

The evolution of cranial design, diet, and feeding mechanisms in batoid fishes

Mason N. Dean,^{1,*} Joseph J. Bizzarro,[†] and Adam P. Summers*

*Ecology and Evolutionary Biology, University of California Irvine, 321 Steinhaus Hall, Irvine CA 92697-2525, USA;

[†]Pacific Shark Research Center, Moss Landing Marine Laboratories, 8272 Moss Landing Road, Moss Landing, CA 95039, USA

Synopsis The batoid fishes (electric rays, sawfishes, skates, guitarfishes, and stingrays) are a trophically and morphologically diverse clade in which the observed range of diets is a product of a feeding mechanism with few parts and therefore a limited number of functional interactions. This system allows an intriguing comparison to the complex network of associations in the feeding apparatus of bony fishes and an anatomically simple framework for investigations of the mechanisms underlying the evolution of functional and phenotypic diversity. We quantified morphology from reconstructed CT scans of 40 batoid species, representing more than half of the extant genera. We used pairwise comparisons to evaluate the extent of coevolution among components of the feeding apparatus and among morphologies and diets. These relationships were then used to predict diets in poorly studied taxa and in a reconstruction of the batoid ancestor. Although functionally there are fewer examples of convergence in the batoid feeding mechanism than in bony fishes, our data show multiple evolutions of similar dietary compositions underlain by a broad morphological diversity. Elements of the feeding apparatus evolved independently of one another, suggesting that decoupling components of the head skeleton created separate but interacting evolutionary modules that allowed trophic diversification. Our data imply that food habits exhibit strong independent and convergent evolution and that suites of morphologies are associated with certain diets; however, lack of behavioral data for this clade, and one example of divergent diets underlain by convergent morphology, caution against the assumption of simplistic relationships between form and function. We therefore urge future work to ground truth our study by testing the functional, dietary and evolutionary hypotheses suggested by our data.

Introduction

The feeding mechanism of the elasmobranch fishes (sharks, rays and their relatives) in general offers few options for interactions among skeletal components: the functionally important elements are simply the mandibular arch, a portion of the hyoid arch and the fused chondrocranium (Fig. 1). The jaws (mandibular arch) are suspended from the chondrocranium at their corners by the paired hyomandibular cartilages and allied portions of the hyoid arch (Motta 2004). In the batoids (electric rays, sawfishes, skates, guitarfishes, and stingrays) (Fig. 2), mandibular arch associations are minimized and almost entirely muscular—only the hyomandibula supports the jaws caudally and is apparently the primary mediator of jaw protrusion, and additional hyoid cartilages are reduced or absent (Wilga and Motta 1998; Dean and Motta 2004a). Batoids also lack the ethmopalatine ligament of sharks that tethers the

upper jaw to the cranium (Fig. 1). This releases a constraint on jaw motion and allows even greater freedom of movement of the upper jaw than in sharks. Perhaps as a result, the extreme jaw protrusion ability of some batoid fishes is far greater than that of sharks and in some batoid species the jaws can be extended ventrally more than 100% of head length (e.g., *Narcine*: Dean and Motta, 2004b; *Hexatrygon*: Dean, pers. obs.).

Although the small number of cranial parts suggests a constrained functional parameter space, it apparently does not limit dietary or behavioral diversity. For a rather small group of fishes, batoids are impressively morphologically and trophically diverse and represent more than half (560 of 1080) of the extant species of cartilaginous fishes. Batoids occupy nearly all the same feeding niches as sharks (except perhaps megacarnivory) (Dean et al. 2005) from generalist predators on modest-sized

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¹E-mail: mdean@uci.edu

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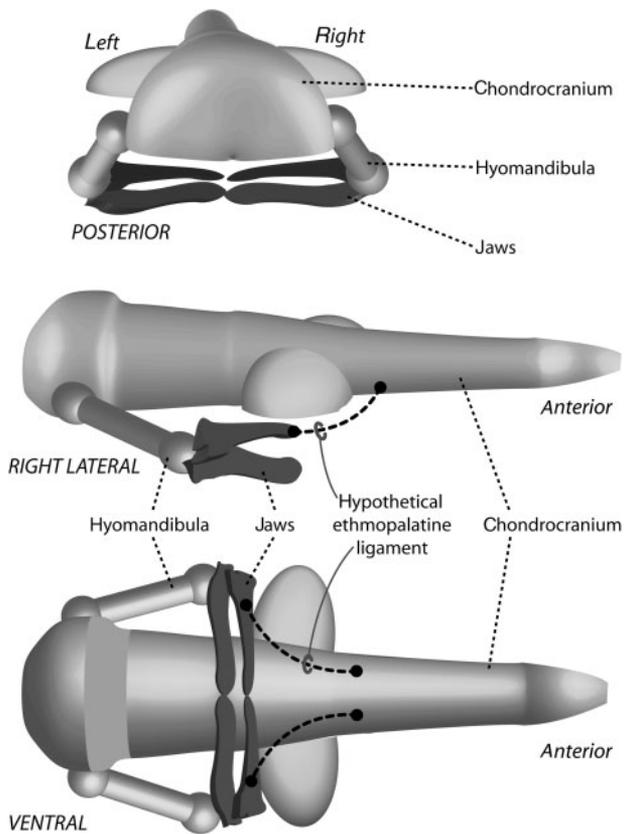


Fig. 1 Schematic model of the feeding apparatus of batoid fishes, shown in posterior, right lateral, and ventral views at the top, middle, and bottom of the figure, respectively. In the latter two views, anterior is to the right. The jaws are suspended from the chondrocranium by the paired hyomandibulae, the dorsalmost hyoid elements. This cranial design is largely similar to sharks except batoids lack an ethmopalatine ligament between the upper jaw and cranium, illustrated in the right lateral and ventral views.

vertebrates and invertebrates to dietary specialists that pursue and filter swarms of krill, crush shelled molluscs, capture fish (including sharks) that are much larger than their own gape, and process prey that require significant manipulation to remove edible from inedible components. The dorsoventrally flattened batoids face a constraint in that the ventral position of the mouth (except in the giant filter-feeding *Manta*) rules out the ram feeding mode, and results in the principal direction of body motion being perpendicular to the direction the mouth will open/protrude during feeding. Yet the batoid fishes include successful benthic, demersal and pelagic predators that span several orders of magnitude in size, from about 100 gm and 8 cm in disc width to several thousand kilograms and 8 m in width. Moreover, these radiations have occurred in a short amount of time relative to the evolutionary timeline of cartilaginous fishes: sharks and chimaeras diverged

in the Devonian (~400 mya), but the batoid body form only arose in the last 200 million years (McEachran and Aschliman 2004).

