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Always chew your food: freshwater stingrays use mastication to process tough insect prey

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Chewing, characterized by shearing jaw motions and high-crowned molar teeth, is considered an evolutionary innovation that spurred dietary diversification and evolutionary radiation of mammals. Complex prey-processing behaviours have been thought to be lacking in fishes and other vertebrates, despite the fact that many of these animals feed on tough prey, like insects or even grasses. We investigated prey capture and processing in the insect-feeding freshwater stingray *Potamotrygon motoro* using high-speed videography. We find that *Potamotrygon motoro* uses asymmetrical motion of the jaws, effectively chewing, to dismantle insect prey. However, CT scanning suggests that this species has simple teeth. These findings suggest that in contrast to mammalian chewing, asymmetrical jaw action is sufficient for mastication in other vertebrates. We also determined that prey capture in these rays occurs through rapid uplift of the pectoral fins, sucking prey beneath the ray's body, thereby dissociating the jaws from a prey capture role. We suggest that the decoupling of prey capture and processing facilitated the evolution of a highly kinetic feeding apparatus in batoid fishes, giving these animals an ability to consume a wide variety of prey, including molluscs, fishes, aquatic insect larvae and crustaceans. We propose *Potamotrygon* as a model system for understanding evolutionary convergence of prey processing and chewing in vertebrates.

1. Introduction

For predators to effectively digest prey, considerable mechanical or chemical processing is often required [1,2]. Chewing is used to break down tissue, expose digestible elements and increase surface area for chemical digestion. Mastication varies across diet, but typically involves multi-axis shearing by dental occlusal surfaces rather than a uniaxial compression-only loading regime. Complex teeth or the possession of multiple types of teeth (heterodonty) are frequently associated with chewing, enabling some teeth to be used for capture (e.g. canines) while others are used for processing (molars) [3]. In terrestrial taxa, mastication is generally considered to be restricted to mammals (the 'definitive chewers' [4]). Chewing is considered an evolutionary innovation in mammals that spurred dietary diversification, allowing exploitation of food sources ranging from insects to grasses. Dietary flexibility and efficiency made possible by chewing is thought to have contributed to the evolutionary radiation of mammals [5].

Many mammals capture and process prey with the oral jaws and associated teeth, with chewing and prey processing therefore occurring within the mouth. However, in many other vertebrates, prey capture and processing are accomplished using anatomically distinct modules, meaning that these functions have become dissociated. For example, birds and some other archosaurs use a beak or jaws to seize and rend prey, while a muscular gizzard is used to grind prey further [4]. Most fishes use expansion of the oral jaws for prey capture through suction feeding, but in many cases use pharyngeal dentition (posterior jaws

derived from gill arches) to crush or grind prey [6,7]. In some cartilaginous fishes, notably batoids (skates, stingrays, etc.), prey capture and processing may be handled by two systems—prey capture is achieved with the disc (the morphological structure derived from encircling pectoral fins [8]), leaving the jaws and teeth for prey processing. In all these cases, different anatomical modules handle different functions, allowing for independent modular evolution [9] and increased evolutionary flexibility.

In fishes, considerable variation in tooth shape, muscle motor activity and jaw kinematics suggests that prey processing is more diverse than previously expected [10,11]. Stingrays (Myliobatiformes) have conspicuously 'loose' jaw joints [12], a trait in common with mammals. Loose jaw joints allow for transverse (medio-lateral translation, as in bovids and cervids) as well as propalinal (longitudinal translation, as in rodents) translation of the jaws against one another [4]. Unlike mammals, stingrays have homodont dentitions; however, some species can reorient the teeth during feeding to a cusped occlusal surface, making them functionally heterodont [13,14]. Batoid fishes also exhibit unilateral, asymmetric muscle activity and asymmetric jaw action during lengthy prey-processing behaviours which strip invertebrate prey of their exoskeleton [15–17]. Loose jaw joints, transverse or longitudinal translation of the lower jaw, asymmetrical (unilateral) jaw activity and heterodonty are all chewing-associated traits shared with mammals [18]. Stingrays then offer an interesting example of convergence in function with other masticating vertebrates, and are an apt model system for comparisons with other vertebrates.

Here, we analyse both prey capture and processing in the Neotropical freshwater stingray *Potamotrygon motoro* (Müller & Henle, 1841). This species is a member of a lineage of stingrays (29+ species) that probably invaded South American freshwaters during the Miocene [19] and diversified across a range of dietary niches. We selected *P. motoro* as a model because it represents one of a few chondrichthyan species whose diet includes insects. Several potamotrygonid stingray species, including *P. motoro*, feed on chironomid, orthopteran, dipteran and odonate larvae [20–23]. Chitin, a main component of insect cuticle, provides considerable toughness (fracture resistance) and strength (elastic energy storage) [24]. For these reasons, insect cuticle generally survives digestion through the gut of many insectivores, albeit in pieces. It is possible that the challenges of insect-feeding in the freshwater stingrays prompted the evolution of mastication in this clade, providing an interesting parallel with early mammals, many of which also fed on insects [25].

