



SYMPOSIUM

Evolution of Muscle Activity Patterns Driving Motions of the Jaw and Hyoid during Chewing in Gnathostomes

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From the symposium “Synthesis of Physiologic Data from the Mammalian Feeding Apparatus Using FEED, the Feeding Experiments End-User Database” presented at the annual meeting of the Society for Integrative and Comparative Biology, January 3–7, 2011, at Salt Lake City, Utah.

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Synopsis Although chewing has been suggested to be a basal gnathostome trait retained in most major vertebrate lineages, it has not been studied broadly and comparatively across vertebrates. To redress this imbalance, we recorded EMG from muscles powering anteroposterior movement of the hyoid, and dorsoventral movement of the mandibular jaw during chewing. We compared muscle activity patterns (MAP) during chewing in jawed vertebrate taxa belonging to unrelated groups of basal bony fishes and artiodactyl mammals. Our aim was to outline the evolution of coordination in MAP. Comparisons of activity in muscles of the jaw and hyoid that power chewing in closely related artiodactyls using cross-correlation analyses identified reorganizations of jaw and hyoid MAP between herbivores and omnivores. EMG data from basal bony fishes revealed a tighter coordination of jaw and hyoid MAP during chewing than seen in artiodactyls. Across this broad phylogenetic range, there have been major structural reorganizations, including a reduction of the bony hyoid suspension, which is robust in fishes, to the acquisition in a mammalian ancestor of a muscle sling suspending the hyoid. These changes appear to be reflected in a shift in chewing MAP that occurred in an unidentified anamniote stem-lineage. This shift matches observations that, when compared with fishes, the pattern of hyoid motion in tetrapods is reversed and also time-shifted relative to the pattern of jaw movement.

Introduction

In many jaw-bearing vertebrates (Gnathostomata), chewing drives the tooth-bearing or bony-plated elements of the feeding apparatus through successive and often rhythmic abduction–adduction cycles during the processing of food (Hiemae 1976, 1978; Herring 1993; Langenbach and van Eijden 2001; Reilly et al. 2001; Ross et al. 2007). Functional studies of chewing have often examined jaw adductor muscle activity, measured via electromyograms (EMG) and mandibular movements during

mastication in mammals as summarized elsewhere in this volume. Despite early work showing a relationship between jaw and hyoid movements during chewing (Crompton et al. 1975) we know very little about the generality of this pattern across gnathostomes compared with the vast amount of knowledge accumulated on the muscle activity patterns (MAP) powering jaw movements during chewing. Moreover, there has been virtually no research on the behavioral characteristics of chewing in gnathostomes other than in lungfishes, one of the most recent common

ancestors of fishes and tetrapods (Bemis and Lauder 1986), in basal bony (osteichthyan) fishes (Lauder 1980; Sanford and Lauder 1990; Konow and Sanford 2008b; Gintof et al. 2010), and in basal amniotes, such as lizards (Smith 1984; Herrel et al. 1997, 1999, 2001; Ross et al. 2007).

There are clear and broad similarities in chewing behaviors across gnathostomes. In both mammals and lizards, mandibular movement involves four phases: slow and fast opening, followed by fast and slow closing (Hiimae 1976, 1978; Bramble and Wake 1985; Ross et al. 2007). Jaw adductor EMG during chewing in basal bony fishes is highly rhythmic (Gintof et al. 2010), less so than in mammals, but significantly more so than in lizards (Ross et al. 2010). In fact, the chewing MAP in basal bony fishes reflects a stereotyped pattern of activity and recruitment of muscles (Konow and Sanford 2008b). While it is well-established that gnathostomes ranging from basal bony fishes to mammals chew their food, amniotes appear to use their feeding apparatus differently than mammals do during chewing. The hyoid forms a part of the principal food processing apparatus in basal bony fishes (Lauder 1979; Bemis and Lauder 1986; Konow and Sanford 2008a). In lepidosaurs, movements of the hyoid are extensive during chewing, but they are associated with transport, repositioning and swallowing, rather than with reduction of food (Smith 1984; Schwenk and Throckmorton 1989; So et al. 1992; Herrel et al. 1996;). In mammals, mastication occurs at occlusal surfaces between the post-canine mandibular and maxillary teeth. However, the idea that the mammalian hyoid merely steadies the tongue (e.g. Klingener 1972; Miller et al. 1979) is challenged by the interspecific differences seen in hyoid movements during chewing (Crompton et al. 1975; Hiimae et al. 1981; Thexton 1984; Franks et al. 1985; but see Anapol 1988). Even so, the paucity of phylogenetic comparative studies makes it difficult to determine if and how jaw and hyoid MAPs have evolved across gnathostomes.

