

## Rhythmic chewing with oral jaws in teleost fishes: a comparison with amniotes

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### SUMMARY

**Intra-oral prey processing (chewing) using the mandibular jaws occurs more extensively among teleost fishes than previously documented. The lack of muscle spindles,  $\gamma$ -motoneurons and periodontal afferents in fishes makes them useful for testing hypotheses regarding the relationship between these sensorimotor components and rhythmic chewing in vertebrates. Electromyography (EMG) data from the adductor mandibulae (AM) were used to quantify variation in chew cycle duration in the bowfin *Amia*, three osteoglossomorphs (bony-tongues), four salmonids and one esocid (pike). All species chewed prey using their oral jaw in repetitive trains of between 3 and 30 consecutive chews, a pattern that resembles cyclic chewing in amniote vertebrates. Variance in rhythmicity was compared within and between lineages using coefficients of variation and Levene's test for homogeneity of variance. These comparisons revealed that some teleosts exhibit degrees of rhythmicity that are comparable to mammalian mastication and higher than in lepidosaurs. Moreover, chew cycle durations in fishes, as in mammals, scale positively with mandible length. Chewing among basal teleosts may be rhythmic because it is stereotyped and inflexible, the result of patterned interactions between sensory feedback and a central pattern generator, because the lack of a fleshy tongue renders jaw–tongue coordination unnecessary and/or because stereotyped opening and closing movements are important for controlling fluid flow in the oral cavity.**

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Key words: stereotypy, flexibility, fish feeding, feeding behavior, neuromotor control, muscle-activity pattern, sensorimotor system, neuromechanics.

### INTRODUCTION

In most vertebrate classes, prey-processing behaviors determine the range of prey that a species can consume (Liem, 1979). Like many activities, prey-processing performance can be improved through appropriate use of sensory information by the central nervous system (CNS) to modify motor-activity patterns (MAPs) in order to meet challenges presented by the prey (Konow and Sanford, 2008a). It has been hypothesized that variation in the sensorimotor systems underlying vertebrate feeding and locomotion is related to variation in the ability and propensity of different vertebrate lineages to modify their MAPs (Ross et al., 2007a).

Chewing is an ideal behavior for investigating the evolutionary relationship between sensorimotor systems and behavior. Chewing is widespread among vertebrates – it has either evolved multiple times or been preserved in multiple lineages (Reilly et al., 2001; Reilly and Lauder, 1990) – and there is variation in both the behavior itself and the morphological and sensorimotor systems involved. Mammalian mastication is a particular form of chewing, which evolved in the context of novel sensorimotor system components, specifically periodontal afferents and  $\gamma$ -motoneurons (Ross et al., 2007a).

Ross and colleagues (Ross et al., 2007a) hypothesized that  $\gamma$ -motoneurons and periodontal afferents allow mammals to chew rhythmically (i.e. with low cycle duration variance) by enabling feed-forward modulation of MAPs during the slow close phase of the gape cycle (SC) in response to variation in food material properties within and between chewing sequences (Hidaka et al., 1997). In support of this hypothesis, Ross and colleagues showed that mammalian chew cycle durations not only are less variable than

those of lepidosaurs but also increase with mandible length, which does not occur among lepidosaurs. These authors hypothesized that improved chewing rhythmicity, a form of stereotypy (*sensu* Wainwright et al., 2008), enables mammals to chew for longer, facilitating the acquisition of the greater amounts of food required by their elevated metabolism (Nagy, 2005). Ross and colleagues also suggested that size-related changes in chew cycle durations in mammals support the hypothesis that energy consumption during chewing is minimized by adjusting chew frequency to the natural frequency of the chewing system (Ross et al., 2007a).

More recent research into patterns of variance in the gape cycle phases calls into question the hypothesized link between chewing rhythmicity and feed-forward modulation of muscle activity during SC (Ross et al., 2010). Primate mammals do not chew with less variable SC durations than lepidosaurs. Rather, primates are distinguished from lepidosaurs by trade-offs in variance between the phases of gape cycles that keep overall cycle durations relatively constant. Lepidosaurs, in contrast, show strong relationships between variance in the slow open phase (SO) of the gape cycle and variance in total cycle duration. Consequently, lepidosaurs chew with variable cycle durations because variance in SO duration is not accompanied by inversely related variance in other phases. This difference suggests that lepidosaurs do not use information on the external properties of the food bolus (e.g. position, size, texture, mobility) collected during SO to modulate durations of subsequent gape cycle phases. This implies that chew cycle durations in lepidosaurs are more variable than in mammals because of differences in CNS processing of afferent information collected during SO.

Fishes provide an interesting opportunity to test these hypotheses regarding the evolution of vertebrate sensorimotor systems. They are outgroup to the tetrapods, and apparently lack the periodontal afferents and muscle spindles implied in the maintenance of chewing rhythmicity (Ballintijn and Bamford, 1975; Ono, 1979) (but see Maeda et al., 1983). Moreover, intra-oral prey processing has been described in several teleost fish orders, where it has been differentiated from prey capture and other prey-processing behaviors on the basis of kinematics (Lauder, 1980; Sanford and Lauder, 1990; Sanford, 2001; Konow and Sanford, 2008b) and MAPs (Konow and Sanford, 2008a). In many fish lineages, intra-oral processing occurs as single bites used to immobilize and reduce prey for intra-oral and pharyngeal transport. However, our observations suggest that intra-oral prey processing in fishes often occurs in more than single bites: fishes employ trains of multiple intra-oral prey-processing cycles, potentially matching the definition of chewing (Reilly et al., 2001; Ross et al., 2007a), which renders both the motor control and temporal characteristics of fish intra-oral prey processing particularly interesting.