We used high-speed videography to investigate feeding behaviour by *Potamotrygon motoro* on different prey types. Our primary objective was to test whether this species uses chewing to process prey, as assessed by asymmetric motions of the jaws that shear and compress food between occlusal surfaces. We predicted that, across a range of prey types, chewing motions would be more exaggerated for more chitinous food items (insects and crustaceans). We also tested the hypothesis that *P. motoro* dissociates prey capture and processing by using the whole body (disc) to capture prey items, and the mouth and jaws for processing, as observed in two other batoid species [8]. Finally, as chewing is typically associated with heterodonty, we determined whether *P. motoro* are capable of reorienting their teeth, to produce a functionally heterodont condition.

2. Material and methods

(a) High-speed videography

Potamotrygon motoro ($n = 4$) were purchased from aquarium wholesalers and kept in 284 l aquaria with sand bottoms. All animals were fed a diet of processed seafood mixes and commercial elasmobranch foods. Rays were kept at a 12 L : 12 D cycle. Food was buried in the sand to promote natural foraging. We chose three frozen, dead experimental prey items: whole silversides (fishes), *Palaemonetes* shrimps, and *Libellula* and *Aeshna* genera odonate larvae. These three prey types span the diversity, in taxonomic and material terms, of *P. motoro* prey in the wild. In general, calcified chitin (crustaceans) is stiffer and less tough than insect chitin, which without mineralization can deform more freely during compression or tension [24]. For feeding trials, rays were introduced into the filming aquarium and allowed to acclimate in water from their home aquarium for at minimum 2 h, with food being withheld for a prior 24 h period. Prey items were weighed prior to being placed in the feeding aquarium.

Stingrays were filmed in a custom 290 l clear acrylic aquarium which allowed viewing from all sides and the bottom, with bare bottom (i.e. no substrate). Rays were filmed feeding either at 250 frames s^{-1} for most prey capture behaviours or, if processing behaviours were particularly lengthy, 150 frames s^{-1} in order to not exceed the integrated memory of the camera system. Three high-speed cameras (model SPR-I, AOS Technologies, Baden Daettwil, Switzerland) were placed at orthogonal angles to the tank in order to capture ventral, lateral and frontal views simultaneously. Only ventral views were analysed in further methods. High-intensity, 70 W (joules per second) mercury halide lights mounted on a retractable gantry could be raised/lowered to provide lighting. Video data were streamed via Ethernet to AOS IMAGING STUDIO software (AOS Technologies, Baden Daettwil, Switzerland), cropped to only the pertinent behaviours, and saved as .RAW files. These files were later converted to .AVI formats and read into ImageJ (ImageJ v. 1.40 (National Institute of Health, Bethesda, MD) as an iterated .TIFF stack for kinematic analysis. A prey capture event was considered successful if prey was captured, processed and then ingested.

(b) Feeding kinematics

To quantify asymmetrical jaw action, we examined the angular deviation of the jaws during feeding relative to a resting state (when the rays were not feeding; figure 1). This metric specifically quantified to what degree jaw protrusion during biting was asymmetrical (i.e. unilateral or restricted to only one side of the jaw). This method was also used to quantify the degree to which the medial symphysis of the lower jaw was flexed and then extended during feeding, which shears prey held in place by the opposing lateral rami of the jaw [17]. In either case, prey is pinched and held in place by one side of the jaw, and either sheared against the opposing upper or lower jaw or pulled in tension when the symphysis is extended. Both cases allow for propalinal translation of the upper and lower jaws against each other as well as transverse shearing of opposing left and right rami of the jaws.