There is a high diversity in hyoid structure and complexity across gnathostomes (e.g. compare Lauder 1980; Smith 1986; Takada et al. 2009; Pérez et al. 2010). This diversity might reflect changes in the relative role of hyoid movements during acquisition and chewing of food, and during other oral behaviors. Hyoid movements are directly powered by ventral, paired strap-like muscles, and across taxa these muscles interconnect the shoulder girdle, hyoid bar and mandible in different ways (Fig. 1A and B) (Lauder 1980; Westneat 1990; Wilga et al. 2000; Anderson 2008). In fishes, opening of the

mouth is initiated by trunk muscles (hypaxial and epaxial), transmitting power through the strap-muscles from the shoulder girdle via the hyoid to the mandible (Muller 1987; Konow and Sanford 2008a). Coupled with the jaw adductors, these strap-muscles can also power occlusion of the jaw—again indirectly—via the hyoid structures (Lauder 1980; Aerts et al. 1987; Van Wassenbergh et al. 2005). The hyoid in bony fishes is suspended by two robust bony flanges (the ceratohyals) and motion is transmitted to both the hyoid and mandible via stout ligaments and a four-bar linkage mechanism (Muller 1987; Anderson 2008). Mechanical constraints inherent to four-bar linkages mean that the hyoid-mandibular apparatus in basal bony fishes functions with a single degree-of-freedom (Westneat 1990). This apparatus is also powered by stereotyped MAPS during acquisition and processing of food (Wainwright et al. 1989; Konow and Sanford 2008b).

Architecture of the jaw and hyoid musculature has changed significantly across gnathostomes. The evolution of a muscular mechanism that abducts the mandible in basal amniotes (Fig. 1B) has potentially decoupled the strap musculature of the hyoid from actuation of the lower jaw (but see Weijs and Muhl, 1987; Konow and Sanford 2008b). Still, there are several clear examples of muscle homology (Fig. 1C and D). The actinopterygian adductor mandibularis (AM) pars 2 is evolutionary homologous, and also functionally analogous to all mammalian jaw adductors, except the medial pterygoid, and possibly part of the temporalis, which arose from the AM pars 3 (Edgeworth 1935; Diogo et al. 2008). In contrast, jaw abductor muscles have evolved repeatedly, as a depressor mandibulae in lungfishes, which is not homologous to the mandible abductor muscle in amphibians and reptiles (Bemis and Lauder 1986), and as the digastricus complex in mammals. In the submandibular musculature, the sternohyoid (hyoid retractor) is homologous across gnathostomes (Edgeworth 1935; Diogo et al. 2008), whereas the hyoid is protracted by functionally analogous muscles (see above).