The aims of this study were to compare the degree of rhythmicity in teleost chewing with that of amniotes, and to determine whether fish chewing shows size-related frequency changes, as in mammals. We relied on a phylogenetically balanced taxon-sampling design, including the bowfin *Amia calva*, representative osteoglossomorph and salmonid taxa, and the pike *Esox americanus*. We investigated the rhythmicity of electromyographic (EMG) activity in the primary chewing muscle, the adductor mandibulae (AM), quantified as the degree of variation in AM cycle duration (EMG burst onset to onset) during processing of a standardized prey type (Ross et al., 2007a).

We hypothesized that, if muscle spindles,  $\gamma$ -motoneurons and periodontal afferents are indeed necessary for high levels of chewing rhythmicity, then the apparent absence of these sensorimotor components from fishes (Ballintijn and Bamford, 1975; Ono, 1979; Ross et al., 2007a) (but see Maeda et al., 1983) means that this Class should exhibit more variable chew cycle durations than lepidosaurs, which possess muscle spindles, and mammals, which possess muscle spindles,  $\gamma$ -motoneurons and periodontal afferents. We also hypothesized that if energy consumption rate in the chewing system is an important determinant of chewing frequency, then fishes should show size-related changes in chewing frequency, as seen in mammals.

## MATERIALS AND METHODS

### Specimens and husbandry

Our taxon sampling focused on basal teleosts, ranging from the bowfin *A. calva*, as outgroup *via* three bony-tongues (Osteoglossomorpha), to four salmonids with one species of pike (Esocidae), *E. americanus*, as outgroup (Fig. 1; Table 1). All specimens were housed individually and studied within the Hofstra University animal care facility in accordance with applicable animal care and use protocols. During acclimation, individuals were fed a varied diet of crickets (*Gryllus* sp.), goldfish (*Carassius* sp.), earthworms (*Lumbricus* sp.) and minnows (*Pimephales* sp.) to avoid induced stereotypy in feeding behavior (Deban et al., 2001). Specimens were not fed for 2 days prior to experiments in order to avoid satiation and to standardize the level of voracity among individuals.

### EMG recording

EMG recordings (Fig. 2) were made from the primary jaw-closing muscle, the adductor mandibulae pars 2 (AM), which is active in all feeding sequences (Lauder, 1980; Lauder and Liem, 1980;

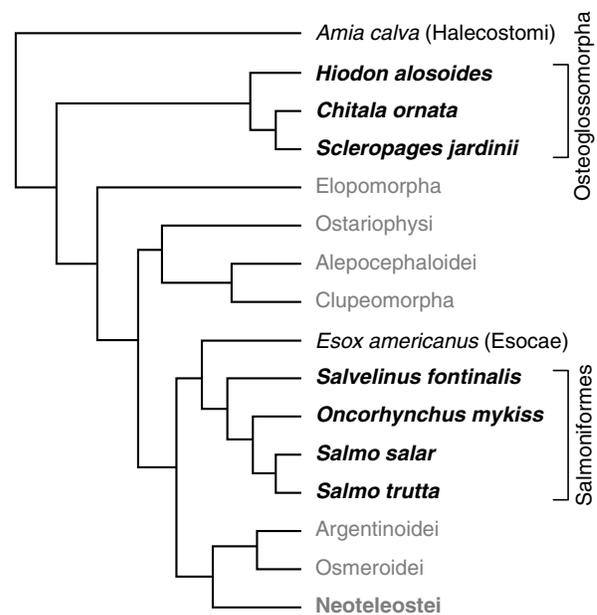


Fig. 1. Composite phylogeny of basal teleost fishes. Protacanthopterygian stem-group inter-relationships from Ishiguro et al. (Ishiguro et al., 2003), species-level osteoglossomorph interrelationships from Lavoue and Sullivan (Lavoue and Sullivan, 2004) and salmoniform inter-relationships from Sanford (Sanford, 2000). Taxa in black were sampled in this study, and taxa in gray served to reflect the phylogeny. Branch-length scaling is for ease of interpretation only.

Wainwright, 1989; Konow and Sanford, 2008a). The experimental protocol followed that described previously (Konow and Sanford, 2008a). Briefly, hooked fine-wire electrodes with 0.5 mm exposed tips were prepared by threading 1.25 m lengths of bi-filar wire (0.05 mm diameter polyethylene-coated stainless steel; California Fine Wire, Grover Beach, CA, USA) through 25 gauge 5/8 hypodermic needles. Electrodes were implanted at a 45 deg. angle to the surface of the left side muscles of anesthetized animals (Eugenol, Rush and Hebble, Edinburgh, IN, USA; 1/10 alcoholic suspension, 40 p.p.m. aqueous solution) (see Munday and Wilson, 1997). The electrode poles were anchored percutaneously into the muscle bellies, parallel to the muscle fiber orientation. Electrode wires were anchored to a mid-dorsal suture on the specimen, the electrode connector ends were crimped into din-25 adaptor pins, and floats on the electrode wires prevented tangling. Surgery lasted approximately 20 min, with complete recovery *ca.* 25 min post-surgery.