Prey processing is a complex routine and the distinction between capture and processing is difficult to pinpoint, but processing typically occurs when prey is ingested (moves beyond the gape into the pharynx). We observed that rays rarely swallowed insect prey immediately after ingestion, and instead spat prey back out of the mouth, then re-ingested it before maceration with the jaws. Thus, the second biting event was designated as the beginning of the prey-processing sequence for analyses, the start of prey winnowing. To determine whether feeding on

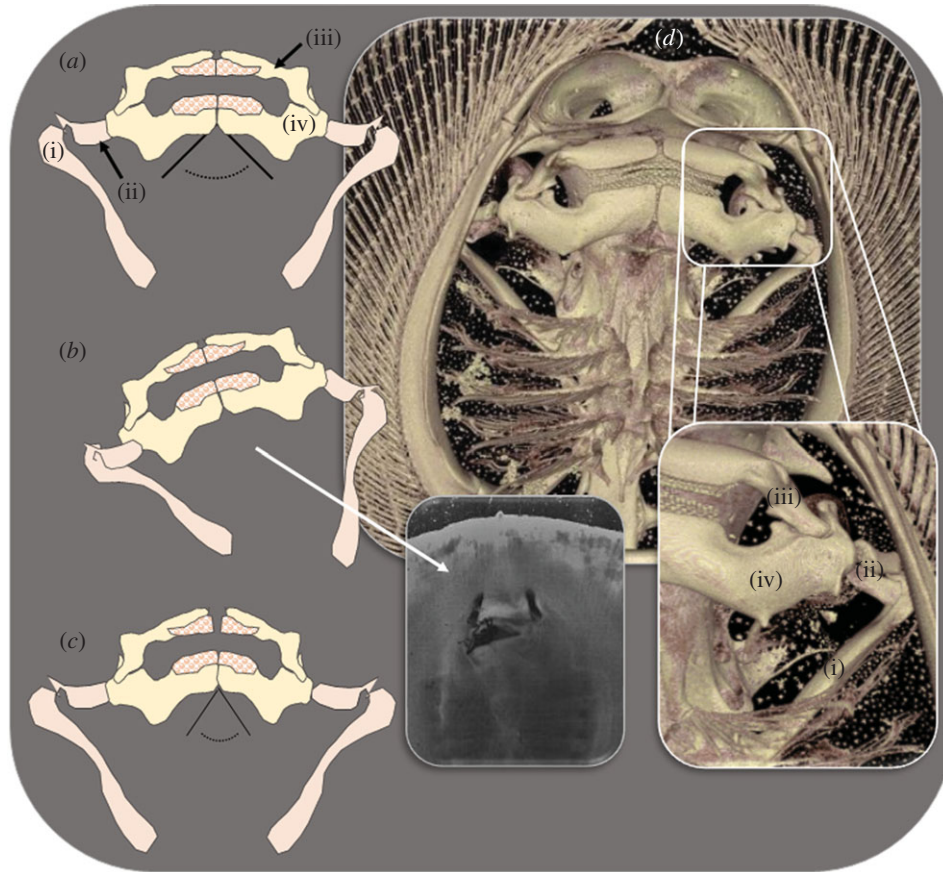


Figure 1. Functional morphology of asymmetrical jaw protrusion in *Potamotrygon motoro*. (a) Resting jaw and hyomandibular articulations; (b) asymmetrical protrusion of jaws relative to kinetics of angular cartilage and hyomandibular articulations (inset and pointer: photo of asymmetrical protrusion of live *Potamotrygon* while feeding on insect larva); (c) medial flexion of mandibular (Meckelian) symphyses; (d) computed tomography scan of *Potamotrygon motoro* crania (inset: articulation of jaws to hyomandibular cartilage via dual angular cartilages). The number of angular cartilages varies in potamotrygonids, in *P. motoro* there are two angular cartilages (ii). These angular cartilages bridge the gap between the hyomandibulae (i) and the palatoquadrate (upper jaw; iii) and Meckel's cartilages (lower jaw; iv). (Online version in colour.)

insect prey required longer prey-handling times and more frequent biting, we tallied the number of bites as well as the duration of prey handling for 15 feeding events per prey type (five per individual) and calculated the bite rate as the number of bites divided by the duration of prey handling for each feeding event. In total, there were 45 analysed feeding events (15 events for each prey item).

For determining whether asymmetrical kinetic action of the jaws occurred more frequently when rays fed on chitinous prey, we analysed the first 15 bites from a processing cycle, for each prey type, per individual. We note that in addition to obvious biting, many more rapid and non-stereotyped motions of the jaws were observed, suggesting that prey processing also occurs deeper in the pharyngeal cavity. During these quick bites, the magnitude of jaw protrusion and gape size was limited, but considerable asymmetrical jaw motion (symphyseal flexion and asymmetrical jaw protrusion; figure 1; electronic supplementary material, video) was observed. These motions presumably reflect internal processing events that cannot be recorded with these methods [26]. Beginning with the onset of lower jaw depression and finishing at time of upper jaw retraction (which proceeded after jaw closure, i.e. the actual 'bite'), we measured the maximum deviations for symphyseal flexion and angle of jaw protrusion from a resting state. For prey capture, the onset of rostral lifting was chosen as time zero, with the rest of the kinematic variables examined occurring relative to this moment. Peak rostral lifting occurred when the anterior edge of the disc, closest to the prey reached peak height.