Here, we study EMG activity in the jaw and hyoid muscles powering chewing behaviors in unrelated gnathostome taxa that bracket the fish-tetrapod split (Reilly and Lauder 1990). First, by comparing MAPs in closely related artiodactyl mammals with different diets, we test whether trophic specialists (the herbivorous goats and alpaca) have more highly coordinated MAPs of the jaw and hyoid muscles than does a trophic generalist (the omnivorous pig). Differences in the coordination of activity in muscle-pairs are quantified as differences in the

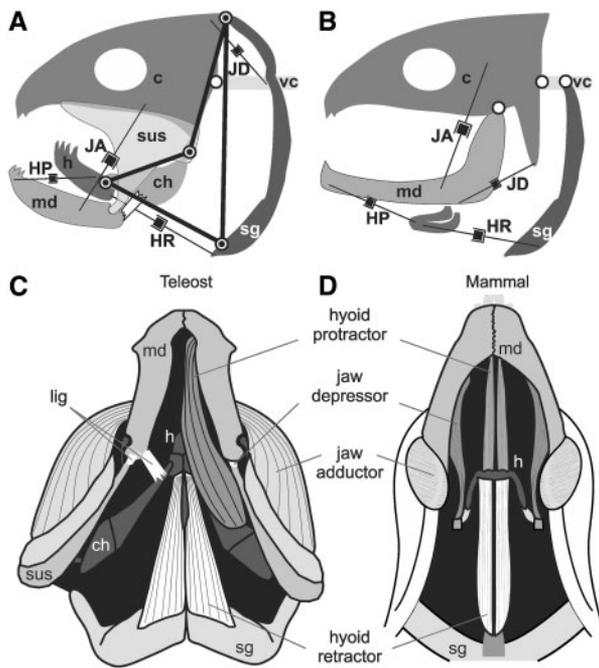


Fig. 1 The feeding apparatus of bony fishes (A) and mammals (B). In fishes, the hyoid (h) and the suspensorial “cheek” (sus) is linked by a bony ceratohyal (ch). The hyoid in mammals is suspended in a muscle sling (black dash-pots) from the mandible (md), cranium (c) and shoulder girdle (sg). In fishes, abduction of the jaw is driven by trunk muscles moving the cranium and shoulder girdle. This motion is transmitted to the hyoid and mandible via ligaments (white) and a four-bar linkage mechanism (black bars and bullets). Mammalian jaw abduction is driven by novel jaw abductor musculature (JD). Other focus muscles: HP, hyoid protractor; HR, hyoid retractor; JA, jaw adductor. Ventral view of feeding apparatus muscles in bony fishes (C) and mammals (D). Evolutionary homologous focus muscles include the hyoid retractor (sternohyoid) and the jaw adductor (Adductor mandibulae pars 2 in fishes and superficial masseter in mammals). Hyoid protractor muscles (protractor hyoideus in fishes versus geniohyoid in mammals) are functional analogs. Jaw abduction relies on mechanisms that differ anatomically and functionally (in fishes; trunk musculature via hyoid-mandibular bones and ligaments versus anterior digastric muscle in mammals).

variance of peak-to-peak timing of EMG. This hypothesis is prompted by Liem’s theorem, that species with generalist diets, such as omnivory, require flexibility during food-processing in order to process foods with different material properties (Liem 1978). One way that this flexibility might manifest itself is through increased variation in coordination of EMG between muscles of the tongue and jaw. In contrast, the energetic requirements of endothermy, coupled with a relatively low-quality diet that requires more food processing, should translate into a need for herbivorous mammals to maintain high energetic efficiency during chewing. We expect this need to be reflected by a heightened degree of rhythmicity in

mastication (Ross et al. 2007; Williams et al. in press), possibly involving a tighter coordination of EMG activity in the jaw, hyoid, and tongue muscles than in omnivores.

We then compare coordination of MAPs in basal bony fishes and artiodactyl mammals during chewing. We hypothesize that there are differences in the degree of coordination between activity in muscles powering movement of the jaw and hyoid in fishes and tetrapods. If coordination of MAP is related to the degrees of freedom of the jaw/hyoid complex and the physical properties of the feeding environment, then the single degree-of-freedom associated with the hyoid mechanism in fishes, coupled with the high viscosity and density of water predicts stereotyped and coordinated hyoid MAPs during chewing in fishes. On the other hand, mammalian jaw and hyoid MAPs should show a higher level of variability and be less coordinated because of their muscular hyoid suspension and the fact that transport of the bolus is governed by the tongue directly, rather than through more stereotyped movements of the tongue, to control flow of fluid as well as transport of food.

