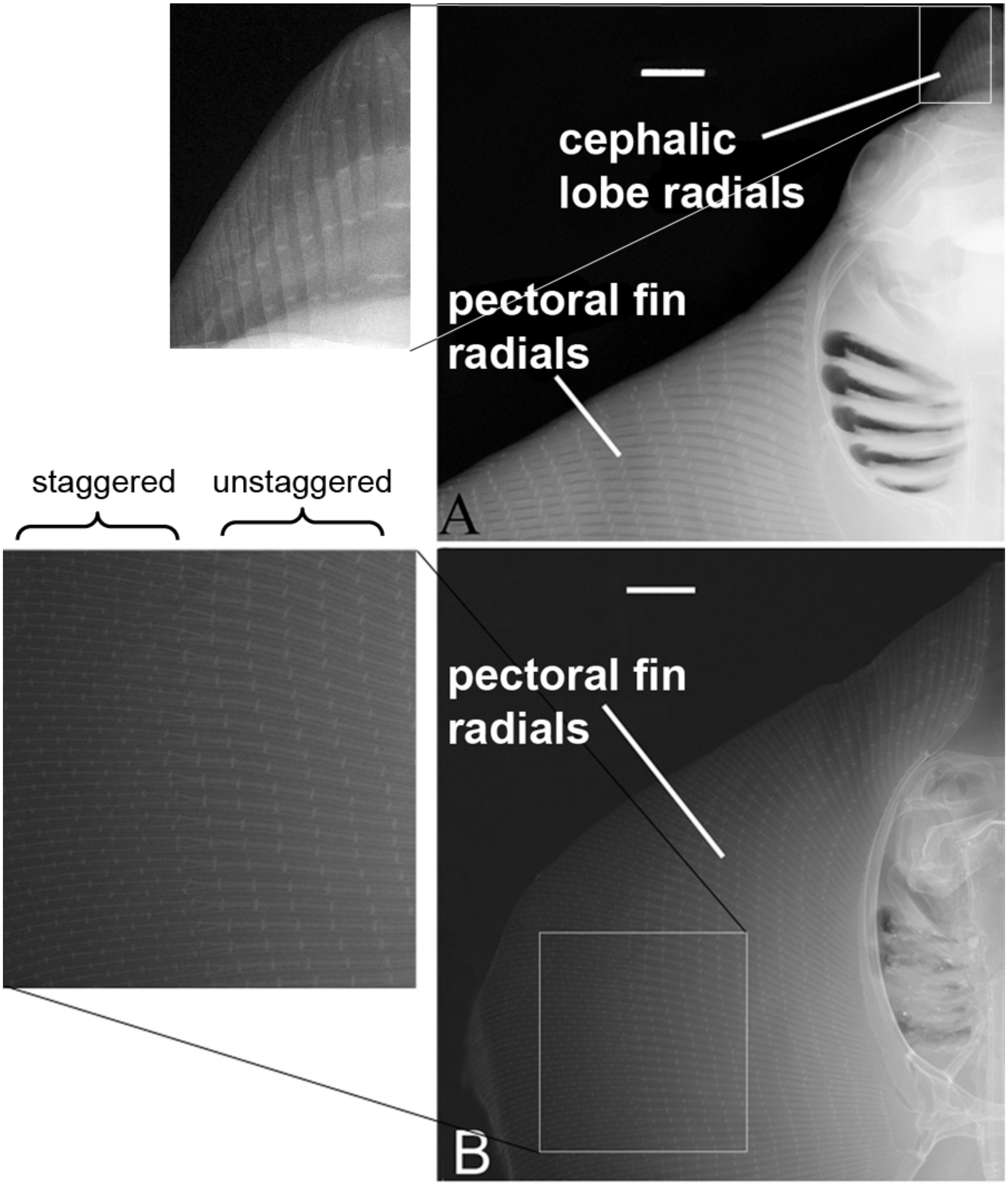

Figure 2.4. Radiographs showing the orientation of the radials of the (A) eagle ray, *A. narinari* pectoral fin and cephalic lobe (upper left); (B) Atlantic stingray, *D. sabina* pectoral fin. Scale bars = 1 cm.

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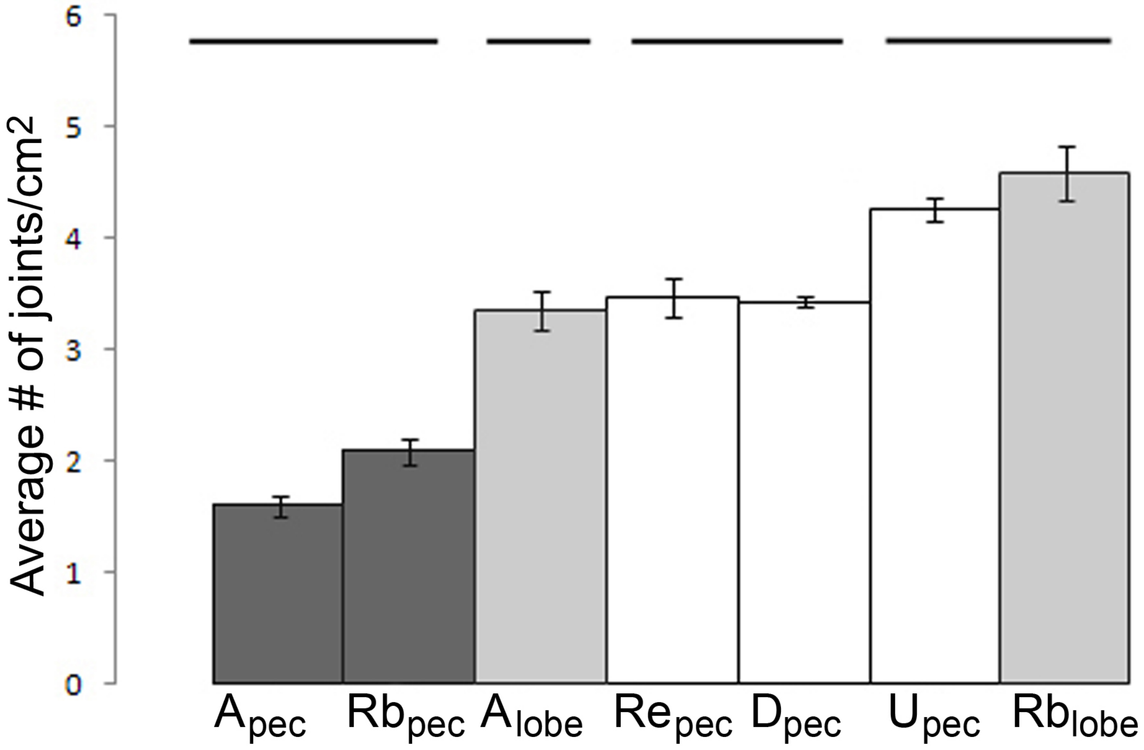

Figure 2.5. Average number of joints/cm$^2$ for the anterior pectoral fins and cephalic lobes. The x axis represents $A_{pec}$ = *A. narinari* anterior pectoral fin, $Rb_{pec}$ = *R. bonasus* anterior pectoral fin, $A_{lobe}$ = *A. narinari* cephalic lobe, $Re_{pec}$ = *R. eglanteria* anterior pectoral fin, $D_{pec}$ = *D. sabina* anterior pectoral fin, $U_{pec}$ = *U. jamaicensis* anterior pectoral fin, and $R_{lobe}$ = *R. bonasus cephalic* lobe. Pectoral fins with oscillatory locomotion = dark grey, pectoral fins with undulatory locomotion = white, cephalic lobes = light grey. Error bars are standard error. Groups within the lines are not significantly different.

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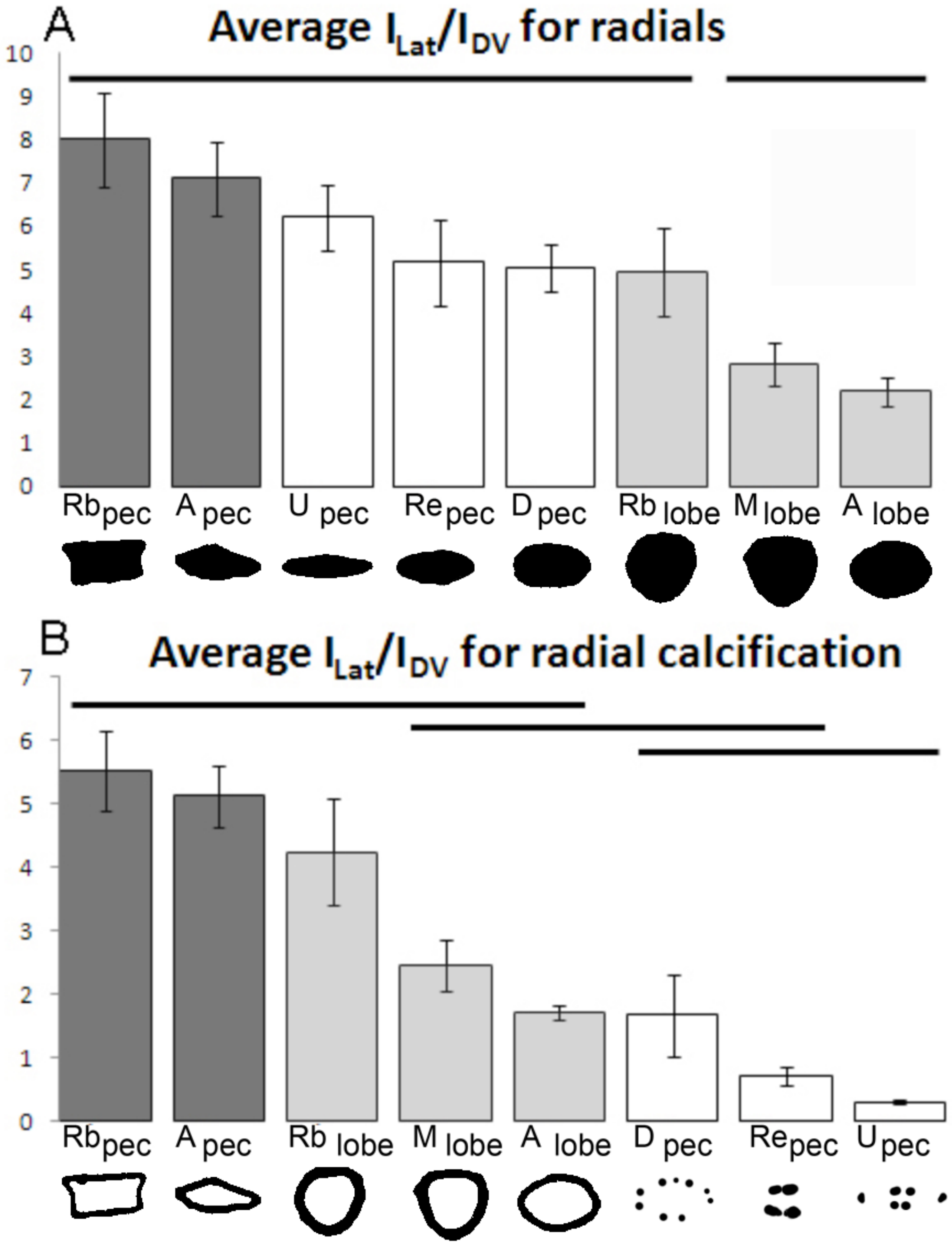

Figure 2.6. Average $I_{Lat}/I_{DV.}$ (A) Average $I_{Lat}/I_{DV}$ for the cross sections of the entire radials and (B) average $I_{Lat}/I_{DV}$ for only the calcified regions of the radials of: $Rb_{pec}$ = *R. bonasus* anterior pectoral fin, $A_{pec}$ = *A. narinari* anterior pectoral fin, $Rb_{lobe}$ = *R. bonasus* cephalic lobe, $M_{lobe}$ = *M. munkiana* cephalic lobe, $A_{lobe}$ = *A. narinari* cephalic lobe, $D_{pec}$ = *D. sabina* anterior pectoral fin, $Re_{pec}$ = *R. eglanteria* anterior pectoral fin, $U_{pec}$ = *U. jamaicensis* anterior pectoral fin. Pectoral fins with oscillatory locomotion = dark grey, pectoral fins with undulatory locomotion = white, cephalic lobes = light grey. Drawings below each bar represent the CSA used to determine the $I_{Lat}/I_{DV}$. Error bars are standard error. Groups within the lines are not significantly different.

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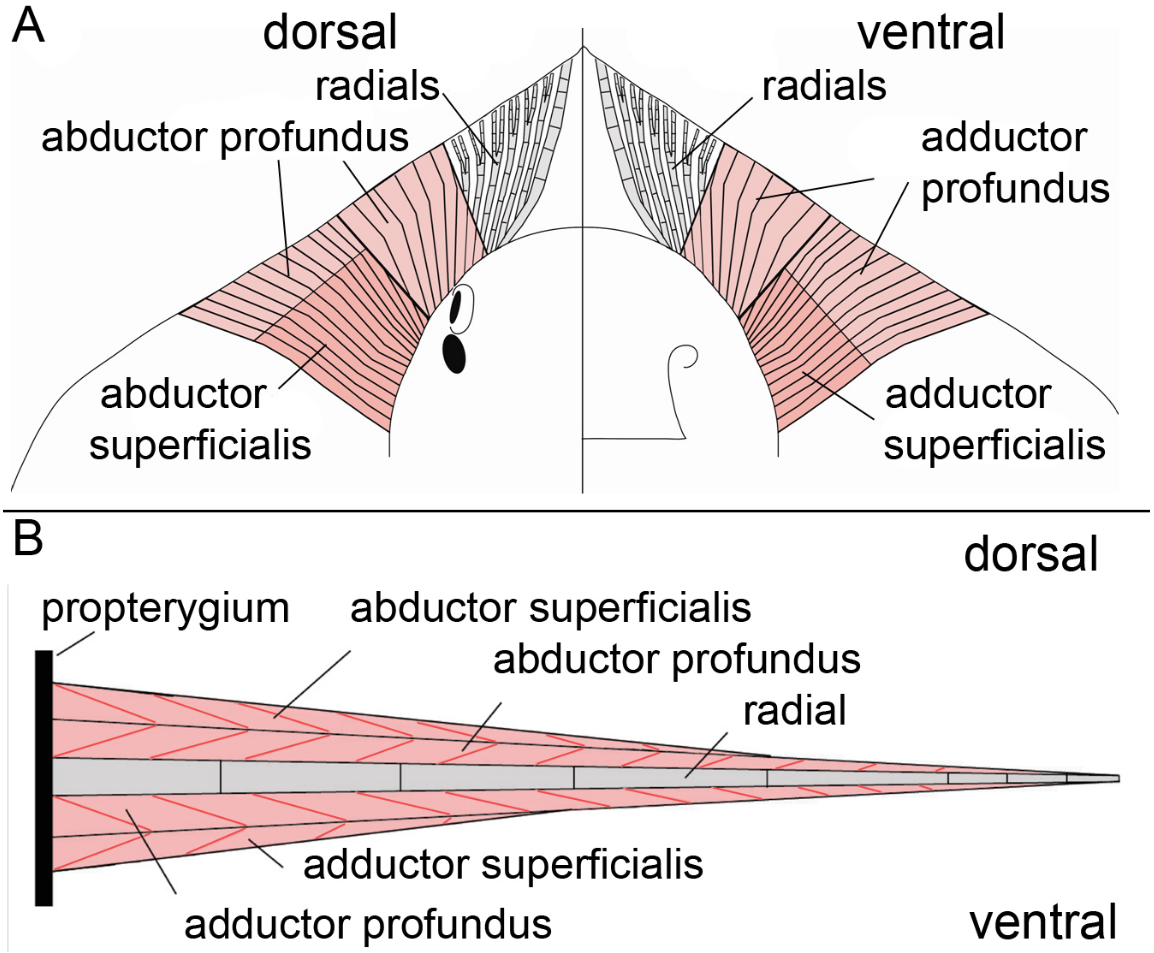

Figure 2.7. Dorsal (left) and ventral (right) views of the pectoral fin musculature in *D. sabina*. The different layers are shown with the lateral and posterior panel as the most superficial layer (A) and a cross sectional view of the pectoral fin of *D. sabina* with dorsal on the top and ventral on the bottom, showing the different muscle layers as well as muscle fiber direction (B).

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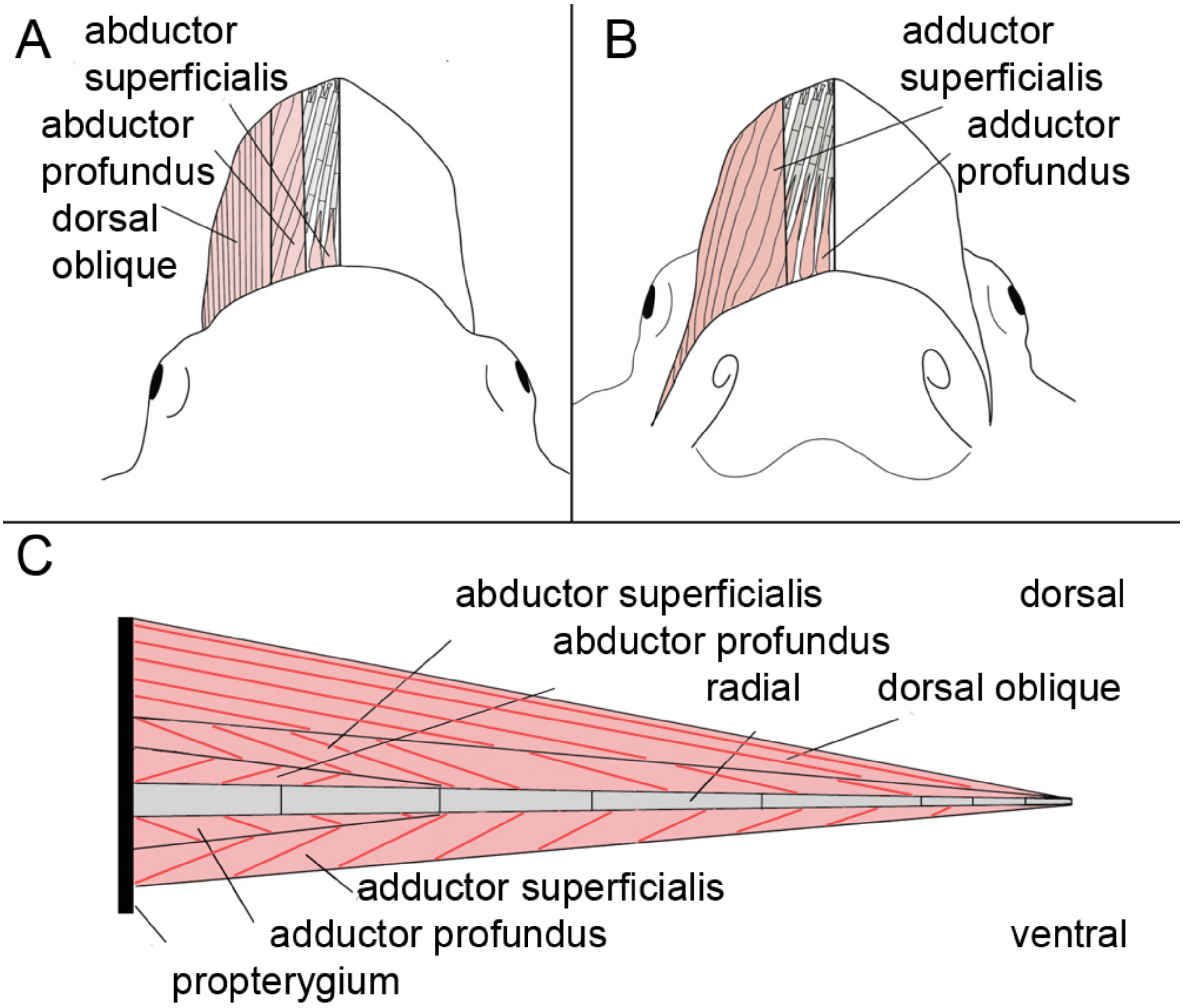

Figure 2.8. Dorsal (A) and ventral (B) views of the cephalic lobe musculature in *A. narinari*. The deepest layer starts medially. Cross sectional view of the cephalic lobe in *A. narinari*, showing the different muscle layers as well as muscle fiber direction (C).

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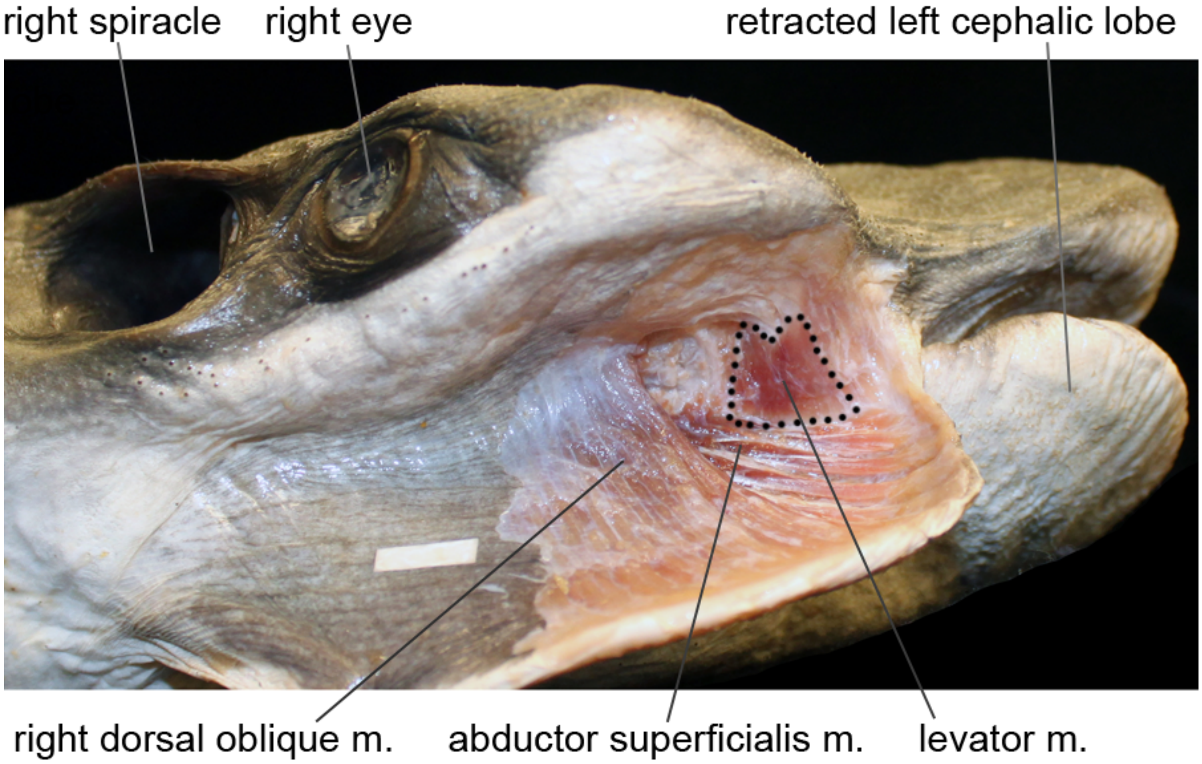

Figure 2.9. Antero-lateral view of the right side of *R. bonasus* with the right cephalic lobe depressed, showing the cephalic lobe levator muscle outlined. The white scale bar is 1 cm.

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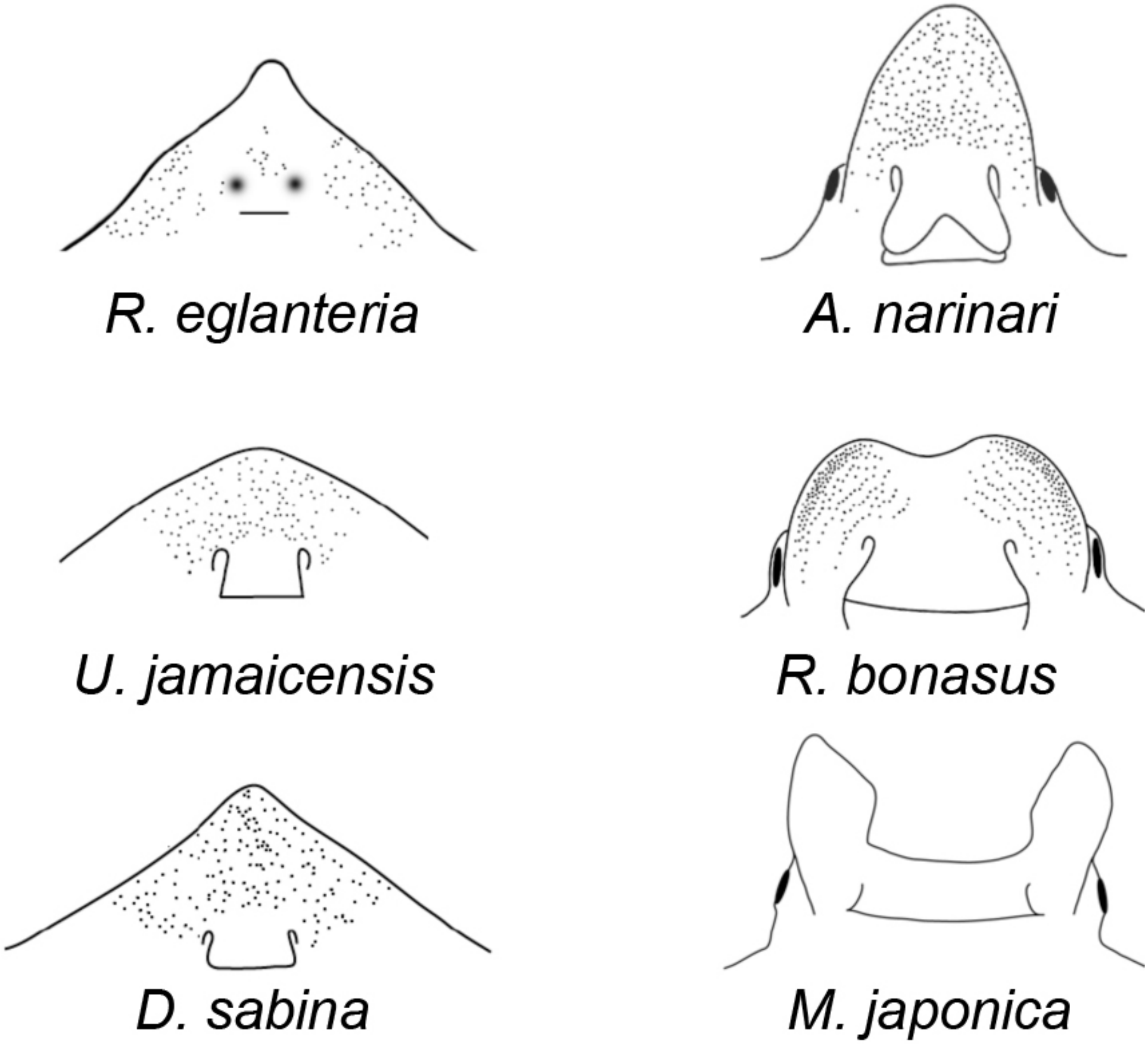

Figure 2.10. Electrosensory pore distribution on the anterior ventral pectoral fins and the ventral cephalic lobes for the six species. Each pore is represented by a black dot.

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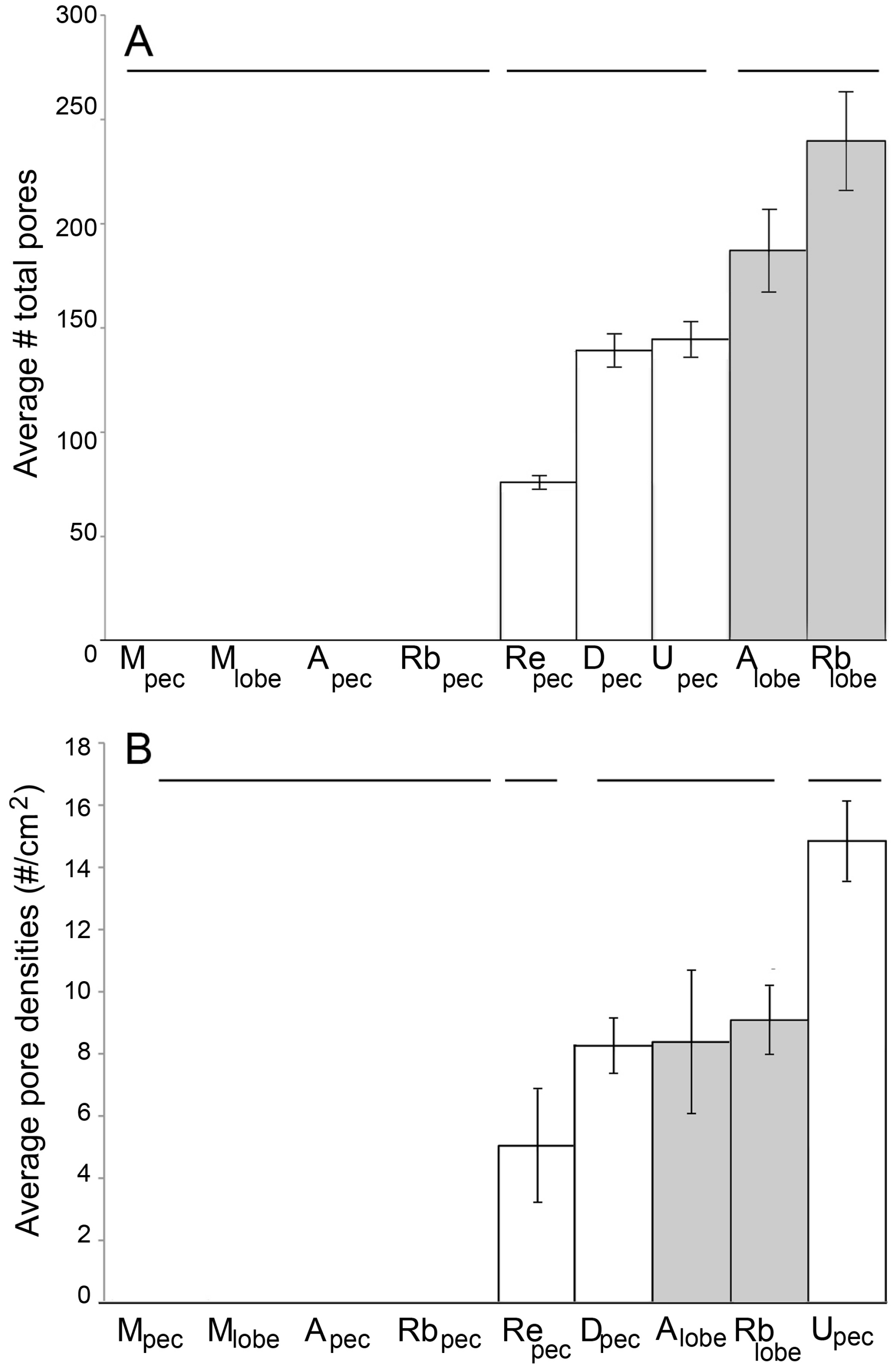

Figure 2.11. The average number of pores for the anterior pectoral fin or cephalic lobes of different species, including standard error (SE). $M_{pec}$ = *Mobula* and *Manta* species pectoral fin, $M_{lobe}$ = *Mobula* and *Manta* species cephalic lobe, $A_{pec}$ = *A. narinari* anterior pectoral fin, $Rb_{pec}$ = *R. bonasus* anterior pectoral fin, $Re_{pec}$ = *R. eglanteria* anterior pectoral fin, $D_{pec}$ = *D. sabina* anterior pectoral fin, $A_{lobe}$ = *A. narinari* cephalic lobe, $Rb_{lobe}$ = *R. bonasus* cephalic lobe, $U_{pec}$ = *U. jamaicensis* anterior pectoral fin. Pectoral fins with undulatory locomotion = white, cephalic lobes = light grey. Groups within the lines are not significantly different.