The small number of component parts in the feeding skeleton and the freedom of movement of the jaws make the feeding mechanism of batoids an interesting comparison to bony fishes and a spring-board for questions regarding the evolutionary history of dietary diversification and for investigating form and function. What morphological characters have been important for functional and ecological evolution in batoid fishes? Is dietary breadth matched by morphological diversity? Is function constrained by the simple skeletal form of the feeding apparatus and are the individual components constrained to evolve in concert?

Although feeding behavior and cranial functional morphology are well studied in a phylogenetic context in other fish taxa (e.g., Alfaro et al. 2005; Bellwood et al. 2006), questions of evolutionary mechanics are difficult to address in batoid fishes given the current literature. There is a scarcity of dietary data and morphological analyses are couched in a phylogenetic rather than in a functional context. Most accounts of feeding behavior are anecdotal with only three kinematic studies of prey capture, including two functional analyses of feeding mechanisms and a single electromyographic experiment (Wilga and Motta 1998; Dean and Motta 2004a, 2004b; Sasko et al. 2006). There is a near complete disconnect between our limited understanding of batoid ecological and morphological diversity, with functional studies being the missing link.

Pharyngeal jaws and protrusible premaxillae are implicated in the diversification of bony fishes (Lauder and Liem 1983; Motta 1984; Bellwood et al. 2006), but we know no similarly important functional suites that can be implicated in the diversification of batoid fishes. Investigations of the evolution of the feeding anatomy of batoids are only recently possible in light of comprehensive phylogenies based on morphology (McEachran et al. 1996; McEachran and Aschliman 2004). Models of constraints and capabilities of the feeding mechanism based on the geometry of cranial anatomy provide an elementary entry to a synthetic view of the feeding mechanism in an evolutionary context. We use these models to provide absolute limits and upper and lower bounds that mechanically and anatomically constrain dietary composition. For example, the jaws can be protruded from the cranium by no more than the length of the hyomandibulae, and the width of the mouth places an upper bound on