Finally, given the differences in the architecture of the musculoskeletal system and the movement patterns of the jaw and hyoid across gnathostomes, we compare onset-timing of jaw and hyoid muscles during chewing in gnathostomes and ask: are there differences between classes in the sequence of hyoid protractor and retractor muscle activity? Such a shift would imply a shift in the jaw and hyoid MAP of chewing during the evolution of gnathostomes, from basal aquatic to derived terrestrial taxa.

Methods

Specimens and instrumentation protocols

EMG of chewing in pigs (*Sus scrofa*), an omnivorous artiodactyl, were collected using a protocol detailed elsewhere (Konow et al. 2010). All individuals ($N=4$) were maintained in the Johns Hopkins Medical Institute animal care facility until they were instrumented under Isoflourane anesthesia and under sterile conditions. The jaw and hyoid area was exposed through a ventral incision and fine-wire bipolar EMG electrodes with the hooked poles offset (Basmajian and Stecko 1962; AS631, Cooner Wire, Chatsworth, CA, USA) were implanted in the target muscles on the animals left. The muscles instrumented were: (1) hyoid protractor (geniohyoideus); (2) hyoid retractor (sternohyoideus); (3) jaw adductor (superficial masseter); and (4) jaw abductor (anterior digastric). Post-mortem dissections followed each experiment to verify the placement of

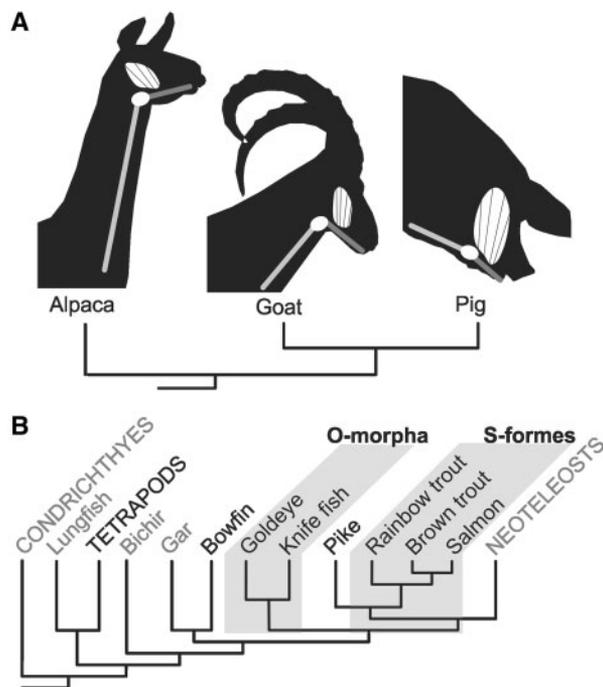


Fig. 2 Interrelationships of taxa. **(A)** Interrelationship of the artiodactyl taxa, following Agnarsson and May-Collado (2008) and Price et al. (2005). Diagrams illustrate differences in neck length and head size. The long neck of alpacas yields a longer hyoid retractor (light grey). The robust head of the pig increases the area of the origin of the jaw adductor (white). The short nose in pigs shortens the hyoid protractor (dark grey). The hyoid is shown as a white ellipse. **(B)** Gnathostome phylogeny following Ishiguro et al. (2003) and Alfaro et al. (2009) with the basal bony fishes under study in black. Grey boxes outline osteoglossomorph (O-morpha) and salmoniform (S-formes) groups that chew but also process food using raking motions of the hyoid. Note that several nodes from source trees were collapsed for ease of interpretation.

electrodes. EMG of chewing in alpacas (*Lama pacos*) and goats (*Capra hircus*) (Fig. 2A), two herbivorous artiodactyls, were collected using a protocol similar to that for pigs, from three individuals of each species, all housed at the Ohio University Large Animal Research Facility. Animals had identically constructed bipolar fine-wire EMG electrodes (0.05 mm \varnothing polyethylene coated stainless steel, California Fine Wire, CA, USA) as in the pigs, but implanted transcutaneously into the target muscles (same as for pigs, but bilaterally) under anesthesia with ketamine, butorphanol and xylazine (alpacas) or medetomidine (goats). Placement of the electrodes were immediately confirmed by stretching the target muscle while back-stimulating it through the electrode using a Grass s88 stimulator.