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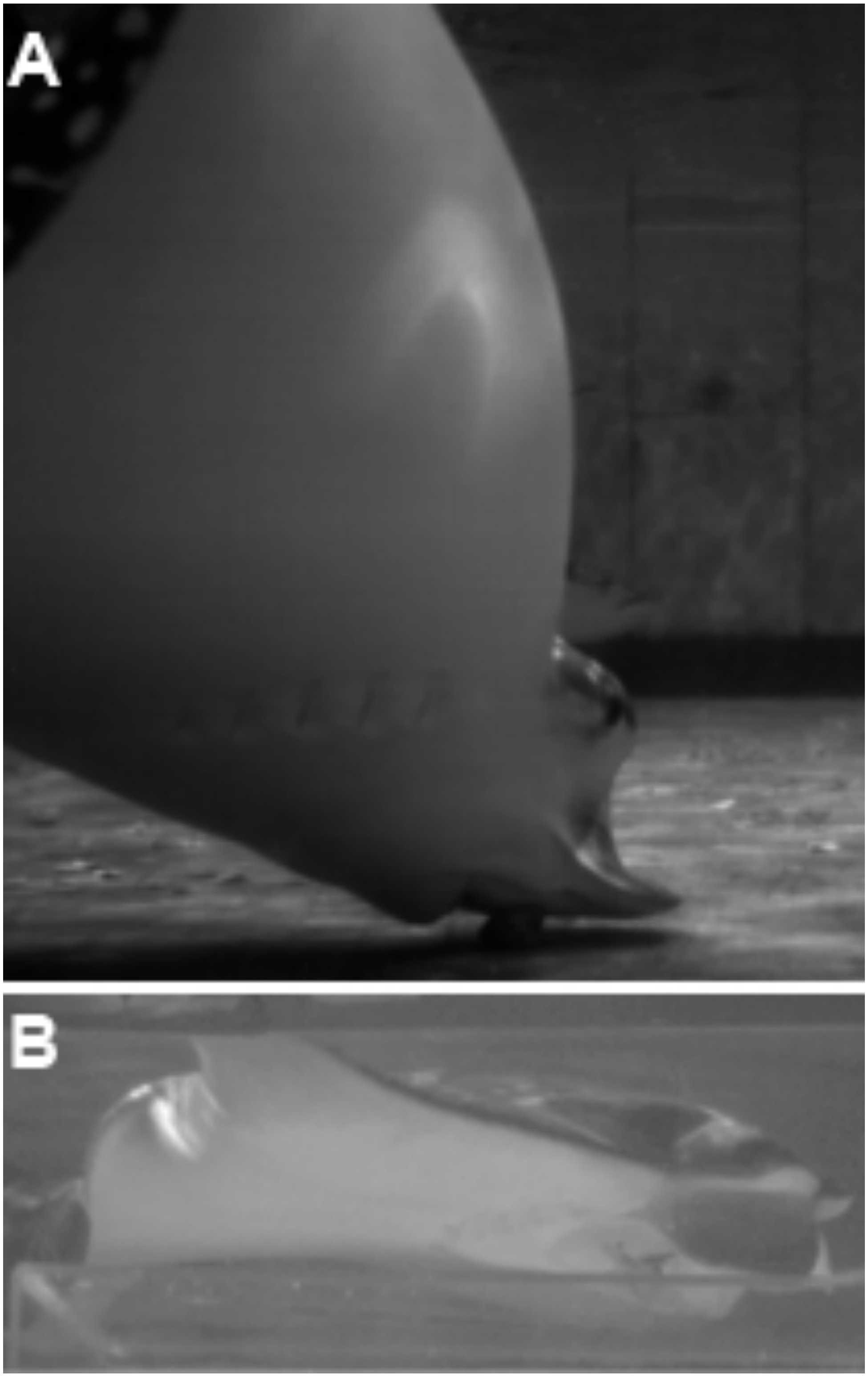

Figure 2.12. Body orientation and cephalic lobe position during feeding in (A) *A. narinari* and (B) *R. bonasus*.

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# CHAPTER THREE: PREY CAPTURE KINEMATICS IN BATOIDS ON DIFFERENT PREY TYPES: THE ROLE OF THE CEPHALIC LOBES

## ABSTRACT

Cephalic lobes are novel structures found in some myliobatid stingrays. While undulatory batoids utilize the pectoral fins for prey capture and locomotion, lobed species partition locomotion to the pectoral fins, utilizing exclusively the lobes for prey capture. We investigated the use of the anterior pectoral fins and cephalic lobes in prey capture in five batoid species. The purpose of this study was to investigate the: 1) prey capture kinematics and use of the cephalic lobes in lobed and lobeless batoids; 2) role of the cephalic lobes in modulating capture behavior based on prey type. It was hypothesized that lobed species would display unique capture behaviors resulting in faster and more successful capture of prey, and display greater modulation in capture behavior. Findings showed that lobed species used only the head region for capture, were faster at pouncing and tenting, but slower at mouth opening. The cephalic lobes were more movable than the anterior pectoral fins of lobeless species. Modulation occurred in all species. Elusive prey increased tent duration for the lobeless species, increased mouth opening duration in the lobed *Aetobatus narinari,* and were farther away from the mouth than non-elusive prey during biting for all species. All species had very few prey escapes. Overall, species with cephalic lobes captured prey faster but did not display increased modulatory ability or feeding success. The cephalic lobes help localize prey capture to the head

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region, speeding up the prey capture event and maintaining an efficient capture rate despite the lack of flexible pectoral fins.

## INTRODUCTION

Novel feeding structures can lead to changes in prey capture and can open up new ecological niches by resolving previously constrained conditions. For example, the evolution of ballistic tongue projection in chameleons and salamanders allows for not only prey capture at greater distances, but also an expanded thermal niche via the ability to capture prey at lower temperatures compared to other lizards (Anderson and Deban, 2010; Deban and Richardson, 2011). Modifications to the upper and lower jaw in loricarioid catfishes, including novel muscle insertions, subdivisions and attachments, results in increased mobility of the premaxillae and independence of functional components of the feeding mechanism, allowing this clade to scrape algae as well as attach to the substrate with an oral sucker (Schaefer and Lauder, '86). Furthermore, novel structures are often associated with increased functional complexity, leading to increased modulation in feeding performance. Multiple subdivisions of the adductor mandibulae jaw muscle complex in tetraodontiform fishes results in novel motor patterns when feeding on different prey types (Turingan and Wainwright, '93).

A group of derived myliobatid rays possess novel structures called cephalic lobes, which may facilitate modifications in prey capture and an expanded ecological niche. These novel appendages are derived from the anterior pectoral fins (Bigelow and Schroeder, '53; Nishida, '90; Miyake et al., '92). The lobes have an additional dorsal muscle layer, with muscles running oblique to the skeletal components, along with more circular supportive cartilaginous radials

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compared to those of the pectoral fins (Mulvany and Motta, 2013). While lobes in some species are stiff and immovable, in many other species they are used to help excavate, grasp/cup prey, maneuver prey into the mouth (Sasko et al., 2006), and can even furl and unfurl, as seen in *Manta birostris (*Notarbartolo-di-Sciara and Hillyer, '89). Electrosensory pores are present on the ventral surface of the cephalic lobes and are thought to aid in prey detection (Mulvany and Motta, 2013). While cephalic lobes are considered primarily to aid in feeding (Moss, '77; Sasko et al., 2006), shifts in habitat and locomotor patterns coincide with the appearance of the cephalic lobes (Fig. 3.1).

Basal batoids that lack cephalic lobes are benthic and exhibit undulatory locomotion (waves traveling posteriorly along the pectoral fins) while derived batoids with cephalic lobes are more pelagic and exhibit predominantly oscillatory locomotion (repeated depression and elevation of the pectoral fins in a flapping motion) (Rosenberger, 2001; Schaefer and Summers, 2005; Sasko et al., 2006). Undulatory species have high maneuverability close to the substrate, but lack the ability to travel extended distances (Rosenberger, 2001). Oscillatory species possess stiffer pectoral fins (Schaefer and Summers, 2005; Mulvany and Motta, 2013), which aid in travelling long distances, though maneuverability is decreased, particularly close to the substrate. Despite these differences, undulatory and oscillatory batoids often feed on similar prey, facilitating comparisons of prey capture and handling.

The majority of batoids feed largely upon benthic or epi-benthic organisms, such as polychaetes and bivalves, as well as more elusive prey (e.g. shrimp or fish) (Smith and Merriner, '85; Michael, '93; Compagno, '97; Ebert and Cowley, 2003; Ebert and Bizzarro, 2007; Collins et al., 2007; Ajemian and Powers, 2012; Jacobsen and Bennett, 2013). Batoids typically pounce on prey, pinning them against the substrate, and use their pectoral fins and body to form a tent over

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the prey to prevent escape (Lowe et al., ‘94; Wilga and Motta, ‘98; Wilga et al., 2012), followed by suction feeding after the prey is positioned near the mouth. Often times, the pectoral fins aid in prey excavation, manipulation of prey toward the mouth and winnowing to separate the prey from the substrate (Lowe et al., ‘94; Maruska and Tricas, ‘98; Dean and Motta, 2004; Wilga et al., 2012). In undulatory batoids, maneuverability during feeding and locomotion are achieved through the flexible pectoral fins. In oscillatory species, the pectoral fins are much less flexible, thus the highly maneuverable cephalic lobes are used in feeding while the pectoral fins are primarily used for locomotion (Smith and Merriner, ‘85; Sasko et al., 2006; Mulvany and Motta, 2013). The use of the novel cephalic lobes may result in unique feeding behaviors, resulting in increased versatility or modulation of prey capture in these derived batoids.

The ability to modulate prey capture behavior can affect feeding success and also expand the diversity of prey. Modulation can be defined as the active modification of movements by the nervous system in response to a changing variable (Liem, ‘78; Deban et al., 2001). The ability of a predator, in this case batoids, to change their feeding behavior in function based on the type or position of the prey constitutes modulation (Van Wassenbergh et al., 2006). Numerous bony fishes are capable of modulating feeding behavior in response to differing stimuli: prey types, prey presentations, elusivity, or changes in environmental conditions (Liem, ‘78; Turingan and Wainwright, ‘93; Frost and Sanford, ‘99; Liem and Summers, 2000; Wainwright and Friel, 2000; Alfaro et al., 2001; Ferry-Graham et al., 2001; Van Wassenbergh and De Rechter, 2011; Gardiner and Motta, 2012). Elasmobranchs are less well studied than bony fish in this regard. Some carcharhinid sharks have shown the ability to modulate feeding behavior based on prey type, size or presentation (Moss ‘72, Tricas and McCosker ‘84, Frazzetta and Prange ‘87; Motta et al., ‘97), although most specialized suction-feeding sharks displayed less modulatory ability

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(Ferry-Graham, ‘97; Ferry-Graham, ‘98; Edmonds et al., 2002; Motta et al., 2002: Matott and Motta, 2005). Indeed, some of the few feeding kinematic studies on batoids illustrate modulation in feeding behavior. For example, modulation in recruitment of muscles that depress the mandible and hyoid during feeding was found in the guitarfish, *Rhinobatos lentiginosus* (Wilga and Motta, ‘98). The little skate, *Leucoraja erinacea,* increases the degree of asynchrony in muscle activation with prey that requires manipulation and processing (Gerry et al., 2008), while the lesser electric ray, *Narcine brasiliensis,* can modify the degree and direction of jaw protrusion during predatory striking and processing (Dean and Motta, 2004). However, modulation in prey capture behavior due to elusive and non-elusive prey types has yet to be investigated in batoids, as well as the role of the pectoral fins or cephalic lobes.

The purpose of this study is to elucidate the role of the cephalic lobes in prey capture behavior and specifically to investigate: 1) the prey capture kinematics of a group of representative batoids that possess and lack cephalic lobes, and 2) the role of the novel cephalic lobes in diversifying and modulating prey capture behavior based on prey type. I hypothesized that the highly maneuverable cephalic lobes would decrease the time required to manipulate prey toward the mouth, that batoids with cephalic lobes would be more successful in preventing prey escape, and that lobed species would demonstrate greater modulation in their capture behavior.

## METHODS

The five species under investigation were the clearnose skate, *Raja eglanteria* (Bosc, 1800); yellow stingray, *Urobatis jamaicensis* (Cuvier, 1816); Atlantic stingray *Dasyatis sabina* (Lesueur, 1824); spotted eagle ray *Aetobatus narinari* (Euphrasen, 1790); and cownose ray

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*Rhinoptera bonasus* (Mitchill, 1815) (Fig. 3.1, Table 3.1). These species are found in the Atlantic Ocean (Bigelow and Schroeder '53; Smith, '97) and their diets all include mollusks, polychaetes, and crustaceans (Bigelow and Schroeder '53; Stehmann and McEachran, '78; Michael, '93; Compagno, '97; Summers, 2000; Jardas et al., 2004; Sasko et al. 2006; Collins et al., 2007).

Animals were collected in Florida from Tampa Bay, Lake Monroe of the St. John's River, the waters off the Florida Keys or the waters near Sarasota Bay. Batoids were either housed in a ~18,000 liter display tank at the Florida Aquarium, in a ~200 liter display tank or a ~151,500 liter holding tank at Mote Marine Laboratory, or in a ~3,700 liter holding tank at the University of South Florida (Tampa, FL). Animals were fed three times a week to satiation with cut Atlantic thread herring, *Opisthonema oglinum*, veined squid, *Loligo forbesi*, live hard clams, *Mercenaria mercenaria*, or pink shrimp, *Penaeus sp.* The prey used in experiments was determined by the regulations of the facilities that housed the batoids, as well as the willingness of the batoids to feed on certain prey in captivity. Salinity was maintained at 31-34 ‰ and temperature at 21-24°C. Experimental procedures for all animals took place during regular feeding times and all prey items were slightly less than the width of the ray's mouth width. For each species, five individuals were imaged ten times for each feeding treatment. A Photron Fastcam 512PCI camera was used to image all species at 125 Hz. Only the first five feeding per imaging day were used to avoid effects of satiation (Sass and Motta, 2002).

During imaging, *R. eglanteria, U. jamaicensis* or *D. sabina* were individually placed in a 60cm x 90cm tank. A Plexiglas box with 45° mirror was placed under the tank to capture both lateral and ventral views simultaneously. Lateral views angled approximately 10° or more to the

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imaging plane were not analyzed. Live, loose ghost shrimp, *Palaeomonetes sp.* were used for elusive prey and pieces of *L. forbesi* were used for non-elusive prey.

*Aetobatus narinari* were imaged in ~151,500 liter tank with a viewing window on one side. Lateral views were imaged with a Photron Fastcam 512PCI camera, while a Sony JVC DVL 9800u high-speed camcorder was used to obtain dorsal views of the eagle rays at 125 Hz, although the videos were not synchronized. *Mercenaria mercenaria* (intact) was used for non-elusive prey. It was not possible to train the *A. narinari* to feed on live shrimp, therefore *M. mercenaria* were tied to a cotton string and haphazardly jerked about 5-15cm across the substrate every 1-3 seconds to mimic elusive prey.

*Rhinoptera bonasus* was imaged in a ~18,000 liter holding tank. A Plexiglas box with 45° mirror was placed in the holding tank to capture both lateral and ventral views simultaneously. Non-elusive prey consisted of dead *Penaeus sp., O. oglinum* and *L. forbesi.* Live *Penaeus sp.* was used as elusive prey. The shrimp were tethered at their thorax to the center of the mirror box using thin strands (~1 mm) of seaweed approximately 30 cm long (species unknown) so that the shrimp were free to move about the length of the mirror box, but not outside of the imaging area.

### *Kinematic capture variables*

Thirteen kinematic variables were calculated for each of prey capture trials using MaxTRAQ v.1.87 software: (1) pounce duration (beginning of the prey capture event as defined by the onset of cephalic lobe depression to the time of maximum cephalic lobe depression in species that possess cephalic lobes; or the onset of rostrum elevation to the time of rostrum

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contact with the substrate in lobeless species); (2) tenting duration (time from the rostrum/lobes touching the substrate to the time the mouth begins to open for a successful bite). During tenting, the batoid is over the prey with its cephalic lobes and/or pectoral fins depressed against the substrate around the prey, preventing escape. The tenting duration measures the amount of time spent manipulating the prey item before successfully consuming the prey (keeping prey trapped, moving prey toward mouth, and even unsuccessful biting attempts prior to consumption); (3) mouth opening duration (time from mouth opening of a successful bite to the last piece of prey entering mouth); (4) mouth closing duration (time of last piece of prey entering mouth to mouth closing); (5) bite duration (from the onset of mouth opening to the time the mouth closes, durations 3 and 4 combined); and (6) time of the prey capture event (durations 1-5 combined). Variables to quantify movement of the cephalic lobes or anterior pectoral fins during a prey capture event included: (7) vertical movement (angle of movement of the tips of the cephalic lobes or rostrum in the vertical plane); (8) horizontal movement (angle of movement of the tips of the cephalic lobes or rostrum in the horizontal plane). Angles were taken by measuring the difference between the tips of the lobes or rostrum in resting position and when maximally depressed or elevated, using the position where the radials pivot on the propterygium as the vertex (Fig. 3.2). Other variables to analyze capture success included: (9) presence/absence of tenting behavior; (10) number of times prey escaped during pouncing; (11) number of times prey escaped during tenting; (12) number of times prey escaped after being grasped by the mouth; and (13) total number of bite attempts (mouth openings) by the batoid.

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### *Mapping distance of prey*

Each attempt at consuming a prey item (a mouth opening), successful or unsuccessful, was recorded and used to create a distance map from the prey to the mouth of each batoid using SigmaScan Pro v4.01.003 (SPSS Inc., Chicago, IL). Still pictures of ventral views were captured from the image sequences at the onset of mouth opening, and the distance from the center of the mouth to the center of the prey was measured for each bite. A line was drawn down the midsagittal plane of the animal to divide the left and right side of the batoid. A perpendicular line was drawn through the mouth to divide the bites anterior and posterior to the mouth. For each bite, prey type, bite success, the distance from the mouth and position of the bite (left/right side and posterior/anterior end) was recorded. The disc width of the batoid was also measured for each image. Because ventral views were not obtained for *A. narinari,* prey distance data were not obtained.

### *Statistics*

Five individuals per species were imaged. For each individual, 10 prey capture events with elusive prey and 10 prey capture events with non-elusive prey were imaged. To avoid pseudoreplication, the ten events in each category were averaged to provide an estimate of a prey capture event for each individual. A multiple regression was used to regress kinematic variables against disc width to determine if any variables correlated with size, as some studies have shown increases in duration variables with increased size (Richard and Wainwright, ‘95; Hernandez, 2000; Robinson and Motta, 2002; Deban and O’Reilly, 2005). Only mouth closing duration was found to correlate with size so this variable was regressed against disc width and the standard

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residuals were used for analysis. Since the same individuals were fed both elusive and non-elusive prey items, kinematic data were analyzed using a 2-way repeated measures ANOVA, investigating differences among species, between prey types and interactions. Data that failed the Shapiro-Wilk test for normality and the Levene median test for equality of variance test were log10 transformed and retested. To correct for multiple comparisons, a Benjamini-Hochberg false discovery rate control was used to ensure a p-value of 0.05 (Benjamini and Hochberg, '95). A Tukey's post hoc test was used determine which specific variables significantly differed.

A regression of prey distance measurements (from prey to the center of the mouth at the start of mouth opening) against disc width was performed to remove the effect of size among all species and the standard residuals were entered into a 3-way ANOVA to determine any differences among prey type, species and biting success. Analyses were conducted with SigmaStat v. 3.1 (SYSTAT Software, San Jose, CA). Animal use for the study was approved by the University of South Florida Institutional Animal Care and Use Committee (IACUC # W3565, W2959) and Mote Marine Laboratory Institutional Animal Care and Use Committee (IACUC # 08-04-PM2,10-03-PM1).

## RESULTS

Prey capture events, both elusive and non-elusive, were always initiated while the batoid was locomoting above the prey for *R. bonasus* and *A. narinari*, while *D. sabina, U. jamaicensis* and *R. eglanteria* were often sedentary on the substrate at the onset of prey capture. Species with cephalic lobes tended to use just the head to tent prey, depressing and fanning out the cephalic lobes during the entire prey capture event. Species lacking lobes used the entire body to capture

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prey, initially elevating the rostrum or entire body to swim over prey before depressing the pectoral fins around the prey using the entire body (Fig. 3.3). Prey were consumed using suction, biting or a combination of both, often with the batoid maneuvering its mouth closer to the prey. The body of *A. narinari* was noticeably pitched forward during all captures, with the head level to the substrate. *Rhinoptera bonasus* was noted to either capture prey with the body pitched forward or with the body level to the substrate. All other batoids maintained a level body position relative to the substrate during prey capture.

### *Prey capture kinematics*

In general, kinematic results showed that species with cephalic lobes had shorter pounce and tent durations, longer mouth opening and closing durations, and overall faster capture events. Pounce duration was not affected by prey type ($p > 0.05$) but showed differences among species after a false discovery rate correction ($p = 0.001$, adjusted critical value = 0.004). *Raja eglanteria* had a significantly longer pounce duration than *A. narinari* and *R. bonasus* ($p < 0.012$) (Fig. 3.4; Table 3.2). Tenting duration showed species differences ($p = .001$, adjusted critical value = 0.008), with *R. eglanteria* and *U. jamaicensis* tenting significantly longer than *D. sabina, R. bonasus* and *A. narinari* ($p < 0.031$). Prey type affected tenting duration ($p = 0.005$, adjusted critical value = 0.013), with *R. eglanteria* and *U. jamaicensis* spending more time tenting elusive prey ($p = 0.005$) compared to non-elusive prey. Mouth opening duration showed species differences ($p = 0.001$, adjusted critical value = 0.013), with greater durations in *R. bonasus, A. narinari* and *U. jamaicensis* compared to other two species ($p = 0.039$ and 0.046). Between prey types, *A. narinari* mouth opening was significantly slower with elusive prey ($p = 0.013$). After

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removing the effect of size from mouth closing duration, no significant differences among species or between prey type were found ($p > 0.05$). When looking at bite duration, prey type had no effect ($p > 0.05$), but there was a difference among species ($p = 0.004$, adjusted critical value = 0.029); *Rhinoptera bonasus* had a significantly longer bite than *R. eglanteria* and *U. jamaicensis* ($p < 0.05$). The overall prey capture event was significantly different among species ($p = 0.001$, adjusted critical value = 0.017), shorter for *A. narinari* and *R. bonasus* compared to *R. eglanteria* and *U. jamaicensis* ($p < 0.026$). Elusive prey increased the overall capture event duration for *R. eglanteria* and *U. jamaicensis* compared to non-elusive prey ($p < 0.05$). The mixed interactions (Table 3.2C) showed no significant differences among species and prey type combined ($p < 0.05$).

Variation in the range of motion of the rostrum or cephalic lobes was found (Fig. 3.5). Movement of the cephalic lobes and rostrum in the vertical plane did not vary by prey type ($p = 0.357$) but was significantly different among species ($p = 0.001$, adjusted critical value = 0.025). *Rhinoptera bonasus* cephalic lobes had a significantly higher angle of vertical movement compared to all other species ($p < 0.05$), with an average angle between 80-90˚. *Aetobatus narinari, D. sabina* and *U. jamaicensis* grouped together, displaying angles around 30-40˚ while *R. eglanteria* did not display any vertical rostral movement. For movement in the horizontal plane, *R. bonasus* showed a significant difference in prey type ($p < 0.05$), with more motion when capturing non-elusive prey. *Rhinoptera bonasus* had the largest range of horizontal motion of the cephalic lobes ($p < 0.05$), while *A. narinari* showed significantly less movement ($p < 0.05$), and the remaining species did not display any horizontal movements.

Tenting behavior was present in all species for every prey capture event. The total number of bites taken for each capture event showed no significant difference among species or

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between prey type (Fig 3.6A), with all species averaging roughly 2 to 3 bites per capture event. No significant difference in number of pounce, tent and mouth escapes for all species were found ($p > 0.05$), with all escape averages below 1 escape per capture event for elusive prey (Fig. 3.6B-D).

### *Mapping distance of prey*

Analysis of prey location during mouth opening (successful and unsuccessful bites) revealed significant species differences, with *R. eglanteria* biting when prey items were closer to the mouth ($p\ 0 < .005$) compared to other species (Fig 3.7; Table 3.3). For all species, elusive prey were farther away from the mouth during mouth opening ($p < 0.001$) and successful bites were closer to the mouth compared to unsuccessful bites ($p < 0.001$). No left/right side differences were found ($p > 0.05$), whereas anterior/posterior differences were found among species ($p < 0.001$). *Urobatis jamaicensis* bit more frequently when prey items were posterior to the mouth ($p < 0.001$) while *D. sabina* and *R. eglanteria* frequently bit when prey items were anterior to the mouth ($p \leq 0.008$) and *R. bonasus* did not show any anterior/posterior preference ($p \leq 0.003$). For elusive prey ($p = 0.005$), non-elusive prey ($p < 0.001$), successful bites ($p \leq 0.043$) and unsuccessful bites ($p \leq 0.006$), *U. jamaicensis* consistently showed a preference for biting when prey were posterior to the mouth. *Raja eglanteria* and *D. sabina* often bit when elusive prey ($p = 0.005$) and non-elusive prey ($p < 0.001$) were anterior to the mouth for successful ($p \leq 0.043$) and unsuccessful ($p \leq 0.048$) bite attempts. *Rhinoptera bonasus* tended to bite when elusive prey were posterior to the mouth ($p \leq 0.033$), and when non-elusive prey were

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anterior of the mouth ($p < 0.001$). Successful bites for *R. bonasus* occurred more anterior to the mouth ($p = 0.043$) while there was no preference for unsuccessful bites ($p \leq 0.048$).

## DISCUSSION

We have found marked differences in prey capture behavior among these batoid species and some differences in capture behavior based on prey type. Overall, species with cephalic lobes localize tenting to the head region, utilize the cephalic lobes to prevent prey escapes, and decrease the duration of a prey capture event. Species that lack cephalic lobes utilize the entire body to subdue prey and overall take a longer time to complete a prey capture event. However, no difference in success of prey capture was observed between lobed and lobeless species in this experimental setting. Some modulation driven by prey type was seen in all batoids, but species with cephalic lobes did not demonstrate overall greater modulation of prey capture kinematics as hypothesized.

### *Cephalic lobes and prey capture kinematics*

It was hypothesized that the cephalic lobes would decrease the time needed to manipulate prey toward the mouth. Overall, this was true, as lobed species tended to pounce and tent prey faster (Fig. 3.4). The faster pouncing was possibly a result of a more mobile lifestyle of these batoids, while lobeless species tended to pounce from a stationary position. *R. bonasus* and *A. narinari* initiated pouncing while cruising, which may increase the velocity of pouncing. As lobed species are more pelagic (Lovejoy, ‘96; Rosenberger, 2001), pouncing was initiated from

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above the prey. The more benthic species, *R. eglanteria, U. jamaicensis* and *D. sabina,* swam up and over prey before descending to form a tent over the prey, possibly increasing pounce duration.

Tenting durations, which include prey handling, were faster for lobed species (Fig. 3.4). Because lobed species are oscillatory swimmers (Rosenberger, 2001), their pectoral fins are stiffer and less maneuverable (Schaefer and Summers, 2005; Mulvany and Motta, 2013), making them less efficient in tenting with their pectoral fins compared to undulatory swimmers with flexible pectoral fins. These oscillatory swimmers avoid this dilemma by localizing prey capture to the head region and utilizing the movable cephalic lobes to tent prey, perhaps decreasing prey handling time by decreasing the tenting area. Species with cephalic lobes might also have an advantage at pinpointing prey once it is tented. Electrosensory receptors are found on all the examined species (Chu and Wen, ‘79; Bedore et al., 2013; Mulvany and Motta, 2013), but with a dorso-ventrally depressed body, batoids are limited to sensitivity in the horizontal plane (Tricas and Sisnero, 2004), as the receptors are all in one plane. Depressing the cephalic lobes, which are covered in electrosensory pores (Chu and Wen, ‘79; Mulvany and Motta, 2013), may help to create a more three-dimensional sensory field by positioning electrosensory canals in a vertical plane, while the other canals remain in a horizontal plane on the body. The high density of pores on the cephalic lobes may also help pinpoint prey by increasing resolution (Raschi, ’86; Bedore et al., 2013).

