

Research



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Rhythmic chew cycles with distinct fast and slow phases are ancestral to gnathostomes

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Intra-oral food processing, including chewing, is important for safe swallowing and efficient nutrient assimilation across tetrapods. Gape cycles in tetrapod chewing consist of four phases (fast open and -close, and slow open and -close), with processing mainly occurring during slow close. Basal aquatic-feeding vertebrates also process food intraorally, but whether their chew cycles are partitioned into distinct phases, and how rhythmic their chewing is, remains unknown. Here, we show that chew cycles from sharks to salamanders are as rhythmic as those of mammals, and consist of at least three, and often four phases, with phase distinction occasionally lacking during jaw opening. In fishes and aquatic-feeding salamanders, fast open has the most variable duration, more closely resembling mammals than basal amniotes (lepidosaurs). Across ontogenetically or behaviourally mediated terrestrialization, salamanders show a distinct pattern of the second closing phase (near-contact) being faster than the first, with no clear pattern in partitioning of variability across phases. Our results suggest that distinct fast and slow chew cycle phases are ancestral for jawed vertebrates, followed by a complicated evolutionary history of cycle phase durations and jaw velocities across fishes, basal tetrapods and mammals. These results raise new questions about the mechanical and sensorimotor underpinnings of vertebrate food processing.

This article is part of the theme issue ‘Food processing and nutritional assimilation in animals’.

1. Introduction

Intra-oral food processing using repeated mandibular motion cycles occurs in jaw-bearing vertebrates (Gnathostomata) from fishes to mammals [1,2], and is key to bolus reduction [3,4] and nutrient assimilation in the gastrointestinal tract [5–7]. Rhythmic chewing—repetitive gape cycles with a duration coefficient of variation (CV) < 25% [1,2]—is thought to optimize energetic efficiency by minimizing disruptions to the natural oscillating frequency of repeatedly elevating and depressing the mandible. Mandibles are typically long and heavy structures [8] and disruptions to the natural oscillation frequency of heavy or rapidly moving structures can incur substantial inertial penalties [9,10]. Repeated (cyclic) power-strokes require energy expenditure for the cyclic muscle contractions that move the mandible and other dentition-lined intra-oral surfaces, and to generate the bite force used for food fracture [11]. Moreover, while chewing promotes bolus control and safe swallowing [12], it also inflicts wear [13–15] and is associated with a risk of dentition fracture or loss [16,17], which is especially problematic

in mammals because they perform a lot of intra-oral food processing and only have one set of molar teeth. Taken together, these factors suggest that, while chewing may expand the range of foods that can be processed to yield nutrition and the efficiency with which this happens, it is not necessarily beneficial for all animals to chew.

Cyclic chewing occurs in disparate clades across the gnathostome phylogeny, including aquatic fishes [1] and terrestrial mammals [8], but also in some lepidosaurs, including *Sphenodon* [18–21] (electronic supplementary material, videos S1–S3). However, most lepidosaurs that chew do so less rhythmically than mammals and fishes [8]. The apparent phylogenetically fragmented distribution of rhythmic chewing prompts several questions. We first ask: (I) Is rhythmic chewing ancestral for gnathostomes? Our second question is: (II) How rhythmic is chewing in amphibians?

Chewing with rhythmic mandible movements has been hypothesized to increase kinematic and kinetic predictability, facilitating motor control and thereby decreasing tooth wear and risk of tooth fracture. Predictability may be generally advantageous in many feeding contexts [22], but may be less important in the aquatic realm, where incompressible fluid inside the oral cavity can buffer against excessively fast jaw closing. However, drag of a viscous fluid may simultaneously increase the risk of food escape [1] and reduce the ability to oscillate the mandible close to its natural frequency, whereas lubrication of the intra-oral cavity by water may suppress friction-based impacts on rhythmicity [23]. The challenges associated with chewing in water beg the question of whether and in what way aquatic chewing resembles terrestrial chewing? As previously suggested [1], we hypothesize that (i) fishes and salamanders lack a phase transition between fast- and slow close—as well as between fast- and slow open—to avoid food escape while the mouth gape is expanded, with the alternative hypothesis that their jaw opening and closing are partitioned into distinct slow and fast phases. Our third question is: (III) How do aquatic-to-terrestrial transitions impact the phase structure and rhythmicity of chew cycles? (ii) Our second hypothesis is that in salamanders the phases of chew cycles during aquatic feeding are retained post-terrestrialization. Here, we make inroads on these questions by studying chewing in four salamander species across ontogenetically and behaviourally mediated terrestrialization.

Mammals have been hypothesized to maintain rhythmic chewing by trading off the durations of gape cycle phases [2,12]. High rhythmicity has also been hypothesized to protect the precisely occluding dentition in mammals during high-force application [24]. Primates have also been hypothesized to maintain rhythmicity by using rate-modulation (i.e. speed-based control) of force application during the slow close phase or ‘power-stroke’, aided by anticipatory—feed-forward control—mediated by γ -motoneurons to muscle spindles [8,25–28]. By contrast, lepidosaurs lack γ -motoneurons and hence lack any feed-forward control of muscle spindle sensitivity, and they accumulate phase variation in slow open [2]. Available evidence suggests that basal aquatic-feeding amniotes lack jaw muscle spindles ([29], but see [30]), hence our fourth question is: (IV) How is variation in jaw movement duration and velocity distributed across chew cycle phases?

To address our four questions and test our two hypotheses, we analyse the CV of jaw cycle duration, and use $CV < 25\%$ as the threshold for rhythmic chewing (a cut-off informed by

comparative data from Gintof *et al.* [1] and Ross *et al.* [8], as advocated by Wainwright *et al.* [31]). We also analyse jaw velocity in statistically distinguishable phases of chew cycles to determine if basal vertebrates maintain distinct chew cycle phases, with potential implications for energy conservation and feeding safety.