EMG of chewing in basal bony fishes were collected using a protocol detailed elsewhere (Konow et al. 2008; Konow and Sanford 2008b). Two to three

individuals of bowfin, *Amia calva* (Amiiformes); the osteoglossomorph goldeye, *Hiodon alosoides*; knife fish, *Chitala ornata* (Osteoglossomorpha); pike, *Esox americanus* (Esociformes); and the salmoniform rainbow trout, *Oncorhynchus mykiss*; brown trout, *Salmo trutta* and salmon, *Salmo salar* (Salmoniformes) (Fig. 2B) were maintained in the Hofstra University animal care facility. Bipolar fine-wire EMG electrodes with non-staggered hooked poles (Konow and Sanford 2008) were implanted in the target muscles on the animals left, following anesthesia by immersion of the animal in 40 p.p.m. Eugenol in alcoholic suspension. The implanted muscles were: (1) hyoid protractor (posterior inter-mandibularis in Knife fish; otherwise protractor hyoideus); (2) hyoid retractor (sternohyoideus); (3) jaw adductor (adductor mandibulae pars II); and (4) jaw abductor (hypaxial and epaxial muscles of the trunk).

Collection of data

Following recovery from anesthesia, artiodactyls fed on and processed commercially available pellets (pigs) or hay (goats and alpacas) and fishes captured and processed goldfish that were matched in size with the mouth-width of the predatory fish. EMGs were recorded at 4 kHz for fishes and at 10 kHz for mammals from all electrodes synchronously with a band-pass filter set at 100–1000 Hz and a 60 Hz notch filter engaged. All data files were carefully annotated during collection of the data to ensure that (1) intercalated behavioral EMGs were omitted from analyses (viz. raking in salmonids and osteoglossomorphs); (2) data with excessive signal noise or artifacts of motion were excluded; and (3) only working-side data were included in analyses of chewing by the herbivorous artiodactyls. We digitized fish and pig EMGs *via* a Powerlab 16/30 (ADInstruments), and artiodactyl EMGs *via* LabView onto hard-drives.

Extraction and processing of data

Following manual screening of data, we extracted chewing sequences that each contained four or more successive cycles (Loeb et al. 1987; Wren et al. 2006). By analyzing data on chewing sequences, rather than on individual cycles (Konow et al. 2010), we aimed to suppress variability related to between-cycle differences in EMG activity and muscle recruitment (Loeb et al. 1987), to focus our analyses on interspecific differences. Consequently, we extracted the longest sequences of uninterrupted chewing possible without breaching the above criteria for analysis

(1–3). Matlab routines were used to pad shorter chewing sequence with zeros, in order to standardize length of the files. Then, all data were rectified and reset-integrated, so that EMGs were converted into positive waveforms. A Matlab routine implementing the “Thextonizer” randomization method (Thexton 1996; Konow et al. 2010) was used to detect the level of baseline noise, and remove this noise along with orphan spikes.