(c) Statistical analyses

Mixed models were used in order to account for multiple recordings from single individuals in our dataset, essentially a one-way ANOVA with fixed and random effects. Linear mixed models (LMMs) account for individual variation as a random effect as long as data follow a normal distribution. The R package *lme4* was used to generate LMMs on our capture dataset with 'Individual' as our random effect, and uses maximum likelihood to estimate the parameters of our equation model. Prey type and prey mass were treated as covarying explanatory variables against separately analysed kinematic response variables. To determine which distribution best fit our data, we visualized each variable using the *MASS* package in R while simulating a normal, lognormal, Poisson, gamma and negative binomial distribution. In the case of non-normal variables, we used generalized mixed models (GLMMs) with the corresponding coefficient modifier to fit the data. Analysis of deviance (Type II Wald χ^2 -test) was used to generate *p*-values for LMM and GLMM results, coupled with Tukey tests for finding significant differences among specific variables.

Because prey are comminuted over time and predator feeding behaviour reflects this change in prey integrity, analysis of how aspects of feeding kinematics change over time are essentially auto-correlated, with measurements closer in time expected to resemble one another more than measurements taken farther apart in time. In order to examine how asymmetrical jaw action varied between prey types generally, as well as how patterns of asymmetrical kinesis change over time when feeding on different prey, we used time-explicit growth models using a

Table 1. Linear mixed model results of prey-processing kinematics for prey type and mass.

model	covariate	Tukey results	estimates	standard error	t-value	p-value
number of bites <i>n</i> = 15 per prey item	prey type	—	17.48	4.41	3.96	<0.001
		fish insect	34.96	4.45	7.86	<0.001
		shrimp fish	10.55	4.60	2.29	0.057
		shrimp insect	−24.41	4.25	−5.74	<0.001
duration of prey handling <i>n</i> = 15 per prey item	prey type	—	−16.75	14.30	−1.17	0.241
		fish insect	7.08	1.79	3.95	<0.001
		shrimp fish	13.31	1.81	7.36	<0.001
		shrimp insect	3.98	1.87	2.13	0.084
bite rate <i>n</i> = 15 per prey item	prey type	—	−9.32	1.73	−5.40	<0.001
		fish insect	−5.55	5.81	−0.96	0.339
		shrimp fish	2.57	0.19	13.26	0.457
		shrimp insect	0.23	0.20	1.16	0.480
significance level ($\alpha = 0.05$)	prey mass	—	−0.18	0.19	−0.94	0.612
		fish insect	−0.55	0.63	−0.87	0.386
		shrimp insect	—	—	—	—

LMM framework. We contrasted increasingly complex models accounting for individual variability, auto-correlated error structure, slope and intercept variability and used the Akaike information criterion (AIC) to determine the models which best fit our data for measurements of symphyseal flexion and asymmetrical protrusion, separately. As with regular LMMs, analysis of deviance (Type II Wald χ^2 -test) was used to generate *p*-values, coupled with Tukey tests for finding significant differences between specific variables [27]. All statistical analyses were performed in R (v. 2.15.0, www.theRproject.org).

(d) Computed tomography scanning

In order to examine tooth morphology, and whether teeth reoriented during jaw protrusion (non-asymmetrical, in this case), we used one of the experimental animals (*P. motoro*) from filming trials in computed tomography (CT) scanning. For comparison, we also examined a specimen of *Potamotrygon orbignyi* (Castelna, 1855), a congeneric insectivorous freshwater stingray species. For both specimens, the oral cavity was filled with flexible hobby foam until the jaws attained a protruded state. Specimens were scanned with a Bruker Skyscan 1173 at the Karel F. Liem Bioimaging Center at Friday Harbor Labs at 60 kV and 100 μ A and a voxel resolution of 35.5 μ m. Specimens had their pectoral fins removed in order to fit within the CT scanner, and were wrapped in alcohol-saturated cheesecloth in large Ziploc bags. The images were reconstructed and visualized with AMIRA (v. 5.0, Mercury Computer Systems, Inc., USA).

3. Results

(a) Prey-processing kinematics

We observed asymmetrical jaw action in all prey-processing trials as evidenced by consistent symphyseal flexion and asymmetrical jaw protrusion across all prey types (tables 1 and 2). During each bite, some asymmetrical jaw action (symphyseal flexion and/or asymmetrical protrusion) is observed.