2. Material and methods

Data collection complied with institutional animal care and use protocols and federal assurances (see Ethics section). Information about our sample for each species is listed in electronic supplementary material, table S1. All data used in this study were collected as part of other studies. However, our taxon sampling sought to achieve a balanced representation of the major aquatic-feeding lineages of sarcopterygians, whilst by necessity excluding some important taxa (*Neoceratodus*, *Andrias*, *Cryptobranchus*) due to their rarity or protection status.

(a) Experiment preparations

We collected jaw kinematics data on intra-oral feeding behaviours using biplanar videofluoroscopy [32] for *Potamotrygon motoro* (ocellate river stingray) [33], *Polypterus bichir* (Bichir), *Protopterus annectens* (African lungfish), *Necturus maculosus* (mudpuppy), *Amphiuma means* (two-toed Amphiuma), *Plethodon glutinosus* (Northern slimy salamander), *Siren intermedia nettingi* (Western lesser siren) [34], *Ambystoma tigrinum* (tiger salamander), *Ambystoma mexicanum* (Axolotl) [35,36], *Triturus carnifex* (Italian crested newt) [37,38] and *Ichthyosaura alpestris* (alpine newt) [39]. Sonomicrometry was used to measure jaw movements in *Chiloscyllium plagiosum* (bamboo shark) [40].

In preparation for biplanar videofluoroscopy, animals were induced into a deep plane of anaesthesia (by immersion in either Benzocaine 1.5–2.5 g l⁻¹ or phosphate-buffered MS222 0.05%) and radio-opaque, tantalum markers (0.5–1.0 mm diameter; Ball-Tec, LA CA. Abbott-balls, West Hartford, CT) were implanted. For this study, we focused on data from a pair of markers, one implanted in the premaxilla (upper jaw) and another in the anterior mandible (Meckel’s cartilage in the rays). All subjects also had other markers implanted for other studies. Skin incisions required for implants were either less than 10 mm long and healed without assistance in 5–6 days or were closed with a single stitch of self-absorbable suture. After marker implantation, subjects were monitored during recovery and fasted for 2–3 days to avoid mechanical influences of chewing that could dislodge implants from bones. An X-ray sequence was recorded to ensure successful marker implantation.

Bamboo sharks were fasted for 2–3 days prior to all procedures. Each was anaesthetized by immersion in 0.1 g l⁻¹ solution of MS-222 in 22–23°C seawater, then moved to a custom temperature-controlled and aerated intubation system containing a diluted solution (0.05 g l⁻¹ MS-222) for surgery. Implants consisted of two 2 mm piezoelectric crystals with suture loops (Sonometrics Corp) positioned on the sagittal plane. Both were sutured immediately lingual to the tooth-pads, one on the palatoquadrate and one on Meckel’s cartilage (upper and lower jaw, respectively). Wires leads were routed through the fifth gill slit and tethered to a suture loop on the animal’s dorsum so they could be connected to the sonomicrometer during experiments. Sharks were allowed to recover for 3–4 h after the implantation procedure before feeding experiments began.

(b) Feeding experiments

Animals with tantalum implants were imaged during feeding in one of the biplanar X-ray facilities at Harvard University

(Concord Field Station), Brown University (W. M. Keck XROMM facility) or University of Jena. Animals were first habituated over several days to testing tanks (910 × 70 × 150 mm $L \times W \times H$) made from 7 mm thick acrylic, which is sufficiently radio-lucent for fluoroscopy imaging. The tanks were designed to fit within the visualization volume of two fluoroscopes that were arranged near-orthogonally.

During experiments, subjects were presented with food that they readily consumed (electronic supplementary material, table S1) but did not vary substantially in hardness and mobility, which could influence feeding kinematics. Elasmobranchs were fed squid pieces, salamandrids were fed maggots and all other species were fed crickets. Food items were carefully size-matched to the gape-width of the subject and worms occasionally had to be cut to avoid food-size effects on kinematics. During experiments, food items were implanted with one or more radio-opaque markers so that intra-oral food movements could be visualized. Rostrocaudal food movement during a cycle was used to distinguish transport (movement > 0.25 s.d. of the mean movement for all cycles) from processing cycles (less than 0.25 s.d.; see details in [41]), with only the latter being analysed here.

Food was presented via plastic forceps and post-capture feeding behaviours were imaged using X-ray techniques ranging from 75 to 85 kVp and 2.5 to 3.0 mA; camera frame rates ranged from 125 to 500 Hz (see electronic supplementary material, table S1). Videos were captured onto PC hard drive via proprietary software from the high-speed video camera manufacturers (Photron; FastCam Viewer v.1.3.40. Photron, Tokyo JP. Phantom; Visart v.5.0, High Speed Vision GmbH, 76275 Ettlingen, Germany). For bamboo sharks, sonomicrometry data were captured at frequencies of 377–415 Hz onto a PC hard drive via the SonoView package (Sonometrics).

After experiments, subjects were euthanized by immersion in an overdose of the anaesthetic agent used for surgeries (see above) and frozen for dissections and morphological measurements.

(c) Extraction of chew cycle data

We limited our analyses to trains of more than two cycles of rhythmic jaw movement following the prey-capture strike. For stingrays, we excluded all shearing overbite cycles and the single recovery cycle immediately following overbite events [33]. For lungfish (*Protopterus*) and mudpuppies (*Necturus*), we excluded the suction cycles that occur between trains of rhythmic processing [42]. An overview of the total chew cycle sample available for analysis from each species is shown in electronic supplementary material, table S2.