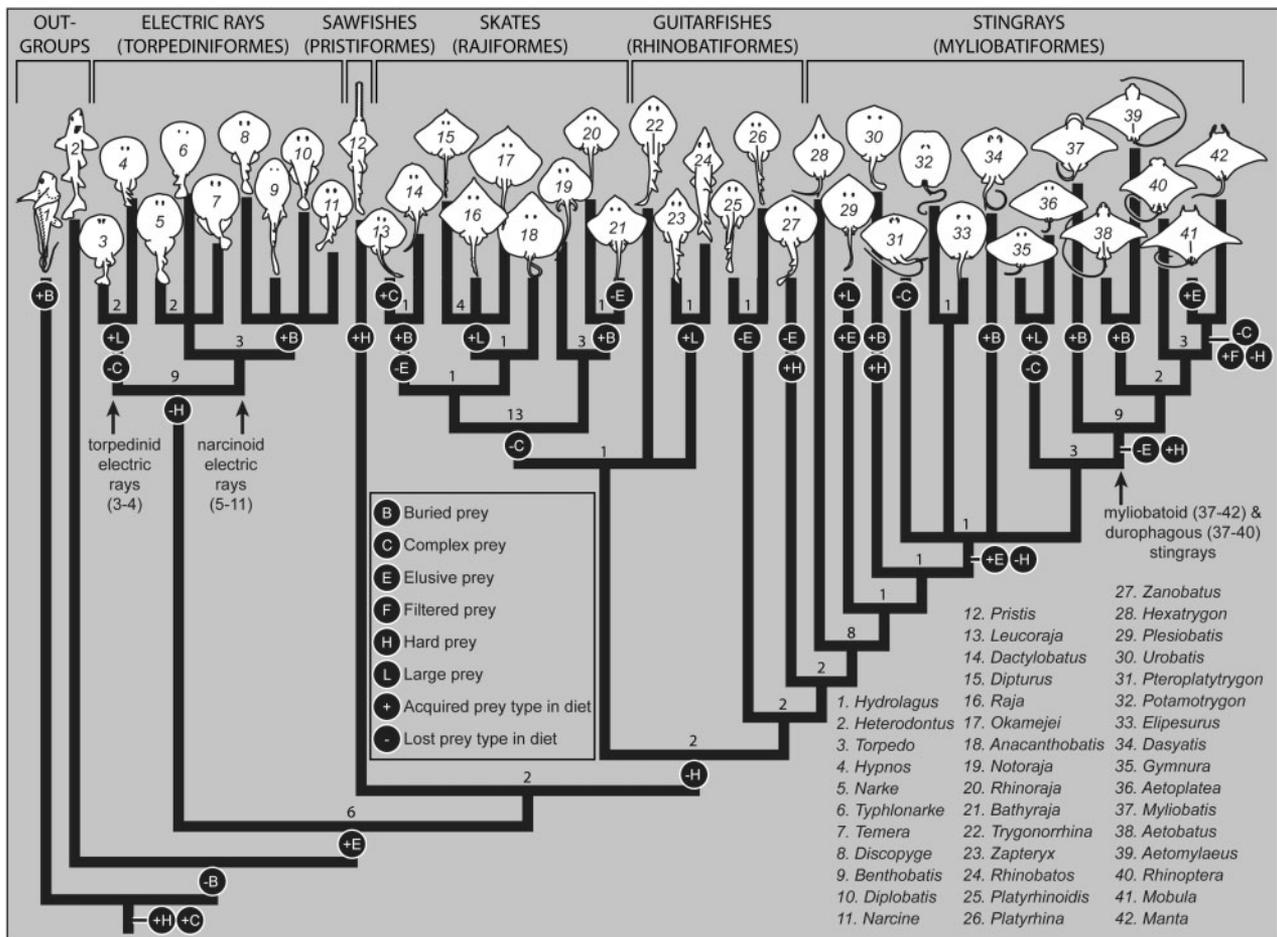


Fig. 2 Phylogeny of the 40 batoid species sampled in this study, including two chondrichthyan outgroups (*Heterodontus* and *Hydrolagus*) and hypotheses for the evolution of dietary characters. Gain and loss of character states are indicated at nodes by a letter and a plus or minus in a black circle (e.g., +B = the gain of buried prey in the diet). Tip taxa are numbered and listed in the lower right corner, with the torpedinid, narcinoid, myliobatoid, and durophagous taxa indicated by arrows. Group names assigned at the top of the figure are to facilitate the broadscale referencing of taxa, but may not be taxonomically accurate (e.g., “guitarfishes” are polyphyletic). Tree topology and Bremer decay indices (displayed above nodes) are from McEachran and Aschliman (2004) with some deletions of taxa for clarity; the addition of *Manta* as the sister taxon to *Mobula* is per Notobartolo-Di-Sciara (1987).

ingestible prey width. To determine morphological parameters that may drive, or be driven by, the diverse batoid diet, we (1) reconstructed from CT scan data the skeletal morphology of 40 genera of batoid fishes, (2) determined which morphological aspects of the batoid feeding mechanism are coevolving, (3) mapped morphological and dietary trends onto the batoid phylogeny, and (4) correlated morphology with dietary specialization. Our clarification of the relationships between morphology, ecology and phylogeny allowed us to extrapolate from our findings to (5) generate predictions of diet for poorly studied taxa, and (6) reconstruct the ancestral batoid diet and feeding apparatus. This broad-scale analysis is the basis for models of the batoid feeding mechanism that provide

predictions of function and highlight important areas for future research.

Materials and methods

Specimens and computed tomography

Adult specimens of 40 batoid genera (Fig. 2), from several collections in United States museums, were chosen to represent a wide taxonomic sampling of genera within the constraint that they were of sufficient size for CT-scanning (about 15 cm disc width minimum) (see Supplemental Material online). The batoid clade is comprised of ~560 species and ~75 genera; our samples represent more than 50% of all genera and at least 50% of genera in each of the major groups except skates for

which we sampled 9 of ~24 genera or 38% (Fig. 2). This undersampling limits our resolution within the skates but does not affect our broader conclusions.

Multiple CT-scanning systems were used to optimize scan quality for the diversity of body shapes and disc widths. The majority of specimens were scanned on a 16-slice medical grade Siemens RS SOMATOM Sensation (MDCT-16) with 0.75-mm slice thickness and helical-spiral scans. Smaller animals for which this system was inadequate (*Anacanthobatis borneensis*, *Dactylobatus armatus*, *Diplobatus ommata*, *Elipesurus* sp., *Potamotrygon* sp., *Rhinoraja longi*, *Temera hardwickii*, *Typhlonarke* sp.) were scanned on an experimental flat panel conebeam CT-scanner operating at a peak tube potential of 70 kVp and reconstructed with a 0.4 mm isotropic voxel size. For these scanning systems, all specimens were wrapped in alcohol-saturated cheesecloth and sealed in large Ziploc™ bags prior to scanning. To reduce scanning time, these bags were layered together into additional larger bags allowing 6–8 animals to be scanned simultaneously. The sawfish, *Pristis perotteti*, was scanned at the University of Texas High-Resolution X-Ray Computed Tomography Facility (Ketcham and Carlson 2001; Summers et al. 2004). The *Narcine brasiliensis* specimen was scanned on a Philips MX-800 imaging system with helical-spiral scans (pitch = 0.875) under a high-resolution dental protocol (1.3 mm slice thickness, 120 kV, 200 mA) with a 50% interleaving of successive scans, as outlined by Dean and Motta (2004a). All specimens were scanned supine or prone with jaws closed and retracted.

Morphological characters

Scan series were reconstructed as 8-bit TIFF stacks and rendered as three-dimensional visualizations using Amira software (v. 4.1.1, Mercury Computer Systems, Inc., USA) to illustrate the morphology and geometric relationships of the jaws, hyomandibulae, and chondrocranium. The following 13 anatomical landmarks were located in three-dimensional space using the PointProbe module in Amira (see Fig. 1 for anatomy): (1) *basal plate*—the flattened medioventral floor of the chondrocranium immediately anterior to the occipital condyles of the skull, (2–3) *left and right craniohyomandibular joints*—the cranial insertion of the hyomandibula, measured at the midpoint between the anterior and posterior edges of the skeletal element, (4–5) *left and right distal hyomandibular articulation*—the distal end of the hyomandibula at its articulation with the

mandible, (6–7) *left and right lateral mandibular edges*—the corresponding lateral edge of the mandible opposite the articular surface of the distal hyomandibula, (8–9) *left and right corners of the mouth*—the lateral edge of the anatomical gape, immediately medial to the jaw joints, (10–11) *left and right labial cartilage boundaries*—the lateral boundaries of the effective gape, defined as the medial edges of the labial cartilages when present, (12–13) *upper and lower jaw symphyses*—the midpoints of the upper and lower jaws, measured at the upper and lower margins of the gape, respectively.

The X, Y, and Z coordinates for all points of each specimen were exported from Amira using a custom script and imported into Microsoft Excel (v. 11.3, 2004 for Macintosh, Microsoft Corporation). The following Euclidean distance measurements were then calculated from the above 13 landmark coordinates: (1) *interhyomandibular distance*—the distance between the left and right craniohyomandibular joints, (2) *hyomandibular length*—the distance between the craniohyomandibular joint and the distal hyomandibular articulation, (3) *hyomandibula-mandibula joint space*—the distance between the distal hyomandibular articulation and the lateral mandibular edge, (4) *quadratomandibular joint width*—the distance between the lateral mandibular edge and the corner of the mouth, a measure of the width of the joint between the upper and lower jaws, (5) *anatomical gape width*—the distance between the left and right corners of the mouth, (6) *effective gape width*—the distance between the left and right labial cartilage edges, a measure of the actual gape in species with labial cartilages, (7) *gape height*—the distance between the upper and lower jaw symphyses.