During experiments, live goldfish with a body length matching the lateromedial gape width of the individual (typically prey of



Fig. 2. Rectified and reset-integrated electromyogram (EMG) from the adductor mandibulae (AM) muscle in *Esox americanus* showing 22 consecutive rhythmic chews on a goldfish prey delivered over a 7 s time period. Cycle durations were extracted using conventional time-series analysis, measuring EMG activity duration from the onset of one AM burst to the onset of the next burst, as indicated by the gray area.

Table 1. Summary of fish species used, sample sizes and descriptive statistics of mandible lengths

Taxa	N	Mandible length (mm)	
		Range	Mean $\pm$ s.e.m.
<i>Amia calva</i> Linnaeus 1766	3	27.7–33.0	29.9 $\pm$ 0.959
<i>Hiodon alosoides</i> Rafinesque 1819	2	19.5–26.5	23 $\pm$ 1.573
<i>Chitala ornata</i> Gray 1831	3	37.0–41.0	38.8 $\pm$ 0.821
<i>Scleropages jardinii</i> Saville-Kent 1892	4	28.0–51.5	44.4 $\pm$ 1.65
<i>Esox americanus</i> Gmelin 1789	3	27.0–78.0	45.5 $\pm$ 3.07
<i>Salvelinus fontinalis</i> Mitchill 1814	4	47.0–54.0	50.8 $\pm$ 0.971
<i>Oncorhynchus mykiss</i> Walbaum 1792	3	30.5–39.1	35.4 $\pm$ 1.21
<i>Salmo trutta</i> Linnaeus 1758	5	26.0–29.0	27.8 $\pm$ 0.477
<i>Salmo salar</i> Linnaeus 1758	5	21.5–36.5	26.7 $\pm$ 1.11

Bold-faced taxa represent members of the osteoglossomorphs (top) and salmonids (bottom). See text for details.

35–40 mm total length, *SL*), were released into the tank. The individual typically caught the prey and immediately commenced vigorous prey processing. *Hiodon*, a micro-invertivore, only accepted crickets, which were also size matched to predator gape width. Crickets, like goldfish, are a hard-bodied prey type that exhibit considerable aquatic motility. *Hiodon* did not exhibit significantly different results from most of the other fishes studied.

EMGs from feeding were sampled at 10 kHz, amplified 1000 times (A-M systems, differential AC amplifier, model 1700; Everett, WA, USA), and conditioned with band-pass at 100–1000 Hz with a 60 Hz (notch) filter engaged. Both EMG signals and a manual trigger code (+5 V) used to label the behaviors during recording were digitized using a PowerLab 16/30 system linked to a PC running Chart v.5.4 to v.7.0 for Windows (PowerLab, Colorado Springs, CO, USA). Immediately following each feeding trial, EMG signals were comprehensively logged for behavior type, prey orientation and predator motivation, using the comments tool in Chart (Konow and Sanford, 2008a). These annotations ensured that

no EMG data from behaviors other than chewing were used in this study.

### Statistical design

Collection of these data added 1339 chewing cycles from 32 individuals belonging to nine species to the dataset of Ross et al. (Ross et al., 2007a), creating an overall sample size of 11,452 chewing cycles by 128 individuals from 45 species of mammals, 8 species of lepidosaurs and 9 species of fishes. These data were used for comparisons of levels of intra-individual variability in species (Fig. 3) and classes (Fig. 4). Mean chew cycle durations are available for larger samples of mammals ( $N=78$  species) and lepidosaurs ( $N=29$  species). These mean cycle durations were used to compare scaling of chew frequency in fish, lepidosaurs and mammals.

Chew cycle duration was measured as the time from AM burst onset to AM burst onset in succeeding cycles (Fig. 2) and mean chew cycle duration was calculated for each species (Table 2). To compare levels of variation, it is necessary to use measures that account for the strong correlation between mean duration of the behavior and the standard deviation for the behavior stemming from the fact that cycle duration variance increases with cycle time (Ross et al., 2007a; Wainwright et al., 2008). This was done in two ways. At the level of the Class (i.e. lepidosaurs, mammals and fishes)

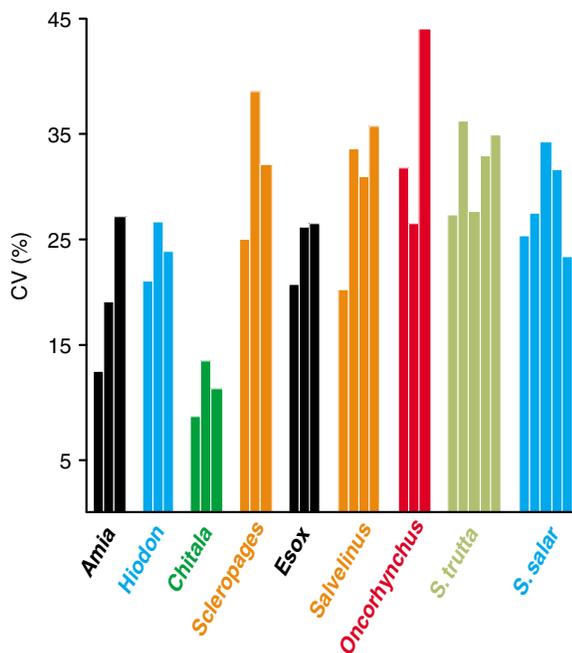


Fig. 3. Levels of intra-individual variation in the fish specimens studied. Each column represents the coefficient of variation (CV) in chew cycle duration for one individual from each of the species indicated (color scheme as in Fig. 4).