(d) Data processing

For each selected trial, the two camera views were un-distorted and spatially calibrated via direct linear transformation, and the upper and lower jaw markers were 3D-tracked in XMALab (v.1.5.5.) [43]. Jaw kinematics were exported as CSV files containing the time-varying inter-marker distances between upper and lower jaw markers. For *P. motoro*, jaw cycles were extracted using a joint-coordinate system for the quadrate-articular jaw joint in the XROMM workflow [33]. All time-varying gape data were imported into IgorPRO (v.8.1; Wavemetrics), scaled to the sample (video or sonomicrometer) frequency for the experiment and filtered using FIR interpolation to the extent that differentiation (see below) would not result in spurious fluctuations in the resulting time-varying velocity profiles.

(e) Analyses of chew cycle duration

To address our first two questions—is rhythmic chewing ancestral for gnathostomes and present in amphibians?—we first identified trains of chew cycles, with a train containing two or

more uninterrupted cycles. We then extracted the duration from max gape to max gape (in milliseconds) [1,8] and calculated the CV (%) as

$$CV = \left(1 + \frac{1}{4} (N) \times \frac{\sigma}{\mu} \right) \times 100,$$

where N is the sample size from that taxon (number of cycles) and σ is the standard deviation around μ , the mean of the variable. To facilitate comparisons with data from previous studies and to correct for the very small sample sizes in some species [44] we followed established protocols of calculating sample-size corrected CV [1,2].

(f) Analyses of chew phase duration and velocity

We used time-varying velocity to objectively identify the inflection points on the gape distance or angle that marked transitions between the four gape cycle phases [45]. We were able to objectively identify all three inflection points (fast close (FC)–slow close (SC), SC–slow open (SO) [=minimum gape], SO–fast open (FO)) in all gape cycles and used them for further analyses (table 1). Using these inflection points, we extracted the duration of each cycle phase (in milliseconds; figure 1). The speed-based (fast and slow) designations in the original analysis of gape opening and closing cycle phases [12], coupled with subsequent ambiguity in the literature about the relative speed of the four different phases [46,47] motivated us to extract mean velocity for each phase for statistical comparisons. Phase duration and jaw velocity are theoretically at least partially independent and related to functionally distinct aspects of food processing [24].

(g) Hypothesis-testing and statistical design

To address our third and fourth questions, we sought to determine the numbers of phases in the gape cycles of each species by comparing jaw movement velocities. Jaw closing velocity was compared across fast- and slow close phases, and jaw opening velocity was compared across slow- and fast open phases. Analysis of mean phase velocities across cycles for a given species involved general linear models (Systat v.12.0) that factored phase velocity as the response variable, phase type (fast or slow) as the independent effect and subject as a random factor, to account for behavioural differences between individuals (i.e. where individual $N > 1$). In our figures, CV data were plotted as mean ± s.e.m. (figure 2) and phase durations and speeds were plotted as median-quartile boxes, with whiskers indicating data-ranges and dot-density distributions of all cycle data (figure 3).

3. Results

(a) Mandibular kinematics and cycle rhythmicity

Intra-oral food processing is widespread among basal gnathostome vertebrates, with trains of cyclic chewing occurring across all species studied here. There is considerable variability in the number of chews used during a given feeding event. Because X-ray videos by necessity are briefer than traditional high-speed video, due to risks of cathode overheating, we do not have entire feeding sequences for most species. However, we observed cyclic movements of the mandible to occur in trains incorporating only three to six cycles in the bamboo shark *C. plagiosum* and the salamander *A. means*, whereas the lungfish *P. annectens* and the stingray *P. motoro* use trains of 60–80+ cycles.

Our data show that the 12 basal gnathostome species in our sample, with very few exceptions (*Amphiuma* and Axolotls), chew with jaw movements that are rhythmic (CV < 25%)