Measurements with values for left and right sides (i.e., all variables except interhyomandibular distance) were averaged to a single measurement and then all variables were scaled to interhyomandibular distance (IHD) to remove variation due to body aspect ratio. There is no universally accepted method for body-size correction across batoids as groups vary widely in rostral, wing and tail length; IHD is a measure that is independent of the morphology of the feeding apparatus and was accessible to us on these scans. The relationship between IHD and more traditional measures of length, disc width and total length for example, will require a larger scale study. Two additional variables were calculated from the above distances: (1) *percent gape width*—the ratio of effective gape to anatomical gape. In species with labial cartilages this measures the occlusion of the

anatomical gape, with a smaller ratio indicating a greater occlusion (e.g., a ratio of 0.30 = an anatomical gape occluded by 70%). In those species without labial cartilages, anatomical gape width equals effective gape width and the ratio is 1.0. (2) *mouth shape*—the aspect ratio (gape height:effective gape). Lower ratios indicate a more rectangular mouth where width is greater than height (e.g., a ratio of 0.50 = a mouth twice as wide as tall), whereas higher ratios indicate “boxier” mouths (e.g., a ratio of 1.0 = a square mouth where width and height are equal). This variable acts as a descriptor of mouth shape; the shapes of batoid mouths at rest vary from nearly circular (e.g., *Narcine*), to rectangular (e.g., *Myliobatis*), to an upside-down U-shape (e.g., *Torpedo*) or V-shape (e.g., *Leucoraja*). Although they drastically simplify the contours of some morphology, our descriptors of mouth geometry are useful parameters for understanding the constraints of the feeding mechanism (e.g., the linear distance between the mouth corners likely delimits maximum width).

Dietary characters

Diet for sampled species was compiled from a synthesis of quantitative data in primary literature sources and from anecdotal information (i.e., field guides, personal communications) (see Supplemental Material online). Dietary items were then classified according to aspects of predator performance necessary for ingesting them (similar to Verwaijen et al. 2002): (1) *buried*—if the prey is excavated, typically indicated by feeding pits in the benthos, (2) *complex*—if the prey is processed in the oral cavity to remove inedible materials prior to swallowing, (3) *elusive*—if the prey is pursued and requires agility on the part of the predator, (4) *filtered*—if the prey is filtered from the water column, (5) *hard*—if the prey’s exoskeleton, prior to being swallowed, is damaged by the predator, (6) *large*—if both the length and width of the prey are greater than ~75% of the width of the predator’s mouth. Each dietary character was classified in binary fashion (0 = absence, 1 = presence), with the presence of any one character having no effect on the presence of any other (i.e., a species could eat both large and hard prey). A given taxonomic prey category (e.g., fish) could represent multiple functional prey categories (e.g., elusive and large), however, each species’ diet was evaluated on a case by case basis and classifications, when possible, were based on the condition of the ingested prey. For example, if a species swallowed whole gastropods

without crushing them it was not considered to eat hard prey, if a species swallowed whole shrimp without processing them it was not considered to eat complex prey.

Species were also coded for foraging habitat using published information, personal observations and anecdotal evidence of diet and behavior. Foraging habitat relates to the region of the water column where the majority of prey items are captured: *benthic* species feed on prey in or on top of the benthos, *demersal* species capture prey within 2–3 m of the benthos and *pelagic* species feed on prey captured more than 3 m above the benthos. These codings were used in reconstructing the hypothetical batoid ancestral state (see following section).

Fifteen species lacked dietary information. For six of these (*Dactylobatus*, *Dipturus*, *Potamotrygon*, *Rhinobatos*, *Rhinoraja*, *Zapteryx*), there was sufficient dietary information available for other species of the same genus to hypothesize that food habits were similar. For the remaining nine species (*A. borneensis*, *Benthobatis yangi*, *Hexatrygon bickelli*, *Narke japonica*, *Notoraja tobituki*, *Okamejei acutispina*, *T. hardwickii*, *Trygonorrhina fasciata*, *Typhlonarke* sp.) dietary information was unknown or unavailable and/or there were no published data available from congeners.

Phylogenetic correlations

We mapped our continuous morphological data and our coded binary dietary characters onto the most recent and comprehensive batoid phylogeny based on morphology (McEachran and Aschliman 2004) using Mesquite OSX (Maddison and Maddison 2006) with *Hydrolagus*, a chimaera, and *Heterodontus*, a galean shark, as outgroups. We set branch lengths to unity because adequate information on length of branches is lacking for this clade. Although less than ideal for reconstruction of ancestral diets, unity branch lengths are an accepted technique and pairwise comparisons do not make assumptions about lengths of branches (Garland et al. 2005; Midford et al. 2005).

We calculated pairwise comparisons for taxa that contrasted in the binary dietary character (i.e., species compared differed in their coding for a particular type of diet) to examine correlations between morphology and diet (Maddison 2000; Midford et al. 2005). This allowed us to ask generalized questions of association: for example, do species with elusive diets tend to have wider or narrower mouths? Only those species with coded dietary information were used in these comparisons, which eliminated from the analysis the narkid

electric ray (Fig. 2: taxa 5–7), narcinid electric ray (Fig. 2: taxa 8–11), and [*Okamejei* + *Raja* + *Dipturus*] (Fig. 2: taxa 15–17) polytomies. For the morphological variables that were significant predictors of a dietary character, we visualized the extent of morphospace occupied by the predator. In other words, if a morphological variable or variables were predictive of a dietary character, we generated a scatterplot of all species' values for those morphological variables and drew a minimum convex polygon around those species with that dietary classification. This method allows us to generate morphologically based hypotheses of dietary composition that are easily tested by gut content studies. Our small sample sizes for each dietary character (e.g., there are comparatively few large prey eaters in our study) preclude rigorous statistical analyses that would allow prediction of diet from morphology.

Continuous-valued characters are expected to differ among groups and therefore it is more important to increase degrees of freedom than to distinguish groups that differ in characters; pairwise comparisons between morphological variables (e.g., gape height and width) were made without regard to character states; instead, contrasts were chosen to maximize pairings for the given tree topology (Maddison 2000; Garland et al. 2005; Midford et al. 2005). Statistical significance for variable associations was tested using Mesquite and accepted for *P*-values <0.05.