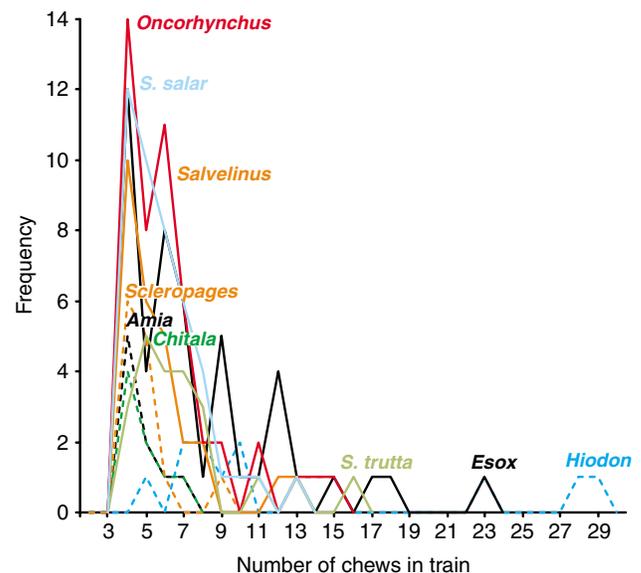


Fig. 4. Frequency distribution of number of gape cycles in chewing trains for the fish taxa studied (color scheme as in Fig. 3).

Table 2. Species mean values for chew cycle duration with corresponding variation values represented as coefficients of variation (CV)

Taxa	No. of cycle durations collected	Species mean cycle duration (ms)	Species mean cycle duration CV (%)
<i>Amia calva</i>	77	496.2	20.17
<b><i>Hiodon alosoides</i></b>	355	301.1	21.33
<b><i>Chitala ornata</i></b>	66	430.1	12.62
<b><i>Scleropages jardinii</i></b>	51	625.2	30.20
<i>Esox americanus</i>	522	610.5	22.92
<b><i>Salvelinus fontinalis</i></b>	280	458.7	26.45
<b><i>Oncorhynchus mykiss</i></b>	531	393.2	30.60
<b><i>Salmo trutta</i></b>	262	398.8	31.47
<b><i>Salmo salar</i></b>	452	465.8	27.76

Bold-faced taxa represent members of the osteoglossomorphs (top) and salmonids (bottom), respectively.

Levene's test for homogeneity of variance was applied to  $\log_{10}$ -transformed data, following Lewontin (Lewontin, 1966) and Sokal and Braumann (Sokal and Braumann, 1980). At the level of the species, levels of variation in cycle duration were compared by comparing coefficients of variation (CV) corrected according to equation 4 in Sokal and Braumann (Sokal and Braumann, 1980):

$$CV = \left(1 + \frac{1}{4n}\right) \frac{s}{\bar{Y}}, \quad (1)$$

where the traditional CV is  $s/\bar{Y}$ , the parenthetical term is the correction factor,  $n$  is sample size,  $s$  is standard deviation of the sample, and  $\bar{Y}$  is the sample mean. Pairwise comparisons of these CVs were performed using  $t$ -tests with Bonferroni corrections of critical  $P$ -values to accommodate multiple comparisons.

To compare chew cycle duration scaling in fishes and mammals, inter-specific scaling relationships between chew cycle duration and mandible length were documented using the slope of the standardized major axis (SMA; the reduced major axis, or RMA). SMA regressions were performed for all three Classes; significant slopes were then compared (Table 3). SMA was preferred over least-squares regression or major axis for the reasons given previously (Ross et al., 2009b). SMA slopes were compared using SMATR Version 2.0 (<http://www.bio.mq.edu.au/ecology/SMATR/>) employing methods described elsewhere (Warton et al., 2006). To compare SMA estimates between groups, tests for a common slope employed a likelihood ratio test compared to a chi-squared distribution, and tests for differences in elevation (SMA-‘ANCOVA’) employed the WALD statistic (Warton et al., 2006).

## RESULTS

EMGs from the AM in nine basal teleost species showed that all taxa process their prey using a rhythmic mandibular chewing behavior (Fig. 2; supplementary material Movie 1). This behavior occurs in trains from 3 to almost 30 cycles with the majority of trains consisting of less than 10 cycles (Fig. 4). Our data show that these fishes chew food with their oral jaws in sequences that at least superficially resemble those of mammals in the number of cycles employed per train (Ross et al., 2009a).

## Variation in chew cycle duration

Fish chew cycle duration CV averaged 24.8% (range 12.6–31.5%) but varied between species, ranging from low values in the osteoglossomorph *Chitala ornata* (13%) to values of more than 30% among both osteoglossomorphs and salmonids (Fig. 5A). The only fish species with chew cycle duration CV outside of the mammal range (mean 18.6%; range 8.3–31%) was *Salmo trutta* (31.5%). By comparison, chew cycle duration CV in lepidosaurs are much higher (mean 60.4%; range 21–193%).