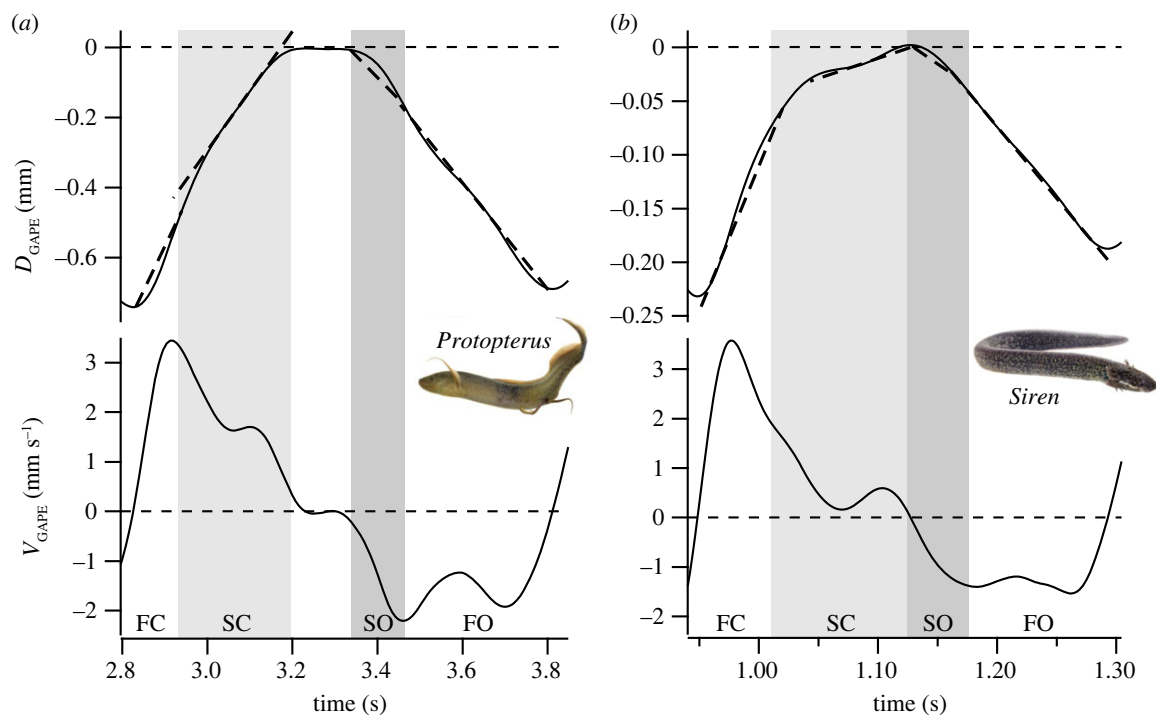


Figure 1. Velocity-based extraction of chew cycle variables, illustrated for a lungfish (*a*) and a salamander (*b*). Thin solid lines are filtered time-varying gape cycles, with thick dashed lines indicating line-fits, the slopes of which were used to determine mean velocity for each phase. Fits were taken between inflection points on the time-varying velocity profile (V_{GAPE} , bottom), as obtained through differentiation of the time-varying gape distance profile (D_{GAPE} , top). White and grey columns indicate FC, fast close; SC, slow close; SO, slow open; FO, fast open.

Table 1. Statistical test results of differences in velocities between fast and slow close and open phases. FC–SC (GLM on mean velocities for fast close versus slow close); SO–FO (GLM on slow open versus fast open mean velocities). AQ, aquatic; TE, terrestrial.

species	fluid	FC–SC (GLM)	SO–FO (GLM)	no. phases
<i>Chiloscyllium plagiosum</i>	AQ	0.0001 ^a	0.01 ^a	4
<i>Potamotrygon motoro</i>	AQ	0.0001 ^a	0.36	3
<i>Polypterus bichir</i>	AQ	0.064	0.01	3
<i>Protopterus annectens</i>	AQ	0.001	0.001	4
<i>Necturus maculosus</i>	AQ	0.001	0.14	3
<i>Amphiuma means</i>	AQ	0.49	0.18	2
<i>Amphiuma means</i>	TE	0.34	0.12	2
<i>Plethodon glutinosus</i>	TE	0.15	0.56	2
<i>Siren intermedia nettingi</i>	AQ	0.0001 ^a	0.0001 ^a	4
<i>Ambystoma tigrinum</i>	AQ	0.0001 ^a	0.0001 ^a	4
<i>Ambystoma tigrinum</i>	TE	0.0001	0.0001	4
<i>Ambystoma mexicanum</i>	AQ	0.0001	0.0001	4
<i>Ambystoma mexicanum</i>	TE	0.65	0.08	2
<i>Triturus carnifex</i>	AQ	0.67	0.01 ^a	3
<i>Triturus carnifex</i>	TE	0.22	0.0001 ^a	3
<i>Ichthyosaura alpestris</i>	AQ	0.01	0.001 ^a	4

^aStatistically significant individual effect.

(figure 2; electronic supplementary material, videos). Chew cycle CVs for these taxa (figure 2*a*) are similar to those of fishes and mammals, and lower than those reported for lepidosaurs (figure 2*b*) [1,2]. Species-level CVs range from $11.6 \pm 2.0\%$ in *Siren* to over +40% in *Amphiuma* and terrestrial ambystomatids. Grand average CVs (\pm s.e.m.) are $21.2 \pm 3.2\%$ for fishes (shark, ray, bichir and lungfish), $20.2 \pm 2.1\%$ for aquatic salamanders and $36.3 \pm 4.5\%$ for terrestrial salamanders.

Terrestrialization results in elevated jaw movement CV (i.e. chewing being less rhythmic on land) for *Amphiuma*, and the ambystomatids but notably not for the newt *T. carnifex*.

(b) Chew cycle phase durations

Phase durations vary considerably through chew cycles and across species (figure 3*a*). In chondrichthyans the