We hypothesized the basal batoid condition for all observed variables and foraging habitat by reconstructing ancestral character states using squared-change parsimony for continuous characters, and squared-change parsimony and maximum likelihood for categorical characters. This is a phylogenetically corrected estimate of a suite of ancestral character states based on our measured characters and chosen tree topology (Garland et al. 2005).

Results

Morphological variation

For the sake of general description, we will focus on exemplar/extreme morphological dietary trends, rather than attempting to outline character states at all nodes. The history of feeding habits is illustrated graphically in Fig. 2; all types arose multiple times in the batoid lineage, with the exception of filter feeding, which is present only in the [*Mobula* + *Manta*] clade. All anatomical distances are normalized to interhyomandibular distance (IHD); for example, a length of 0.700 IHD is 70% of the distance between the cranial ends of

the hyomandibulae. Hyomandibula length (HYMavg) is relatively uniform throughout the batoid clade (~0.600–0.750 IHD), with notable increases in length (>90% IHD) in the narcinoid electric rays (i.e., non-torpedinid electric rays; Fig. 2), sawfish, and stingrays. In particular, the hyomandibula of the stingray *Dasyatis kuhlii* is an extreme outlier (1.891 IHD; Fig. 3) and is more than 135% the length of the next shortest hyomandibula.

In torpedinid electric rays, the sawfish, skates, and demerso-pelagic piscivores (*Pteroplatytrygon*,

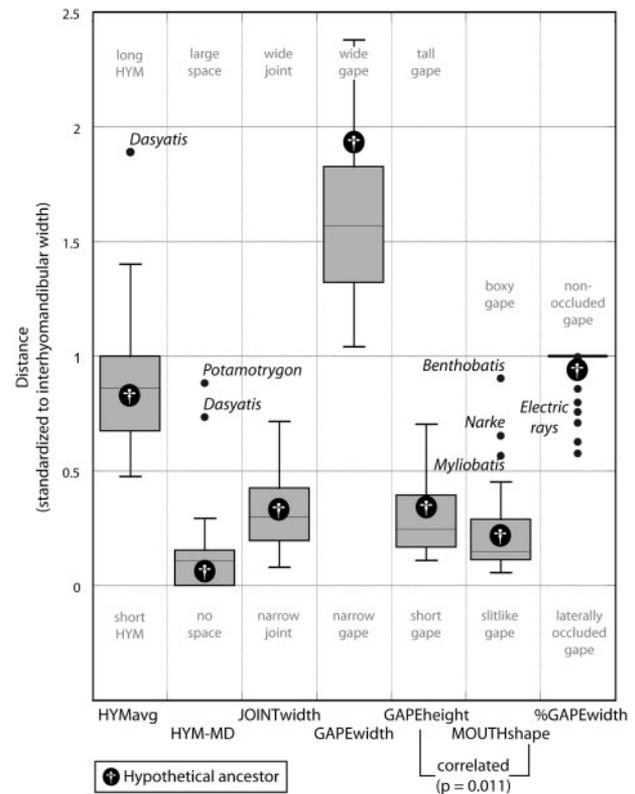


Fig. 3 Graphical summary of cranial morphological variables of the cranium for 40 species of batoid fishes as measured from CT scan data. Morphological variables (described in text) are listed on the x-axis, their values on the y-axis are scaled to IHD and the qualitative descriptors of high and low variable states are listed at the top and bottom of each column. For example, in the case of hyomandibula length (HYMavg), higher and lower values indicate longer and short hyomandibulae, respectively. The final two morphological variables, GAPEshape and %GAPEwidth are percentages that do not exceed 1.0. The boxplot for each variable encloses 50% of the data with the median value of the variable displayed as a line and the top and bottom of the box the upper and lower quartiles, respectively. The whiskers extending above and below the box represent the data range, with outliers displayed as individual points and labeled by species. The morphologies of the hypothetical ancestor as determined by squared-change parsimony are indicated for each variable by a white dagger in a black circle.

Gymnura, *Aetoplatea*) the hyomandibula abuts the mandible, leaving no space between the elements in CT scans (i.e., $\text{HYM-MD}=0.0$ IHD). In narcinid electric rays, most guitarfishes, and some stingrays, these skeletal elements are slightly separated (~ 0.080 – 0.275 IHD), but this variable is most pronounced in the stingrays *Elipesurus*, *Potamotrygon*, and *Dasyatis* in which the distance may be more than three times that of other species (e.g., IHD in *Potamotrygon* is 0.884) (Fig. 3).

The width of the quadratomandibular joint is typically $<40\%$ of interhyomandibular distance and is notably smaller ($<\sim 0.200$) in many demerso-pelagic and/or large prey eaters (torpedinid rays, *Raja*, *Anacanthobatis*, *Plesiobatis*, *Gymnura*, *Aetoplatea*, *Mobula*, *Manta*). These joints are stouter (joint width >0.400) in narcinoid electric rays, durophagous myliobatoid stingrays and the six-gilled stingray, *Hexatrygon*.

Gape width is highly variable, but exhibits a notable dichotomy in the electric rays, in which the narrowest torpedinid ray gape (*Torpedo*: 2.022 IHD) is approximately twice that of the narrowest gape of a narcinoid ray (*Discopyge*: 1.042 IHD). This split is the result of the labial cartilages of narcinoid electric rays, which serve to laterally occlude the mouth and reduce the effective gape. As a result, in these species the lateral span of the gape is reduced by as much as $\sim 40\%$ (*Narke* and *Benthobatis*). All other batoid groups lack these elements and therefore their effective and anatomical gapes are equal. In general, skates and guitarfishes have wider gapes (>1.650 IHD) than do stingrays (>1.285 IHD), and demerso-pelagic species in all groups (*Torpedo*, *Hypnos*, *Pristis*, *Raja*, *Dipturus*, *Pteroplatytrygon*, *Gymnura*, *Aetoplatea*, *Mobula*) tend to have wider mouths (>1.65 IHD). The gapes of the coffin ray, *Hypnos* (2.370 IHD), and the sawfish, *Pristis* (2.380 IHD) are the widest of the species we examined.