Levene's test for homogeneity of variance revealed that fishes have significantly lower levels of variance in chew cycle duration than lepidosaurs (Levene's statistic, 80.726; d.f.1, 1; d.f.2, 5,735;  $P < 0.001$ ) (Fig. 5B). Pair-wise comparisons of all fish and lepidosaur CV were performed using one-tailed  $F$ -tests (Lewontin, 1966). Fish chew cycle durations were only more variable than those of lepidosaurs in a few cases: *Scleropages jardinii*, *Oncorhynchus mykiss* and both *S. trutta* and *S. salar* (Fig. 1; Table 1) chew cycle durations were more variable than in adult *Sphenodon punctatus*. Otherwise, the fishes sampled here were less variable in chew cycle duration than lepidosaurs.

As a group, fishes have significantly higher levels of chew cycle duration variance than mammals (Levene's statistic, 10.6; d.f.1, 1; d.f.2, 6,812;  $P = 0.001$ ) (Fig. 5B). Pair-wise comparisons of mammal and fish CV using one-tailed  $F$ -tests (Lewontin, 1966) with Bonferroni correction for multiple comparisons ( $P$ -crit  $< 0.00007$ ) revealed that chew cycle durations in *A. calva* were not more variable than those in most mammals. *Amia calva* CV were only significantly more variable than those of the goat, *Capra capra*, the rabbit, *Oryctolagus cuniculus*, the springhare, *Pedetes capensis*, and the opossum, *Didelphis virginiana*.

The chew cycle durations in the goldeye, *Hiodon alosoides*, were more variable than those of only 11 of the 45 mammal species, the chew cycle durations of the arowana, *S. jardinii* were more variable than those of 30 mammals, while the chew cycle durations of the knife fish, *C. ornata*, were not significantly more variable than those of any mammals. In contrast with the basal osteoglossomorphs, the more derived salmonids exhibited more variable chew cycle durations than most of the mammal species sampled. At  $P < 0.0006$ ,

Table 3. Standardized major axis regression statistics for regression of mean chew cycle duration against mandible length

Group	$N$	$R^2$	$P$	Slope	Lower 95% CI	Upper 95% CI	Intercept	Lower 95% CI	Upper 95% CI	Y mean	X mean
Mammals	78	0.547	<0.0001	0.4903	0.4207	0.5715	-1.351	-1.499	-1.202	-0.415	1.909
Lepidosaurs	29	0.053	0.228	1.5212	1.0452	2.2141	-2.135	-2.925	-1.344	-0.101	1.337
Fishes	9	0.449	0.048	0.8234	0.4419	1.5345	-1.611	-2.454	-0.767	-0.343	1.54
Common slope	87			0.5086	0.4335	0.5865					

WALD-test statistic for differences in slopes: 2.66,  $P = 0.113$ ; i.e. slopes are not significantly different. CI, confidence interval.