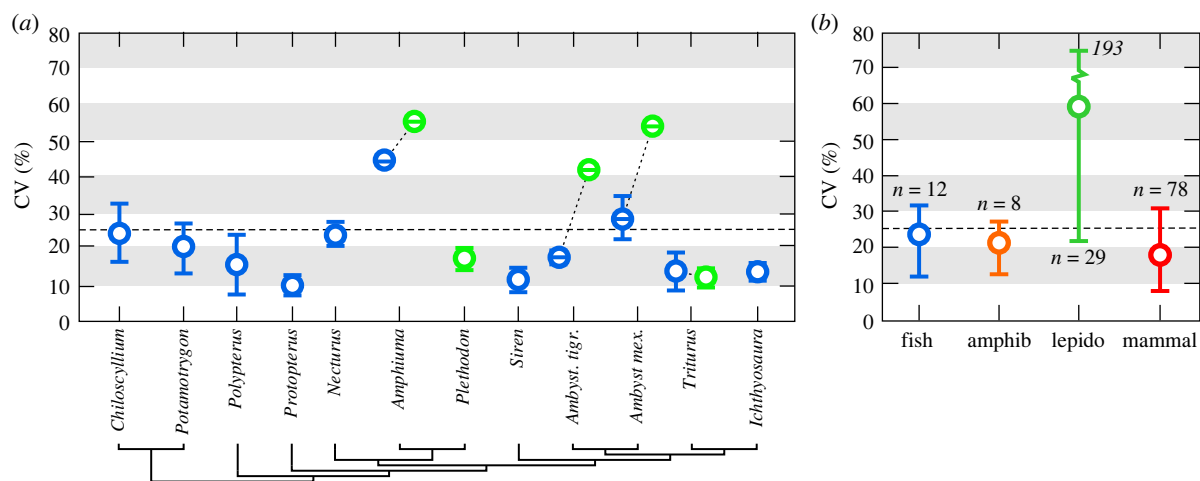


Figure 2. Anamniotes are rhythmic chewers. (a) Species-level CV's (mean \pm s.e.m.) for chew cycle durations collected from 12 species of basal aquatic-feeding anamniotes (mapped onto phylogeny pruned from [48]); chondrichthyans (*Chiloscyllium* and *Potamotrygon*), a basal actinopterygian (*Polypterus*), a basal sarcopterygian (*Protopterus*) and eight salamander species. Blue symbols = aquatic feeding, green symbols = terrestrial feeding. The dashed line indicates the nominal cut-off of rhythmic chewing of CV < 25%. (b) Grand average chewing CVs (mean \pm s.e.m.) for mandibular jaw cycles, incorporating data from [1,2] for fishes ($N = 12$ spp.), salamanders ($N = 8$ spp.), lepidosaurs ($N = 29$ spp.) and mammals ($N = 78$ spp.). Note the interrupted upper whisker for the lepidosaur data.

fastest phases tend to have shorter durations than the slow phases. In *Polypterus* and among most salamanders, slow open is the longest and most variable phase. Across terrestrialization (in ambystomatids, newts and *Amphiuma*), terrestrialized tiger salamanders retain the phase duration pattern of their larval morphs, with slow-open having a longer and more variable duration. In the chemically metamorphosed Axolotl [49] fast open lasts longer and has a more variable duration than the other phases. In *Amphiuma* and the multiphasic newt *Triturus* there are no distinguishable changes in phase duration in response to terrestrialization.

(c) Chew cycle phase velocities and variance

The gape velocities of phases often follow the four phase names: fast close is the fastest, and also has the most variable velocity (figure 3b). In the chondrichthyans, there are distinct fast and slow close phases, and slow and fast open phases, with statistically significantly different velocities (figure 3 and table 1). In basal fishes and aquatic salamanders, there are usually four phases, but slow and fast open phase velocities are not always distinct. In some, but not all terrestrialized salamanders, a break-down of the fast-to-slow transitions results in a biphasic (close-open) chew cycle pattern.

4. Discussion

Our analyses show that cyclic intra-oral food processing—chewing—is widespread among gnathostome vertebrates. We previously documented trains of intra-oral food processing cycles in several species of teleost fish [1], and here we complement that prior analysis with data from sharks, rays, bichirs, lungfish and salamanders. Analyses of chew cycle duration CV answered our first question by revealing that rhythmic jaw movements during chewing occur across basal aquatic-feeding anamniotes, in at least some of the most ancestral extant species of chondrichthyans (bamboo sharks and stingrays), in the basal-most extant actinopterygian (bichirs) and in basal sarcopterygians (lungfish). These analyses also answered our second question by showing

that extant salamanders (Caudata) are capable of chewing rhythmically, similar to ancestral (fish) and derived (amniote) taxa. In combination with previously published results for fishes, lepidosaurs and mammals [1,2], our data demonstrate that rhythmic chewing (chew cycle duration CV < 25%) is the ancestral condition for gnathostome vertebrates.

Our velocity-based approach was able to statistically partition chew cycles into their constituent phases of fast close, slow close, fast open and slow open [12]. This analysis rejected our first and previous working hypothesis [1], that fishes and salamanders would lack fast-to-slow close and slow-to-fast open phase transitions, instead demonstrating that transitions between fast and slow jaw movements are not exclusive to mammals. In fact, our data suggest that the fast-to-slow transition in jaw closing may be ancestral to gnathostome vertebrates, whereas a slow-to-fast transition in jaw opening may have been established in aquatic-feeding anamniotes, prior to the origin of Tetrapoda.

(a) Evolution of chewing rhythmicity across Gnathostomata

Our rhythmicity data (figure 2) show that rhythmic chewing (cycle duration CV < 25%) is widespread across basal, aquatic-feeding anamniotes (chondrichthyans, sarcopterygians and salamanders). Indeed, chewing in many of these taxa is highly rhythmic (CV \leq 20%). Although more extensive sampling of chondrichthyans (sharks and rays) and basal sarcopterygians (lungfishes) is clearly needed, the data presented here, in combination with those from bony fishes [1] and amniotes [8], suggest that rhythmic chewing is the ancestral condition for gnathostomes.

As groups, fishes and salamanders chew rhythmically ($23.7 \pm 2.1\%$ and $21.2 \pm 3.2\%$, respectively), even compared to mammals (23.05 ± 12.19 ; $n = 78$ [8]). A recent analysis of chew and locomotion cycle CVs for six salamander species reported a significantly higher chewing grand average ($50.44 \pm 33.12\%$) [50] than our data for the same six species ($18.55 \pm 2.53\%$), a difference that may be due to small sample sizes in that study. In that context, it is important to note that CV can be significantly affected by small sample sizes [44], and