In general, batoid gapes are wide and short or “slitlike” (Fig. 3); the resting distance between the symphyses of the upper and lower jaws is typically short (<0.275 IHD), with notable exceptions in the narcinoid electric rays, *Pristis*, *Zanobatus*, *Elipesurus*, and the myliobatoid stingrays (>0.275 IHD). These differences are further reflected in the ratio of gape height to gape width (or mouth shape); narcinoid electric rays, *Zanobatus*, and the myliobatoid stingrays possess gapes with heights $>35\%$ of their widths, whereas the rest of the clade exhibits gapes only 5–20% tall as wide. The lateral occlusion of the gape in the narcinoid electric rays nearly equates gape height and width in some

cases, forming a roughly circular mouth opening (e.g., the resting gape width of *Benthobatis* is $\sim 90\%$ of gape height).

Morphological and dietary correlations

Pairwise comparisons of morphological characters indicate a single significant positive association between morphological variables from 13 pairs examined in each comparison (Fig. 3). The height and shape of the gape are positively associated ($P=0.011$), meaning taller gapes are also “boxier” (height more similar to width). This indicates that height of the gape rather than its width is responsible for changes in mouth shape.

Four significant positive associations also exist between morphological and dietary characters (Fig. 4). Correlations were determined from five or six pairwise comparisons for each morphological-dietary character pairing, except in cases of pairs including the “filter feeding” dietary character where only a single pair was possible per morphological character due to the limited number of filter feeding species. Stouter quadratomandibular joints ($P=0.016$) and “boxier” gapes ($P=0.016$) both predict diets including complex prey, whereas larger quadratomandibular joint widths ($P=0.031$) and longer hyomandibulae ($P=0.031$) predict diets including hard prey. Hyomandibular length is also negatively associated with large prey ($P=0.031$), predicting shorter hyomandibulae in species with large prey items in their diet. Notably, several variables exhibited nearly significant correlations ($P=0.063$; hyomandibula length and buried prey, quadratomandibular joint width and buried prey, hyomandibular-mandibular distance and complex prey), which may become more prominent when more species are added to future analyses (see “Discussion” section).

Ancestral reconstruction

Reconstruction of ancestral diets depicts the hypothetical diet of the basal batoid. Squared-change parsimony analysis of dietary and foraging habitat suggest that the basal batoid was a benthodemersal predator that fed on complex and elusive prey (perhaps prey similar to cephalopods, fish and shrimp). Although these predictions are also implied by maximum likelihood estimates, only the diet of elusive prey is statistically likely ($P<0.05$).

Squared-change parsimony reconstructions of ancestral morphology suggest a batoid ancestor with a wide gape (~ 5.5 times its height), without labial cartilages (the percent gape width approaching 1.0) and with little space between the