We then carried out cross-correlation analyses on pairs of muscle activity waveforms using a MATLAB routine (Konow et al. 2010). For these pairs, we used the jaw abductor waveform as the reference wave, and the hyoid protractor, hyoid retractor and jaw adductor EMG waveforms as response waves. A cross correlation analysis returns a lag-score indicating the best pair-wise goodness-of-fit between response and reference waves, as close in time to zero-lag as possible. If peak-EMG in the response wave precedes the reference wave, a negative lag-score is returned. If peak-EMG in the response wave follows the reference wave, a positive lag-score is returned. Each cross-correlation analysis also returns a cross correlation coefficient (*R*-value) indicating the strength of the cross-correlation at the optimum time-lag encountered. We constrained our cross-correlation analyses to seek fits inside a lag-envelope corresponding with the mean duration of one chew cycle for each species (Ross et al. 2007; Gintof et al. 2010). Lag-scores were standardized by duration of chew-cycle for each species for a duration-independent measure of MAP coordination (a lag-phase) i.e. directly comparable between unrelated taxa with different chew-cycle durations.

Hypothesis testing and statistical design

Three sets of analyses were used to address our hypotheses about evolution of the jaw and hyoid MAPs powering chewing in gnathostomes. To test the hypothesis that the level of coordination in jaw and hyoid MAPs differs between herbivorous and omnivorous mammals, we used EMG data from closely related artiodactyls (Fig. 2), the herbivorous domestic goat (*Capra*) and alpaca (*Lama*), and the omnivorous domestic pig (*Sus*). We first determined onset-timing patterns to detect reorganization of the MAP at this phylogenetic tip-level. Then, we looked for differences in the peak-timing of activity in the jaw and hyoid musculature. We ran cross-correlation analyses of jaw adductor, hyoid protractor and hyoid retractor peak-timing relative to jaw abductor peak-timing. The cross-correlation results are displayed for each pair-wise comparison using median

quartile box and whisker plots. We then used ANOVA on the lag-scores from the cross-correlation analyses to determine if there was reorganization of the MAP. In these analyses, individual was nested within species to parse out differences resulting from intra-specific variability, and post-hoc testing was used to establish whether pair-wise differences existed between the three taxa. Finally, we examined the variance in lag-scores from cross-correlation analyses (Wainwright et al. 2008) to evaluate the level of coordination in jaw and hyoid MAP.

To test the hypotheses that the fish hyoid, which is suspended in a bony and robust apparatus, is powered by a less variable MAP than the hyoid in mammals, which is more gracile and suspended muscularly, and whether the MAP powering movement of the mandible is more variable for the gracile mandible suspension in fishes, than for the robust mandible suspension in mammals, we compared the variance associated with the lag-scores from each of the cross-correlation analyses. Finally we conducted a meta-analysis of data from the literature on EMG onset-timing sequencing with our artiodactyl data to determine whether there are differences between fishes and tetrapods in the MAPs of their jaw and hyoid during chewing.

Results

Reorganization of jaw and hyoid MAP during chewing in artiodactyls

Interspecific differences in the onset timing of jaw and hyoid muscles during chewing in artiodactyls are illustrated by comparing a sample of MAPs (Fig. 3). In the alpaca and pig, the onset of activity in the hyoid retractor (sternohyoid) follows the onset of activity in the hyoid protractor (geniohyoid), but these muscles fire together in the goat. In the alpaca, the jaw abductor (anterior digastric) and the jaw adductor (superficial masseter) fire almost in synchrony. In the goat, onset of activity in the jaw abductor clearly precedes that of the jaw adductor, and there is almost complete lack of overlap in activity of the jaw abductor and jaw adductor muscles in the pig. The pig has more sustained activity in the jaw abductor and hyoid retractor, and shorter duration of the hyoid retractor burst than in the alpaca or goat.

Interspecific differences are evident when comparing peak-to-peak muscle activity across the three artiodactyls (Fig. 4). Most cross-correlation coefficients exceeded 0.6, indicating a high pair-wise goodness of fit in the cross-correlations. Based on the lag-scores, peak activity in the hyoid retractor precedes peak-activity in the jaw abductor in alpacas and