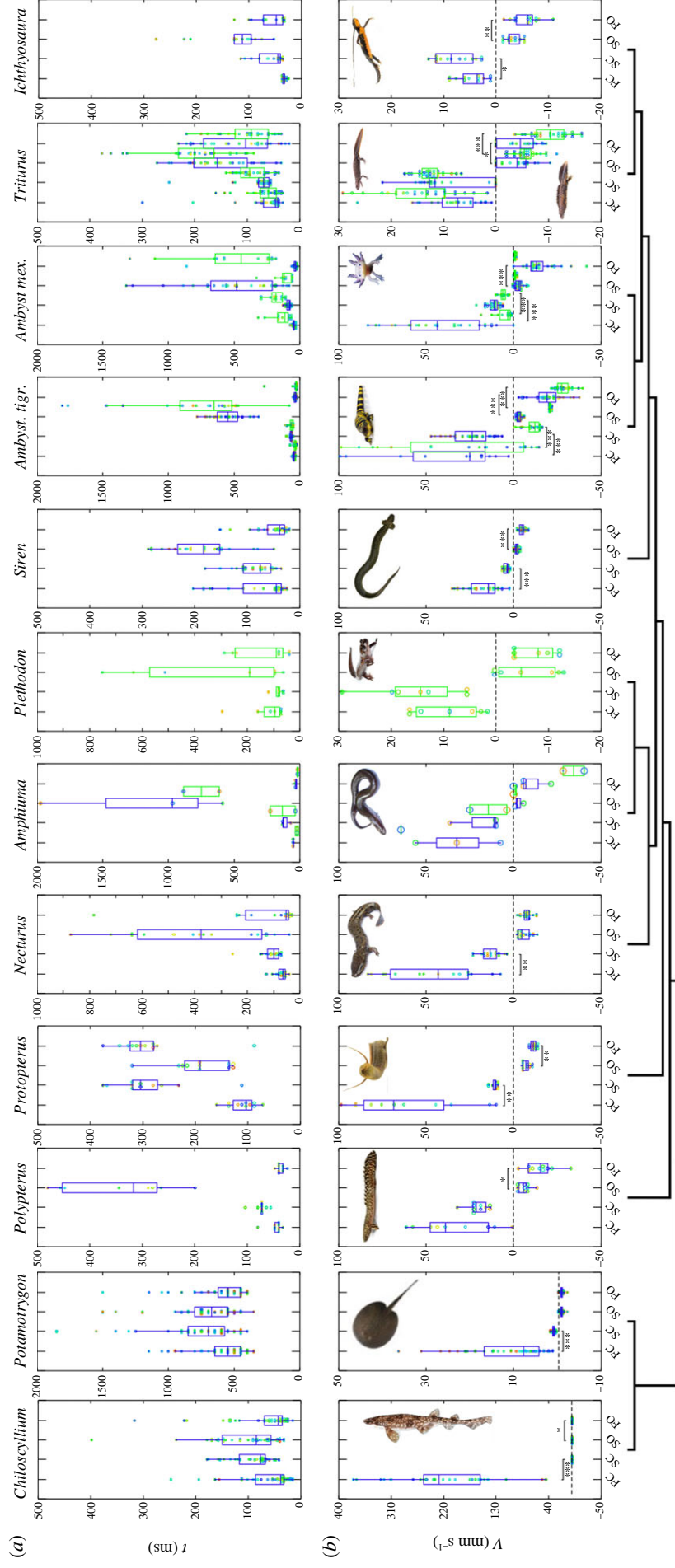


Figure 3. Evolution of chew phases across amniotes. Top row shows duration (ms) and bottom row mean velocities (mm s^{-1} , with negative velocities indicating gape opening) for the four phases of a chew cycle (x-axes). Data are median-quartile boxes with range-whiskers and dot-density distributions of data from individual chew cycles, with the colour of each dot corresponding to an individual. Results from GLM comparisons of fast-to-slow transitions in dosing and opening velocities: * $p = 0.01$, ** $p = 0.001$, *** $p = 0.0001$. Note that the longest and most variable phase duration is usually SO, whereas the fastest but also most variable velocities are seen during FC. Also note the different y-axis ranges across taxa, and that the thumbnails of each study taxa are not shown to scale. FC, fast close; SC, slow close; SO, slow open; FO, fast open.

consequently, our data for *Amphiuma* and terrestrialized ambystomatids (all $n = 1$) should be considered with caution. Using our results, the analysis of Faltings *et al.* [50] would suggest that chewing and locomotion are equally rhythmic ($22.20 \pm 5.66\%$) in those six amphibian species.

Our rhythmicity results for basal aquatic-feeding amniotes raise new questions about how the rhythmicity of gnathostome jaw systems is controlled. Our data show that chewing can be rhythmic in the absence of the sensorimotor traits hypothesized to facilitate rhythmic chewing in mammals (muscle spindles, as well as γ -motoneurons and periodontal afferents that engender feed-forward control) [8,25–28]. Earlier studies have also shown that some lepidosaurs, including the basal *Sphenodon*, chew highly rhythmically [18–20], presumably only with sensorimotor aid from α - and β -motoneurons [8]. Moreover, fishes that chew rhythmically lack the sensorimotor traits that are thought to govern chewing rhythmicity [29,51], so how do basal gnathostomes chew so rhythmically?

Rhythmic chewing may be a relatively unmodulated manifestation of central-pattern-generation [52], and possibly a carry-over from the ancestral pulsing constrictions of the branchial apparatus [53], which according to the serial homology hypothesis [54] gave rise to vertebrate mandibular and hyoid arch elements [53]. The location of the ventilatory pattern generator in lampreys, adjacent to the trigeminal motor nucleus responsible for innervating jaw muscles across gnathostomes, provides neuroanatomical support for the serial homology hypothesis [55].