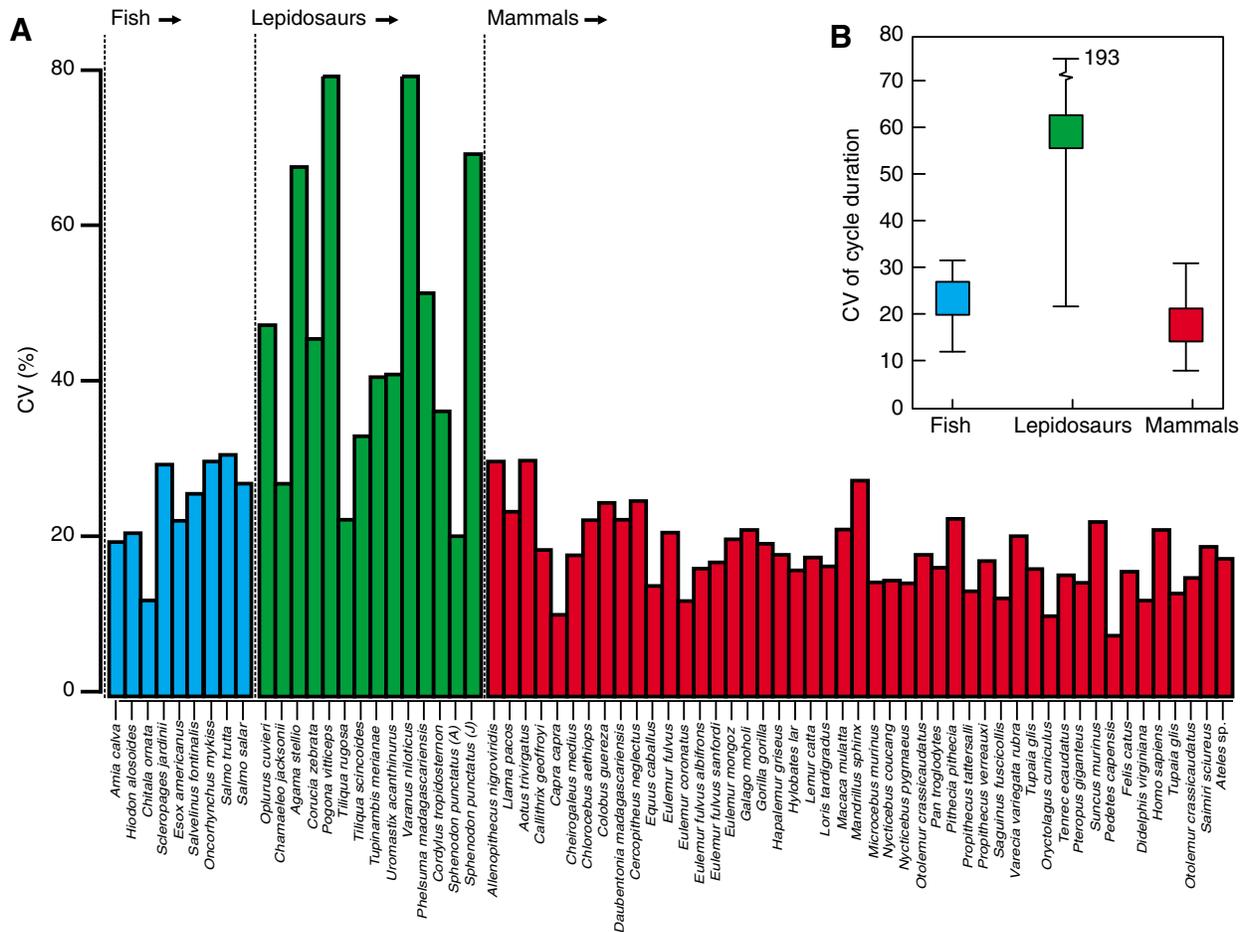


Fig. 5. (A) Bar plot of CV in AM cycle duration, and (B) box and whisker plot of grand mean AM cycle duration CV in fishes, lepidosaurs and mammals.

the rainbow trout, *Oncorhynchus*, and the brown trout, *S. trutta*, were more variable than 39 mammal species, the salmon, *S. salar*, was more variable than 35 and the brook trout, *Salvelinus fontinalis*, was more variable than 20 mammal species. The pike, *E. americanus* exhibited more variable cycle lengths than 15 of the mammal species. All salmonid chew cycle durations were more variable than those of 40+ mammal species when the *P*-value accepted as significant was relaxed to 0.01.

To test the hypothesis that fishes show size-related changes in chewing frequency, SMA equations for scaling of mean chew cycle duration against mandible length were calculated across mammals, lepidosaurs and fishes (Table 3). The regressions were significant: i.e. in fishes, like in mammals, mean chew cycle duration increased with mandible length (Fig. 6). The slopes for the two groups were not significantly different, so comparisons of intercepts were possible using an SMA-‘ANCOVA’ employing the WALD statistic (55.757,  $P < 0.001$ ). There was a significant difference in elevations, with fishes having significantly longer mean cycle times for their mandible length than mammals (Fig. 6).

## DISCUSSION

Whereas previous studies of chewing rhythmicity only examined chewing in amniotes (lepidosaurs and mammals), our data demonstrate that basal fishes also engage in trains of cyclic intra-oral processing, and that these trains often are lengthy (Fig. 4).

Moreover, chewing in basal fishes resembles chewing in amniotes in occurring intra-orally, rather than in the pharyngeal region, as in neoteleosts (e.g. Sibbing, 1982; Vincent and Sibbing, 1992; Claes and DeVree, 1991). Fishes appear to lack muscle spindles, which are characteristic of amniotes: only a single muscle spindle has been reported in a single species of fish, the Japanese landlocked trout *Oncorhynchus masou* (see Maeda et al., 1983). And, even if some spindles are present, the presence of  $\gamma$ -motoneurons seems unlikely in fishes, given the absence of  $\gamma$ -motoneurons in lizards, turtles and amphibians. Periodontal afferents have never been reported in fishes (Ballintijn and Bamford, 1975; Ono, 1979) and their presence seems unlikely given that many fishes have acrodont teeth or teeth with rapid turnover.

These differences in sensorimotor anatomy between fishes and amniotes make studies of chewing in fishes important when testing hypotheses regarding the evolution of the sensorimotor components of rhythmic intra-oral motor behaviors. In particular, hypotheses regarding the evolution of intra-oral food processing in vertebrates should be broad enough to include chewing in fishes.

We tested two hypotheses: that fishes should exhibit more variable chew cycle durations than amniotes, because fishes lack muscle spindles,  $\gamma$ -motoneurons and periodontal afferents, and that fishes should show size-related changes in chewing frequency resembling those seen in mammals, if energy expenditure in the feeding system is indeed an important performance criterion.