Prey handling, including degree of asymmetric protrusion and symphyseal flexion, differed between insect prey and less tough prey like fish and shrimp. Handling durations were, on average, at least twice as long for insects (18.6 ± 1.76 s.e.) compared with other prey (shrimp, 9.19 ± 0.12 s.e.; fish, 5.84 ± 0.71 s.e.; table 1). Linear mixed model results show that the duration of prey processing differed significantly among prey types ($p < 0.0001$), regardless of prey mass ($p = 0.241$). LMM results also showed handling duration times for insect prey to be significantly different from other prey types (insects | fish, $p < 0.0001$; insects | shrimp, $p < 0.0001$), while fish and shrimp prey did not differ from one another ($p = 0.084$) (table 1). Insect prey incurred twice as many bites on average as shrimp prey (insects, 47.13 ± 4.21 s.e.; shrimp, 22.40 ± 3.07 s.e.), and more than three times the number of bites compared with feeding on fish (fish, 13.70 ± 1.32 s.e.). The number of bites during a prey handling bout also significantly differed among prey types ($p = 1.5 \times 10^{-15}$), but did not differ significantly with prey mass ($p = 0.241$) according to LMM analyses. LMM results confirmed that the number of bites during feeding on insects was significantly different from other prey types (insects | fish, $p < 0.0001$; insects | shrimp, $p < 0.0001$), while fish and shrimp prey did not differ from one another ($p = 0.241$) (table 1). Finally, bite frequency did not differ significantly among prey types ($p = 0.46$) or according to prey mass ($p = 0.39$) (table 1) and averaged about 2.5 Hz (± 0.08 s.e.) across prey types.

Time-explicit growth modelling revealed similar trends as general LMM results for the effect of prey type on asymmetrical jaw protrusion during prey processing (figure 2 and table 2). For both asymmetrical jaw protrusion angle and symphyseal flexion, we found that models which incorporated an autoregressive structure with serial correlations incorporating how feeding variables changed over the course of the processing event were necessary to improve model fitting (AIC = 3792 versus 3847). The random effect of individual variation explained approximately 0.9–6.0% of the variance

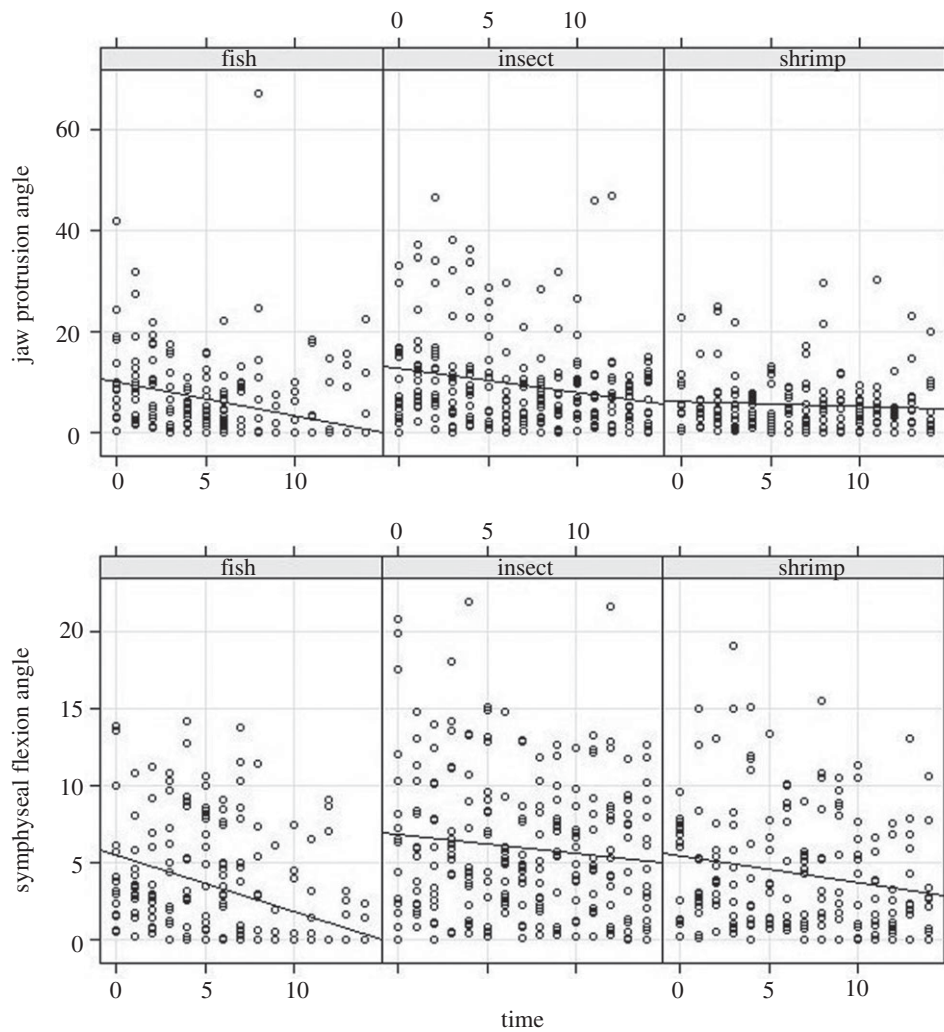


Figure 2. Growth model plots for angular deviations of the jaws in *P. motoro* during prey-processing behaviour for three different prey types.