In bony fishes (Actinopterygii), there are up to three separate jaw systems, associated with the mandibular-arch, the basihyal (tongue) and buccal cavity roof [56], and/or the pharyngeal arches [57,58]. These three systems provide alternative sites for intra-oral food processing, but the CV of their cycle kinematics is generally low (less than 20%) [57–59]. Consequently, it is reasonable to speculate that chewing with *arrhythmic* cycles (i.e. $CV > 30\%$) may have evolved once as a derived condition in lepidosaurs (figure 2b). Among lepidosaurs, gape cycle duration is heavily influenced by the duration of slow open, which is not the case in mammals. During slow open, the tongue engages the food bolus in the oral cavity in preparation for its repositioning during fast open. Why might lepidosaurs have more variable slow open phases than mammals? Our data suggest that a highly variable slow open duration is ancestral to gnathostomes, but this is not itself an explanation for its retention in lepidosaurs. Among lepidosaurs, squamates often feed on large prey that are rarely processed intraorally and sometimes transported directly to the oesophagus for swallowing via *inertial* (throw-and-catch) food handling behaviours [60,61]. This feeding style has been argued to be associated with altered tongue morphology, which might compromise the ability of some lizards to effectively move food items intraorally during chewing [60,62]. Lepidosaurs also use their tongues for chemoreception, possibly enforcing a compromise in tongue function between sensation of odorants and intra-oral bolus manipulation [63,64]. Further work on tongue–jaw coordination in lizards is clearly needed.

(b) Evolution of chew cycle phases

In mammals, the chew cycle is partitioned into four phases: fast close, slow close, slow open and fast open [12,65]. Our data from chondrichthyans (bamboo sharks and stingrays)

suggest that both fast close and slow close phases are ancestral to gnathostomes (figure 3). Our analysis also suggests that both slow open and fast open phases, as seen in the bamboo shark, the bichir and the lungfish (figure 3), predate the origin of tetrapods.

Among tetrapods, our analysis of chew cycle phases in two salamander clades (figure 3) established differences in the extent to which chew cycles were partitioned into phases: one clade (*Necturus*, *Amphiuma*, *Plethodon*) often lacked fast-to-slow transitions (although *Necturus* retains a fast-to-slow close transition), whereas the other clade (*Siren*, ambystomatids and salamandrid newts) usually showed four phases (figure 3). The basis for this difference between salamander clades is unknown but worthy of further study as it may be related to aquatic versus terrestrial lifestyles (see below). The fast-to-slow close transition may simply owe to mechanical interactions between the teeth and the food coming into occlusion, and the slow-to-fast open transition may owe to adhesive or drag forces between the food, jaws and surrounding fluid [23]. The mere *existence* of chew cycle phases, however, is ancient and, if the slow-to-fast open transition in mammals is triggered by sensory feedback driving lingual manipulation of the intra-oral bolus, then the functional underpinnings of this transition in mammals are not the same as those underlying it at its origin.

(c) Variation in chew cycle phases durations and speeds

In order to discriminate phases within a given chew cycle, we took two approaches. The first approach simply evaluated phase durations, as was done previously for lepidosaurs and primates [2]. Across our sample, the duration of slow open was generally the longest and most variable for aquatic chewing (figure 3). This finding runs counter to the hypothesis that aquatic-feeding species would typically lack slow open due to the inherent risks of food escape associated with maintaining jaw gape in a viscous dense fluid [1]. The slow open phase is precarious in the aquatic environment, is a precarious phase in the aquatic environment, where both food and fluid forces may be acting to slow down mandible movements, potentially explaining why its duration is both prolonged and variable. Whereas prolonged and variable slow open phases also are seen in lepidosaurs [2], mammals (with primarily primates analysed to date) either partition variability in chew phase duration across both fast open and fast close (primates) [2] or in the case of flying foxes, into slow close [66]. In the case of bats, this result may be a secondary effect of hanging inverted whilst chewing.

Our second approach to discriminating chew cycle phases drew inspiration from the velocity-based approach to determining chew phase durations in an earlier study [2]. Whereas that study only used time-varying velocity to objectively identify inflection points in the time-varying gape kinematics that delineated boundaries between cycle phases, we chose to extract mean velocities for each phase for statistical comparisons. These data provide a functionally relevant metric that (for jaw closing) may correspond better than phase duration to the rate of force application by jaw muscles on the food via the mandible [24]. We also considered evidence suggesting that the relative velocity of the four phases may not correspond to their nominal labels, with for instance ‘fast open’ in some mammals being slower than ‘slow open’ [47,67].

Our velocity-based analyses provide new insight into partitioning of within-cycle variation across phases: for nearly all species analysed, fast close was indeed the fastest phase, but also the phase into which the most variability in velocity was partitioned across cycles. Again, this result likely reflects challenges associated with fast jaw closing in a dense viscous fluid, coupled with mechanical interactions between the jaw and the food upon occlusion.

Reliable analyses of phase velocity require precise and accurate data, such as those obtained from XROMM and sonomicrometry. In the many studies where gape cycles have been imaged using standard or infrared light, the results may have been impacted by soft-tissue artefacts [68] and our derivative approach may yield spurious results for visual light-based motion capture data. Fortunately, X-ray-based data on gape kinematics are rapidly becoming available from a swath of lepidosaurs and mammals (e.g. [69–71]), which will facilitate future velocity-based analyses of chew cycle partitioning into phases.

One related shortcoming of our study is that we did not extract six degree-of-freedom mandible movement from XROMM models (except for *P. motoro*) and instead relied on a fluoromicrometry-based approach [32] to extract the vertical (arcuate) component of jaw movement. Thus, our gape analyses ignored transverse and propalinal jaw movements, as well as hemi-mandibular roll [69,72], which were recently demonstrated for *Siren* chewing [34]. However, the contribution of mandible roll to the vertical gape distance is likely small and we are confident that this methodological limitation does not impact the general take-homes of our study.