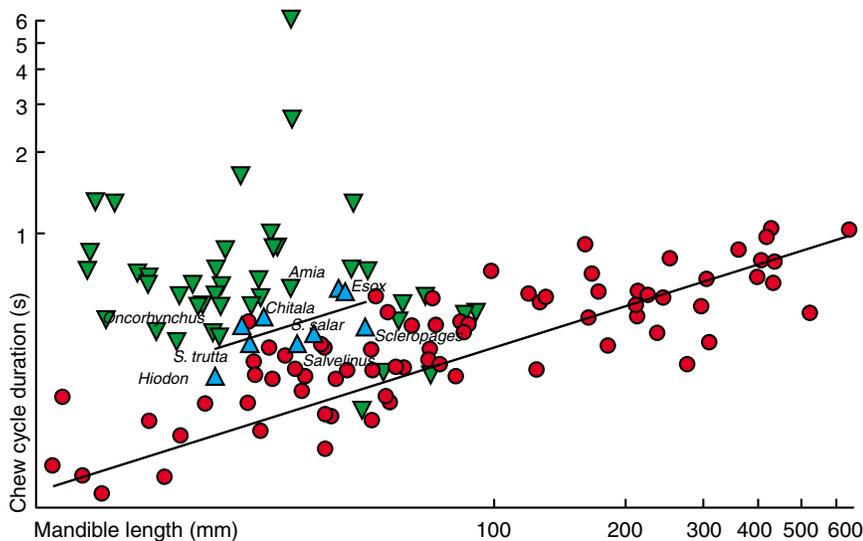


Fig. 6. Bivariate plot of mean chew cycle duration and mean mandible length in fishes (blue), lepidosaurs (green) and mammals (red). Best-fit standardized major axis (SMA) lines for mammals and fishes are shown (see Table 3). The relationship across lepidosaurs is not significant (Ross et al., 2007b).

### Rhythmicity, efficiency and sensorimotor evolution in vertebrates

The data presented here suggest that it is in fact possible for a gnathostome vertebrate to chew rhythmically without possessing muscle spindles,  $\gamma$ -motoneurons and periodontal afferents. Low cycle duration CV, not exceeding those seen in a majority of the 45 mammals sampled, are found in the bowfin *Amia*, and in the osteoglossomorphs *Hiodon* and *Chitala*. Conversely, higher CV than those in most mammals are characteristic of all the salmonids sampled in this study. Cyclic intra-oral food processing in fishes is also significantly more rhythmic than in most lepidosaurs. What are the implications of these results for the hypothesized relationships between chewing rhythmicity and sensorimotor evolution in vertebrates?

The presence of rhythmic chewing in fishes falsifies the hypothesis that periodontal afferents and  $\gamma$ -motoneurons are necessary for rhythmic chewing in vertebrates (Ross et al., 2007a). This result supports more recent work (Ross et al., 2010) suggesting that chew cycle durations in lepidosaurs are more variable than those of mammals because of differences in the way afferent information is processed by the CNS, rather than differences in the afferent information itself.

If rhythmic chewing is primitive for fishes, then it is also primitive for gnathostomes. If so, periodontal afferents and  $\gamma$ -motoneurons must have evolved to serve other functions in mammals [e.g. feed-forward control of mammalian chewing enabled by periodontal afferents and  $\gamma$ -motoneurons (Hidaka et al., 1997) likely reduces the degree of tooth wear and the probability of tooth fracture during mastication], leaving lepidosaurs unusual in their low degree of rhythmicity. In support of this hypothesis, chewing rhythmicity is seen in the basal osteichthyan lungfish *Lepidosiren paradoxa* (Bemis and Lauder, 1986). Data on chewing rhythmicity from a broader taxon range may help determine the actual distribution of chewing rhythmicity in the gnathostome clade.

However, it is important to consider the possibility that the rhythmic chewing characterizing the fishes studied here is qualitatively different from that seen in mammals. High rhythmicity of chewing among fishes might reflect high stereotypy but a lack of flexibility, whereas rhythmic chewing among mammals might be indicative of stereotypy in the context of flexibility. Our use of the terms stereotypy and flexibility follow Wainwright et al., where

stereotypy is 'the extent of variation in a behavior under a given set of conditions' and flexibility is 'the extent to which the behavior is altered in response to a change in stimulus' (Wainwright et al., 2008). Chewing in mammals is stereotyped in the sense that it is rhythmic, i.e. mammalian chewing shows low variation in cycle durations, but it is also flexible in that mammals appear to trade-off variance in the durations of gape cycle phases to maintain low variance in chew cycle durations (Yamada and Yamamura, 1996; Thexton and Hiiemae, 1997; Ross et al., 2010).

Chewing in some fishes is also stereotyped in the sense of being rhythmic, but the available data suggest this rhythmicity is accompanied by less flexibility than in mammals. For example, *Ctenopharyngodon idella* exhibits low variance in pharyngeal jaw cycle duration even in the face of variance in food material properties (Vincent and Sibbing, 1992). In a slightly different manner, *Chitala* exhibits low levels of variation in oral jaw chewing cycle durations, although it clearly displays flexibility in muscle activity patterns during other prey-processing behaviors (Konow et al., 2008a). In contrast, *Oreochromis niloticus* shows significant effects of prey type on its pharyngeal jaw cycle durations despite exhibiting stereotypy in chew cycle durations, matching that of many mammals (CV 11%) (Claes and De Vree, 1991). If some fishes do indeed combine stereotypy and flexibility in chewing behavior, the likely absence of muscle spindles and periodontal afferents suggests that this relationship is governed by fundamentally different sensory mechanisms than in amniotes. Determining exactly what those mechanisms are will require detailed anatomical study of sensorimotor systems of fish feeding systems, similar to those conducted previously by Ono (Ono, 1979; Ono, 1982).

The observation that lepidosaurs chew with more variable cycle durations than either mammals or the teleosts studied here prompts a new hypothesis regarding sources of variance in vertebrate chewing. Among lepidosaurs, variance in SO duration is the most important determinant of variance in chew cycle duration (Ross et al., 2010). During this gape cycle phase, the fleshy tongue is protracted to engage the food item in preparation for food transport. The absence of a fleshy tongue in fishes, and consequently a likely absence of the need to coordinate jaw and tongue movement during food transport, may well explain the low levels of variability and the heightened stereotypy of chewing in fishes compared with lepidosaurs. Indeed, highly stereotyped jaw movements may be

important for generating and maintaining the water flow needed for intra-oral food manipulation in fishes. We are currently evaluating this hypothesis using a combination of EMG and kinematics of jaw movements in teleost fishes.