Table 2. Growth model results for angular deviations of the jaws during prey processing.

model	covariate	Tukey results	value	standard error	t-value	p-value
angular jaw protrusion <i>n</i> = 15 per prey item	prey type	—	8.48	1.37	6.17	<0.001
		fish insect	4.52	1.06	4.27	<0.001
		shrimp fish	−0.09	1.08	−0.08	0.996
		shrimp insect	−4.61	1.02	−4.53	<0.001
symphyseal flexion <i>n</i> = 15 per prey item	prey mass	—	1.97	3.64	0.54	0.588
	time	—	−0.39	0.08	−5.17	<0.001
	prey type	—	4.33	0.49	8.77	<0.001
		fish insect	3.34	0.52	6.46	<0.001
		shrimp fish	1.35	0.53	2.54	0.030
	shrimp insect	−1.99	0.50	−4.02	<0.001	
	prey mass	—	−0.61	0.53	2.54	0.721
	time	—	−0.21	0.04	−5.33	<0.001

significance level ($\alpha = 0.05$)

in symphyseal flexion and asymmetrical jaw protrusion, respectively. Feeding bouts on insect prey were characterized by a greater significant (mean $4.5^\circ \pm 1.06$ s.e.; $p < 0.0001$) asymmetrical angular deviation from other prey types, decreasing over the extent of the prey-processing event (mean $-0.3^\circ \pm 0.08$ s.e.; $p < 0.0001$). Effect of prey mass on

asymmetrical protrusion was not found to vary significantly with time or prey type ($p = 0.588$). Flexion at the medial jaw symphysis was significantly greater (insects, $3.3^\circ \pm 0.52$ s.e., $p < 0.0001$; shrimp, $1.3^\circ \pm 0.53$ s.e., $p = 0.01$) than during prey processing on other prey, and the amount of flexion decreased over the extent of the prey-processing event (mean

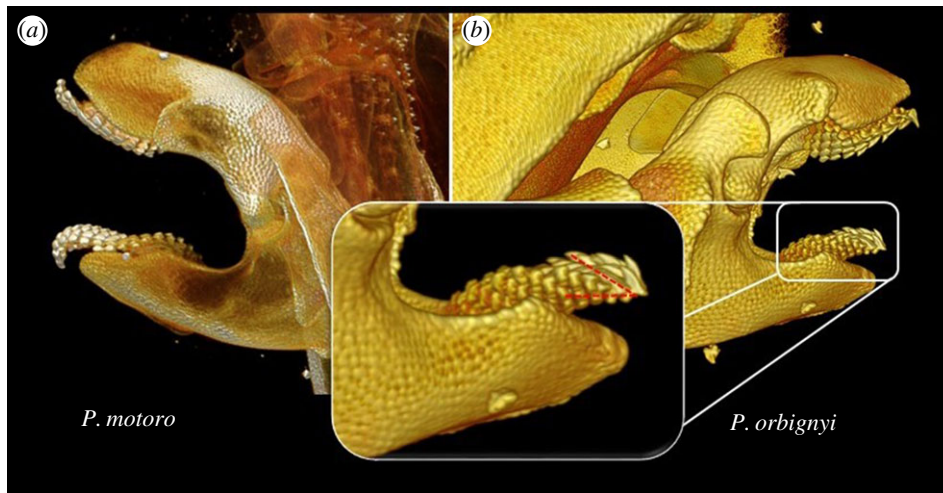


Figure 3. Computed tomography scans of (a) *P. motoro* teeth (the animal analysed in this study, an insect-feeding generalist predator) and (b) *P. orbignyi* (a sympatric, insect-feeding specialist). Jaws were protruded normally (as opposed to asymmetrically), flexing the mandibular symphises. This action pinches the dental ligament, reorienting the teeth in some batoids [14]. (Online version in colour.)

$-0.2^\circ \pm 0.04$ s.e.; $p < 0.0001$). As with asymmetrical protrusion, prey mass was not found to contribute to symphyseal flexion during prey processing ($p = 0.72$).