Despite size differences among species, the only kinematic variable that correlated with size was mouth closing duration. After removing the effect of size, no differences in mouth closing were seen among species or between prey types. Mouth opening and bite duration, however, were slower in lobed species compared to lobeless species (Fig. 3.4). *Raja eglanteria,*

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*U. jamaicensis* and *D. sabina* had bite duration values comparable to other batoid feeding kinematic studies (Wilga and Motta, '98; Dean and Motta, 2004) while species with cephalic lobes, *R. bonasus* and *A. narinari,* took almost twice as long to open and close their mouth. It is possible that *A. narinari*, in particular, relies more on biting and less on suction during feeding, as suction feeding requires rapid jaw expansion to generate negative pressure (Lauder, '85, Holzman et al., 2012). In addition, *R. bonasus* and *A. narinari* are known to feed on hard prey, such as bivalves and crustaceans, as well as polychaetes, fish and squid (Smith and Merriner, '85; Jardas et al., 2004; Collins et al., 2007), which may lessen the need for rapid jaw expansion (Alfaro et al., 2001). The jaws of durophagous species may also be more biomechanically force efficient than speed efficient (Turingan et al., '95; Huber et al., 2005; Westneat, 2006).

Movement of the cephalic lobes and/or anterior pectoral fins in the vertical plane was prevalent for all species except *R. eglanteria* (Fig. 3.5A). *Raja eglanteria* has panes of stiff rostral tissue on either side of the rostrum (Smith, '97; McComb and Kajiura 2008), which, to our knowledge, has not been investigated in detail. Only a small amount of the anterior pectoral fins extend anterior to the mouth, lateral to these panes, perhaps accounting for the inflexibility of the rostrum in both the horizontal and vertical plane. Pouncing and tenting durations were the longest for *R. eglanteria* (Fig. 3.4), presumably because of this inflexibility of the rostrum. Instead of moving the rostrum, *R. eglanteria* maneuvered its whole body to trap prey. The anterior pectoral fins or cephalic lobes in other batoid species extended well beyond the mouth (Schaefer and Summers, 2005; Mulvany and Motta, 2013), allowing movement in the vertical plane. Lobed species depressed the lobes to search and trap prey against the substrate (Fig. 3.3D-E), elevating the lobes after consuming prey. Lobeless species utilized the anterior pectoral fins

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to elevate the rostrum up over the prey and depress the rostrum to trap the prey against the substrate (Fig. 3.3A-C).

Lobeless species exhibited no anterior pectoral fin movement in the horizontal plane, or fanning out. As the pectoral fins extend in all directions from the body, horizontal movement may not be possible. The pectoral fins can depress against the substrate, sufficient to fully surround prey under the body without any horizontal movement. Since the cephalic lobes are distinct from the pectoral fins in *R. bonasus* and *A. narinari,* there is a physical gap between the fins and the lobes where prey could escape. As the cephalic lobes are depressed, they undergo horizontal movement, helping to occlude the lateral portion of the head as well as the anterior portion. The radials (skeletal elements of the lobes and fins) of the *R. bonasus* cephalic lobes are rounded at the proximal ends and attach to the propterygium via round sockets (Mulvany and Motta, 2013) conferring flexibility to the cephalic lobes.

The distance of the prey to the mouth during biting (mouth opening for successful and unsuccessful bites combined) was significantly closer for *R. eglanteria* compared to other species examined (no data for *A. narinari*) (Fig. 3.7, Table 3.3). While the tenting duration of *R. eglanteria* was the greatest of the species examined, there was no difference in the number of bites taken to ingest prey, indicating that *R. eglanteria* bit relatively less often than other batoids, seemingly waiting until prey were very close to the mouth before striking. Mouth opening has been linked to electroreception in sharks (Gardiner et al., in prep). Compared to other species in this study, *R. eglanteria* has relatively fewer electrosensory pores on the anterior pectoral fins (Chu and Wen, ‘79; Mulvany and Motta, 2013) and mouth opening cues may depend primarily on the ampullae surrounding the mouth, where the pores are highly concentrated (Chu and Wen, ‘79; Montgomery and Bodznick, ‘99). The other batoid species might detect prey and engage

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biting behavior when prey is farther from the mouth because of numerous electrosensory pores on the pectoral fins and cephalic lobes.

Elusive prey were farther away from the mouth for all species when biting occurred (Fig. 3.7; Table 3.3). Maneuvering elusive prey toward the mouth is complicated, as batoids move prey toward the mouth by manipulating water flow under the body (Wilga et al., 2012) yet also firmly pin prey to the substrate to prevent escape movements. The combination of these opposing actions, along with movements from the prey trying to escape likely made maneuvering elusive prey difficult, likely resulted in elusive prey being greater distances from the mouth during biting.

Successful bites occurred when prey were closer to the mouth for all species examined (Fig. 3.7; Table 3.3). The examined species utilized primarily suction feeding to move prey into the mouth, which is most efficient at short distances, as water flow velocity into the mouth decreases exponentially with distance (Svanback et al., 2002). Some individuals of *R. eglanteria, U. jamaicensis, D. sabina* and *R. bonasus* were noted to utilize a strategy of repeated biting, and presumably sucking, while attempting to maneuver prey toward the mouth, regardless of proximity of prey to mouth. Suction generated from mouth opening would help move water, and consequently prey, toward the mouth.

*Urobatis jamaicensis* displayed a preference of biting when both prey types were posterior to the mouth, while *D. sabina* and *R. eglanteria* had the opposite preference (Fig. 3.7; Table 3.3). While there is no clear explanation for these results, sensory differences may be driving anterior/posterior preferences. In addition to electrosensory pores, batoids possess ventral nonpored canals and vesicles of Savi, mechanotactile receptors used to detect and capture

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prey (Chu and Wen, ‘79; Maruska and Tricas, ‘98). Studies have shown that the canals run anterior and posterior of the mouth in these three genera (Chu and Wen, ‘79; Montgomery and Bodznick, 2004; Maruska and Tricas, 2004; Jordan, 2008), with the canals seemingly more concentrated near the rostrum, though this has not been specifically investigated. Variation in the distributions and densities of these canals and electrosensory pores could account for the different preferences. There is also a possibility that other factors such as fluid dynamics, body shape or the way prey is pinned to the substrate were causing this anterior/posterior preference.

### *Modulation of prey capture kinematics*

Modulation, noted by a significant change in the kinematic variables when switching between prey type, was seen in all batoids during certain stages of prey capture (Table 3.4), not supporting our hypothesis that lobed species would modulate prey capture behavior more than lobeless species. All batoids initiated mouth opening when elusive prey were farther from the mouth compared to non-elusive prey. With the exception of *D. sabina,* all batoids displayed modulation during one other stage of prey capture. For instance, *R. eglanteria* and *U. jamaicensis* increased tenting duration, or prey handling duration, for elusive prey (Fig. 3.4). Increased handling time of elusive prey was also found in herring, *Clupea harengus,* sprat, *Sprattus sprattus,* (Brachvogel et al., 2013) and the whitespotted bambooshark, *Chiloscyllium plagiosum,* (Lowry and Motta, 2007).

The only batoid to display modulation of mouth opening duration was *A. narinari,* with greater durations for elusive prey compared to non-elusive prey (Fig. 3.4), possibly in response to prey moving away from them. Similarly, prolonged mouth opening was seen in cyprinid fish

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(Van Wassenbergh and Rechter, 2011), perch (Osse, ‘69; Elshoud-Oldenhave, ‘79) and cichlid fish (Aerts, ‘90) when feeding on elusive prey, compensating for prey movement away from the mouth. In the case of these batoids, the mimicked prey was always pulled away from the approaching *A. narinari*, unlike real elusive prey, which can move in any direction, including toward the mouth. These results could be an over-emphasis of natural behavior with this “elusive” prey.

*Rhinoptera bonasus* tended to bite at elusive prey when they were posterior to the mouth, while non-elusive prey was usually anterior of the mouth. *Rhinoptera bonasus* may be relying on different sensory receptors or modifying fluid dynamics involved in prey capture with different prey types. However, this may not be the result of modulation. Unlike the other batoids, which usually pinned prey to the substrate while tenting, the depression of the cephalic lobes in *R. bonasus* created a vertical wall anterior and lateral to the mouth, leaving space for the prey to move around within the tent (Fig. 3.2E). This space may allow elusive prey to move posteriorly in response to the cephalic lobes rapidly depressing in front of them, resulting in this posterior preference.

### *Feeding success*

The hypothesis that lobed batoids would be more successful in preventing prey escapes was not supported. All batoid species were equally successful in capturing elusive prey. There were very few prey escapes during pouncing, tenting and biting durations for all species (Fig. 3.6), with no difference among species. This suggests that the different strategies these batoids utilize make them very successful predators with these prey types, under these experimental

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conditions. Modulation has been shown to increase capture success of elusive prey in bony fishes (Norton ‘91, Wainwright and Turingan, ‘93; Nemeth, ‘97). In the leopard shark, *Triakis semifasciata,* and the whitespotted bamboo shark, *C. plagiosum*, slight modulation was seen with elusive prey while still maintaining high capture rates (Ferry-Graham, ’98; Lowry and Motta, 2004). The flexibility of the pectoral fins or cephalic lobes (Mulvany and Motta, 2013) forming a tent around prey, sensory receptors (Maruska and Tricas, ‘98; Chu and Wen, ‘79), manipulating water flow (Wilga et al., 2012), and modulatory ability all aid in the success of these organisms. However, it should be noted that capture success in the wild may be different, as division of foraging time and watching for predators may differ among species, more complex substrates may reduce tenting efficiency, and buried prey as well as different prey types may elicit different capture behaviors.

The cephalic lobes may have evolved to help maintain feeding performance as locomotor modes shifted. One advantage to undulatory locomotion in basal batoids during prey capture is the ability to maintain maneuverability (Rosenberger, 2001) while keeping the entire body close to the substrate. Consequently, derived oscillatory batoids have less maneuverability while gliding along the substrate and may not keep their entire body as close to the substrate. This could lead to difficulty in detecting a prey item, as the electrosensory receptors would be further away from the substrate. The ability to depress the cephalic lobes may allow closer placement of these receptors to the substrate, as well as the maneuverability needed to manipulate prey toward the mouth. The evolution of these kinetic cephalic lobes may have accompanied morphological changes related to locomotor styles, helping to retain feeding performance while allowing the exploitation of a more pelagic habitat.

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## CONCLUSIONS

In summary, we have found that lobed species predominantly use the head region for prey capture and not the entire body. Lobed species handle prey faster and have a greater range of movement but have a slower bite duration compared to lobeless species. All batoids were able to modulate prey capture behavior with different prey types. Lobeless species had variability in tenting duration while lobed species modulated mouth opening (*A. narinari)* and anterior/posterior biting preference (*R. bonasus)*. Despite these morphological and behavioral differences, all species were equally successful in prey capture, attesting to the availability of multiple strategies that maintain success in these predators under these laboratory conditions.

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## TABLES AND FIGURES

Table 3.1. List of batoid species studied. Both males and females were used for all species.

| Species | Average DW (cm ± SE) | | |
|---|---|---|---|
| *Raja eglanteria* | 20.6 | ± | 1.25 |
| *Urobatis jamaicensis* | 19.5 | ± | 1.29 |
| *Dasyatis sabina* | 23.2 | ± | 2.16 |
| *Aetobatus narinari* | 88.6 | ± | 6.23 |
| *Rhinoptera bonasus* | 53.6 | ± | 1.41 |

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Table 3.2. Statistics for all the prey capture kinematic events. Arranged by (A) species, (B) prey type and (C) both species and prey type. Shaded values show significant differences. For ANOVA results, cv = adjusted critical value from false discovery rate correction. For mixed interactions, significant p-values for prey type are designated with “n” for non-elusive prey and “e” for elusive prey. *Ra. = R. eglanteria; U. = U. jamaicensis,; D. = D. sabina; A. = A. narinari; Rh. = R. bonasus.* Species with cephalic lobes are in bold.

| A. Species | **Pounce** | **Tenting** | **Mouth Opening** | **Mouth Closing** | **Bite** | **Overall Event** |
|---|---|---|---|---|---|---|
| 2 way repeated ANOVA | p-value, cv for species differences | | | | | |
| | p = .001, cv = .004 | p = .001, cv = .008 | p = .014, cv = .033 | p = .001, cv = .0125 | p = .004, cv = .029 | p = .001, cv = .017 |
| Tukey | p-value for differences among species | | | | | |
| *Ra.* *U.* | p >.05 | p >.05 | p >.05 | p >.05 | p >.05 | p >.05 |
| *Ra.* *D.* | p >.05 | p <.031 | p >.05 | p >.05 | p >.05 | p >.05 |
| *U.* *D.* | p >.05 | p <.031 | p >.05 | p >.05 | p >.05 | p >.05 |
| *Ra.* ***A.*** | p <.012 | p <.031 | p = .039 | p <.005 | p >.05 | p <.026 |
| *Ra.* ***Rh.*** | p <.012 | p <.031 | p >.05 | p <.005 | p <.05 | p <.026 |
| *U.* ***A.*** | p >.05 | p <.031 | p >.05 | p <.005 | p >.05 | p <.026 |
| *U.* ***Rh.*** | p >.05 | p <.031 | p >.05 | p <.005 | p <.05 | p <.026 |
| *D.* ***A.*** | p >.05 | p >.05 | p = .046 | p <.005 | p >.05 | p >.05 |
| *D.* ***Rh.*** | p >.05 | p >.05 | p >.05 | p <.005 | p >.05 | p >.05 |
| ***A.*** ***Rh.*** | p >.05 | p >.05 | p >.05 | p >.05 | p >.05 | p >.05 |

B. Prey type

| | | | | | | |
|---|---|---|---|---|---|---|
| 2 way repeated ANOVA | p-value, cv for prey type differences | | | | | |
| | p = .09, cv = .029 | p = .005, cv = .013 | p = .828, cv = .05 | p = .23, cv = .033 | p = .772, cv = .042 | p = .003, cv = .004 |
| Tukey | p-value for prey type for each species | | | | | |
| *Ra.* | p >.05 | p = .003 | p >.05 | p >.05 | p >.05 | p = .006 |
| *U.* | p >.05 | p = .026 | p >.05 | p >.05 | p >.05 | p = .008 |
| *D.* | p >.05 | p >.05 | p >.05 | p >.05 | p >.05 | p >.05 |
| ***A.*** | p >.05 | p >.05 | p = .013 | p >.05 | p >.05 | p >.05 |
| ***Rh.*** | p >.05 | p >.05 | p >.05 | p >.05 | p >.05 | p >.05 |

C. Mixed interactions

| | | | | | | |
|---|---|---|---|---|---|---|
| 2 way repeated ANOVA | p-value for differences among species and prey type | | | | | |
| | p = .24 | p = .119 | p = .066 | p = .051 | p = .118 | p = .098 |

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Table 3.3. Average raw distance of prey from the batoid's mouth at the start of a bite and % of bites anterior to the mouth for each species, elusive and non-elusive prey, successful and unsuccessful bites ± standard error (SE).

| **Species** | **Prey Type** | **Bite** | **Average distance (cm** ± SE) | **% of anterior bites** ± SE |
|---|---|---|---|---|
| *R. eglanteria* | elusive | successful | 1.12 ± 0.13 | 0.72 ± 0.08 |
| | | failed | 1.97 ± 0.10 | 0.82 ± 0.04 |
| | non-elusive | successful | 0.59 ± 0.06 | 0.78 ± 0.06 |
| | | failed | 1.69 ± 0.10 | 0.91 ± 0.03 |
| *U. jamaicensis* | elusive | successful | 1.30 ± 0.13 | 0.59 ± 0.09 |
| | | failed | 2.49 ± 0.27 | 0.42 ± 0.06 |
| | non-elusive | successful | 1.50 ± 0.19 | 0.27 ± 0.12 |
| | | failed | 2.00 ± 0.24 | 0.08 ± 0.08 |
| *D. sabina* | elusive | successful | 1.29 ± 0.23 | 0.80 ± 0.09 |
| | | failed | 2.12 ± 0.33 | 0.72 ± 0.09 |
| | non-elusive | successful | 0.78 ± 0.09 | 0.93 ± 0.05 |
| | | failed | 1.58 ± 0.23 | 0.84 ± 0.09 |
| *R. bonasus* | elusive | successful | 4.96 ± 0.72 | 0.67 ± 0.13 |
| | | failed | 9.96 ± 0.98 | 0.35 ± 0.10 |
| | non-elusive | successful | 4.23 ± 0.62 | 0.70 ± 0.11 |
| | | failed | 5.68 ± 0.68 | 0.73 ± 0.10 |

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Table 3.4 Occurrence of modulation during stages of prey capture, noted by a significant change between elusive and non-elusive prey.

| | *R. eglanteria* | *U. jamaicensis* | *D. sabina* | *A. narinari* | *R. bonasus* |
|---|---|---|---|---|---|
| Pouncing | | | | | |
| Tenting | X | X | | | |
| Mouth opening | | | | X | |
| Mouth closing | | | | | |
| Bite duration | | | | | |
| Prey distance to mouth | X | X | X | X | X |
| Prey position during bite | | | | | X |
| Vertical movement | | | | | |
| Horizontal movement | | | | | X |
| # of bites | | | | | |

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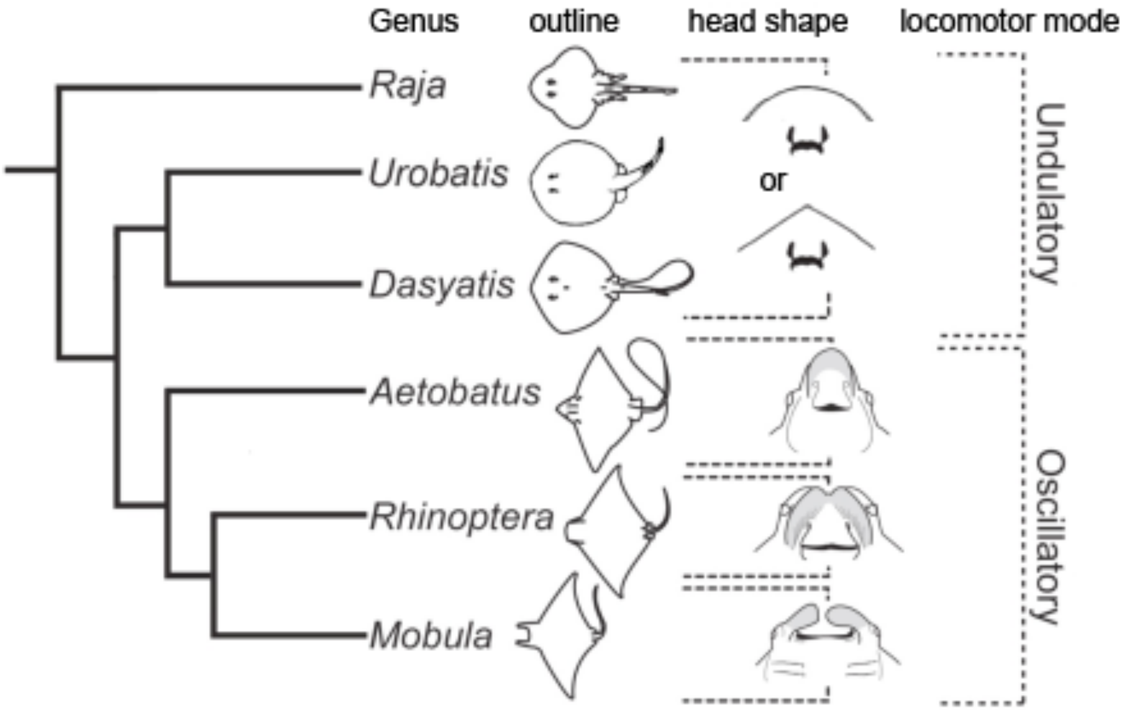

Figure 3.1. Phylogeny of batoids. Based on Aschliman *et al.* (2012) showing presence/absence of cephalic lobes and primary locomotor mode, modified from Sasko *et al.* (2006).

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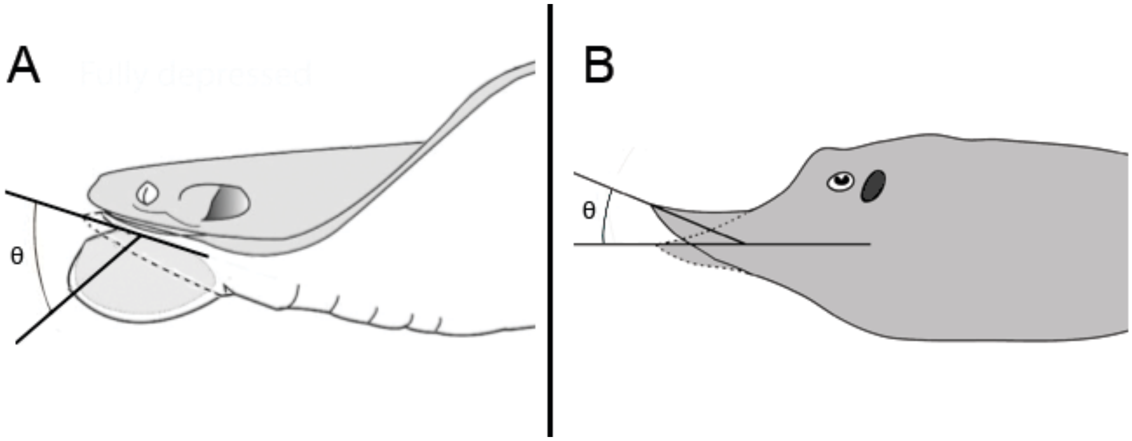

Figure 3.2. Measurement of vertical angle of movement. (A) *Rhinoptera bonasus* with cephalic lobes depressed, dotted line indicating lobe placement while retracted. Modified from Sasko *et al*., (2006). (B) *Dasyatis sabina* with anterior pectoral fins elevated and the dotted line indicating the fins while depressed. The lines extend from the vertex, where the radials of the cephalic lobes or anterior pectoral fins attach to the propterygium, through the tips of the cephalic lobes or pectoral fins in elevated and depressed position. ϴ indicates the vertical angle of movement.

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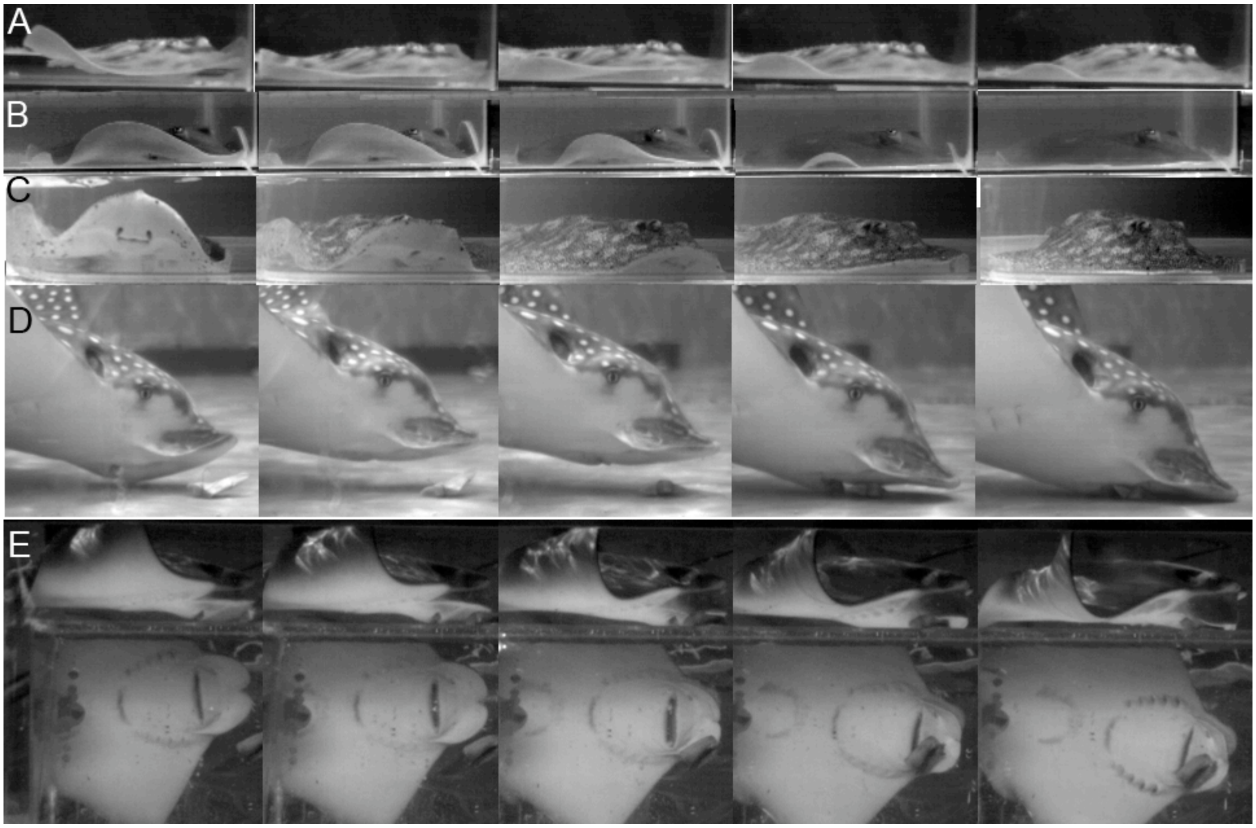

Figure 3.3.  Pounce duration.  Five sequential pictures from left to right showing lateral view of the pounce duration (initiation of pounce to the onset of tenting behavior) in *R. eglanteria* (A); *D. sabina* (B); *U. jamaicensis* (C); *A. narinari* (D) and both lateral (top half) and ventral (bottom half) views in *R. bonasus* (E).

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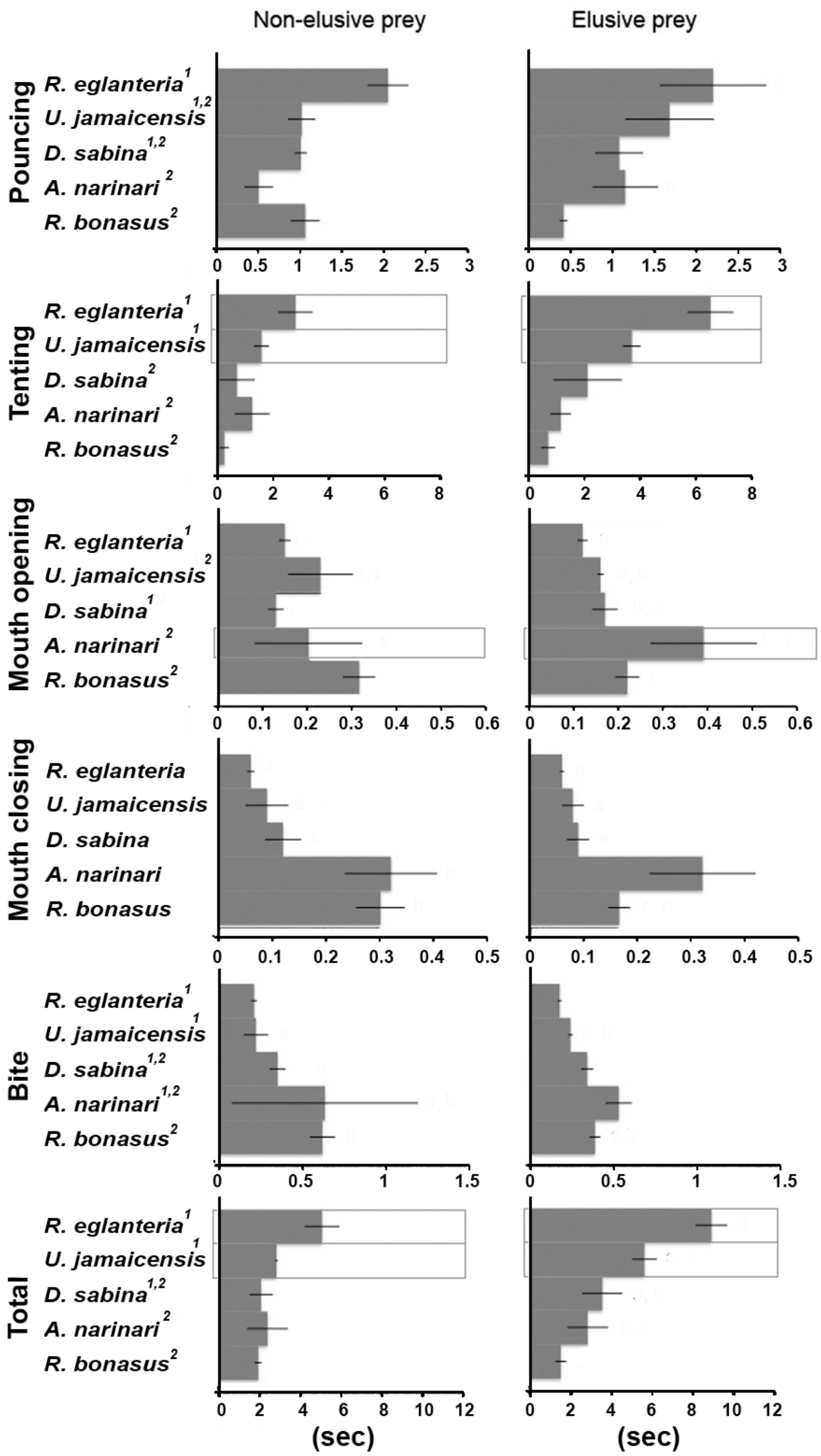

Figure 3.4. Results of kinematic capture analysis. Groups that share the same label are not significantly different. Significant differences among species are marked with numbers ($^1$ and $^2$) next to the species names. Boxes around the data bars indicate significant differences in prey type within species. Error bars are standard error.