#### Fish cycle-length duration and variation compared with amniotes

Our data show that fishes resemble mammals in having chew cycle durations that scale positively with mandible length. In addition, fishes have longer cycle durations than mammals with similar mandible lengths, and shorter cycle durations than similarly sized lepidosaurs (Fig. 6). In mammals, increases in mandible length are associated with an increase in chew cycle duration (Druzinsky, 1993; Shipley et al., 1994; Gerstner and Gerstein, 2008; Ross et al., 2009b) (Fig. 6). Size-related increases in chew cycle durations among fishes may be expected, as they are likely to be dependent on other factors: for instance, macro-predators, like *Esox*, *Scleropages* and *Chitala* (Konow and Sanford, 2008a; Konow and Sanford, 2008b), have significantly longer cycle durations than *Hiodon*, an invertivore (Little et al., 1998) that never accepted the fish prey presented in our experiments. Nevertheless, size-related changes in chew cycle time of fishes suggest similar effects of size on chewing biomechanics in fishes and mammals. In contrast, chew cycle duration does not vary consistently with mandible length in lepidosaurs (Ross et al., 2007a).

Size-related changes in chewing frequency were explained by Ross et al. (Ross et al., 2009b) with reference to the natural frequency of a forced mass-spring system in which jaw length, the spring constants of the jaw muscles and their reduced physiological cross-sectional area are important model components. The demonstration of size-related changes in chew frequency in fishes provides a new opportunity to test this hypothesis using data on moments of inertia and jaw muscle architecture.

Such studies might demonstrate that the significantly longer cycle durations in fishes than in mammals may result from intrinsic biomechanical differences between their feeding systems. Alternatively, differences between fish and mammal tongue and jaw kinematics might be attributable to differences between fluid and aerial environments. In a viscous, fluid environment, higher drag forces acting on a fish mandible during chewing might well result in longer chewing cycle durations. Moreover, the presence of water in the oral cavity may make intra-oral prey manipulation more difficult and precarious, and thus slower. Exactly which of these factors accounts for the slower chewing of fishes cannot be resolved with the currently available data.

#### What is the mechanism underlying rhythmic prey processing in fishes?

Rhythmicity of chewing in fishes may provide insight into the origin and evolution of stereotyped, rhythmic feeding behaviors in vertebrates. The presence of central pattern generators in lampreys (Guimond et al., 2003; Huard et al., 1999; Viana Di Prisco et al., 2005) makes it reasonable to suggest that central pattern generators also could control chewing behavior in fishes (Konow and Sanford, 2008a). In the absence of periodontal afferents, and  $\gamma$ -motoneurons to muscle spindles (should the latter be present in some teleost taxa), chewing behaviors in fishes are unlikely to be actively modulated (*sensu* Deban et al., 2001) *via* feed-forward control. Instead, this feeding system could be operating using feedback information on jaw displacement and muscle force to modulate central pattern generator output in response to food properties, such as size, position, mobility, hardness and toughness. Given the absence of muscle

spindles in fishes, displacement of the jaws could be detected by other peripheral sensors, such as joint receptors, and muscle force could be monitored either by a central mechanism such as efference copy, or by peripheral sensors, such as Golgi tendon organs or slowly adapting touch receptors. Better anatomical data on peripheral sensory organs in fish feeding systems are clearly needed.

Another possibility is that in fishes, active modulation may be less important in driving variation in cycle duration than the requirements imposed by external conditions. The effect of drag in the fluid environment, coupled with the requirement of maintaining an anterior-posterior water flow through the oropharynx to prevent prey escape might pose more powerful constraints on variance in chew cycle duration than variance in prey properties. Specifically, we hypothesize that a rapid, stereotyped fast open phase initiates this fluid flow, and a rapid stereotyped fast close phase secures the prey item prior to crushing [figure 5 in Reilly and Lauder (Reilly and Lauder, 1990)]. Moreover, because intra-oral prey manipulation is not achieved using a large fleshy tongue, the slow open phase that contributes so extensively to the variance of cycle duration in lepidosaurs should be completely absent. Future research should investigate whether fish jaw adductors harbor any specialized physiology for regulating these feeding behaviors and account for the intermediate level of cycle duration variation in fishes relative to amniotes.

#### CONCLUSIONS

We have presented evidence that basal teleost fishes commonly process prey using their mandibular jaws and perform cyclic and rhythmic chewing in repetitive trains, resembling the food processing behaviors of amniotes. The probable absence of periodontal afferents, muscle spindles and  $\gamma$ -motoneurons in teleosts suggests that these sensorimotor components are not necessary for rhythmic chewing. Fishes may chew rhythmically because (a) their chewing behavior is stereotyped and inflexible, (b) their chewing behavior is flexible but actively modulated, and/or (c) fishes lack the fleshy tongues used for intra-oral transport among terrestrial vertebrates. The mechanisms by which chewing and other cyclic feeding behaviors in teleosts are controlled represent promising avenues for future research.

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