(b) Prey capture kinematics

Potamotrygon motoro captures prey with a rapid uplift of the anterior disc region, drawing prey beneath the body. Once prey is 'corralled' beneath the disc, subsequent body repositioning manoeuvres prey towards the mouth, assisted by pelvic fin 'punting' [28]. After the disc was sealed against the substrate, prey was either sucked into the mouth by lower jaw depression or gripped by simultaneous lower jaw depression and upper jaw protrusion. Mouth closure occurred quickly (mean = 0.09 s \pm 0.02 s.e.), before jaw protrusion concludes. Once mouth closure occurs, jaws are retracted and then almost immediately (less than 0.01 s) re-extended, opened and closed again in a sequence which marks the beginning of prey processing. Capture kinematics, such as jaw protrusion, disc-lifting and jaw closure, were broadly comparable across prey categories; however, feeding events on larger prey items were associated with more failed capture attempts and involved repositioning of the body over the prey item. In all cases, across all prey types, we observed asymmetric, unilateral jaw motions during capture (electronic supplementary material, table S1).

(c) Tooth reorientation

We did not observe noticeable reorientation in the teeth of *P. motoro* when the jaws were protruded and during symphyseal jaw flexion. However, in the related insectivore *Potamotrygon orbignyi*, we observed noticeable tooth reorientation during jaw protrusion. The average angle of tooth cusp reorientation in *P. orbignyi* varied from 8.5° to 28.1° (mean = 15.8°) from the resting angle (figure 3).

4. Discussion

(a) Do stingrays use mammal-like chewing to process prey?

While several definitions of chewing have been proposed [2,4,29,30], we define chewing as an interaction of upper and lower teeth which both compresses and shears food between

occlusal surfaces during asymmetric motion of the jaws. Although one of the most frequently cited hallmarks of mammalian chewing is translational, opposing motion of the upper versus lower jaws (i.e. the 'power stroke'), some of the most successful mammalian chewers, rodents, lack a translational action of the jaw, relying instead on propalinal, longitudinal shearing instead [4]. When processing all types of prey, *P. motoro* stingrays show clear evidence of asymmetrical, transverse motion of the jaws, owing to loose mandibular symphises and jaw joints, which allow the left and right rami of the jaws to move independently. Videos also appear to show longitudinal translation of the upper versus lower jaws, although confirming these kinematic sequences requires detailed imaging of internal anatomy, perhaps through X-ray videography. The freshwater stingray *P. motoro* uses asymmetric, unilateral movements of the jaws to successfully reduce tough insect cuticle during feeding, suggesting that stingrays and mammals have found a similar kinematic solution for feeding on tough prey. Loose jaw and symphyseal joints coupled with documented unilateral muscle activity [17], allow for independent movement of the upper and lower jaws relative to one another in batoid fishes, a hallmark of mammalian-style chewing kinematics.

Another hallmark of mammal-like chewing is the use of high-crowned tribosphenic molars, which raises the question: does chewing really require complex teeth? Our results indicate that complex, asymmetrical jaw motions enable insect consumption by *Potamotrygon motoro*, despite the homodont dentition of this species. This contrasts with mammals, which rely on similar kinematic behaviours, but also have intricate tooth morphologies [31–33]. However, *P. motoro* is a dietary generalist, and there is some evidence suggesting that larger individuals include insects in their diet, while smaller individuals eat more crustaceans and fishes [22]. We note that the congeneric and sympatric *Potamotrygon orbignyi* includes more insects in its diet than *P. motoro* [34], and can reorient its teeth, making it facultatively heterodont. Thus, it may be that true or obligate insectivores require both complex kinematic jaw function and teeth with complex tooth shapes.

Our finding of chewing behaviour in freshwater stingrays adds this taxon to a list of other non-mammalian animals that use complex jaw kinematics to process complex prey [4]. Gerry *et al.* [17] documented that both sharks and skates use asynchronous muscle motor patterns during prey

processing. Skates in particular use unilateral jaw muscle activation and simple, piercing teeth to effectively ‘pinch’ prey on one side of the jaw, and use hydrodynamic jetting to disassemble these prey. In fact, there are many examples of vertebrates which feed on tough, stiff or other manners of complex prey, which do so with relatively simple teeth and complex jaw kinematics, including tuataras and *Uromastix* lizards. Some herbivorous pleurodiran turtles and tortoises process food with an absence of teeth entirely [4,35–37]. Translational motion alone, we suggest, is sufficient for shearing complex prey apart—while complex tooth morphologies in mammals are a reflection of highly stereotyped jaw motions, which lead to regimented, low-variability mastication cycles [38,39].