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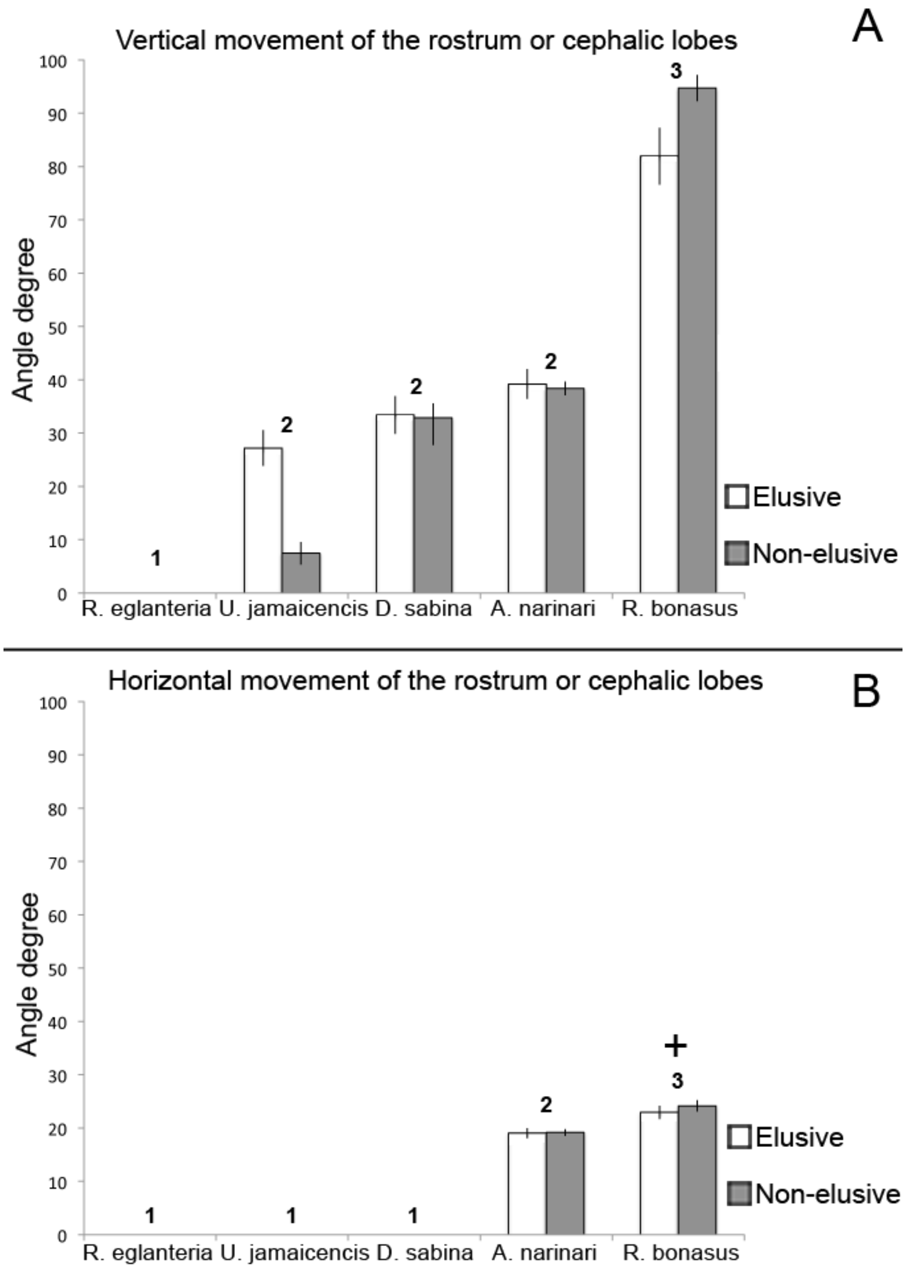

Figure 3.5. Angle of movement of the rostrum or cephalic lobes during prey capture in the vertical plane (A) and horizontal plane (B). Groups belonging to the same number label ([1], [2] or [3]) are not significantly different. Significant differences between prey type within species are marked with +. Error bars are standard error.

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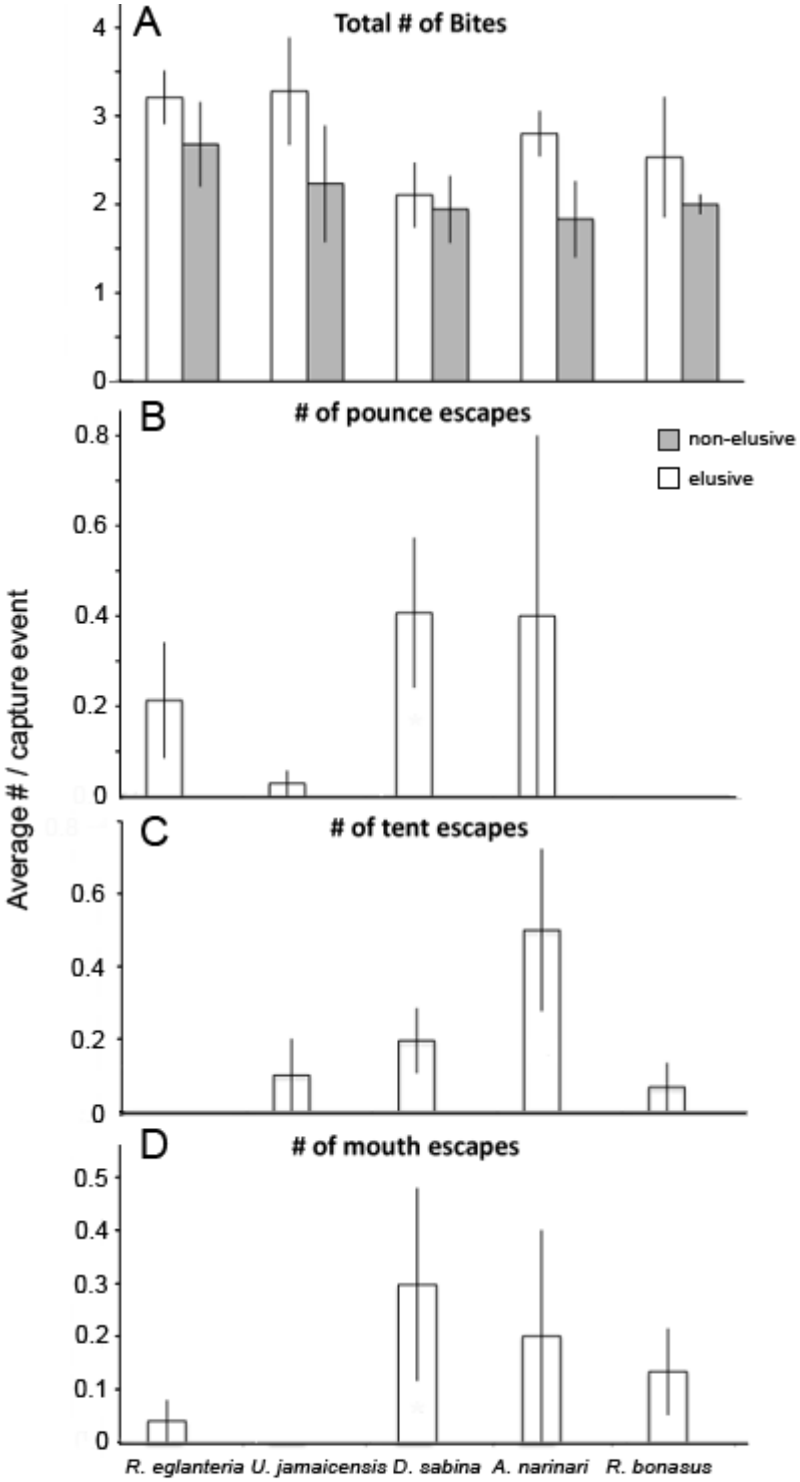

Figure 3.6. Number of bites and escapes. Average number of bites per capture event needed to ingest elusive and non-elusive prey (A), and average number of prey escapes per capture event for elusive prey during pouncing (B), tenting (C) and while in the mouth (D). Species without bars indicate zero values. White bars indicate elusive prey, grey bars indicate non-elusive prey. Error bars are standard error.

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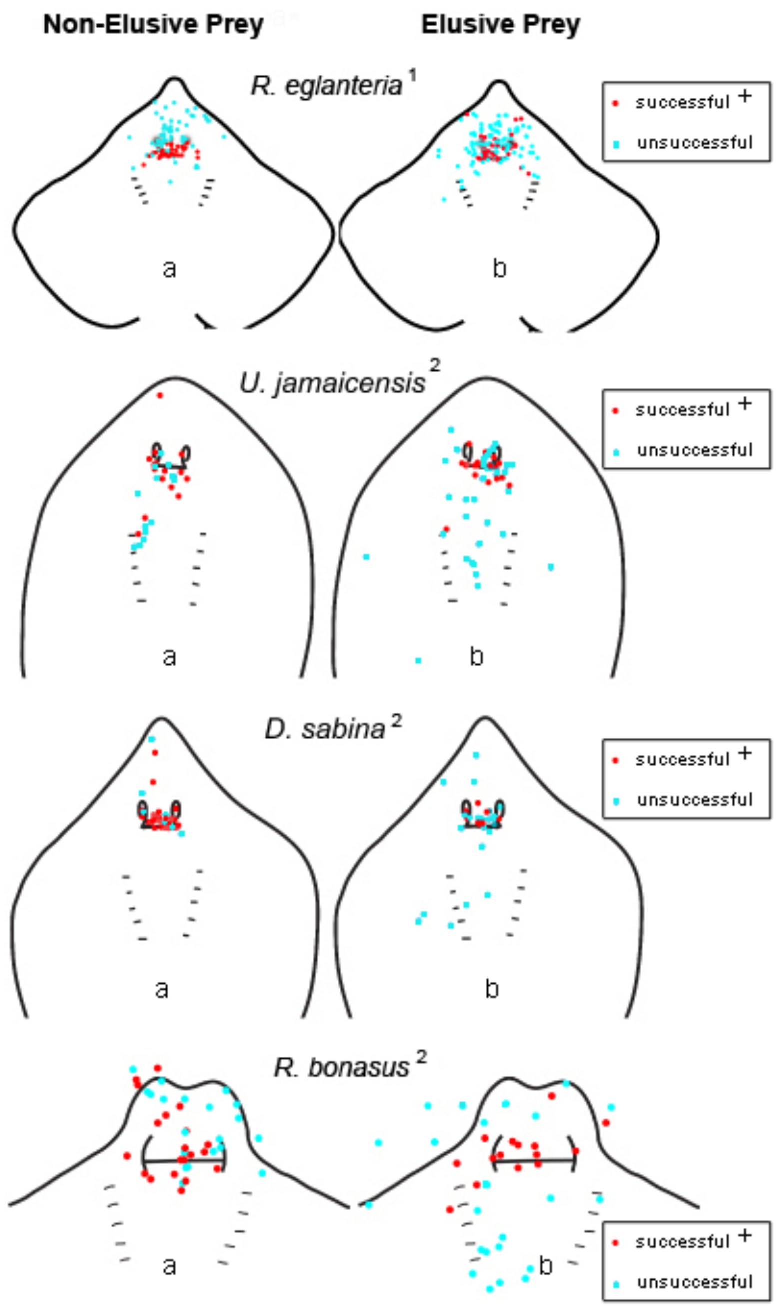

Figure 3.7. Map of prey location at the time of bite attempt. Red dots signify successful bites, blue dots unsuccessful bites. Significant differences in species for prey distance from mouth are indicated by [1] and [2]. For each species, a significant difference between prey type for all bites is indicated by a and b. For each species, a significant difference between bite success is indicated by +.

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# CHAPTER FOUR: CORRELATION OF MORPHOLOGICAL AND BEHAVIORAL VARIABLES TO THE CEPHALIC LOBES: TAKING PHYLOGENY OUT OF THE PICTURE

## ABSTRACT

Some derived, pelagic myliobatid rays possess cephalic lobes. These lobes are modified portions of the anterior pectoral fins. In lobeless batoids, the pectoral fins are used during locomotion and prey capture. In lobed species, locomotion is partitioned to the pectoral fins while the cephalic lobes are used in prey capture. Differences in habitat, locomotor style, morphology, and prey capture behavior may be associated with the cephalic lobes. The aim of this study was to assemble morphological and behavior data and determine which variables correlated to the presence of the cephalic lobes. The independent contrast method was used to phylogenetically correct the data. After phylogeny was accounted for, most of the morphological variables correlated with the presence/absence of cephalic lobes while only one kinematic variable showed a correlation. This supports the idea that changes in the pectoral fins associated with the shift to oscillatory locomotion and consequently a pelagic habitat are linked to the evolution of the cephalic lobes. Changes in prey capture behavior, however, are associated with a factor other than the presence/absence of the cephalic lobes.

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## INTRODUCTION

The cephalic lobes are unique structures found in 39 myliobatid species, derived from the anterior pectoral fins (Bigelow and Schroeder, 1953; Nishida, 1990, Miyake et al., 1992). Rays can have a single continuous lobe, as seen in *Myliobatis*, a single discontinuous lobe, as seen in *Aetobatus*, or two discontinuous lobes, as seen in *Rhinoptera*, *Mobula* and *Manta* (McEachran et al., 1996). Radials (skeletal elements) of the anterior pectoral fins in lobeless species are similar to the cephalic lobe radials in lobed species, while the anterior pectoral fin radials in lobed species are quite distinct (Mulvany and Motta, 2013). The fin rays (chains of radials extending from the propterygium) of the anterior pectoral fin of lobeless species and the cephalic lobe fin rays have a higher number of joints/cm and more circular cross sectional areas compared to the fin rays of the anterior pectoral fins in lobed species. Furthermore, the musculature of the cephalic lobes is similar to the pectoral fin musculature, although an extra muscle layer running obliquely to the radials is found in the lobes. The cephalic lobes have a higher electrosensory pore count compared to the anterior pectoral fins of lobeless species, while the anterior pectoral fins in lobed species do not appear to have any electrosensory pores.

The cephalic lobes play a role in prey detection and capture, as they are covered with electrosensory and mechanotactile receptors, and are depressed over the substrate when searching for prey (Chu and Wen, 1979; Sasko et al., 2006; Mulvany and Motta, 2013); used for digging into the substrate by repeatedly depressing and retracting them to create feeding pits; and used to grasp/cup prey and maneuver or even channel prey toward the mouth (Notarbartolo-di-Sciara, 1988; Sasko et al., 2006; Fisher et al., 2011; Mulvany and Motta, 2013). Kinematic data show that species with cephalic lobes pounce on prey faster, handle prey faster and have an overall faster prey capture event compared to lobeless species (Chapter 2). While lobeless

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species utilize the pectoral fins for locomotion and prey capture, lobed species partition prey capture to the cephalic lobes and locomotion to the pectoral fins.

Typical batoid locomotion consists of axial-undulatory locomotion or undulatory locomotion, where waves of bending propagate down the body or pectoral fins (Rosenberger and Westneat, 1999; Rosenberger, 2001). The appearance of cephalic lobes coincides with a shift to oscillatory locomotion (Rosenberger, 2001; Schaefer and Summers, 2005; Sasko et al., 2006), in which the pectoral fins are depressed and elevated in a flapping motion. Oscillatory swimmers have stiffer, more inflexible pectoral fins with skeletal cross-bracings compared to undulatory swimmers (Schaefer and Summers, 2005; Mulvany and Motta, 2013). While undulatory locomotion offers flexibility and maneuverability, oscillatory locomotion produces lift and allows for sustained swimming at high speeds (Rosenberger, 2001).

Locomotor differences can be linked to changes in habitat for batoids. Most undulatory batoids are benthic (McEachran and Carvalho, 2002), utilizing low speeds to maneuver close to the substrate. Oscillatory batoids, however, are more pelagic, cruising at higher speeds in the water column (Rosenberger, 2001). Though some oscillatory batoids such as mobulids and mantas are truly pelagic and feed in the water column, using the cephalic lobes to channel plankton into the mouth (Notarbartolo-di-Sciara and Hillyer, 1989), many others feed on the same benthic organisms as undulatory, benthic batoids (Bigelow and Schroeder, 1953; Compagno, 1977), predominantly polychaetes and crustaceans as well as fish, bivalves and squid (Bigelow and Schroeder, 1953; Compagno, 1977; Ebert and Cowley, 2003; Collins et al., 2007; Ebert and Bizzarro, 2007). Whereas benthic species possess the flexibility and maneuverability in the pectoral fins needed for both prey capture and undulatory locomotion, pelagic species have

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stiff oscillatory pectoral fins for locomotion and flexible cephalic lobes for prey capture (Mulvany and Motta, 2013).

The phylogeny of batoids has been documented using morphological characteristics (Nishida, 1990; Lovejoy, 1996; McEachran et al., 1996; Shirai, 1996; Gonzalez-Isais and Dominguez, 2004), and more recently with molecular data (Doudy et al., 2003; Dunn, 2003; Winchell et al., 2004; Naylor et al., 2005; Rocco et al., 2007; Aschliman et al., 2012). Though the studies differ in the number of species used and the species themselves, a general pattern of batoid phylogeny is confirmed. Batoids are a monophyletic group with mobulids (devil rays), rhinopterids (cownose rays) and myliobatids (eagle rays) consistently shown as the most deeply nested group. These pelagic batoids also possess cephalic lobes. Rajids (skates) are the most basal, benthic group of batoids while dasyatids (stingrays/whiprays) and urobatids (round rays) are shallowly nested.

Some morphological studies (Lovejoy, 1996; McEachran et al., 1996) show urobatids as basal to dasyatids (Fig. 4.1A), while one study (Gonzalez-Isais and Dominguez, 2004) showed rhinopterids and myliobatids as sister taxa (Fig. 4.1B). The most supported tree, with both morphological (Nishida, 1990; Shirai, 1996) and molecular data (Dunn et al., 2003; Aschliman et al., 2012), include urobatids and dasyatids as sister taxa as well as rhinopterids and mobulids as sister taxa (Fig. 4.1C). Aschliman et al. (2012) paired molecular data with fossil records, yielding a phylogenetic tree with branch lengths for 37 batoid species.

One important factor in comparative studies is that closely related species tend to have similar phenotypes when compared to more distantly related species. This is due to the comparatively brief time since speciation, the tendency of organisms to conserve their niche, and

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consequently the tendency to have similar adaptive responses to environmental changes (Harvey and Pagel, 1991). An important statistical assumption in any study is that all observations are independent of each other. For species, the assumption of independence would be satisfied if the evolution of each species was independent and the divergence times were identical for all species. A phylogenetic tree of independent species would have all branches radiating from a single node with equal branch lengths (Felsenstein, 1985; Fig. 4.1A). Actual relationships among species are nested hierarchies, with some species more closely related to others (Fig. 4.1B-D). To satisfy the assumption of independence, the independent contrast method (Felsenstein, 1985) can be used to remove the effect of phylogeny by accounting for the relatedness among taxa, using the topography of a phylogenetic tree and branch lengths. Contrasts generated by this method are regarded as independent and can be used in statistical analyses. For instance, multiple studies using uncorrected data found a strong correlation between genomic size and effective population size in fish, plants and even across kingdoms (Lynch and Conery, 2003; Albach and Greilhuber 2004; Yi and Streelman, 2005), positing that genetic drift accounted for maladaptive genome sizes. A more recent study found similar results when analyzing uncorrected data in 205 plant species, but the correlation between genome size and effective population size disappeared after retesting the data using phylogenetically independent contrasts (Whitney et al., 2010), suggesting that relatedness caused the correlation.

Recent comparative studies on batoids have noted links between characteristics such as locomotor mode and habitat (Rosenberger, 2001; Macesic and Kajiura, 2010), skeletal calcification patterns and locomotor mode (Schaefer and Summers, 2005), feeding and habitat (Sasko et al., 2006), visual fields and habitat (McComb and Kajiura, 2008), but to our knowledge no studies have attempted to remove the effect of phylogeny to determine if these trends are a

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result of relatedness or if these traits truly correlate to each other. The purpose of this study is to analyze a suit of morphological and behavioral characters to determine if they correlate to the evolution of cephalic lobes. It is hypothesized that the cephalic lobes will correlate to both the morphological and behavioral traits after removing the effects of relatedness, demonstrating the association of the cephalic lobes with a morphological and behavioral shift to a pelagic lifestyle.

## METHODS

Twenty kinematic and morphological variables were examined for correlations to the presence or absence of cephalic lobes in five batoid species: *Raja eglanteria* (Bosc, 1800), *Urobatis jamaicensis* (Cuvier, 1816), *Dasyatis sabina* (Lesueur, 1824), *Aetobatus narinari* (Euphrasen, 1790) and *Rhinoptera bonasus* (Mitchill, 1815). All variables, with the exception of habitat and the cephalic lobes, were continuous. The use of discrete and continuous variables when generating independent contrasts is acceptable and does not violate any assumptions (Garland et al., 1992). Discrete variables were coded following their evolutionary trajectories (more ancestral traits were coded as “0”, more derived traits as “1”) based on Nishida (1990), Lovejoy (1996) and Shirai (1996).

Twelve continuous kinematic variables from Mulvany (Chapter 2) were used: 1.) pounce duration: the beginning of cephalic lobe depression to maximum depression for lobed species, the beginning of rostral elevation to when the rostrum touches the substrate in lobeless species; 2.) tent duration: from the time of maximum lobe depression or when the rostrum touches the substrate until the mouth begins to open for a successful bite; 3.) mouth opening duration: from the onset of mouth opening to the time when the last part of the prey enters the mouth; 4.) mouth

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closing duration: from the time when the last part of the prey enters the mouth until the mouth closes; 5.) bite duration: from mouth opening to mouth closing (parts 3 and 4 combined); 6.) total capture event: from onset of pounce to mouth closing (parts 1-5 combined); 7.) pounce escape: the number of times a prey escaped during the pouncing duration; 8.) tent escape: the number of times a prey escaped during the tenting duration; 9.) mouth escape: the number of times a prey escaped during mouth opening or closing duration; 10.) the number of bites: the average number of bites for a prey capture event; 11.) vertical movement: angle movement of the tips of the cephalic lobes or rostrum in the vertical plane; 12.) horizontal movement: angle movement of the tips of the cephalic lobes or rostrum in the horizontal plane.

In addition, six continuous morphological variables from Mulvany and Motta (2013) were used: 13.) number of pectoral fin skeletal fin ray joints per cm; 14.) $I_{lat}/I_{DV}$ calcified: for the calcified potions of the pectoral fin radials, this is a measurement of the resistance to bending in the lateral plane over the resistance to bending in the dorsoventral plane. A ratio higher than one would indicate higher resistance in the lateral plane, a ratio below one would indicate higher resistance in the dorsoventral plane while a ratio of 1 would indicate equal resistance in both planes; 15.) $I_{lat}/I_{DV}$ whole: the same calculations as the former variable, but examining the entire radial instead of solely the calcified portions; 16.) the insertion point of the abductor superficialis muscle on the pectoral fin radial; 17.) the insertion point of the adductor superficialis muscle on the pectoral fin radial; 18.) the number of electrosensory pores on the pectoral fin.

Data quantifying the continuum of undulatory to oscillatory locomotion for several batoid species and the habitats according to Rosenberger (2001) were used for 19.) locomotor mode: the number of waves present per fin length. Missing locomotor data for *U. jamaicensis* and *A. narinari* were replaced with data from their closest relative. Data on *Taeniura lymma* was used

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as proxy for *U. jamaicensis* and data from *R. bonasus* was used as a proxy for *A. narinari*; and 20.) habitat: benthic = 0, pelagic = 1.

All variables were tested for a correlation to the presence or absence of cephalic lobes, with each species coded as 0 = lobes absent; 1 = lobes present. The full correlation analysis of all variables is found in Appendix A and B. However, because this study focuses on the cephalic lobes, primarily correlations with the cephalic lobes are included in the results section and discussed. Data from individuals of each species were averaged and all data were normalized by subtracting the mean from each value and dividing by the standard deviation. A Pearson correlation was performed to determine correlations between any variables and the cephalic lobes using Sigmastat v. 3.1 (SYSTAT Software, San Jose, CA).

In order to account for phylogenetic relationships among the data, the phylogenetic independent contrast method (Felsenstein, 1985; Garland et al., 2005) was used. A resolved phylogenetic tree of the six batoid species based on morphological and molecular data was utilized (Nishida, 1990; Shirai, 1996; Dunn et al., 2003; Aschliman et al., 2012). Branch lengths were taken from Aschliman et al. (2012), using estimated divergences times under Bayesian approaches (Fig. 4.2). The averaged, normalized data were entered into Mesquite v.2.75 (Maddison and Maddison, 2011). The PDAP:PDTREE package of Mesquite (Midford et al., 2005) was used to generate the independent contrasts using the aforementioned constructed tree.

The absolute values of the standardized contrasts for each variable were regressed against the square root of the sum of the corrected branch lengths (their standard deviation) to verify that the branch lengths corresponded to the data, indicated by a slope not significantly different from 0. The raw, positivized contrasts were exported from Mesquite and divided by their standard

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deviations (Midford et al., 2005). A Pearson correlation was run using the phylogenetically independent contrasts (PICs) to determine which variables were correlated to the presence or absence of cephalic lobes.

## RESULTS

The phylogenetically uncorrected correlations showed that 9 out of the 20 variables correlated to the presence of cephalic lobes (Table 4.1). Locomotion (p = .026), pounce duration (p = .022), number of joints/cm (p = .017) and number of electrosensory pores (p = .021) all correlated negatively to the presence of cephalic lobes. Habitat (p <.001), horizontal movement (p <.001), $I_{lat}/I_{DV}$ calcified (p = <.001), abductor superificalis insertion (p = .031), adductor superficialis insertion (p = .002) were all positively correlated with the presence of cephalic lobes.

The regression of the raw contrasts against their standard deviation for each variable confirmed that the branch lengths corresponded to the data (p >.05). Scatterplots of each variable's contrast against positived contrast can be found in Appendix C. The phylogenetically corrected Pearson correlations showed that 6 of the 20 variables correlated to the cephalic lobes (Table 4.1). Locomotion (p = .027) and number of electrosensory pores negatively correlated to the presence of cephalic lobes while habitat (p <.001), horizontal movement (p= .008), $I_{lat}/I_{DV}$ calcified (p = .003) and adductor superficialis insertion (p = .014) positively correlated with the cephalic lobes. Interestingly, habitat correlated to the same 6 variables as the cephalic lobes, while locomotion correlated to 5 of the 6 variables (Appendix B).

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## DISCUSSION

Our hypothesis that the presence/absence of cephalic lobes would correlate to morphological variables before and after correcting for phylogeny was supported while the correlation to kinematic variables was not supported before or after relatedness of the taxa was taken into account. Overall, the uncorrected correlations showed that five of the six morphological variables and two of the 12 behavioral variables correlated with the presence of the cephalic lobes. After removing the effect of phylogeny, three morphological and one behavioral variable still showed a significant relationship with the cephalic lobes. Habitat and locomotion were correlated to the presence of the cephalic lobes before and after correcting for phylogenetic inertia.

### *Morphological variables*

Two morphological traits correlated to the presence of cephalic lobes using uncorrected data, but showed no relationship after phylogenetic corrections: the number of fin ray joints/cm in the pectoral fin and the insertion of the abductor superficialis muscle (Table 4.1). Because closely related species tend to share similar characteristics (Harvey and Pagel, 1991), correlations using uncorrected data can occur that are simply due to the relatedness among species. Though the most deeply nested, lobed species (*Aetobatus narinari* and *Rhinoptera bonasus*) exhibited the lowest number of joints/cm, *Raja eglanteria,* the most basal species, possessed fewer joints/cm compared to more derived lobeless species (*Urobatis jamaicensis* and *Dasyatis sabina*) (Mulvany and Motta, 2013). The lack of unidirectional change in the number

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of joints/cm through phylogeny likely accounts for the lack of the correlation to the cephalic lobes.

The $I_{lat}$ /$I_{DV}$ for calcified portions of the pectoral fin radials correlated to the presence/absence of cephalic lobes after taking phylogeny into account (Table 4.1). $I_{lat}$ /$I_{DV}$ was higher in lobed species, *A. narinari*, *R. bonasus* and *Mobula munkiana,* indicating more resistance to bending in the lateral plane compared to the radials of the lobeless species, *R. eglanteria, U. jamaicensis* and *D. sabina,* which had better resistance bending in both planes (Mulvany and Motta, 2013). Lobed species exhibit crustal calcification, with a layer of calcification surrounding the perimeter of the radials, while lobeless species exhibit catenated calcification, with struts of calcification running along the edges of the radials (Schaefer and Summers, 2005; Mulvany and Motta, 2013). The number and placement of the struts can vary in lobeless species, meaning the $I_{lat}$ /$I_{DV}$ of the calcified struts is independent of the overall radial cross sectional shapes. Crustal calcification in lobed species, however, is based on the cross sectional shape of the radials, which are more dorso-ventrally flattened. The higher $I_{lat}$ /$I_{DV}$ values for lobed species may reflect differences in calcification patterns. For a given amount of calcified material, the crustal calcification pattern yields higher stiffness than the catenated pattern, which is important for oscillatory swimmers (Schaefer and Summers, 2005). As oscillatory locomotion also correlates to higher $I_{lat}$ /$I_{DV}$ values as well as the presence of cephalic lobes (Appendix B), this shows that the examined morphological variable involved with stiffness ($I_{lat}$ /$I_{DV}$) is linked to locomotor mode and the presence/absence of cephalic lobes.

Insertions of the adductor superficialis were also correlated to the presence/absence of cephalic lobes (Table 4.1), with the muscle inserting ½ to 3/5 down the fin rays in lobeless species and down the length of the entire fin ray in lobed species (Mulvany and Motta, 2013).

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As locomotor mode is also correlated to the adductor superficialis and the cephalic lobes (Appendix B), this may be the driving force behind the correlation of the cephalic lobes to the adductor superficialis muscle. During the upstroke and downstroke of oscillatory locomotion, the fin rays are moving collectively, which requires ample stiffness (Schaefer and Summers, 2005) and force generated by muscles. Cross sectional thickness of the pectoral fins, in effect muscle cross sectional area, was higher in oscillatory batoids compared to undulatory batoids (Fontanella et al., 2013). As the cross sectional area of muscles, sharing the same architecture, is proportional to the force generated (Huber and Motta, 2004), oscillatory species generate more force. Attachment of the muscles along the length of the fin rays helps to increase the lever arm, thus increasing force efficiency. The presence of the cephalic lobes shifted prey capture behavior from whole body tenting to cephalic tenting (Chapter 2), presumably allowing a concomitant modification of the pectoral fin morphology to better suite oscillatory locomotion while still retaining the ability to capture benthic prey.