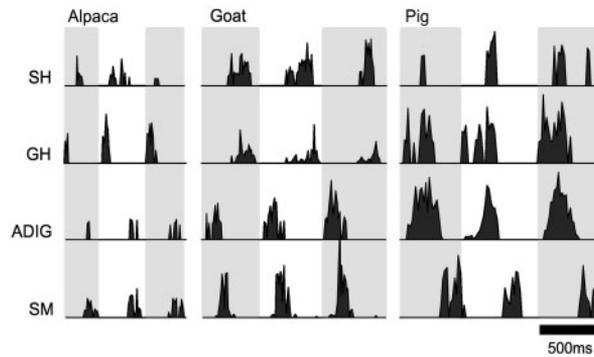


Fig. 3 Onset and duration of activity in the jaw and hyoid muscles during chewing differ between artiodactyls. In the herbivorous alpaca and the omnivorous pig, onset of activity in the hyoid retractor (SH, sternohyoid) follows that in the hyoid protractor (GH, geniohyoid), but in the herbivorous goat, these muscles fire together. In the alpaca, the jaw abductor (ADIG, anterior digastic) fires in near synchrony with the jaw adductor (SM, superficial masseter), while these onsets of activity are more distinct in the goat and there is little overlap of activity in the pig. The pig has more prolonged activity in the GH and ADIG, and shorter duration of activity in the SH than do the other two artiodactyls.

goats, whereas in pigs peak timing of the hyoid retractor and jaw abductor coincides (ANOVA, $F_{2,34} = 3.83$; $P = 0.032$). Peak activity in the hyoid protractor follows peak activity in the jaw abductor in goats, a significant difference from alpacas and pigs, in which peak activity of the hyoid protractor precedes that of the jaw abductor (ANOVA, $F_{2,32} = 4.5$; $P = 0.016$). The time between peak jaw adductor activity and peak jaw abductor activity is similar in all three species, but in the goat and pig the activity of the jaw adductor follows that of the jaw abductor whereas the reverse is true in the alpaca (ANOVA, $F_{2,24} = 0.52$; $P = 0.52$). The variance in peak to peak timing differences in the hyoid or jaw muscles based on normalized lag-scores (Table 1) shows that hyoid muscle activity is less variable, and thus more tightly coordinated with the jaw abductor musculature in the omnivorous pig than in the herbivorous goat and alpaca (light boxes in Fig. 4). This is in contrast with the coordination of the activity of the jaw-abducting and jaw-adducting muscles, which is more variable in the omnivore than in the herbivores (Table 1; dark boxes in Fig. 4).

Differences in coordination of jaw and hyoid MAP during chewing in fishes

The inter-specific differences in coordination among basal bony fishes are less clear than in artiodactyls, when comparing peak-to-peak muscle activity during chewing (Fig. 5). The taxa with tight coordination between jaw and hyoid peak EMGs (goldeye, rainbow trout, brown trout and salmon) all use an alternative food processing behavior in addition to chewing, namely raking (Konow and Sanford 2008b). In contrast, taxa that only chew (bowfin

and pike) have significantly more variable coordination of peak MAP. An exception is the Knife fish, which both chews and rakes, but still shows high MAP variance during chewing (Konow et al. 2008). The lack of offset between peak activity in jaw abductors and jaw adductors is attributable to patterns of prolonged and low-level EMG activity to which the cross correlation analyses are insensitive.

Differences in coordination of jaw and hyoid MAP between artiodactyls and fishes

There were differences between basal bony fishes and artiodactyl mammals in the variability of peak activity timing in jaw and hyoid muscles during chewing. Variance from pair-wise comparisons of peak-timing in EMG from jaw and hyoid muscles consistently exceeds 0.1 in mammals, whereas 55% of the pair-wise comparisons for fish taxa are associated with variance an order of magnitude lower (Table 1).

Shift in chewing MAP during gnathostome evolution

The results from meta-analyses of the onset-timing of EMG during chewing in gnathostomes are shown in Table 2. In basal bony fishes, notably including the lungfish, (anamniotes), the hyoid retractor muscle fires before the hyoid protractor muscle or the jaw adductor. In lizards and mammals (amniotes), the hyoid protractor typically fires simultaneously with the jaw abductor muscle, and prior to the hyoid retractor. Moreover, as reflected by the numerous row-wise numerical reorganizations, the onset-sequencing of jaw and hyoid muscle activity varies more in mammals than in fishes.