The decoupled nature of the cranial skeleton (euhyostyly) in skates and rays allows these fishes to independently position the jaws relative to one another, as well as the cranial skeleton [15–17]. A similar effect of decoupled feeding structures is evident in some teleost fishes, notably black carp and grass carp (Cyprinidae), which use the independent rami of the lower pharyngeal jaws to shear prey against upper pharyngeal dentition, and show complex asymmetrical muscle activation patterns [6,7]. The anatomical traits and behaviours characterizing ‘chewing’ as a prey-processing behaviour are found in a broader context than just mammal (or even amniote) systems. Stingrays have many of the hallmarks of chewing: loose jaw joints, medio-lateral movement of occluding tooth surfaces, asymmetrical jaw and muscle action, and, at least in the insect-feeding specialist *P. orbignyi*, heterodont dentitions. But in the case of large generalist insectivores like *P. motoro*, as well as many reptiles and archosaurs, complex heterodont tooth morphologies are not required to process tough prey.

(b) Why do stingrays chew?

Batoid fishes represent much of the trophic ecological diversity within the elasmobranch fishes, perhaps facilitated by behavioural plasticity of the feeding apparatus [40–42]. Stingrays spend a puzzling amount of time processing prey, during which they are conspicuous to predators and competitors in the wild. Gerry *et al.* [17] documented that both sharks and skates change prey-processing behaviour to suit complex or tough prey, as documented here for *Potamotrygon*. Greater overall jaw kinesis and longer prey-handling times occurred when *Potamotrygon* fed on chitinous prey, markedly greater for insects over shrimp, and for both kinds of chitinous, arthropod prey over fish. The rate of biting during processing remained constant regardless of prey, while asymmetrical action of the jaws and frequency of biting decreased over the duration of the feeding event. This suggests that *P. motoro* uses some stereotypy for feeding on complex prey, biting at comparable rates regardless of prey, but change the manner in which jaw action occludes against prey, as well as how long processing cycles continue. The decreasing extent to which these rays use abrupt, asymmetrical action of the jaws suggests that *Potamotrygon* modifies its behaviour to reflect the progressive dismantling of prey during processing. This mechanical feedback allows predators to respond to nuances of prey material and structural toughness during feeding, another trait in common with mammalian chewers [38]. This ability to modulate prey processing has allowed stingrays to access myriad trophic niches across numerous habitats, faced with novel prey like aquatic insect larvae. This plasticity of feeding behaviour may explain why, despite

entrenched competitors in novel habitats, freshwater rays were successful in making the transition from marine to freshwater environments [43].

(c) ‘Look Ma, no hands!’—decoupling prey capture from prey processing

We determined that *Potamotrygon motoro* uses its appendicular skeleton (its disc) for prey capture and its jaws for prey processing, effectively dissociating the functional anatomy of these two processes. In other fishes, decoupling of prey capture from prey processing is a function of having two sets of jaws, oral and pharyngeal, of which the latter performs the majority of processing [44]. In some fish lineages, decoupling of anatomical modules during feeding behaviour has led to radical changes in the morphology of formerly integrated cranial modules, correlated with increased diversification in these lineages [9]. Using the body as a method of prey restraint or to outright capture prey is prominent in other vertebrates outside fishes (e.g. predatory birds use talons to hold and suffocate prey, bats capture fish from rivers with their hindlimbs, and small and large mammals alike often grasp and rend prey using their forelimbs [45–47]). Although rare in bony fishes, prey capture using the pectoral fins occurs in other batoids, such as guitarfishes and skates [8,15,26,48]. We suggest that using the appendicular skeleton to trap prey is an innovation that was made possible by the evolution of the pectoral fins to encircle the front of the head, forming a flexible, flattened disc, and has evolved at least twice, independently in modern stingrays (Myliobatiformes) and skates (Rajiformes) [41]. In turn, this innovation may have facilitated the evolution of extreme jaw kinesis and chewing behaviour in these stingrays. We suggest that asymmetrical jaw kinematics are a frequent motif of stingray and, more generally, batoid feeding—a function of their decoupled, loose jaws and cranial skeletal architecture. These properties establish *Potamotrygon* as a model system for understanding evolutionary convergence of prey processing and chewing in vertebrates.

Data accessibility. All primary datasets regarding prey processing have been uploaded to Dryad digital database, available at <http://dx.doi.org/10.5061/dryad.1bt27> [49].

Authors’ contributions. M.A.K. carried out videography filming trials, under supervision of K.C.W. M.A.K. headed data analysis, with input from K.C.W. M.A.K. and A.P.S. scanned and rendered computed tomography scans. M.A.K. drafted the manuscript, with equal contributions by K.C.W., A.P.S. and N.R.L. All authors gave final approval for publication.

Competing interests. We declare we have no competing interests.

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