It has been shown that morphological differences in the pectoral fin reflect the requirements for locomotor modes in batoids (Schaefer and Summers, 2005; Fontanella et al., 2013) as well as other fishes (Webb, 1984; Drucker and Lauder, 2002). Flying squirrels that aerially locomote have long forelimbs for shock absorption when landing and short hindlimbs that reduce drag while gliding, while chipmunks that move on the ground have short forelimbs for digging and long hindlimbs that increase stride length (Essner Jr., 2007). The climbing gecko has adhesive toe pads and a sprawled posture, advantageous for vertical climbing, while ground geckos lack adhesive toe pads and have erect posture, advantageous for movement on the ground (Aerts et al., 2000). $I_{lat} / I_{DV}$ and the insertion of the adductor superficialis in these batoids both correlated to locomotion as well as habitat (Appendix B). This suggests that the evolution of the

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cephalic lobes, which is closely tied to changes in pectoral fin morphology, subsequently links to the shift from undulatory to oscillatory locomotion in pelagic species that feed on predominantly benthic organisms.

The pectoral fin electrosensory pore counts showed a significant negative correlation to the presence of cephalic lobes (Table 4.1), indicating that the anterior pectoral fins in lobed species are not often used in prey detection and handling. Electrosensory receptors function in prey detection and localization (Kalmijn, 1971) and the strength of the electric field generated by a prey item rapidly decreases with distance, limiting electrosensory detect to around 5-10 cm (Kalmijn, 1988; Tricas and Sisnero, 2004; Jordan et al., 2009). Lobeless species that utilize the pectoral fins for prey capture and handling have significantly more electrosensory pores on the pectoral fins that can help localize prey under the body, whereas lobed species that utilize the pectoral fins for primarily locomotion have no pectoral fin electrosensory pores (Mulvany and Motta, 2013). The cephalic lobes in *A. narinari* and *R. bonasus*, which are used for prey handling, are covered with electrosensory pores, emphasizing their role in prey localization as well as capture (Mulvany and Motta, 2013). Mobulid and manta species lack electrosensory pores on the cephalic lobes (Chu and Wen, 1979; Mulvany and Motta, 2013), and these species utilize the lobes to channel water into their mouths as they filter-feed on plankton (Notarbartolo-di-Sciara and Hillyer, 1989).

### *Kinematic variables*

Very few kinematic variables correlated to the presence/absence of the cephalic lobes, before and after the phylogenetic correction. For many kinematic variables with significant

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differences among species, at least one lobeless species, usually *D. sabina,* grouped with lobed species, *A. narinari* and *R. bonasus*. *Rhinoptera bonasus* displayed a significantly higher degree of vertical movement of the cephalic lobes compared to *A. narinari* and the anterior pectoral fins of lobeless species. Differences among species for kinematic results appear to be less linked to the evolution of the cephalic lobes, but rather other factors such as jaw morphology, hydrodynamics when manipulating water flow or the use and distribution different sensory receptors. Jaw protrusion can vary in batoids, with some species able to protrude the jaws 100% of their head length (Dean and Motta, 2004), while other species show less than 1 cm of protrusion (Wilga and Motta, 1998; Summers, 2000). Skates are known to manipulate water flow under the body using the pectoral fins (Wilga et al., 2012). Other batoids may do the same, though morphological differences among batoids could result in different flow manipulation techniques. The integration of electroreception, olfaction, mechanotactile, vision and lateral line sensors has recently been studied in sharks (Gardiner et al., in prep), showing that species-specific hierarchies exist for various stages of feeding. Utilization of different senses or combinations of senses during prey capture, as well as differences in the distribution of the receptors may correspond to kinematic differences.

Pounce duration negatively correlated to the presence of cephalic lobes (Table 4.1), though this trend vanished after correcting for phylogeny. Lobed species, *A. narinari* and *R. bonasus,* tended to exhibit faster pouncing durations compared to lobeless species, but due to the relatedness of the species rather than the presence/absence of cephalic lobes. Pounce duration is likely influenced by locomotor speed. Lobed species can cruise at speeds around 2.06-2.57 m $s^{-1}$ (Webb, 1984; Smith and Merriner, 1987; Fontanella et al., 2013) and often initiate pounces while cruising. Although lobeless species, like *D. sabina,* show cruising speeds of .87 m $s^{-1}$ (Wilborn,

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2007), lobeless species often pounce from a standstill and may be using burst locomotion which can faster than normal cruising speeds (Bainbridge, 1962; Barnett et al., 2010). The use of burst locomotion in some species may be enough to narrow the gap in pounce durations between lobed and lobeless species, rendering an insignificant correlation between the presence/absence of lobes and pounce duration.

The amount of horizontal movement of the cephalic lobes or anterior pectoral fin during prey capture was significantly correlated to the presence/absence of cephalic lobes with phylogenetically corrected data (Table 4.1). Because the pectoral fins extend anteriorly, laterally and posteriorly in lobeless species, there are no gaps when forming a tent over a prey item with the fins. There is, however, a gap between the pectoral fins and cephalic lobes which could provide prey with a sizeable access of escape. Horizontal movement of the cephalic lobes fans out the lobes to block this gap, preventing escape, particularly in *Rhinoptera bonasus* (Sasko et al., 2006; Chapter 2). High mobility in cephalic appendages used for prey capture and handling can be seen in other species. The Florida manatee, *Trichechus manatus,* utilizes its highly mobile, muscular snout and perioral bristles to trap and manipulate food into the mouth (Marshall et al., 1998). Centipedes have forcipules, modified anterior legs with multiple joints and a wide range of motion, that are used to grasp, manipulate as well as invenomate prey (Bonato and Minelli, 2009; Dugon et al., 2012). The prominent trunk in elephants is comprised of radially, transversely and longitudinally arranged muscles that allow a wide range of movement used for multiple behaviors, including handling food (Boas and Pauli, 1908).

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### *Habitat* and *locomotion*

Relationships among morphology, locomotion and habitat have been found in various species. Among six species of Jamaican bats, those with short, rounded wings fly at slower speeds and forage in edge habitats while bats with long, pointed wings fly at high speeds and forage in open habitats (Emrich et al., 2013), though no correction for phylogeny was performed. Differences in body shape and tail/flipper aspect ratio correlate to habitat and prey type in four species of baleen whales, though phylogenetic effects were not accounted for (Woodward et al., 2006). After accounting for phylogeny, *Anolis* lizards with longer limbs were found to run faster on broad surfaces and prefer tree trunk habitats, compared to lizards with shorter limbs, which run faster on narrow surfaces and prefer small branches (Irschick and Losos, 1999; Calsbeek and Irschick, 2007). Pectoral fin musculature in Lake Malawi cichlids is correlated to bethic/limnetic habitat and feeding behavior, taking into consideration the effect of phylogeny (Husley et al., 2013). Benthic species have larger pectoral fin musculature compared to limnetic species, possibly for locomoting through more complex environments as well as increased locomotor force needed to scrape or remove attached prey off the substrate. Labrids found in different reef habitats show high correlations between locomotor and feeding morphology, after correcting for phylogenetic effects (Collar et al., 2008). Labrid fishes in open habitats possess cranial traits that increase striking speed and pectoral fin traits that increase swimming speed, while benthic labrids that pick attached prey off the substrate possess traits that increase bite force and locomotor maneuverability.

This present study also found a relationship among morphology, locomotion and habitat. The presence of cephalic lobes correlated to oscillatory locomotion and a pelagic habitat using both uncorrected and corrected data (Table 4.1). Oscillatory locomotion is defined as having less

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than half a wave on the fins (Rosenberger, 2001). This locomotor mode requires stiff pectoral fins to transmit the force of the downward and upward strokes, with the fin rays moving more or less in unison (Schaefer and Summers, 2005), as opposed to undulation in which some fin rays are depressed while others are elevated to form more than one wave across the fins. The dexterity needed for prey capture seemingly conflicts with the rigidity needed for oscillatory locomotion. The cephalic lobes provide a way to both utilize oscillatory locomotion and successfully capture benthic prey by taking on the role of prey capture. *Gymnura micrura,* a predominantly benthic, lobeless batoid, utilizes undulatory locomotion on the substrate but a more oscillatory locomotion in the water column (Rosenberger, 2001), though are not known to travel long distances. Their diet consists of 89-99% teleosts (Jacobsen et al., 2009; Jacobsen and Bennett, 2013; Yokota et al., 2013), suggesting that the cephalic lobes may play a key role in maintaining the feeding success of specifically benthic prey.

Though *A. narinari* and *R. bonasus* spend most of the time in the water column and are classified as pelagic, they feed on benthic prey (Bigelow and Schroeder, 1953; McEachran and Carvalho, 2002). The only other pelagic stingray known thus far is the pelagic stingray (*Pteroplatytrygon violacea*), a lobeless dasyatid ray with an intermediate locomotor mode, between true undulation and true oscillation (Rosenberger, 2001). Cross-bracings, which help reinforce and stiffen the pectoral fin radials, are found in *A. narinari* and *R. bonasus* (Mulvany and Motta, 2013) but are absent in *P. violacea,* along with undulatory species (Schaefer and Summers, 2005). Also unlike *A. narinari* and *R. bonasus, P. violacea* feeds in the water column, wrapping its pectoral fins around fish (Jordan et al., 2009). Having a different feeding strategy may have facilitated an intermediate morphology, where some pectoral fin flexibility is maintained. It is not certain if *G. micrura* migrates long distances (Neer, 2008) though some

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distribution data suggests that females may pup off the coast of Central America and then migrate to Southern California (Mollet, 2002).

## CONCLUSIONS

After accounting for similarities due to phylogeny, most of the morphological variables correlated to the presence/absence of cephalic lobes whereas only one kinematic variable showed a relationship to the cephalic lobes. This signifies that the morphological changes in the pectoral fins, attributed to changes in locomotor style, are associated with the evolution of the cephalic lobes. As the function of prey capture was delegated to the maneuverable cephalic lobes, the pectoral fins were free to evolve in previously constrained ways. The lack of correlation with the majority of kinematic variables suggests that differences in prey capture kinematics are not a result of the presence/absence of cephalic lobes but some other driving factor(s). Expanding this study to include more species, particularly the lobeless *G. micrura,* which feeds in the water column and the lobeless, pelagic *P. violacea*, may help to elucidate the link between the cephalic lobes, feeding behavior, morphology and ecology. However, the need for a complete, continuous dataset to run the independent contrast method limits the ability to greatly expand this study. Overall, this study supports the idea that the cephalic lobes played a role in the shift to a pelagic habitat while maintaining the ability to feed on benthic prey.

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## TABLES AND FIGURES

Table 4.1. List of continuous behavioral and morphological variables used in a Pearson correlation analysis. Uncorrected values do not account for phylogeny. Corrected values generated using phylogenetically independent contrasts. * denotes a significant correlation to the presence of cephalic lobes.

| Variable | Uncorrected Correlation Coefficient (r) | p-value | Corrected Correlation Coefficient (r) | p-value |
|---|---|---|---|---|
| Habitat | 1 | <.001* | 1 | <.001* |
| Locomotion | -.921 | .026* | -.974 | .027* |
| Pounce duration | -.93 | .022* | -.911 | .090 |
| Tenting duration | -.835 | .079 | -.809 | .191 |
| Mouth opening duration | .798 | .11 | .522 | .478 |
| Mouth closing duration | .791 | .11 | .843 | .157 |
| Bite duration | .741 | .15 | .334 | .67 |
| Total capture duration | -.867 | .057 | -.866 | .134 |
| Pounce escape | -.046 | .94 | -.043 | .96 |
| Tent escape | .517 | .37 | -.0125 | .99 |
| Mouth escape | .247 | .69 | .336 | .66 |
| Ave # bites | -.224 | .72 | -.495 | .51 |
| Vertical movement | .748 | .15 | .762 | .24 |
| Horizontal movement | .993 | <.001* | .992 | .008* |
| Ave. # of joints/cm | -.943 | .017* | -.92 | .08 |
| $I_{lat}/I_{DV}$ whole | -.0621 | .92 | -.0561 | .94 |
| $I_{lat}/I_{DV}$ calcified | 994 | <.001* | .997 | .003* |
| Abductor superficialis insertion | .913 | .031* | .844 | .16 |
| Adductor superficialis insertion | .987 | .002* | .986 | .014* |
| # of electrosensory pores | -.931 | .021* | -.967 | .033* |

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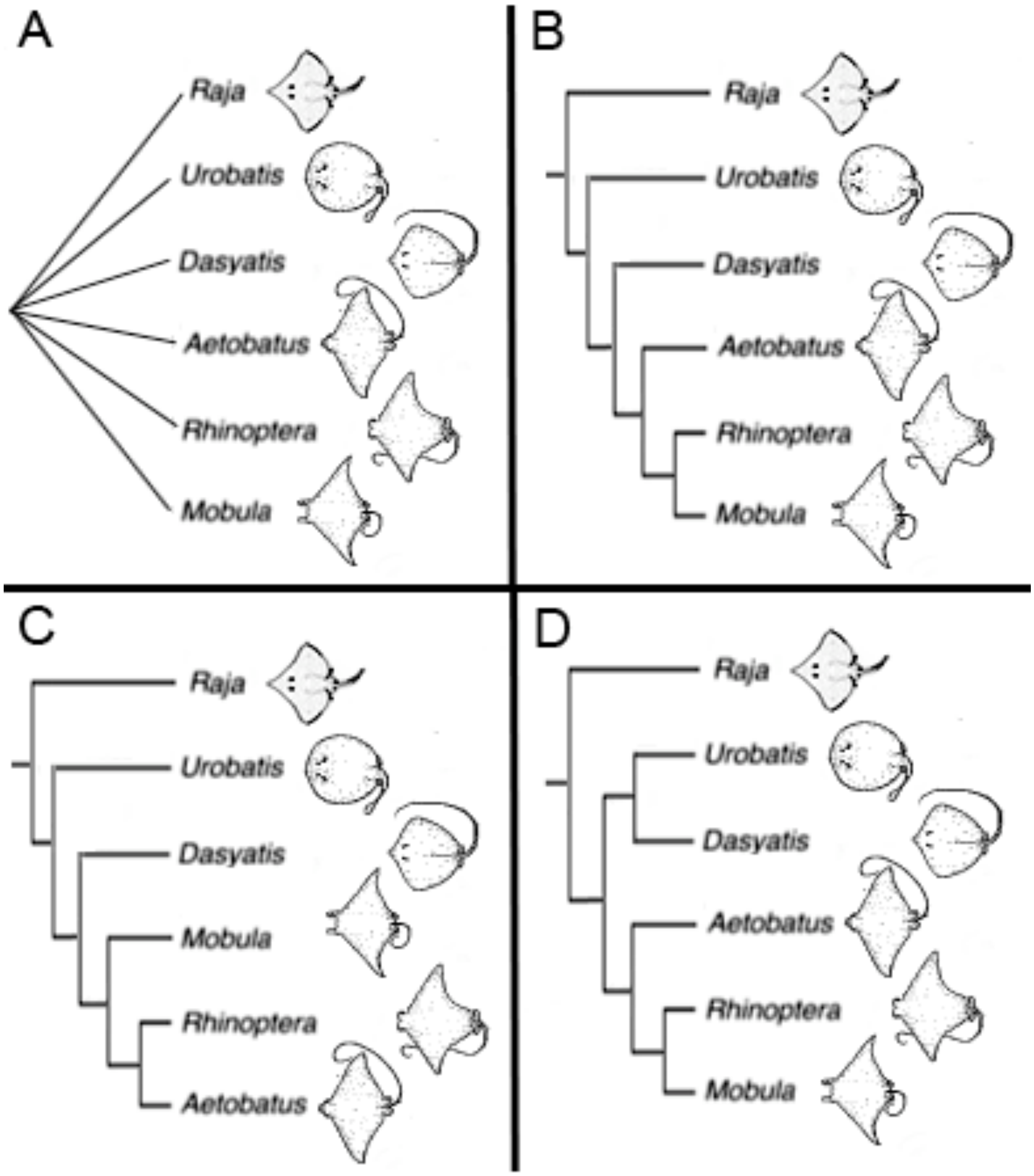

Fig. 4.1. Differing batoid phylogenies. (A) The phylogeny if all species were statistically independent of each other; (B) The phylogeny based on 39 and 65 morphological characters from Lovejoy (1996) and McEachran et al., (1996), respectively; (C) the phylogeny based on 77 morphological characters from Gonzalez-Isais and Dominguez (2004); (D) the phylogeny base on 104 and 105 morphological characters from Nishida (1990) and Shirai (1996) and the mitochondrial DNA and tRNA data from Dunn et al. (2003) and mitochondrial and nuclear DNA data from Aschliman et al. (2012).

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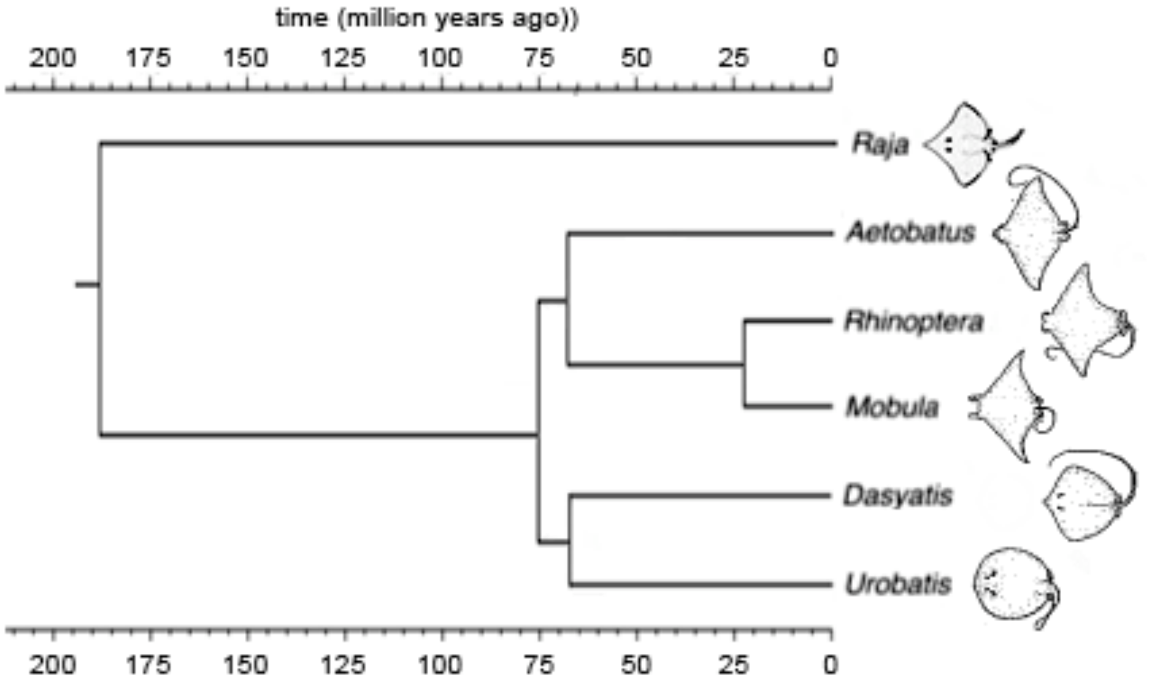

Fig. 4.2. Phylogeny of select batoids with branch lengths. Modified from Aschliman et al., 2012.

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# CHAPTER FIVE: FINAL CONCLUSIONS

This research examines the relationship between the morphology and function of the cephalic lobes in batoids, with respect to ecology and phylogeny. The study of novel structures often illustrate how changes in morphology correspond to ecological changes (Lachaise et al., 2000; Widelitz et al., 2007; Konow et al., 2008; Hernandez et al., 2009). The cephalic lobes in myliobatid rays present an interesting situation where novel structures arose to maintain benthic feeding while a concomitant shift in locomotor mode and habitat occurred, changing the overall ecology of these batoids. In the most derived batoids, the mobulids and mantas, the cephalic lobes have again functionally shifted to a role in pelagic prey capture, making these species truly pelagic (Notarbartolo-di-Sciara and Hillyer, 1989).

The first goal of this study was to examine the morphology of the anterior pectoral fins and cephalic lobes in six phylogenetically representative groups of batoids that differ in locomotor ability, habitat, and the presence or absence of cephalic lobes: the clearnose skate, *Raja eglanteria,* the yellow stingray, *Urobatis jamaicensis* and the Atlantic stingray, *Dasyatis sabina* represented lobeless, benthic batoids that primarily utilize undulatory locomotion; the spotted eagle ray, *Aetobatus narinari* and cownose ray, *Rhinoptera bonasus* represented lobed, pelagic batoids that utilize oscillatory locomotion and feed on benthic organisms; the spinetail mobula, *Mobula japonica,* the smoothtail mobula, *Mobula thurstoni,* the Monk's devil ray,

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*Mobula munkiana,* and the giant manta, *Manta birostris* represented lobed, pelagic batoids that utilize oscillatory locomotion and filter feed in the water column. The cephalic lobes were found to have numerous morphological modifications to the skeleton, musculature, and electrosensory pores that correspond with a functional shift from locomotion to prey detection and capture. The second goal of this study was to examine the use of the cephalic lobes in prey capture in a subset of these species: *R. eglanteria, U. jamaicensis, D. sabina, A. narinari,* and *R. bonasus*. In the lobed species, *A. narinari* and *R. bonasus,* prey capture was found to be localized to the cephalic region, pounce and tent durations were faster, but capture success was equal to lobeless species. Modulation, indicated by a significant change in the kinematic variables with a change in prey type, was seen in all species, although contrary to expectation, lobed species did not display greater ability to modulate prey capture behavior. The final goal of this study was to determine if the presence/absence of cephalic lobes correlated to any of the morphological and behavioral variables, accounting for the relatedness of species. Phylogenetically corrected correlations showed that the majority of the morphological variables, along with locomotor mode and habitat, had a relationship with presence/absence of the cephalic lobes while only one kinematic variable displayed a correlation with the presence/absence of cephalic lobes.

## MORPHOLOGY

Variation in the cross sectional area, second moment of area, calcification patterns, and flexibility of skeletal elements revealed distinct differences between the pectoral fin radials in oscillatory swimmers, *A. narinari, R. bonasus* and *M. munkiana* and the radials of the cephalic lobes and pectoral fins of undulatory species, *R. eglanteria, U. jamaicensis,* and *D. sabina*. The

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morphology of the pectoral fins of oscillatory swimmers showed increased stiffness through inter-radial cross bracings, crustal calcification patterns and decreased number of radial joints. Stiffness and increased resistance to bending is needed for oscillatory locomotion (Schaefer and Summers, 2005), as well as other forms of locomotion that put a high amount of force on the skeleton (Lauder et al., 2006). The cephalic lobes and pectoral fin radials of undulatory species showed adaptations to increase maneuverability, with more circular radial cross sectional areas, lower $I_{\mathrm{Lat}}/I_{\mathrm{DV}}$, increased number of joints/cm and a lack of cross bracings. High maneuverability is needed for prey capture and undulatory locomotion, as the fin rays independently move to bend certain portions of the fins/lobes (Rosenberger, 2001) to locomote, manipulate and excavate prey (Gudger, 1914; Sasko et al., 2006) and even control water flow underneath the body during prey capture (Wilga et al., 2012).

The pectoral fin musculature for all examined species is comprised of two dorsal (abductor superficialis and profundus) and two ventral (adductor superficialis and profundus) muscles that insert along the radials. The cephalic lobes also contain these muscles, though the adductor and abductor superficialis muscles insert at the very distal edge of the lobes via tendons, much like the flexor and extensor digitorum muscles in humans (Gray, 1977). In addition to these four previously described muscles (Rosenberger and Westneat, 1999), the dorsal oblique muscle, a novel muscle running at an oblique angle to the radials, was found in the cephalic lobes, possibly increasing dexterity and control of the lobes. Increases in muscle numbers or subdivisions, along with diversity in muscle orientation have been linked to the ability to perform more complex and dexterous movements (Boas and Pauli, 1908; Friel and Wainwright, 1998; Marshall et al., 1998).

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Electrosensory pores were found on the cephalic lobes of *Aetobatus narinari* and *Rhinoptera bonasus* and the pectoral fins of lobeless species. This indicates that the pectoral fins in lobeless species and the cephalic lobes of *A. narinari* and *R. bonasus* are used in prey capture, as the electrosensory receptors are used in part for prey detection (Tricas and Sisnero, 2004). The paddlefish similarly utilizes electrosensory receptors on the rostrum to detect plankton in turbid, low visibility rivers (Nachtrieb, 1910; Wilkens et al., 1997). Electrosensory pores were absent in the pectoral fins in lobed species, which are not used for prey capture but primarily for locomotion. The cephalic lobes in mobulid and manta species did not have electrosensory pores, but cephalic lobes in these species are used hydrodynamically to channel water and entrained plankton into the mouth (Notarbartolo-di-Sciara and Hillyer, 1989), not prey detection. Similarly, the megamouth shark, *Megachasma pelagios,* and basking shark, *Cetorhinus maximus*, which also filter feed, have relatively few electrosensory pores on the head (Kempster and Collin, 2011a; Kempster and Collin, 2011b). The distribution of examined ampullary pores was uniform for all species except on the cephalic lobes of *R. bonasus,* where the distal edges of the lobes had higher concentrations of pores. Because the lobes are laterally placed on *R. bonasus*, it is not possible to have both lobes parallel to the substrate when depressed. The distal edges of the lobe are closest to the substrate, and thus having a higher concentration of electrosensory pores may increase detection ability and spatial resolution (Raschi, 1978).

## KINEMATICS

As suggested by the electrosensory pore distributions and the skeletal morphology, lobeless species utilized the pectoral fins for prey capture and manipulation while lobed species

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utilized the cephalic lobes to capture and manipulate prey. Pouncing and prey handling time tended to be faster in lobed species, perhaps due to the high cruising speeds when initiating prey capture, and the fact that prey capture was localized to the head and cephalic lobes instead of the entire body. The increased complexity of the cephalic lobe musculature corresponded to a wider range of movement seen in the horizontal plane for lobes species compared to lobeless species. However, only the cephalic lobes of *R. bonasus* showed greater movement in the vertical plane compared to the anterior pectoral fins in lobeless species. Bite duration tended to be longer for lobed species, perhaps a consequence of having more force efficient, thus less speed efficient, jaws to consume hard prey (Turingan et al., 1995; Huber et al., 2005; Westneat, 2006).

With increased functional morphological complexity of the cephalic lobes, it was hypothesized that there would be increased modulation during prey capture for these species, as has been reported for other fishes (Liem, 1979; Turingan and Wainwright, 1993; Wilga and Motta, 1998). This hypothesis was not supported as modulation based on prey elusivity was seen in all batoids during certain stages of prey capture. Modulation of prey capture with different prey types was seen in the lobeless *R. eglanteria* and *U. jamaicensis* during tenting duration, taking longer to handle elusive prey before successfully feeding. Longer handling times have been noted in other cartilaginous (Lowry and Motta, 2007) and bony (Brachvogel et al., 2013) fishes. This most likely reflects the increased complexity in handling elusive prey. The shorter duration of prey handling time in lobed species may be due to the reduced tented area available for prey to move around, as tenting is only in the head region. The cephalic lobes also have higher electrosensory pore counts than the pectoral fins, possibly increasing spatial resolution (Raschi, 1978). For all species, elusive prey was at a greater distance from the mouth compared to non-elusive prey at the beginning of mouth opening (for both unsuccessful and successful

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bites). This may indicate increased complexity in manipulating elusive prey, as pinning prey to the substrate to prevent movement also prevents repositioning prey closer to the mouth before biting. These batoids could also be initiating biting, and thus suction, when elusive prey are farther away in an attempt to prevent prey escapes. The elusive prey may also have simply evaded the mouth during biting, increasing distance.

Ultimately, even with increases in handling times and prey distance from the mouth, batoids were very successful at feeding on prey that had been tented, either by the body or cephalic lobes. Using the head region and cephalic lobes for prey capture in lieu of the pectoral fins and consequently the entire body appeared to have no effect on prey capture success, as there were very few prey escapes under laboratory conditions for all batoids. As prey capture became confined to the head region, the pectoral fins of lobed species were free to shift to an oscillatory locomotor style that suited the shift to pelagic habitats.

## CORRELATIONS AMONG MORPHOLOGICAL, KINEMATIC AND ECOLOGICAL VARIABLES AND THE CEPHALIC LOBES

The independence of data is an assumption of statistical tests. However, when examining trends among groups of species, the relatedness of taxa violates that assumption, as some species are more closely related than others (Felsenstein, 1985). More closely related species often share similar characteristics due to the shorter divergence time (Harvey and Pagel, 1991). Therefore, the expectation that closely related taxa should be more similar while divergent taxa should be more different must be accounted for, as statistical analyses assume taxa to be equally related. The independent contrast method uses phylogenetic tree topography to account for the

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evolutionary relationship among species, generating contrasts that are statistically independent of each other and thus do no violate the assumption of independence (Felsenstein, 1985).

The phylogenetically corrected data showed that most of the skeletal, musculature and electrosensory pore variables correlated to the presence/absence of the cephalic lobes. Less circular radial cross sections, muscle insertions on the radials of the anterior pectoral fins, and a decrease in the number of electrosensory pores are all morphological modifications for oscillatory locomotion in the pectoral fins of lobed species. Fin morphology is often linked to locomotor mode in fishes (Webb, 1984; Drucker and Lauder, 2002; Schaefer and Summers, 2005). The presence of cephalic lobes also showed a direct correlation to oscillatory locomotion as well as a pelagic habitat.

Only two of the twelve kinematic variables (pounce duration and horizontal movement) correlated with the presence/absence of the cephalic lobes, before correcting for phylogeny. However, after the phylogenetic correction, pounce duration did not correlate to the presence/absence of cephalic lobes. This indicates that although pounce duration was longest in the most basal species, *R. eglanteria,* of intermediate duration in the more derived species, *U. jamaicensis* and *D. sabina,* and shortest in the most derived species, *A. narinari* and *R. bonasus,* the trend was not strong enough after the phylogenetic correction to show a significant correlation. Pounce duration may instead be dependent on other variables, such as jaw morphology, locomotor strategy during prey capture, mechanotactile reception, or the ability to manipulate water flow.

The single phylogenetically corrected kinematic variable that correlated to the presence of cephalic lobes was increased horizontal movement of the cephalic lobes during prey capture.

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Lobed species have a gap between the cephalic lobes and pectoral fins, where no radials are present. As the cephalic lobes are depressed, they fan out horizontally to form a barrier at the anterior and lateral part of the head, helping to occlude this gap to prevent prey from escaping. Lobeless batoids that utilize the entire body for prey capture do not need to fan out the pectoral fins, as the pectoral fins expand anteriorly, laterally and posteriorly from the rostrum to the pelvic fins and tail, preventing prey from escaping in all directions.