(d) Terrestrialization-related changes to chew cycles

We analysed data from eight salamander species, systematically sampled from two clades of their phylogeny to provide repeated examples of terrestrializing taxa. One clade contains the obligately aquatic *Necturus* as a basal sister to a clade containing *Amphiuma* and plethodontid (lungless) salamanders. *Amphiuma* is known to venture on land [73], and adult plethodontids (as studied here) are highly terrestrial [74]. The other clade contains *Siren*, which also ventures on land [73], and is a basal sister to the ambystomatid ‘mole’ salamanders that often, but not always, terrestrialize [49], and salamandrid newts with a multiphasic lifestyle that involves seasonal switches between aquatic (summer) and terrestrial (winter) stages [37,38,75,76]. We did not study terrestrialized *Ichthyosaura* nor did we coerce *Siren* onto land, but we generated data for four independent comparisons of chew cycles between aquatic stage salamanders and their terrestrialized conspecifics. These data allowed us to address our third question: how do aquatic-to-terrestrial transitions impact the phase structure and rhythmicity of chew cycles?

Our second hypothesis, that the chew cycle phases of aquatic salamanders are retained post-terrestrialization, was only partially supported by our data. Our CV data revealed a near-doubling in cycle variability as ambystomatids and *Amphiuma* chewed terrestrially, compared to aquatically, a change that was notably absent for the multiphasic newt *Triturus*. Similar pronounced taxon-specific differences were seen in the effect of terrestrialization on the phase pattern. Adult tiger salamanders retained the larval phase duration pattern but significant redistribution in phase speeds was observed. In the Axolotl, sister taxon to the tiger salamander,

chemically induced metamorphosis led to an extended and more variable fast open phase as opposed to slow open in most other taxa, and we also observed a complete breakdown of the chew cycle phase pattern, resulting in a biphasic (open–close) chew cycle, similar to terrestrialized newts and *Plethodon*.

The breakdown of the four-phase gape cycle with terrestrialization, resulting in a biphasic (open–close) chew cycle, is interesting as it may signal complications in maintaining jaw kinematics associated with this pivotal transition in vertebrate evolution. There are many potential factors explaining the observed changes in chew cycle rhythmicity, duration and phasing, including anatomical, physiological and mechanical ones. Future work should model the changes in food and jaw (reaction) forces to determine how chewing is affected by the collapse of the oral volume that results from replacing its filling of water with air [77,78], and how changes to lubrication (by water versus saliva) of the oral space affect chewing [23]. There is clearly a need to better understand the basis for adjustments to coordination of jaw and tongue kinematics between aquatic and terrestrial food processing. Multiphasic newts experience these changes seasonally, yet anatomical analyses of their feeding system have only revealed subtle changes in oral morphology associated with these habitat switches [37,38,76]. However, the pattern of food processing in newts changes from chewing in the larval form to a ‘tongue-palate rasping’ strategy during metamorphosis [37,38,76]. Regardless, the available data suggest that both plethodontids and salamandrid newts maintain a rhythmic jaw cycle ($CV < 25\%$) across terrestrialization, whereas ambystomatids and *Amphiuma* do not. Clearly, more work is needed to determine how morphological, physiological or mechanical traits might explain the differences in cycle properties we observed in salamanders across terrestrialization. Additional promising areas of future investigation include studies of the modulation of jaw and tongue kinematics, as well as of activity in the muscles that drive them, to address the question of how sensory feedback and central-pattern generators interact to control chewing rhythmicity. In this context, the receptor arsenal in the muscles and oral cavities of nonmammalian vertebrates clearly needs closer examination.

5. Conclusion

We show that rhythmic chewing is the norm for gnathostome vertebrates, with notable exceptions being lepidosaurs and some post-metamorphic, terrestrialized salamanders. The subdivision of chew cycles into phases is also ancestral, at least predating Tetrapoda, and therefore not to be interpreted as a special condition in tetrapods. Indeed, this analysis instills caution about *a priori* accepting functional traits as synapomorphies for a given clade without deeper evolutionary analyses as this increases the risks of misunderstanding evolutionary drivers of functional changes. We also note that analysing duration or speed of cycle phases likely results in different interpretations of the constraints on maintaining rhythmicity as our duration analyses suggested that slow open is most variable, whereas average phase speed was most variable for fast close. Finally, we have made inroads on determining how a major life-history transformation—terrestrialization—influences the

biomechanics of chewing, a crucial survival system in gnathostome vertebrates.

Ethics. UMass Lowell: 12-06-19-KON; Harvard University: 20_09-03; Brown University: IACUC Protocol no. 1509000157; University of Rhode Island: AN05-07-001; University of Jena: 02-042/14, 02-008/15 (experiments), J-SHK-2684-05-04-05-07/14 (husbandry).

Data accessibility. Raw kinematics data will be available upon publication of this article from the XMAportal: <https://xmaportal.org/webportal/>; study no. BROWN47.

The data are provided in electronic supplementary material [79].

Authors' contributions. B.A.R.: data curation, formal analysis, methodology, writing—review and editing; M.S.: methodology, resources, writing—review and editing; M.R.-G.: data curation, resources, writing—review and editing; Y.T.R.: resources, writing—review and editing; D.S.: methodology, resources, writing—review and editing; J.B.R.: resources, writing—review and editing; J.D.L.-C.: resources, writing—review and editing; C.F.R.: writing—review and editing; N.K.: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration,

supervision, validation, visualization, writing—original draft, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

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