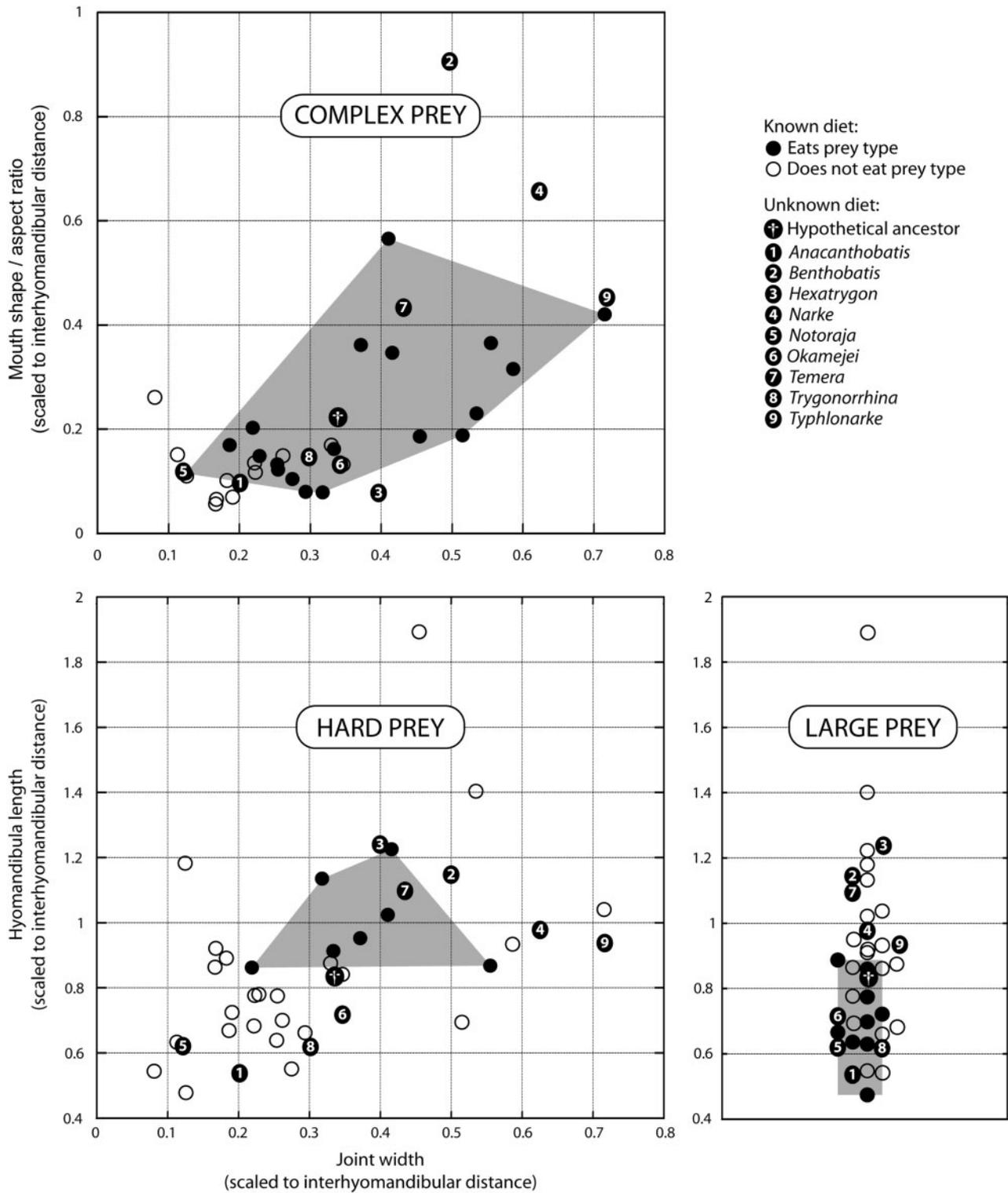


Fig. 4 Correlations between cranial morphology and dietary composition for 40 species of batoid fishes as indicated by pairwise comparisons. For the “complex prey” and “hard prey” graphs, width of the quadratomandibular joint (JOINTwidth) is indicated by the x-axis and the “hard prey” and “large prey” graphs both have hyomandibular length (HYMavg) indicated by the y-axis. Each graph represents a particular prey type (“complex”, “hard” or “large”); solid and empty circles indicate taxa that do or do not, respectively, eat that prey type. The predicted morphologies of the hypothetical ancestor are indicated by a white dagger in a black circle and species with unknown diets are marked as black circles with white numbers. Species feeding on a particular prey type (i.e., black dots) are bounded by minimum convex polygons (gray shading); taxa found within shaded regions are suggested by their morphologies to eat that type of prey. Diets including large prey are predicted by a single morphological variable and therefore, only hyomandibular length (the y-axis variable) is meaningful.

hyomandibulae and jaws. This morphological suite is similar to most batoids other than dasyatid stingrays (Fig. 3). The quadratomandibular joints are predicted to be wider than those present in most extant skates, guitarfishes and basal stingrays, and slightly less stout than those of either narcinoid electric rays or specialists on hard prey. The hyomandibulae were most similar to those of the gymnurid and myliobatid stingrays (~80% of interhyomandibular distance). Overall, of the species studied, the feeding mechanism of the hypothetical ancestor most resembles the sawfish, *P. perotteti*, except that the latter has slightly longer hyomandibulae (by ~10% of interhyomandibular width).

The reconstructed diet of the hypothetical ancestor is also supported by the morphological-dietary correlations generated by pairwise comparisons (Fig. 4). Mouth shape and joint width predict a diet of complex prey; when the ancestral condition is plotted for these variables, it is surrounded by known eaters of complex prey. The reconstructed joint width and hyomandibular length of the ancestor lie very close to the minimum convex polygon of the hard prey eaters. However, the ancestor is predicted by maximum likelihood to be non-durophagous, so these stout joints are likely associated with benthic predation on complex prey (as in narcinoid electric rays) (see “Discussion” section). Reconstructed ancestral hyomandibular length agrees with maximum likelihood estimates in suggesting a diet that does not include large prey.

Dietary predictions

We used the three morphological characters with significant dietary correlations (joint width, mouth shape and hyomandibula length; Fig. 4) to hypothesize about the feeding habits of those nine species with unknown dietary composition. The minimum convex polygon for complex prey joint width/mouth shape morphologies is considerably larger than that for hard prey joint width/hyomandibula length morphologies, but both polygons are reasonably exclusive (they include few species that do not feed on the dietary character in question). In contrast, the range of morphologies that suggest diets including large prey also includes several species that do not feed on large prey, indicating that hyomandibula length is likely not the only anatomical feature linked to this dietary character (see Discussion).

Our data suggest that *Anacanthobatis*, *Okamejei*, *Temera*, and *Trygonorrhina* (and perhaps *Hexatrygon* and *Typhlonarke*) feed on complex prey and *Temera* (and perhaps *Hexatrygon* and *Benthobatis*) feed on

hard prey. In the case of the two electric rays (*Benthobatis* and *Temera*) this hard prey association is likely confounded by the mechanical requirements for excavating buried prey (see “Discussion” section). All skates (*Anacanthobatis*, *Notoraja*, *Okamejei*) and *Trygonorrhina* are predicted to have diets that include large prey.

Discussion

The batoid clade demonstrates diversity in both cranial morphology and feeding ecology. Our data suggest multiple and not always coordinated evolutions of specialized morphology and diet, yielding multiple evolutionary pathways leading to similar trophic endpoints. Complicated mechanical systems, such as the crania of bony fishes, are expected to be highly functionally convergent—similar diets are supported by a range of morphologies. The link between morphology and function can be complicated by incongruence between morphological and functional diversity (Collar and Wainwright 2006). This many-to-one mapping of morphology to function is implicated in teleost phenotypic diversity (e.g., Alfaro et al. 2005) and is also suggested by our analysis of the batoid feeding mechanism, although the range of morphologies with convergent diets is unlikely to be as broad as in bony fishes.

It is tempting to imagine that the link between morphological and functional diversity is tight and therefore that variation is somehow a coordinated affair. Functional interpretations of morphological data, however, are dangerous because anatomical variation likely does not predict functional diversity and unique morphologies may not yield specialized diets (Bellwood et al. 2006; Collar and Wainwright 2006). Nevertheless, in light of the absence of data on batoid performance, our associations of morphology and diet suggest important functional parameters and allow predictions of dietary compositions of species with unknown trophic ecology. Our analysis should not stand on its own but rather should serve as a clarion call for more kinematic, electromyographic, dietary, and functional morphological data to test the predicted associations.

The length of the hyomandibula plays an important role in mediating capture of prey and illustrates that small changes in each of the few elements in this feeding mechanism may have profound effects on dietary niche. Shorter hyomandibulae are correlated with diets including large food items, whereas long hyomandibulae suggest durophagy. Hyomandibular length is not correlated with any other morphological character but the *P*-values ($0.065 > P > 0.05$)

suggest that additional anatomical data will define clusters of morphological characters that together have greater predictive power. These clusters may be particularly easy to define with the addition of certain key taxa to clarify regions of the phylogeny that were underrepresented in the present study (e.g., skates, sawfishes, dasyatid stingrays, and rhynchobatiform guitarfishes). For example, our data suggest that short hyomandibulae and diets including large prey may also be associated with smaller distances between the hyomandibula and mandible, implying a bracing function with little room for bending or movement. Concomitantly, longer hyomandibulae may also be associated with complex prey and larger distances between the hyomandibula and mandible, especially in dasyatid and freshwater stingrays (*Elipesurus*, *Potamotrygon*), suggesting a greater range of motion at the articulation between the hyoid and jaw.