## CONCLUSIONS

The cephalic lobes are novel structures found exclusively in pelagic, oscillatory myliobatids (*Aetobatus*, *Aetomylaeus*, *Manta*, *Mobula*, *Myliobatis* and *Rhinoptera*). With the evolution of the cephalic lobes came a concomitant shift to oscillatory locomotion and a pelagic habitat (Fig. 5.1). Morphological changes to flatten and stiffen the pectoral fins occurred in response to shifts in locomotor style and the different demands. As the requirements for oscillatory locomotion contradict the requirements needed for prey capture, the shift to oscillatory locomotion while maintaining the same feeding strategy would have been extremely difficult without the cephalic lobes. Other batoid species have developed different strategies for feeding in the water column despite the lack of cephalic lobes. One out of approximately 90 species in the family Dasyatidae, the pelagic stingray, *Pteroplatytrygon violacea*, is pelagic and moves in the water column using locomotion intermediate between undulation and oscillation (between half to one wave length along the pectoral fin) but feeds in the water column, wrapping its pectoral fins around fish or squid (Jordan, 2008). Torpedo rays, of the family Torpedinidae, are pelagic, but utilize body-caudal-fin locomotion (Roberts, 1969) and feed in the water column,

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wrapping their pectoral fins around prey and stunning them via electric organs (Wilson, 1953; Lowe et al., 1994). Butterfly rays, of the family Gymnuridae, are benthic and utilize undulatory locomotion on the substrate but shift to locomotion intermediate between undulation and oscillation when feeding on fish in the water column (Rosenberger, 2001), presumably using their pectoral fins to wrap around fish. While other batoids have utilized different strategies to feed in the water column, the myliobatids are the only pelagic batoid species that utilize oscillatory locomotion (less than half a wave along the pectoral fin), and possess cephalic lobes, with the aetobatids and rhinopterids feeding on benthic organisms and mobulids filter feeding on plankton in the water column. The ability to partition locomotor function to the pectoral fins and prey capture to the cephalic lobes allowed these batoids to exploit pelagic habitats, attaining high, sustained cruising speeds that increase their home ranges and possibly expand their niches and resources while still maintaining the ability to successfully feed on benthic organisms with the dexterous cephalic lobes. The cephalic lobes provide an interesting and innovation solution to the trade-off between the high maneuverability needed for prey capture and the stiffness required for oscillatory locomotion.

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## TABLES AND FIGURES

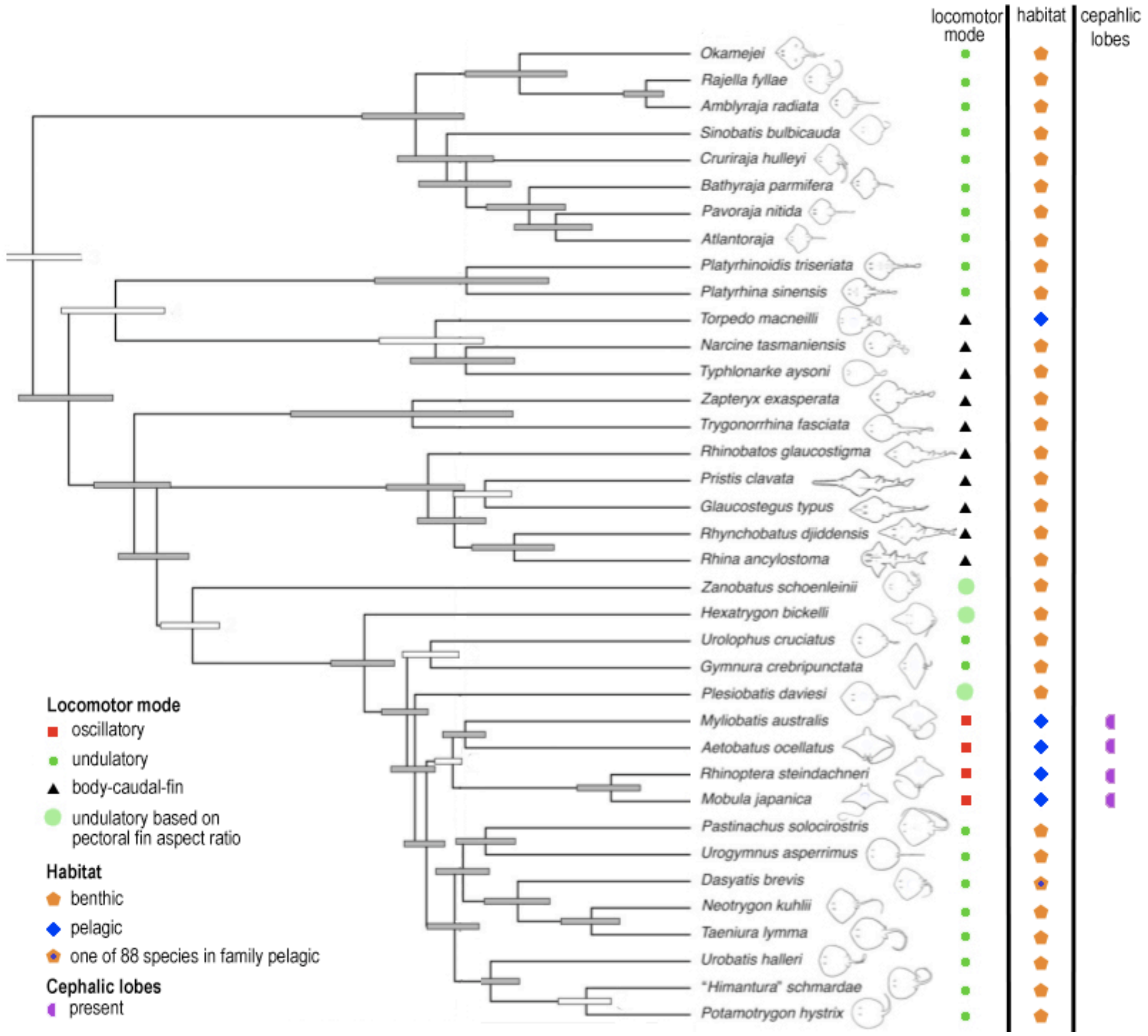

Fig. 5.1 Batoid phylogeny from Aschliman et al. (2013) with 22 of the 23 families represented. Locomotor data based on Rosenberger and Westneat (1999), Rosenberger (2001), Schaefer and Summers (2005), Rosenblaum et al. (2011), Blevins and Lauder (2013). Missing locomotor data was estimated using the pectoral fin aspect ratio as a predictor of locomotor mode (Fontanella et al., 2013). Habitat data was compiled from Bigelow and Schroeder (1953) and Compagno (2009). Cephalic lobe data was compiled from Bigelow and Schroeder (1953), McEachran and Carvalho (2002).

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# APPENDICES

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## APPENDIX A

Results of a Pearson correlation using uncorrected data. The correlation coefficient (r) is the shaded value, p-value is unshaded. Significant correlations are highlighted in red.

| | Locomotion | Habitat | # Bites | # of pounce escapes | # of mouth escapes | # of tent escapes | Pounce duration | Tent duration | Mouth opening duration | Mouth closing duration | Bite duration | Capture event duration | Horizontal movement | Vertical movement | $I_{lat}/I_{DV}$ whole | $I_{lat}/I_{DV}$ calicified | Abductor superficialis insetion | Adductor superficialis insetion | # joints/cm | # electrosensory pores |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cephalic lobes | -0.921 | 1.000 | -0.224 | -0.046 | 0.247 | 0.517 | -0.930 | -0.835 | 0.798 | 0.791 | 0.741 | -0.867 | 0.993 | 0.748 | -0.062 | 0.994 | 0.913 | 0.987 | -0.943 | -0.931 |
| | 0.026 | 0.000 | 0.717 | 0.942 | 0.689 | 0.373 | 0.022 | 0.079 | 0.106 | 0.111 | 0.152 | 0.057 | 0.001 | 0.146 | 0.921 | 0.001 | 0.031 | 0.002 | 0.016 | 0.021 |
| Locomotion | | -0.921 | 0.071 | 0.020 | -0.117 | -0.363 | 0.768 | 0.578 | -0.677 | -0.944 | -0.563 | 0.631 | -0.914 | -0.529 | -0.220 | -0.947 | -0.748 | -0.873 | 0.925 | 0.997 |
| | | 0.026 | 0.910 | 0.975 | 0.852 | 0.548 | 0.130 | 0.308 | 0.210 | 0.016 | 0.323 | 0.254 | 0.030 | 0.359 | 0.722 | 0.014 | 0.146 | 0.053 | 0.024 | 0.000 |
| Habitat | | | -0.224 | -0.046 | 0.247 | 0.517 | -0.930 | -0.835 | 0.798 | 0.791 | 0.741 | -0.867 | 0.993 | 0.748 | -0.062 | 0.994 | 0.913 | 0.987 | -0.943 | -0.931 |
| | | | 0.717 | 0.942 | 0.689 | 0.373 | 0.022 | 0.079 | 0.106 | 0.111 | 0.152 | 0.057 | 0.001 | 0.146 | 0.921 | 0.001 | 0.031 | 0.002 | 0.016 | 0.021 |
| # Bites | | | | -0.438 | -0.922 | -0.243 | 0.344 | 0.587 | -0.174 | 0.107 | -0.051 | 0.556 | -0.245 | -0.510 | 0.890 | -0.192 | 0.009 | -0.139 | 0.356 | 0.019 |
| | | | | 0.460 | 0.026 | 0.694 | 0.571 | 0.298 | 0.779 | 0.864 | 0.935 | 0.330 | 0.691 | 0.380 | 0.043 | 0.757 | 0.989 | 0.824 | 0.556 | 0.976 |
| # of pounce escapes | | | | | 0.735 | 0.663 | 0.265 | -0.083 | 0.419 | 0.148 | 0.291 | 0.029 | -0.132 | -0.350 | -0.407 | -0.100 | -0.283 | -0.138 | -0.253 | 0.073 |
| | | | | | 0.157 | 0.223 | 0.667 | 0.895 | 0.482 | 0.812 | 0.634 | 0.963 | 0.833 | 0.564 | 0.496 | 0.873 | 0.645 | 0.824 | 0.681 | 0.907 |
| # of mouth escapes | | | | | | 0.543 | -0.238 | -0.566 | 0.410 | 0.045 | 0.274 | -0.494 | 0.222 | 0.303 | -0.842 | 0.194 | -0.009 | 0.153 | -0.452 | -0.062 |
| | | | | | | 0.344 | 0.700 | 0.320 | 0.493 | 0.942 | 0.655 | 0.397 | 0.720 | 0.620 | 0.074 | 0.755 | 0.989 | 0.806 | 0.444 | 0.921 |
| # of tent escape | | | | | | | -0.321 | -0.598 | 0.921 | 0.439 | 0.905 | -0.498 | 0.420 | 0.128 | -0.348 | 0.436 | 0.474 | 0.511 | -0.619 | -0.363 |
| | | | | | | | 0.599 | 0.287 | 0.026 | 0.459 | 0.035 | 0.393 | 0.481 | 0.837 | 0.566 | 0.463 | 0.420 | 0.379 | 0.266 | 0.548 |
| Pounce duration | | | | | | | | 0.897 | -0.608 | -0.536 | -0.589 | 0.946 | -0.958 | -0.936 | 0.231 | -0.925 | -0.903 | -0.939 | 0.798 | 0.785 |
| | | | | | | | | 0.039 | 0.276 | 0.352 | 0.296 | 0.015 | 0.010 | 0.019 | 0.708 | 0.024 | 0.036 | 0.018 | 0.105 | 0.116 |
| Tent duration | | | | | | | | | -0.736 | -0.369 | -0.719 | 0.990 | -0.832 | -0.869 | 0.577 | -0.788 | -0.795 | -0.837 | 0.774 | 0.584 |
| | | | | | | | | | 0.156 | 0.541 | 0.171 | 0.001 | 0.080 | 0.056 | 0.309 | 0.114 | 0.108 | 0.077 | 0.124 | 0.301 |
| Mouth opening duration | | | | | | | | | | 0.695 | 0.969 | -0.683 | 0.723 | 0.368 | -0.178 | 0.743 | 0.743 | 0.794 | -0.838 | -0.685 |
| | | | | | | | | | | 0.193 | 0.006 | 0.203 | 0.167 | 0.542 | 0.775 | 0.151 | 0.150 | 0.109 | 0.076 | 0.201 |

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Results of a Pearson correlation using uncorrected data (continued). The correlation coefficient (r) is the shaded value, p-value is unshaded. Significant correlations are highlighted in red.

| | Locomotion | Habitat | # Bites | # of pounce escapes | # of mouth escapes | # of tent escapes | Pounce duration | Tent duration | Mouth opening duration | Mouth closing duration | Bite duration | Capture event duration | Horizontal movement | Vertical movement | $I_{lat}/I_{DV}$ whole | $I_{lat}/I_{DV}$ calicified | Abductor superficialis insetion | Adductor superficialis insetion | # joints/cm | # electrosensory pores |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mouth closing duration | | | | | | | | | | | 0.573 | -0.401 | 0.755 | 0.229 | 0.388 | 0.816 | 0.607 | 0.737 | -0.855 | -0.938 |
| | | | | | | | | | | | 0.312 | 0.504 | 0.140 | 0.711 | 0.519 | 0.092 | 0.278 | 0.156 | 0.065 | 0.018 |
| Bite duration | | | | | | | | | | | | -0.662 | 0.666 | 0.365 | -0.157 | 0.676 | 0.789 | 0.775 | -0.716 | -0.590 |
| | | | | | | | | | | | | 0.224 | 0.220 | 0.545 | 0.801 | 0.210 | 0.113 | 0.124 | 0.174 | 0.295 |
| Capture event duration | | | | | | | | | | | | | -0.878 | -0.922 | 0.510 | -0.833 | -0.824 | -0.869 | 0.784 | 0.639 |
| | | | | | | | | | | | | | 0.050 | 0.026 | 0.380 | 0.080 | 0.086 | 0.056 | 0.116 | 0.246 |
| Horizontal movement | | | | | | | | | | | | | | 0.803 | -0.066 | 0.995 | 0.906 | 0.980 | -0.918 | -0.925 |
| | | | | | | | | | | | | | | 0.102 | 0.916 | 0.000 | 0.034 | 0.003 | 0.028 | 0.025 |
| Vertical movement | | | | | | | | | | | | | | | -0.436 | 0.742 | 0.742 | 0.761 | -0.594 | -0.542 |
| | | | | | | | | | | | | | | | 0.463 | 0.151 | 0.151 | 0.135 | 0.291 | 0.345 |
| $I_{lat}/I_{DV}$ whole | | | | | | | | | | | | | | | | 0.006 | 0.006 | -0.037 | 0.126 | -0.248 |
| | | | | | | | | | | | | | | | | 0.993 | 0.992 | 0.953 | 0.840 | 0.688 |
| $I_{lat}/I_{DV}$ calcified | | | | | | | | | | | | | | | | | 0.894 | 0.977 | -0.935 | -0.956 |
| | | | | | | | | | | | | | | | | | 0.041 | 0.004 | 0.020 | 0.011 |
| Abductor sup. insertion | | | | | | | | | | | | | | | | | | 0.966 | -0.742 | -0.790 |
| | | | | | | | | | | | | | | | | | | 0.008 | 0.151 | 0.112 |
| Adductor sup. insertion | | | | | | | | | | | | | | | | | | | -0.885 | -0.896 |
| | | | | | | | | | | | | | | | | | | | 0.046 | 0.039 |
| # joints/cm | | | | | | | | | | | | | | | | | | | | 0.912 |
| | | | | | | | | | | | | | | | | | | | | 0.031 |

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## APPENDIX B

Results of a Pearson correlation using phylogenetically corrected data. The correlation coefficient (r) is the shaded value, p-value is unshaded.  Significant correlations are highlighted in red.

| | Locomotion | Habitat | # Bites | # of pounce escapes | # of mouth escapes | # of tent escapes | Pounce duration | Tent duration | Mouth opening duration | Mouth closing duration | Bite duration | Capture event duration | Horizontal movement | Vertical movement | $I_{lat}/I_{DV}$ whole | $I_{lat}/I_{DV}$ calicified | Abductor superficialis insetion | Adductor superficialis insetion | # joints/cm | # electrosensory pores |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cephalic lobes | -0.974 | 1.000 | -0.495 | -0.043 | 0.336 | 0.013 | -0.911 | -0.809 | 0.522 | 0.843 | 0.334 | -0.866 | 0.992 | 0.762 | -0.056 | 0.997 | 0.844 | 0.986 | -0.920 | -0.967 |
| | 0.027 | 0.000 | 0.505 | 0.957 | 0.664 | 0.988 | 0.090 | 0.191 | 0.478 | 0.157 | 0.666 | 0.134 | 0.008 | 0.238 | 0.944 | 0.003 | 0.156 | 0.014 | 0.080 | 0.033 |
| Locomotion | | -0.974 | 0.353 | 0.006 | -0.258 | -0.062 | 0.814 | 0.671 | -0.577 | -0.937 | -0.419 | 0.731 | -0.950 | -0.613 | -0.108 | -0.975 | -0.810 | -0.956 | 0.916 | 0.997 |
| | | 0.027 | 0.647 | 0.994 | 0.742 | 0.938 | 0.186 | 0.329 | 0.423 | 0.063 | 0.581 | 0.269 | 0.051 | 0.387 | 0.892 | 0.025 | 0.190 | 0.044 | 0.084 | 0.003 |
| Habitat | | | -0.495 | -0.043 | 0.336 | 0.013 | -0.911 | -0.809 | 0.522 | 0.843 | 0.334 | -0.866 | 0.992 | 0.762 | -0.056 | 0.997 | 0.844 | 0.986 | -0.920 | -0.967 |
| | | | 0.505 | 0.957 | 0.664 | 0.988 | 0.090 | 0.191 | 0.478 | 0.157 | 0.666 | 0.134 | 0.008 | 0.238 | 0.944 | 0.003 | 0.156 | 0.014 | 0.080 | 0.033 |
| # Bites | | | | -0.547 | -0.928 | -0.532 | 0.454 | 0.880 | -0.617 | -0.248 | -0.499 | 0.770 | -0.458 | -0.482 | 0.892 | -0.430 | -0.090 | -0.385 | 0.659 | 0.293 |
| | | | | 0.453 | 0.072 | 0.468 | 0.546 | 0.120 | 0.383 | 0.752 | 0.501 | 0.230 | 0.542 | 0.518 | 0.108 | 0.570 | 0.911 | 0.615 | 0.341 | 0.707 |
| # of pounce escapes | | | | | 0.819 | 0.996 | 0.349 | -0.174 | 0.812 | 0.217 | 0.887 | 0.015 | -0.160 | -0.435 | -0.576 | -0.117 | -0.571 | -0.210 | -0.351 | 0.078 |
| | | | | | 0.181 | 0.004 | 0.651 | 0.826 | 0.188 | 0.783 | 0.113 | 0.985 | 0.840 | 0.565 | 0.424 | 0.883 | 0.429 | 0.790 | 0.649 | 0.922 |
| # of mouth escapes | | | | | | 0.809 | -0.167 | -0.686 | 0.794 | 0.286 | 0.745 | -0.529 | 0.258 | 0.143 | -0.858 | 0.259 | -0.179 | 0.186 | -0.623 | -0.184 |
| | | | | | | 0.191 | 0.833 | 0.314 | 0.206 | 0.714 | 0.255 | 0.471 | 0.742 | 0.857 | 0.142 | 0.741 | 0.821 | 0.814 | 0.377 | 0.816 |
| # of tent escape | | | | | | | 0.315 | -0.184 | 0.851 | 0.293 | 0.922 | -0.002 | -0.108 | -0.424 | -0.528 | -0.059 | -0.522 | -0.154 | -0.403 | 0.009 |
| | | | | | | | 0.685 | 0.816 | 0.149 | 0.707 | 0.078 | 0.998 | 0.892 | 0.576 | 0.472 | 0.941 | 0.478 | 0.846 | 0.597 | 0.991 |
| Pounce duration | | | | | | | | 0.823 | -0.169 | -0.558 | 0.050 | 0.918 | -0.954 | -0.957 | 0.094 | -0.922 | -0.924 | -0.946 | 0.709 | 0.823 |
| | | | | | | | | 0.177 | 0.831 | 0.442 | 0.950 | 0.082 | 0.046 | 0.043 | 0.906 | 0.079 | 0.076 | 0.054 | 0.291 | 0.177 |
| Tent duration | | | | | | | | | -0.500 | -0.474 | -0.309 | 0.980 | -0.807 | -0.811 | 0.613 | -0.773 | -0.552 | -0.756 | 0.811 | 0.638 |
| | | | | | | | | | 0.500 | 0.526 | 0.691 | 0.020 | 0.193 | 0.189 | 0.387 | 0.227 | 0.448 | 0.244 | 0.189 | 0.362 |
| Mouth opening duration | | | | | | | | | | 0.735 | 0.975 | -0.383 | 0.410 | -0.027 | -0.370 | 0.464 | 0.000 | 0.377 | -0.811 | -0.518 |
| | | | | | | | | | | 0.265 | 0.025 | 0.617 | 0.590 | 0.973 | 0.630 | 0.536 | 1.000 | 0.623 | 0.189 | 0.482 |

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Results of a Pearson correlation using phylogenetically corrected data (continued). The correlation coefficient (r) is the shaded value, p-value is unshaded. Significant correlations are highlighted in red.

| | Locomotion | Habitat | # Bites | # of pounce escapes | # of mouth escapes | # of tent escapes | Pounce duration | Tent duration | Mouth opening duration | Mouth closing duration | Bite duration | Capture event duration | Horizontal movement | Vertical movement | $I_{lat}/I_{DV}$ whole | $I_{lat}/I_{DV}$ calcified | Abductor superficialis insetion | Adductor superficialis insetion | # joints/cm | # electrosensory pores |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mouth closing duration | | | | | | | | | | | 0.640 | -0.497 | 0.781 | 0.299 | 0.192 | 0.837 | 0.592 | 0.794 | -0.887 | -0.925 |
| | | | | | | | | | | | 0.360 | 0.503 | 0.219 | 0.701 | 0.808 | 0.163 | 0.408 | 0.206 | 0.113 | 0.075 |
| Bite duration | | | | | | | | | | | | -0.173 | 0.211 | -0.247 | -0.320 | 0.275 | -0.192 | 0.181 | -0.667 | -0.359 |
| | | | | | | | | | | | | 0.827 | 0.789 | 0.753 | 0.680 | 0.725 | 0.808 | 0.819 | 0.333 | 0.641 |
| Capture event duration | | | | | | | | | | | | | -0.882 | -0.904 | 0.468 | -0.845 | -0.700 | -0.844 | 0.792 | 0.711 |
| | | | | | | | | | | | | | 0.118 | 0.096 | 0.532 | 0.155 | 0.300 | 0.156 | 0.208 | 0.289 |
| Horizontal movement | | | | | | | | | | | | | | 0.829 | -0.030 | 0.995 | 0.898 | 0.997 | -0.865 | -0.951 |
| | | | | | | | | | | | | | | 0.171 | 0.970 | 0.005 | 0.102 | 0.003 | 0.135 | 0.049 |
| Vertical movement | | | | | | | | | | | | | | | -0.222 | 0.773 | 0.840 | 0.813 | -0.530 | -0.625 |
| | | | | | | | | | | | | | | | 0.778 | 0.227 | 0.160 | 0.187 | 0.470 | 0.375 |
| $I_{lat}/I_{DV}$ whole | | | | | | | | | | | | | | | | 0.013 | 0.292 | 0.051 | 0.255 | -0.171 |
| | | | | | | | | | | | | | | | | 0.987 | 0.708 | 0.949 | 0.745 | 0.829 |
| $I_{lat}/I_{DV}$ calcified | | | | | | | | | | | | | | | | | 0.882 | 0.995 | -0.889 | -0.975 |
| | | | | | | | | | | | | | | | | | 0.118 | 0.005 | 0.111 | 0.025 |
| Abductor sup. insertion | | | | | | | | | | | | | | | | | | 0.922 | -0.568 | -0.845 |
| | | | | | | | | | | | | | | | | | | 0.078 | 0.432 | 0.155 |
| Adductor sup. insertion | | | | | | | | | | | | | | | | | | | -0.842 | -0.963 |
| | | | | | | | | | | | | | | | | | | | 0.158 | 0.037 |
| # joints/cm | | | | | | | | | | | | | | | | | | | | 0.883 |
| | | | | | | | | | | | | | | | | | | | | 0.117 |

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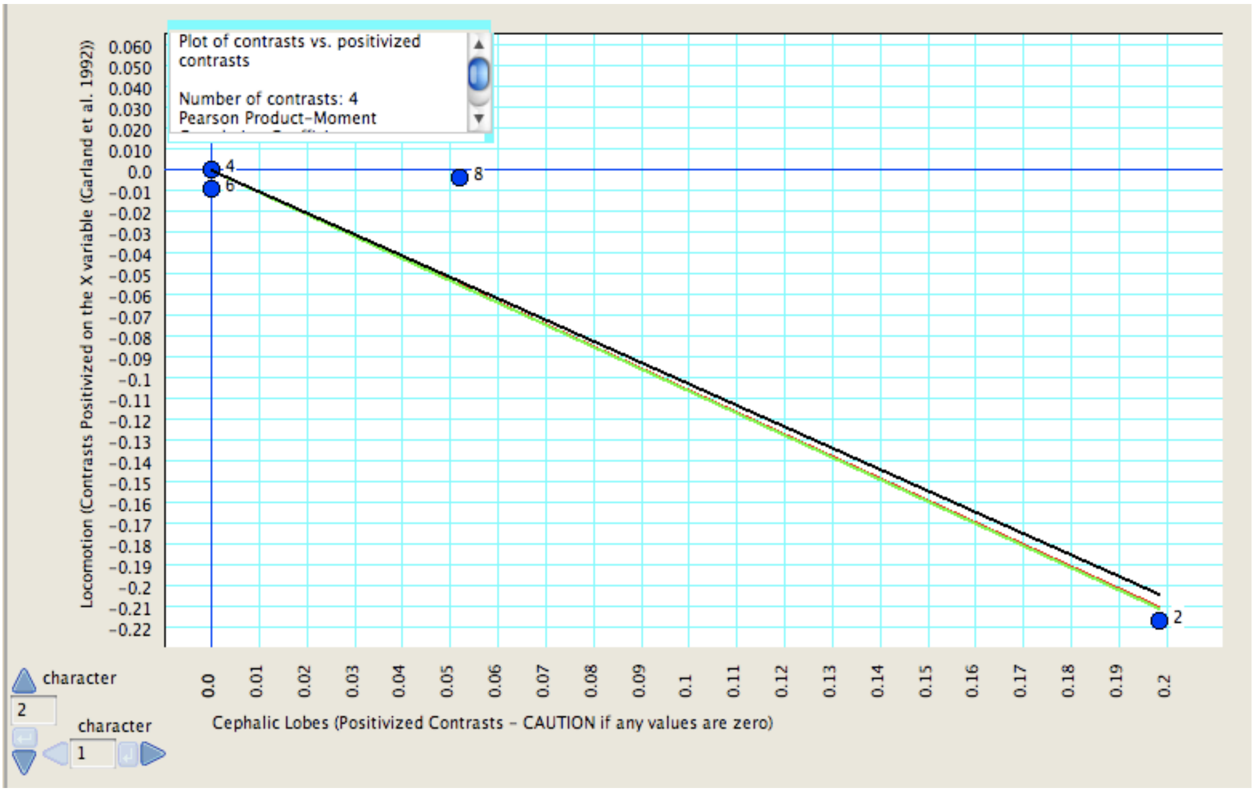
# APPENDIX C

Scatterplots of the x positivized contrasts versus y contrasts.



Plot of contrasts vs. positivized contrasts

Number of contrasts: 4
Pearson Product-Moment

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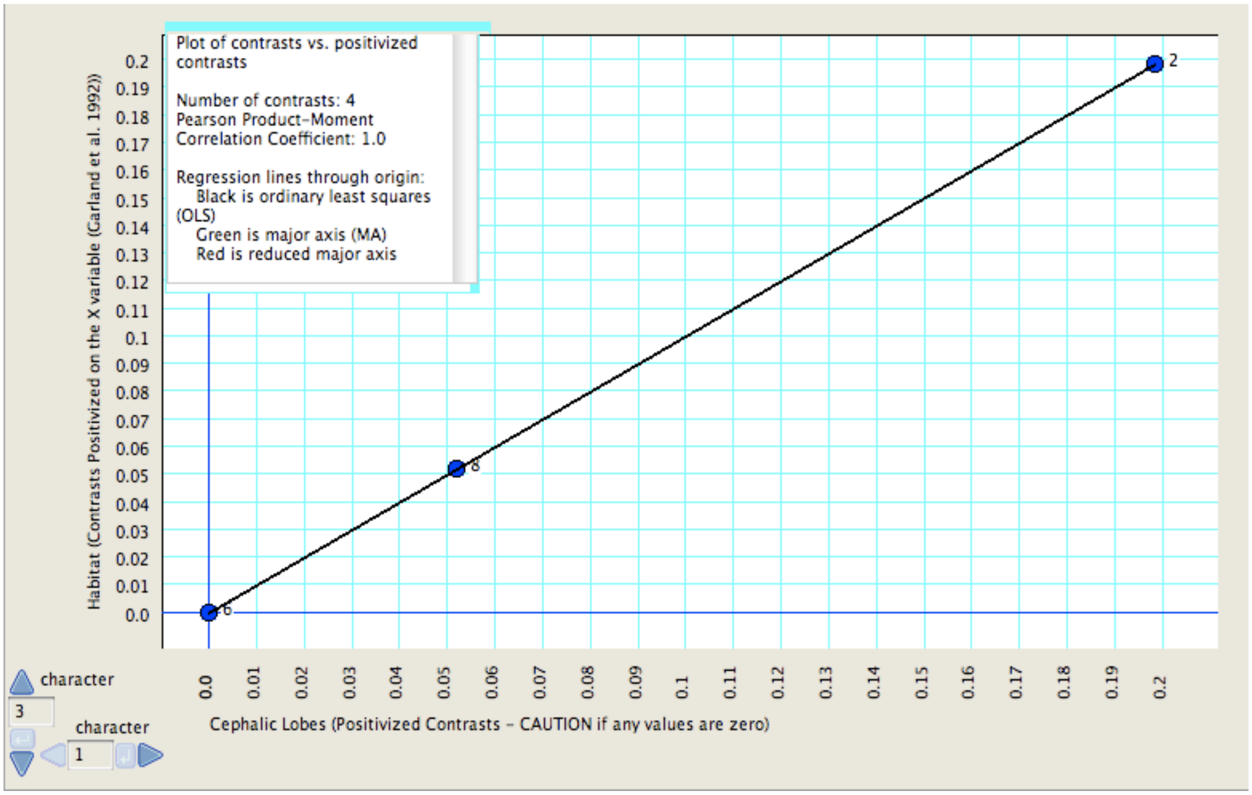
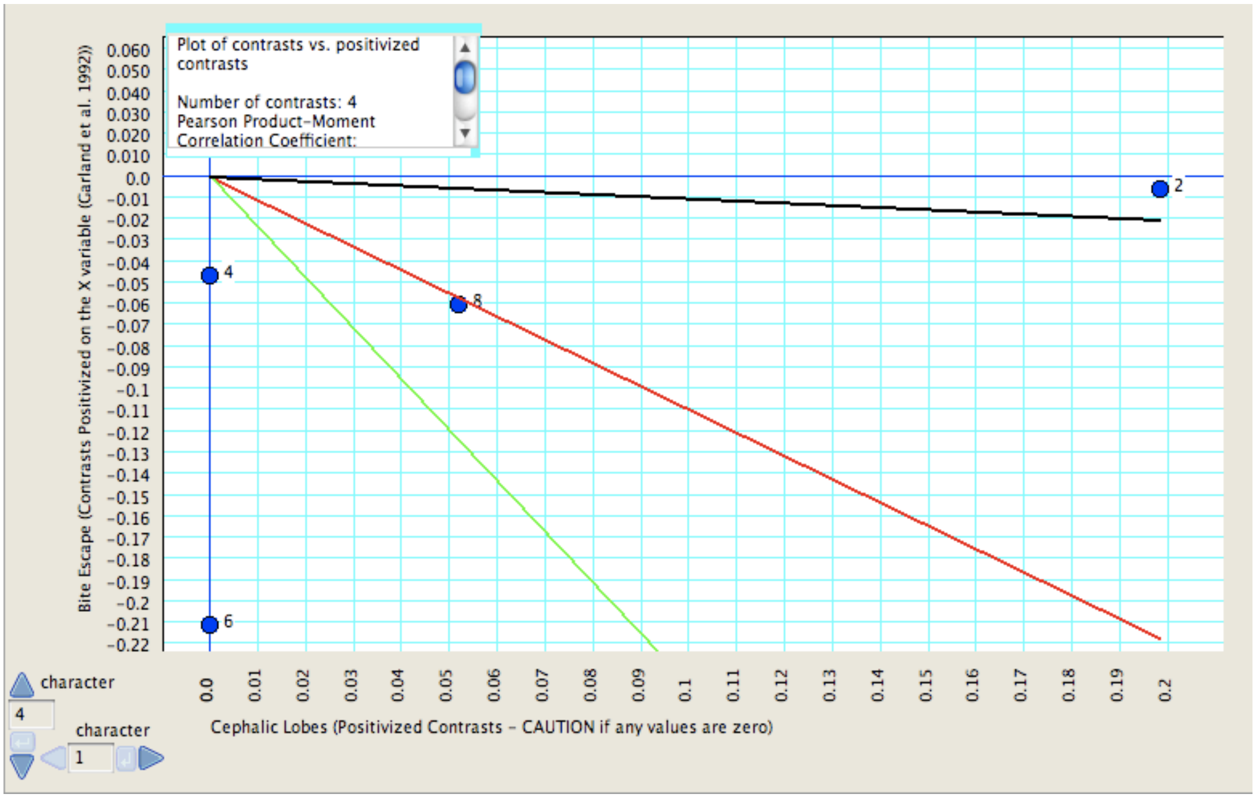


Plot of contrasts vs. positivized contrasts

Number of contrasts: 4
Pearson Product-Moment Correlation Coefficient: 1.0

Regression lines through origin:
Black is ordinary least squares (OLS)
Green is major axis (MA)
Red is reduced major axis



Plot of contrasts vs. positivized contrasts

Number of contrasts: 4
Pearson Product-Moment Correlation Coefficient:

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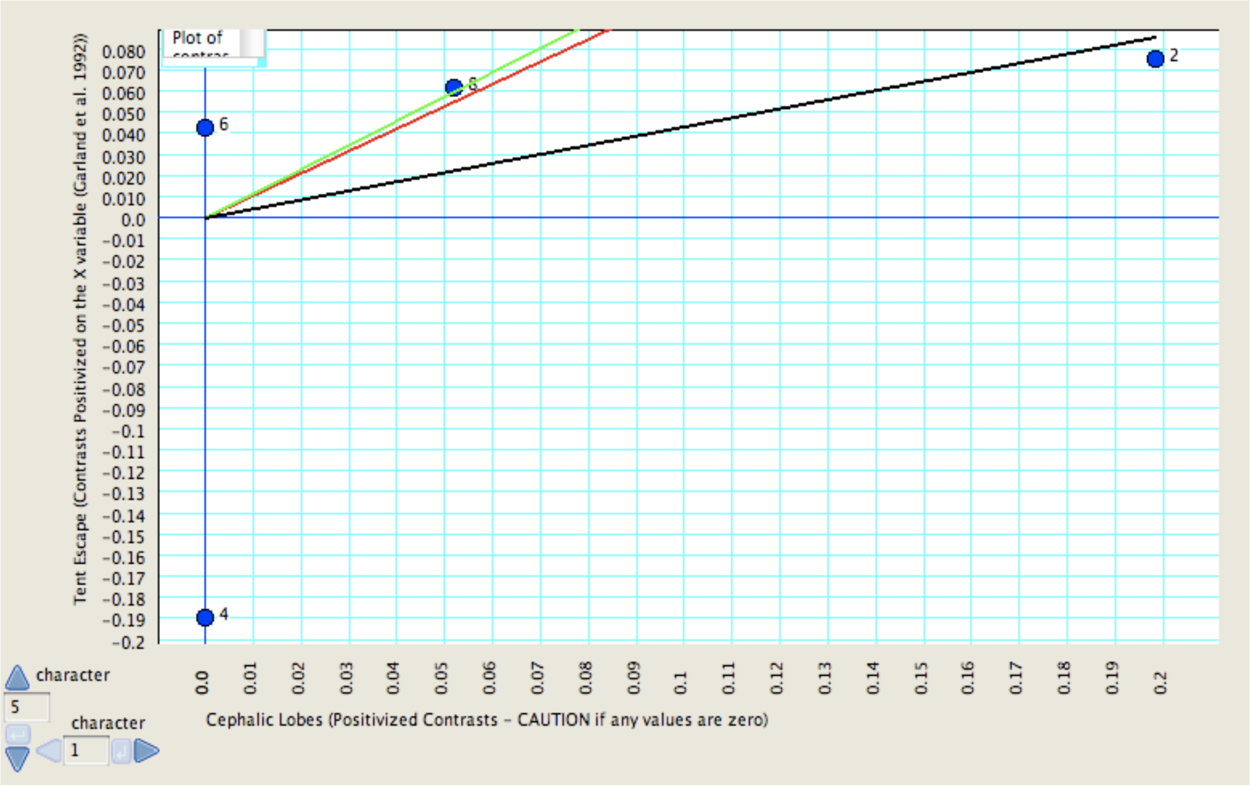

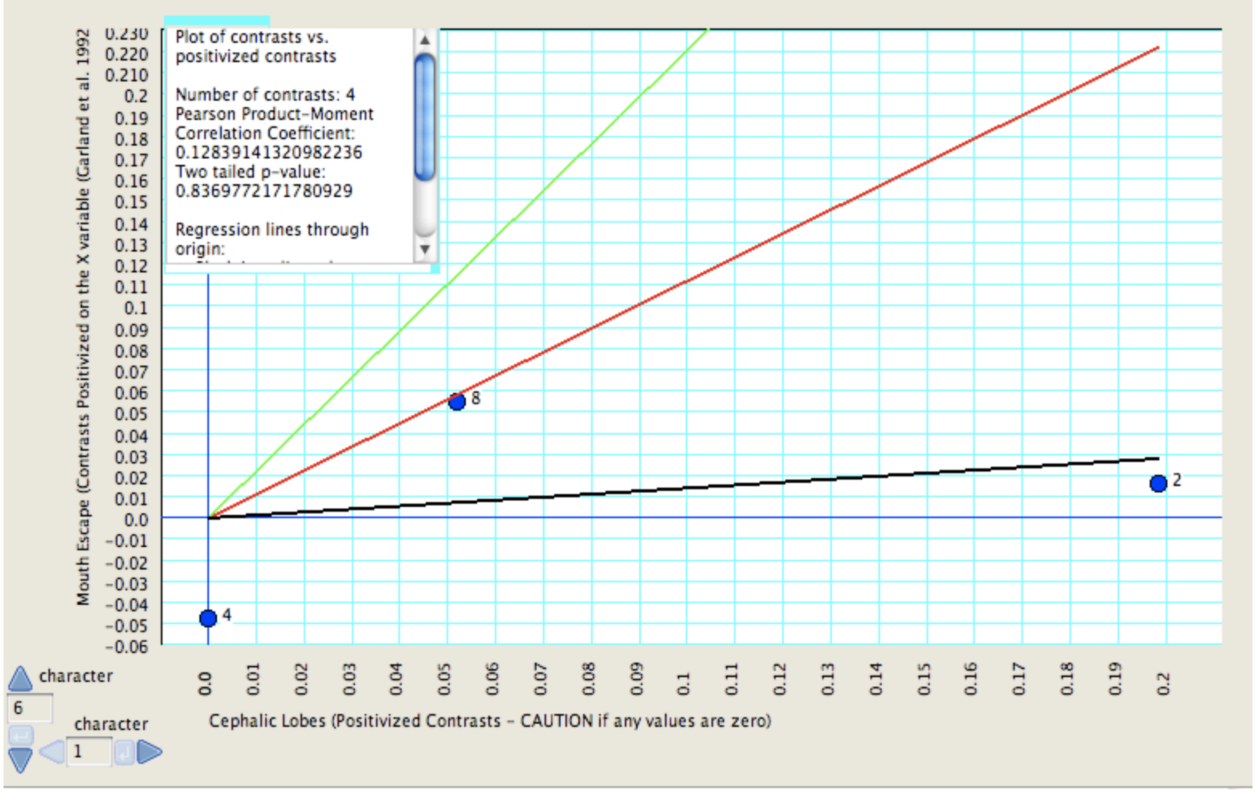
Plot of contrasts vs. positivized contrasts

Number of contrasts: 4
Pearson Product-Moment Correlation Coefficient:
0.12839141320982236
Two tailed p-value:
0.8369772171780929

Regression lines through origin:

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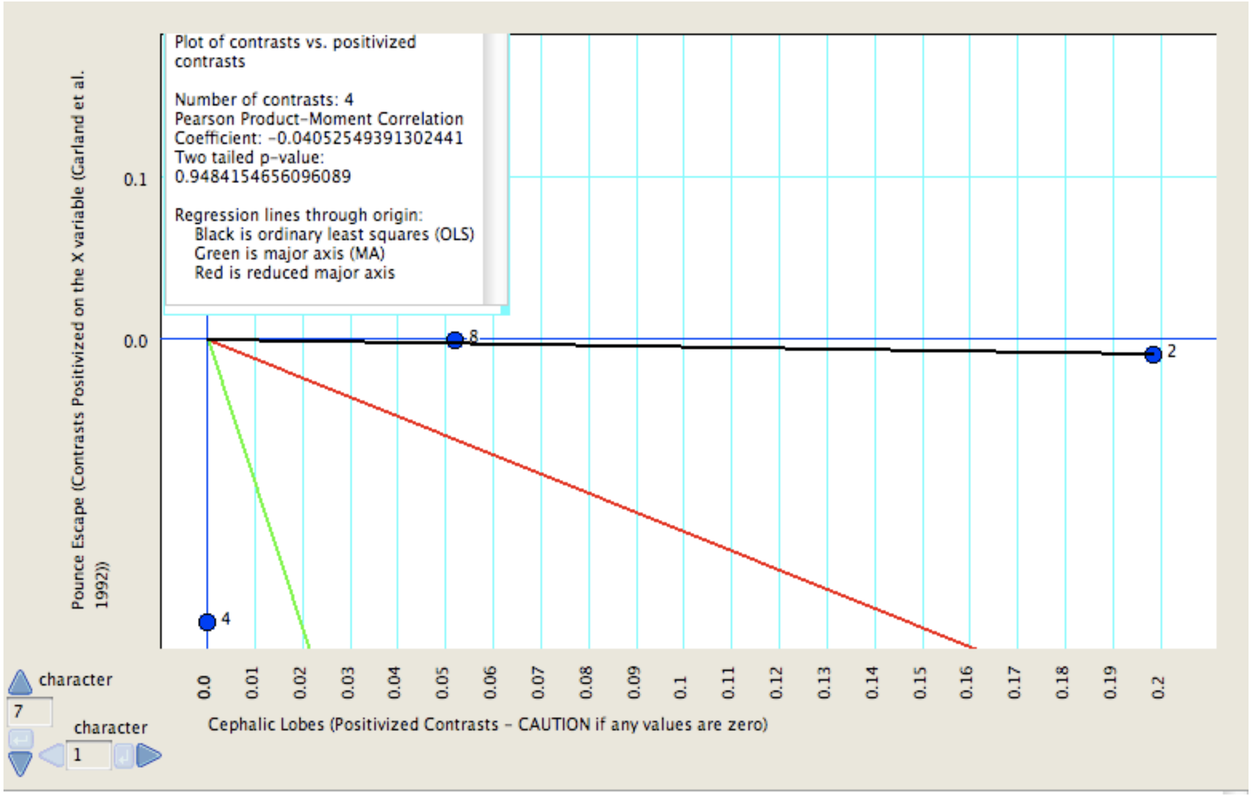
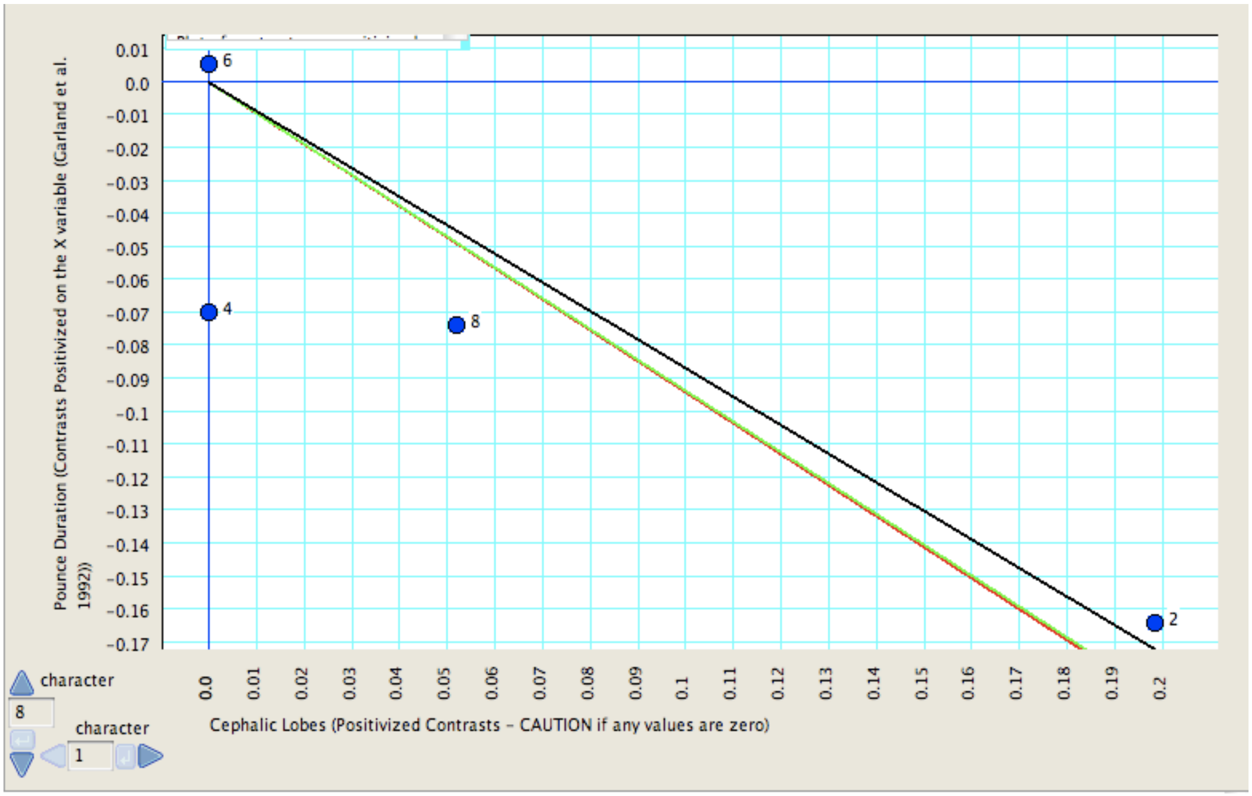
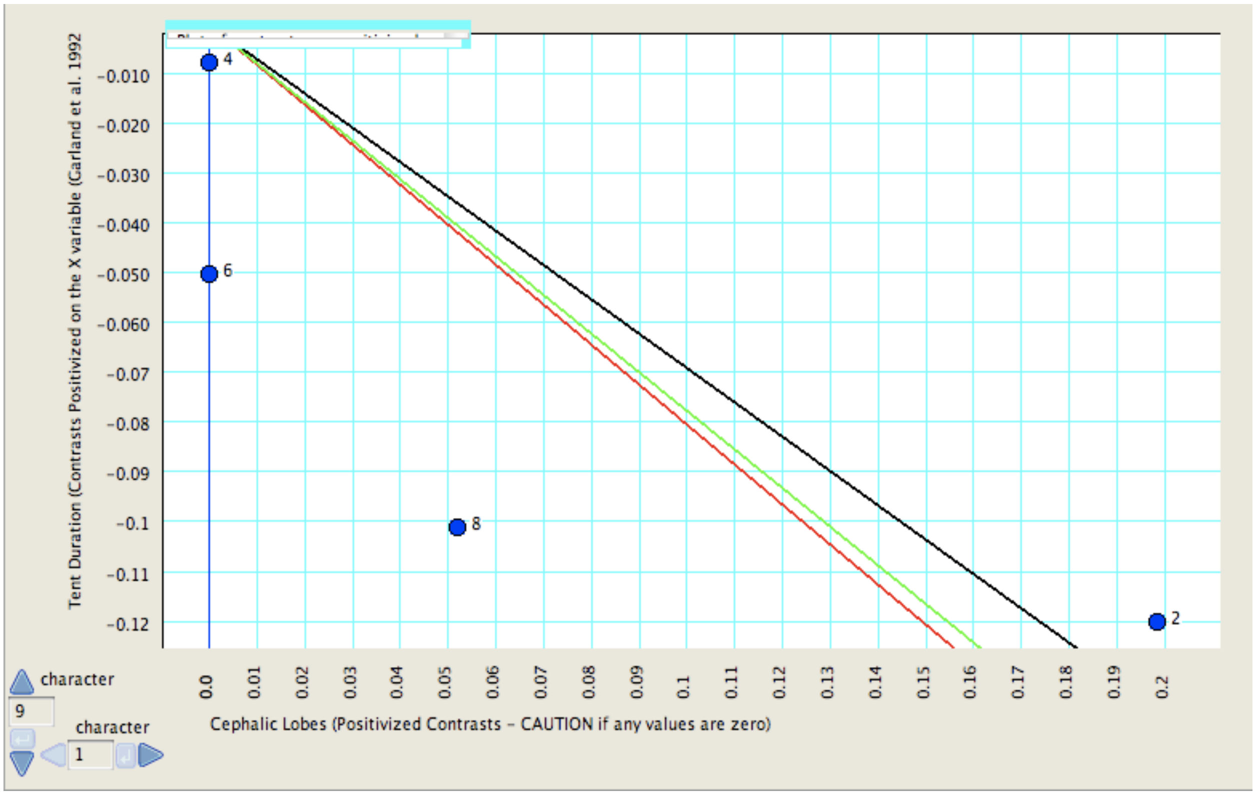


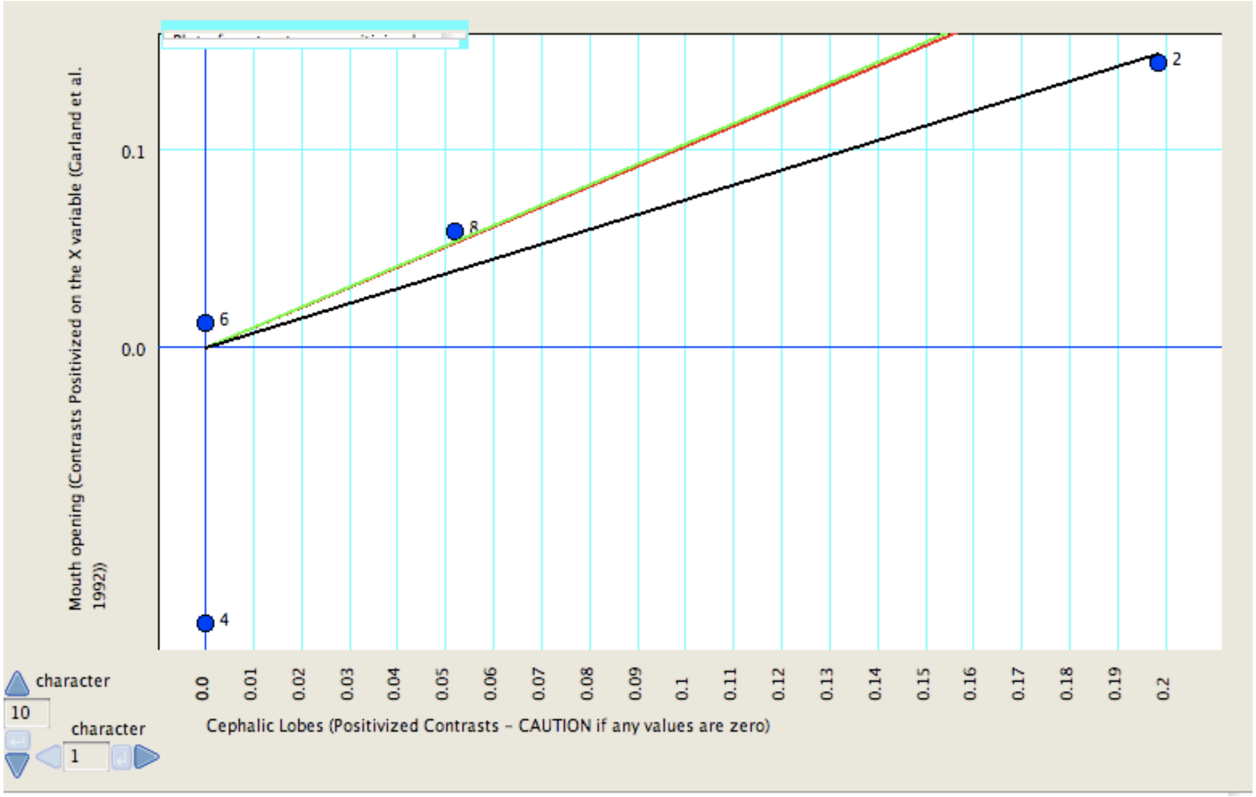
Plot of contrasts vs. positivized contrasts

Number of contrasts: 4
Pearson Product-Moment Correlation Coefficient: -0.04052549391302441
Two tailed p-value: 0.9484154656096089

Regression lines through origin:
Black is ordinary least squares (OLS)
Green is major axis (MA)
Red is reduced major axis







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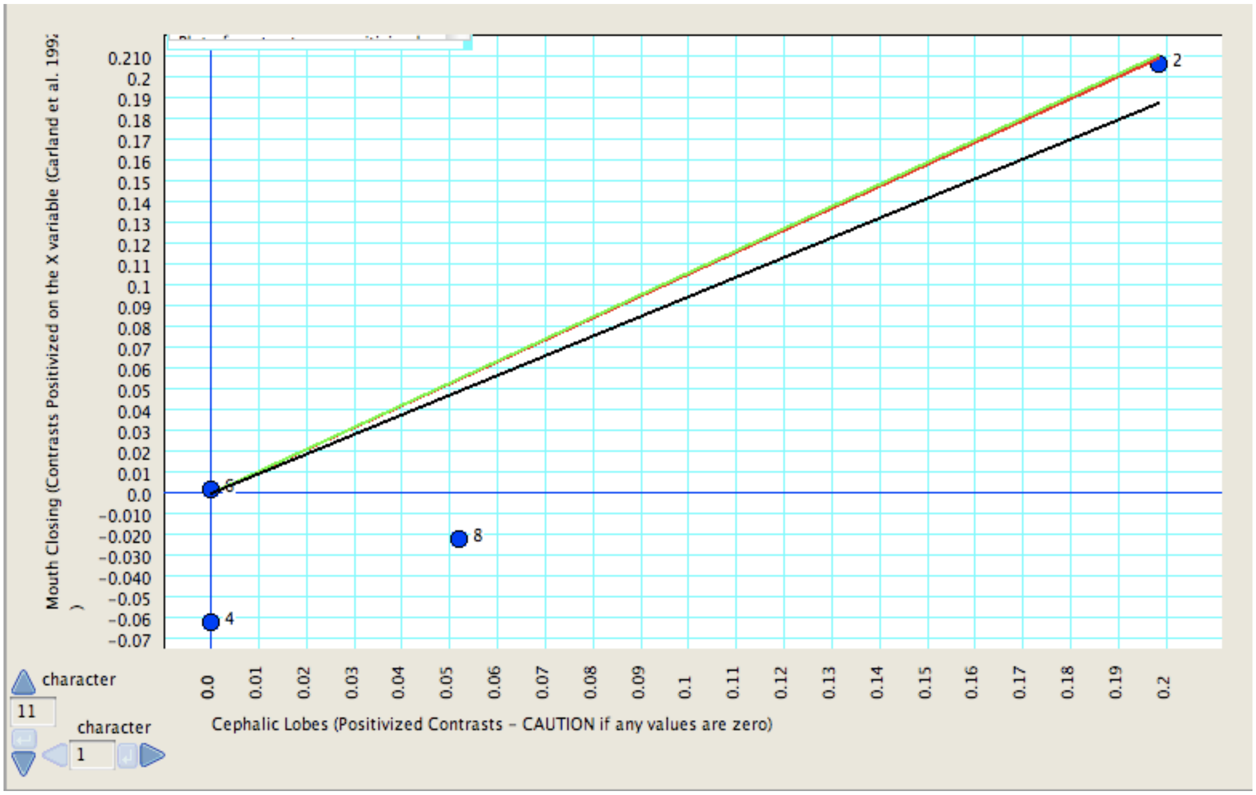