Our preliminary predictions of feeding habits for species of unknown dietary composition are limited by our sample sizes for species coding positively for each dietary character. As such, in cases where a species of unknown dietary composition is not surrounded by a cluster of species that include that prey in their diets, our inferences of feeding ecology are tenuous. Our minimum convex polygons, however, provide interesting hypotheses regarding the relationship between batoid cranial morphology and dietary composition. For example, the morphospace for species feeding on hard prey is much more condensed than that for species with complex prey in their diets, suggesting a degree of morphological specialization for durophagous species. Also, although hyomandibula length is significantly correlated with diets including large prey, the considerable overlap of species that do and do not include large prey in their diets suggests that a currently non- or nearly-significant variable (e.g., hyomandibular-mandibular distance) or an unexamined aspect of cranial morphology also plays a role in mediating this dietary character.

A comparison of narcinoid and durophagous myliobatoid rays illustrates the pitfalls of inferring performance from morphology and also the importance of an informed choice of variables. Both groups (Fig. 2) exhibit long hyomandibulae and reinforced joints. Hyomandibular length is positively correlated with a diet of hard prey (Fig. 4), suggesting that some narcinoids for which diets are unknown (*Benthobatis*, *Temera*) are also durophagous. However, based on the presence of labial cartilages and consistent cranial morphology among narcinoids (which include the lesser electric ray,

Narcine brasiliensis, one of two species for which extensive behavioral, mechanical and dietary information exists) (Dean and Motta 2004a, 2004b) it is more likely that they excavate buried polychaete prey and winnow away ingested sediment. Prey are excavated by folding the jaw arch medially at the symphyses (bringing the left and right sides of the jaw closer together) (Dean and Motta 2004a) and protruding the jaws into the sediment. In this case, the jaw symphyses are flexible and not fused, adductor muscles and dentition are likely too weak for durophagy, hyomandibular length relates directly to protrusion distance, and joint reinforcement helps the jaws resist buckling forces associated with excavation. A similarly spurious prediction is likely for the six-gilled stingray, *Hexatrygon*, which, by its joint width and hyomandibular length, might be inferred to eat hard prey; however the poor mineralization of its skeleton surely precludes this.

In the case of durophagy, our choice of some morphological variables over others may have led to a mistaken prediction of diet from morphology. The jaws of both narcinoid rays and durophagous myliobatoid rays, however, are reinforced by struts of trabecular cartilage, suggesting that at the tissue level the mechanical demands of these strikingly different diets may in fact be similar (Summers 2000; Dean et al. 2006).

Our data imply that batoid cranial elements and functional geometries evolved independently (evidenced by the lack of correlation among the majority of morphological variables), resulting in a highly diverse system despite the apparent constraint of a limited number of interactions between few component parts. In other words, the position of a given functional element (e.g., hyomandibula) within its morphospace is less contingent on the morphology of any of the other elements and the communicating channels between morphospaces (e.g., the interaction of the hyomandibular and mandibular morphospaces) are broader. A similar evolutionary independence has been shown among the mechanical elements of the feeding apparatus of centrarchid and labrid fishes (Wainwright et al. 2004; Collar and Wainwright 2006). With selection working at the level of function, this decoupling allows a diversity of morphological solutions at underlying levels (Wainwright et al. 2004; Wainwright et al. 2005).

The decoupling of functional elements, both evolutionarily and anatomically, is a predominant motif in the evolution of the batoid cranium (Wilga and Motta 1998; Dean and Motta 2004a; Dean et al. 2005). It is possible that the limited association of jaws and cranium was a key

innovation in the evolution of a functionally and evolutionarily flexible feeding mechanism (Schaefer and Lauder 1986, 1996). The euhyostylic jaw suspension of batoids releases functional constraints on the jaw arch, namely the limitation of the ethmopalatine ligament (Fig. 1), permitting dynamic feeding behaviors such as extreme (>100% head length) and/or ventro-lateral jaw protrusion (Wilga 2002; Dean and Motta 2004b). Less appreciated is the decoupling of the jaws and hyomandibulae from the branchial arches, which mechanically isolates structures used in breathing from those used in feeding (Dean and Motta 2004a). Unlike that of sharks, the batoid hyoid arch is divided in its associations—the dorsalmost portion (hyomandibulae) is linked to the jaws and, in all but two cases, the ventral ceratohyal–basihyal unit is lost or coupled to the branchial complex (Miyake and McEachran 1991; McEachran et al. 1996).

This release of jaw constraint may have increased the role of the branchial arches in feeding. Although batoids, like all elasmobranchs, lack pharyngeal jaws and tongues, prey can be processed by rhythmic contractions of the branchial and jaw arches to create a coordinated flow of water through the mouth and a “hydrodynamic tongue” for delicate separation of edible and inedible materials (Dean and Motta 2004b; Dean et al. 2005). By comparison, the jaws of sharks serve double-duty and are primary in both capture and processing.

Processing of prey is arguably more important than its capture for some prey types but has been overlooked in the literature on vertebrate feeding; however, the evolution of a dynamic and functionally plastic processing mechanism in batoids may have further released constraints on the jaws and facilitated the rapid evolution of batoid dietary niches. Shark feeding mechanisms are well studied (e.g., Motta 2004) and provide anatomical and functional context for understanding the importance of branchial-hyoid-jaw coupling. The range of morphologies within the batoid fishes provides further context for examining these associations. For example, we expect that jaw and branchial motion will be constrained in some electric rays (e.g., *Narke*, *Typhlonarke*) that retain a connection between the elements of their hyoid arch whereas in the more derived myliobatoid stingrays the basihyal is completely replaced by medial extensions of branchial and jaw musculature, suggesting a finer control of water flow through the throat (Miyake and McEachran 1991; McEachran et al. 1996).

Investigation of processing mode and the functional coupling of the jaws, hyoid, and branchial

apparatus is a rich area for future research within this clade and will provide insights into important constraints in the feeding mechanism and in the evolutionary consequences of decoupling. Our estimations of ancestral states and unknown trophic ecologies will become more powerful with the inclusion of additional taxa, especially given the small size of this clade and the narrow phylogenetic distribution of some dietary characters (e.g., filter feeding). Morphological data are comparatively easy to collect but the study of batoid trophic evolution is *most* in need of functional, behavioral, and dietary analyses. Although analyses of gut contents and of some tissue-level phenomena are possible from museum specimens, kinematic, electromyographic, and functional morphological studies are only viable using live animals under natural conditions or simulations of natural conditions.

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