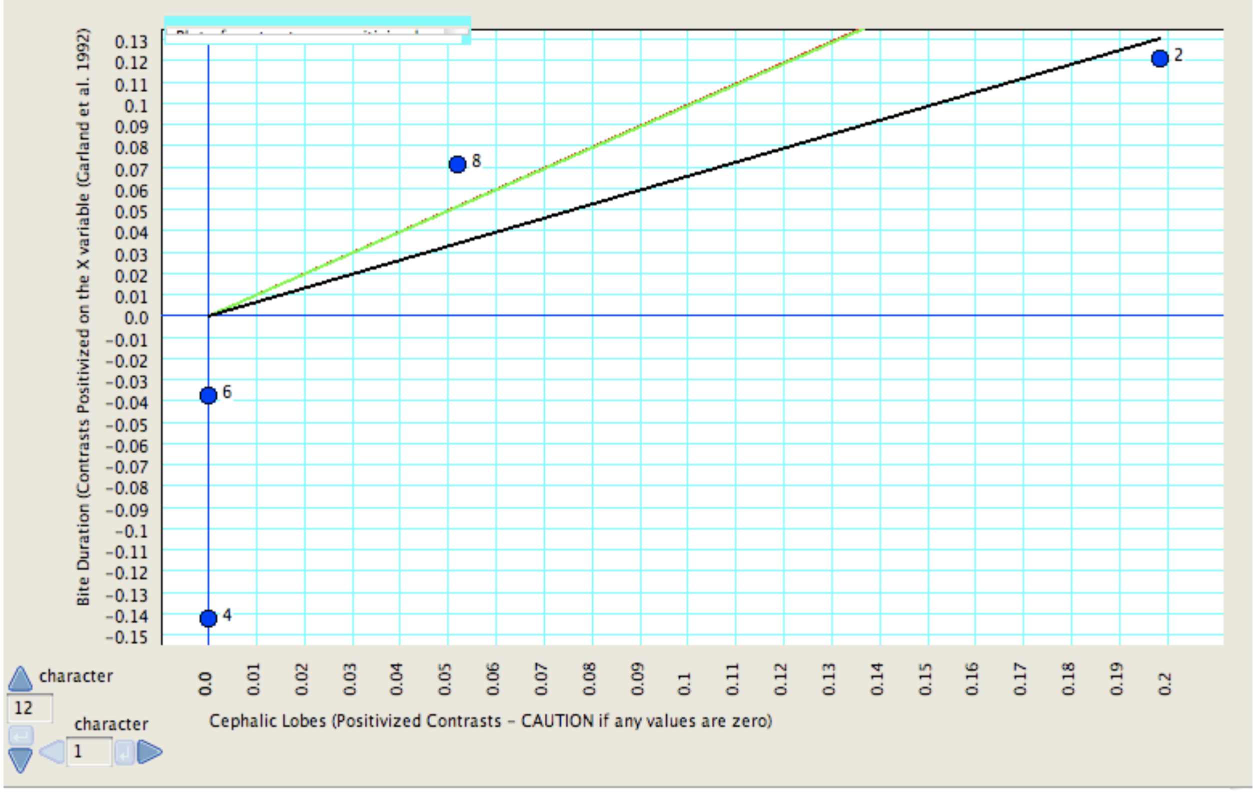

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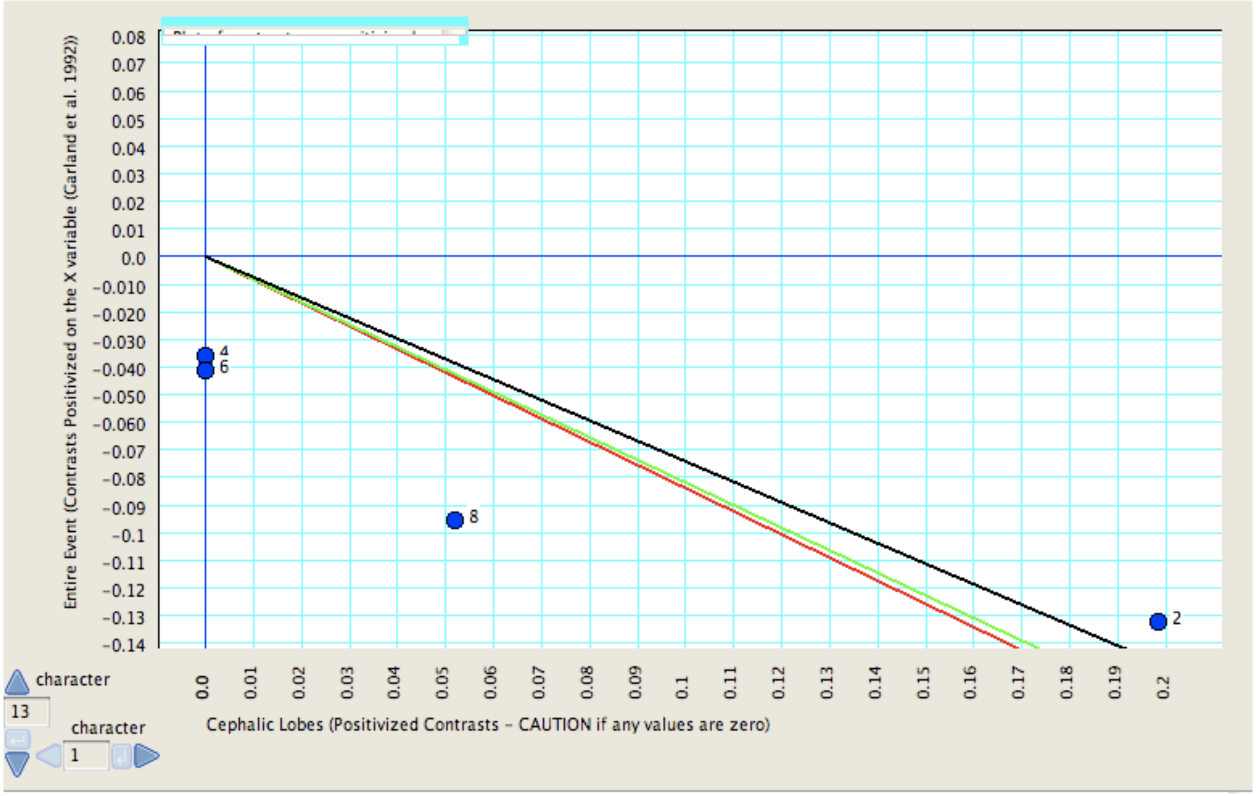
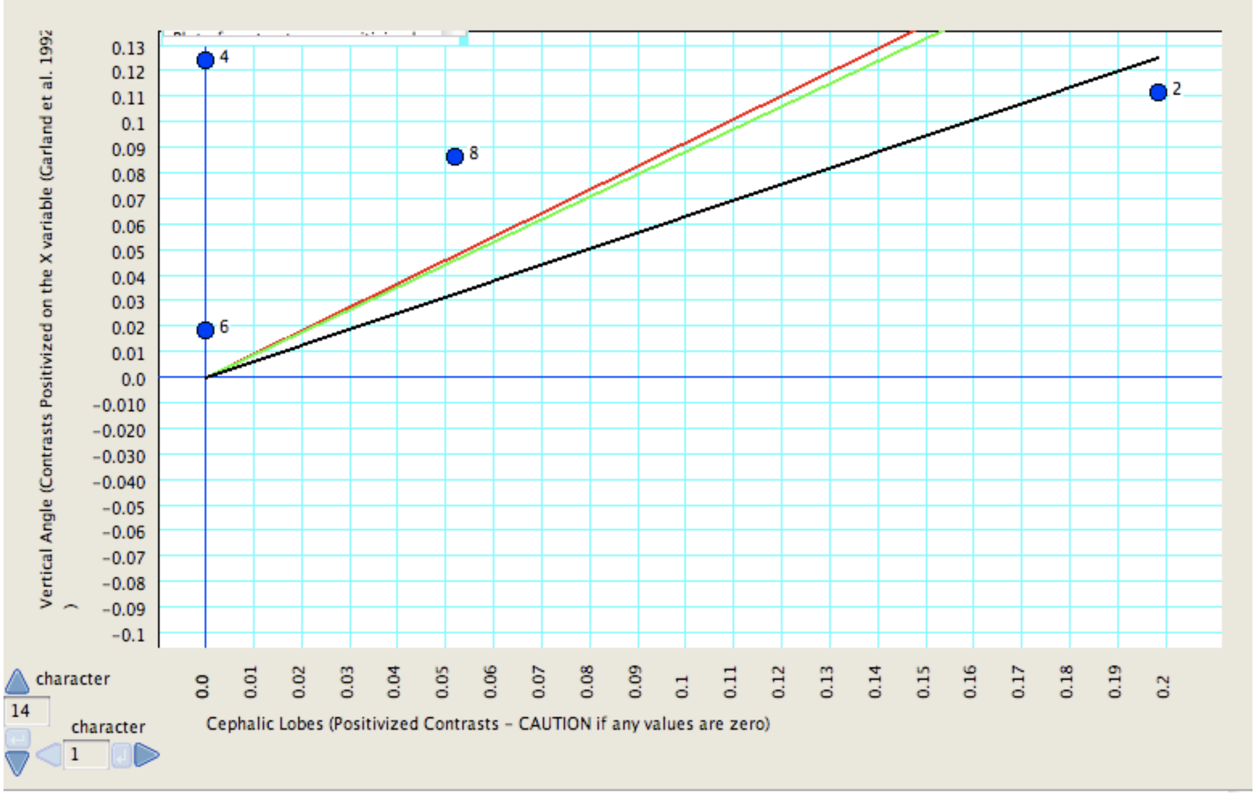
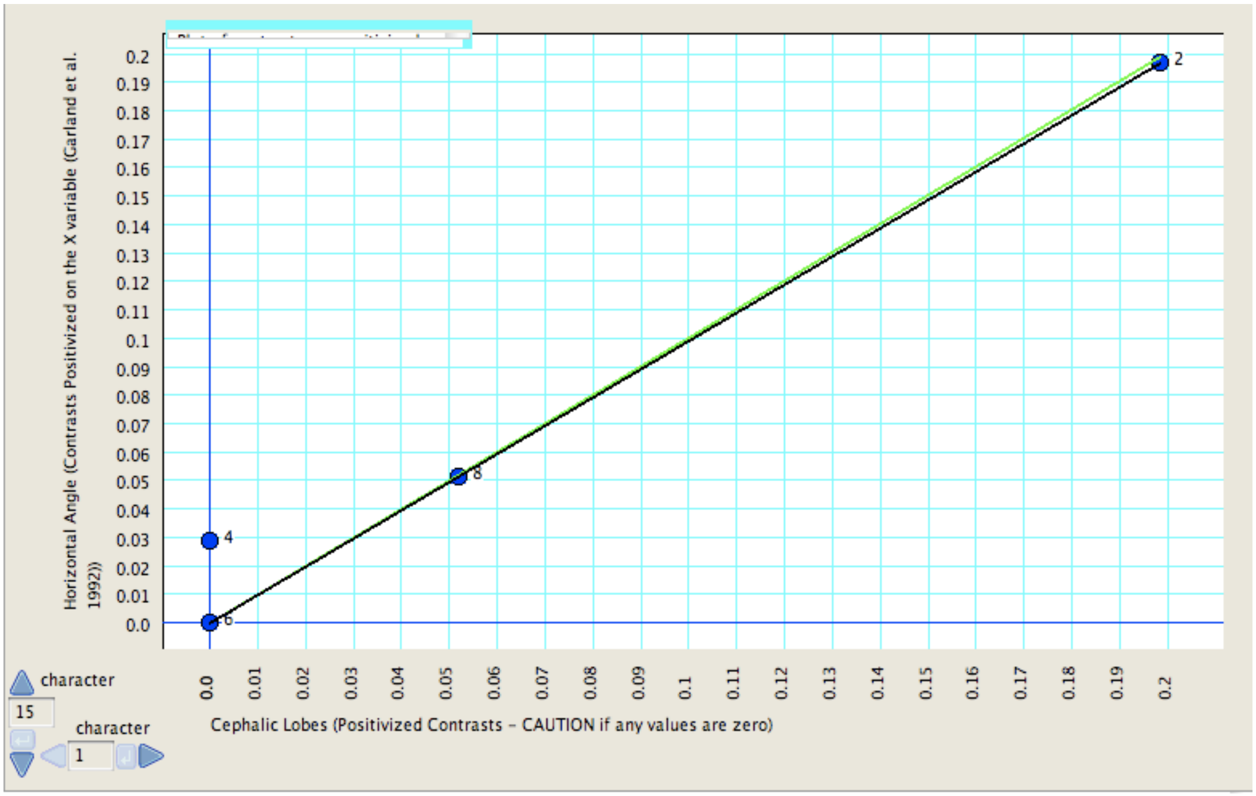
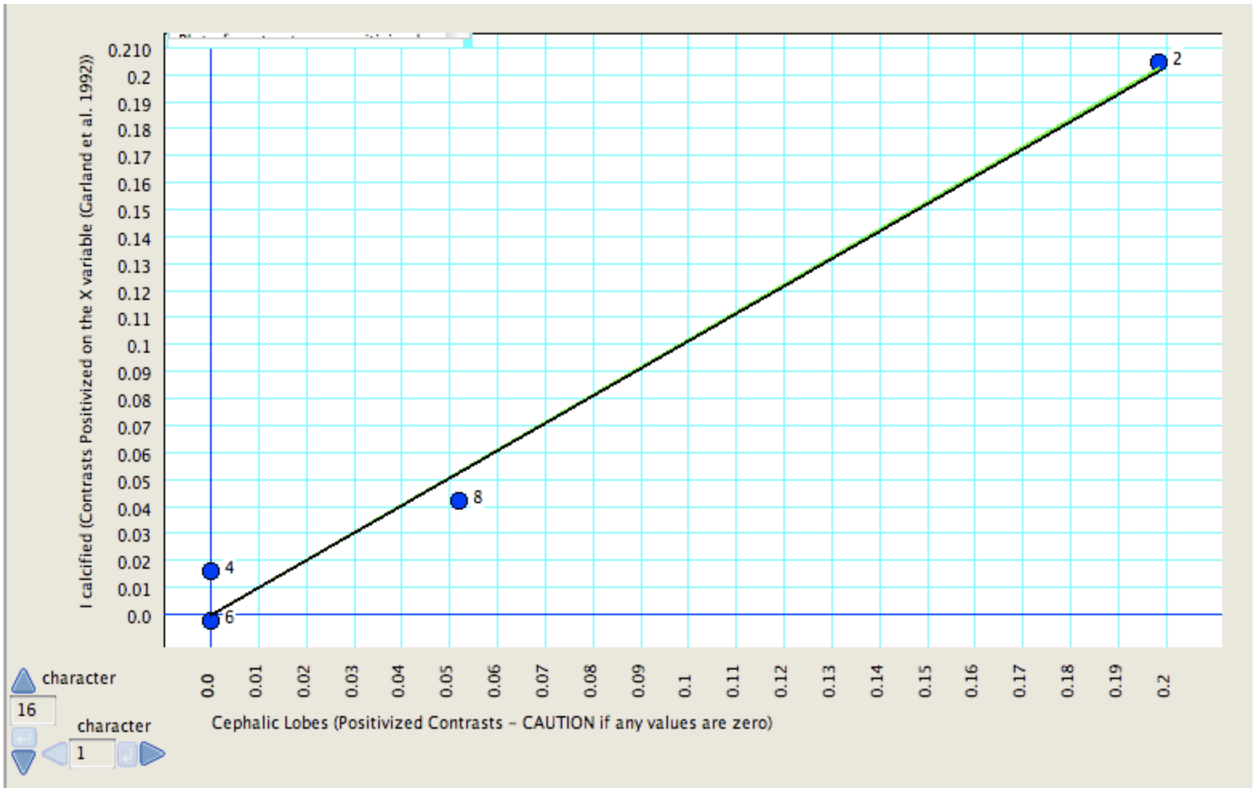

Entire Event (Contrasts Positivized on the X variable (Garland et al. 1992))
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.0
−0.010
−0.020
−0.030
−0.040
−0.050
−0.060
−0.07
−0.08
−0.09
−0.1
−0.11
−0.12
−0.13
−0.14
4
6
8
2
0.0
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
0.09
0.1
0.11
0.12
0.13
0.14
0.15
0.16
0.17
0.18
0.19
0.2
Cephalic Lobes (Positivized Contrasts – CAUTION if any values are zero)
character
13
character
1


Vertical Angle (Contrasts Positivized on the X variable (Garland et al. 1992 )
0.13
0.12
0.11
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.0
−0.010
−0.020
−0.030
−0.040
−0.05
−0.06
−0.07
−0.08
−0.09
−0.1
4
8
2
6
0.0
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
0.09
0.1
0.11
0.12
0.13
0.14
0.15
0.16
0.17
0.18
0.19
0.2
Cephalic Lobes (Positivized Contrasts – CAUTION if any values are zero)
character
14
character
1





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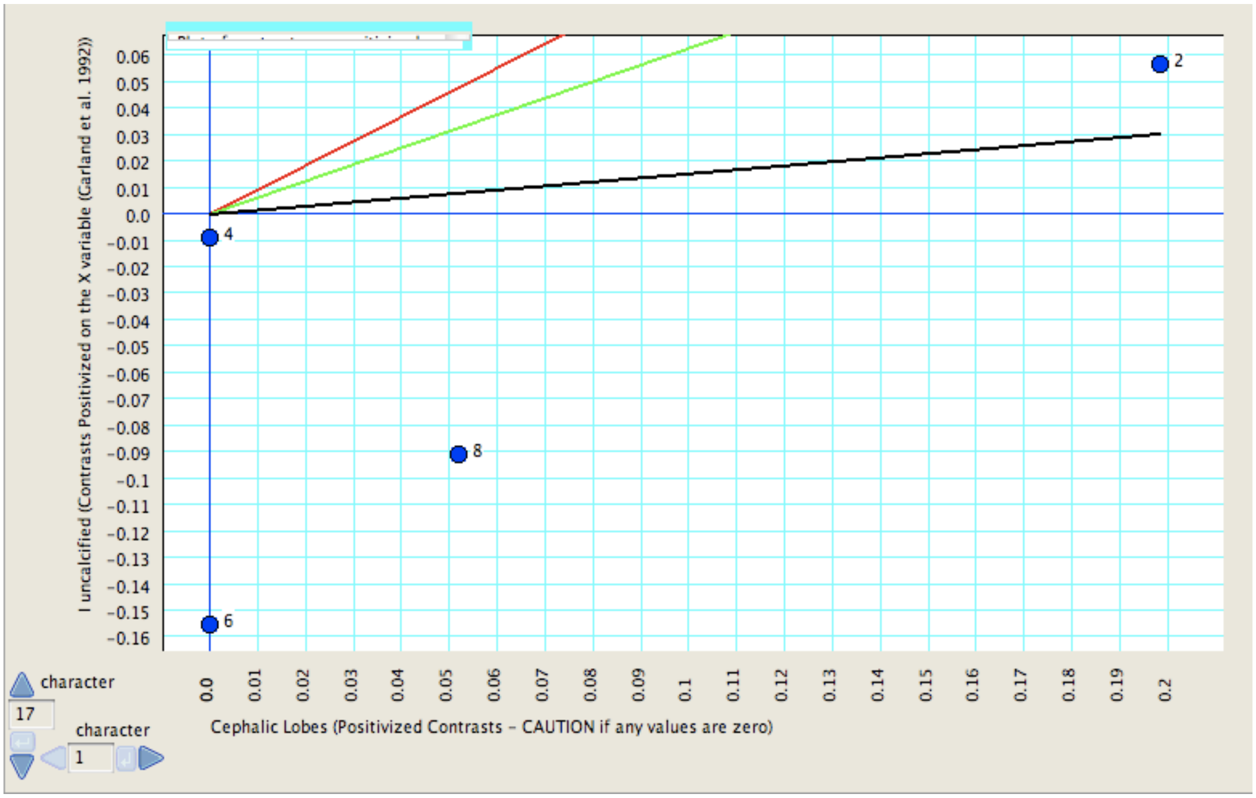
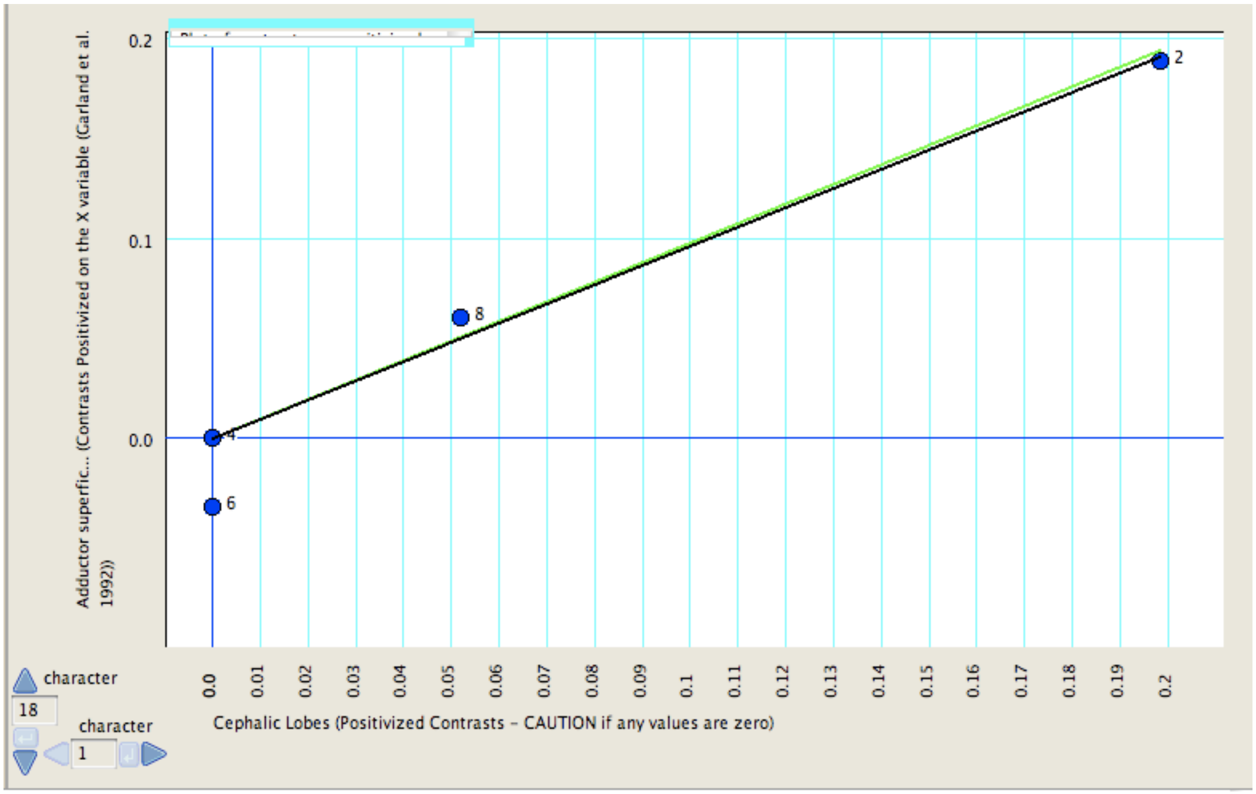
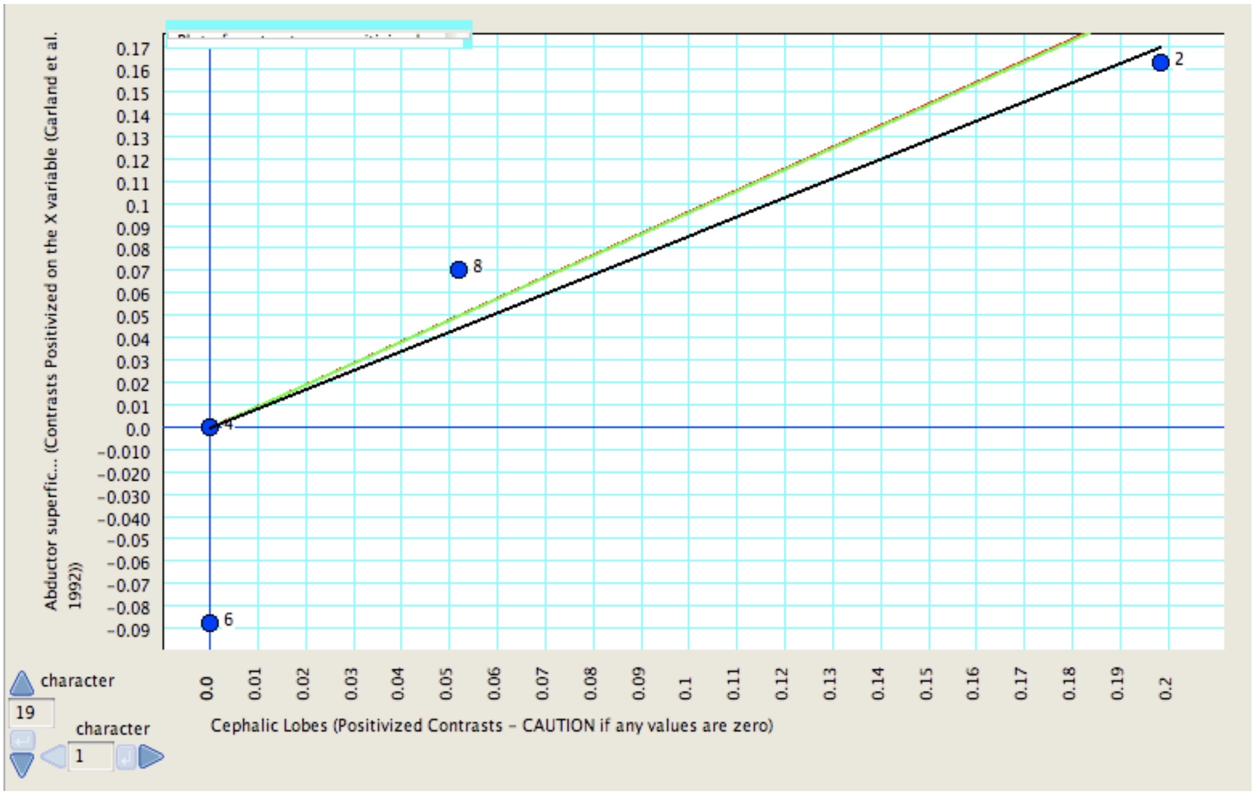
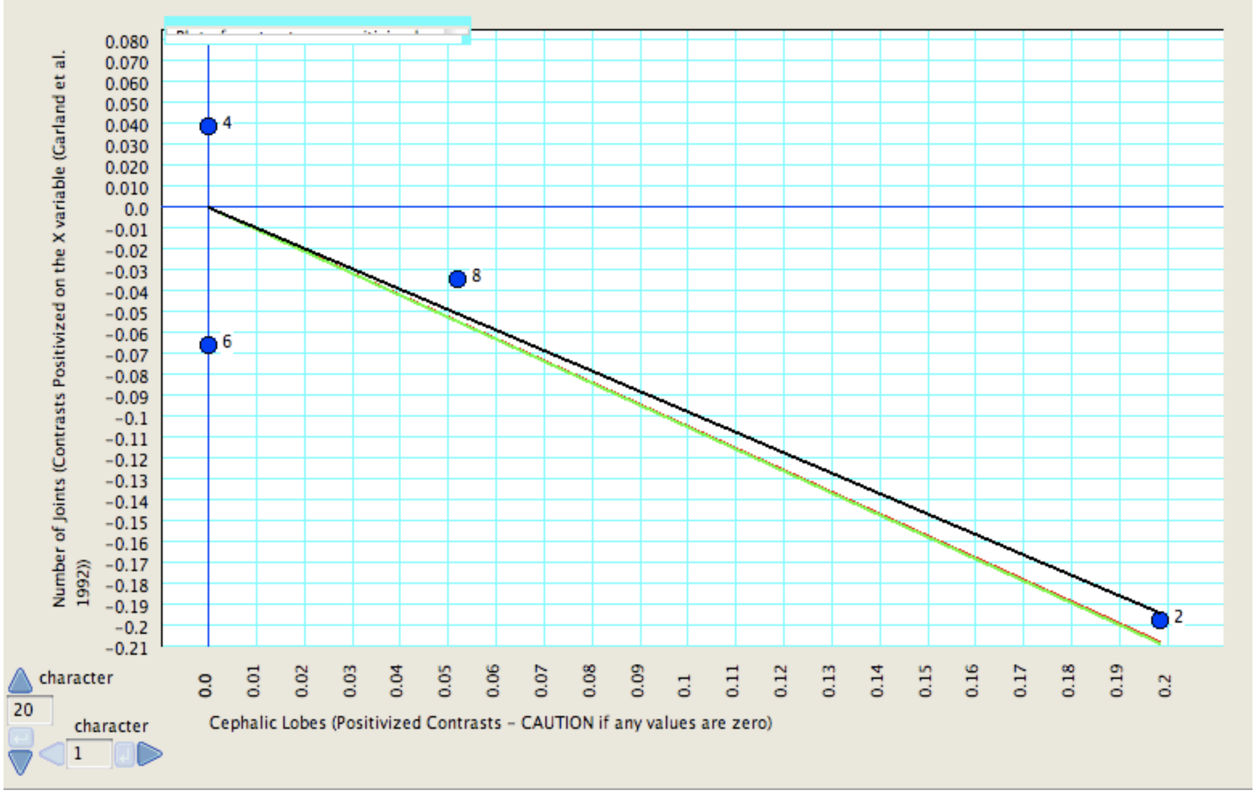

l uncalcified (Contrasts Positivized on the X variable (Garland et al. 1992))
0.06
0.05
0.04
0.03
0.02
0.01
0.0
-0.01
-0.02
-0.03
-0.04
-0.05
-0.06
-0.07
-0.08
-0.09
-0.1
-0.11
-0.12
-0.13
-0.14
-0.15
-0.16
2
4
8
6
0.0
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
0.09
0.1
0.11
0.12
0.13
0.14
0.15
0.16
0.17
0.18
0.19
0.2
Cephalic Lobes (Positivized Contrasts - CAUTION if any values are zero)
character
17
character
1


Adductor superfic... (Contrasts Positivized on the X variable (Garland et al. 1992))
0.2
0.1
0.0
2
8
4
6
0.0
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
0.09
0.1
0.11
0.12
0.13
0.14
0.15
0.16
0.17
0.18
0.19
0.2
Cephalic Lobes (Positivized Contrasts - CAUTION if any values are zero)
character
18
character
1





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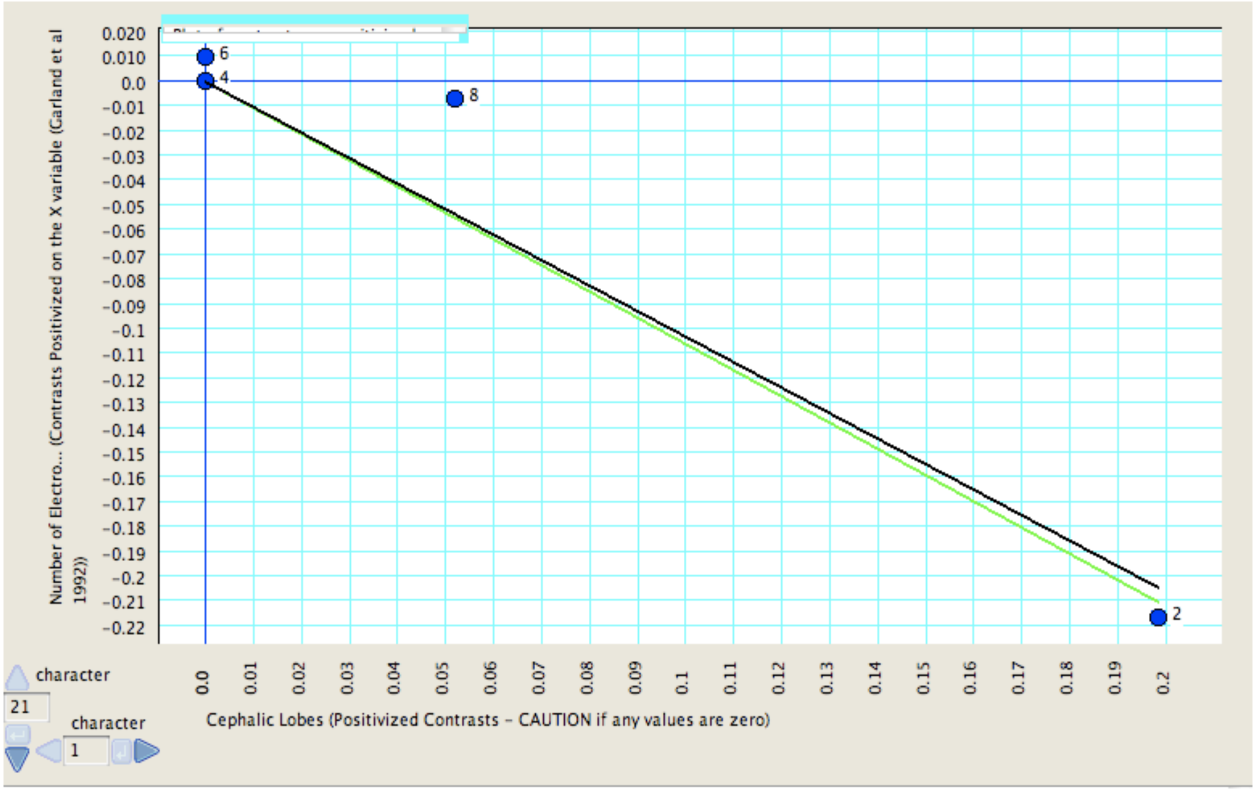

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