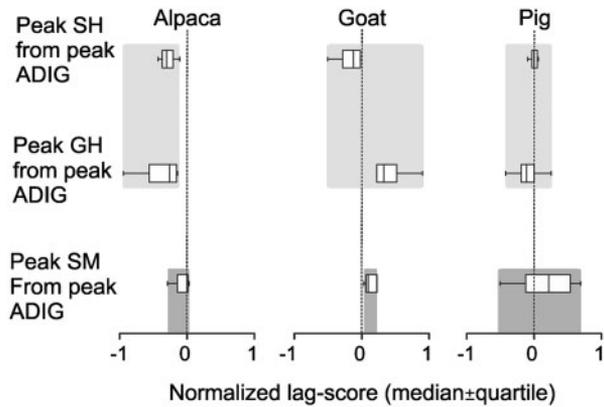


Fig. 4 Coordination of peak-timing of activity in the jaw and hyoid muscles differs between artiodactyls. The normalized cross-correlation lag-scores show that lead in peak-timing of the hyoid retractor (SH) from the jaw abductor (ADIG) in the trophic specialists (herbivorous alpacas and goats) is statistically different from the coincidence of peak-activity of SH and ADIG in the trophic generalist pigs. The lag in hyoid protractor (GH) peak-timing from ADIG in goat is statistically different from the lead in alpacas and pigs. Jaw adductor (SM) peak-activity leads from ADIG in alpacas, and lags in goats and pigs, but this difference is not statistically significant. Alpaca, $N=13$; Goat, $N=6$ and Pigs, $N=26$ chew sequences. The cross-correlation coefficients (vertical dot and whisker plots) indicate a high goodness-of-fit and thus a good reliability of the comparisons of lag. Studying jaw and hyoid muscle activity separately or together clearly influences our understanding of how tightly MAPs are coordinated. Peak-timing of hyoid muscles (light boxes) is more tightly coordinated in the omnivorous pig than in the herbivorous artiodactyls. In contrast, the herbivores have more tightly coordinated peak-timing of their jaw muscle activity than does the omnivore (dark boxes).

Discussion

Our analyses of MAPs reveal aspects of the evolution of chewing at the tip- and stem-level of gnathostome vertebrates that only materialize when patterns of EMG activity in both the jaw and the hyoid musculature are studied together. Tip-level phylogenetic differences in the coordination of jaw and hyoid MAP in artiodactyls suggest that the hyoid serves different functions in trophic specialists and generalists. Generally, MAPs appear more variable in artiodactyls than in basal bony fishes. A meta-analysis of the onset-timing of activity in the jaw and the hyoid musculature show that a shift in the MAP governing hyoid movement must have occurred in a stem-tetrapod.

Cross-correlation analyses reveal differences in MAP at the phylogenetic tip-level

Among artiodactyls, we found clear differences in the coordination of peak-timing of hyoid muscle activity

Table 1 Variance in peak-timing of activity in the jaw and hyoid musculature calculated from cross-correlation analysis lag-scores

Comparison	Hyoid retractor	Hyoid protractor	Jaw adductor
	Jaw abductor	Jaw abductor	Jaw abductor
Alpaca	0.11	0.28	0.11
Goat	0.19	0.39	0.16
Pig	0.09	0.26	0.54
Mammal, average	0.13	0.31	0.27
Brown trout	0.00	0.09	0.00
Rainbow trout	0.00	0.04	0.02
Salmon	0.19	0.54	0.38
Pike	0.01	0.01	0.02
Mooneye	0.00	0.00	0.01
Knife fish	0.46	0.63	0.43
Bowfin	0.00	0.65	0.49
Fish, average	0.10	0.28	0.19
Rakers, average	0.05	0.17	0.10

Note. Values are averages of individual variance within species.

during chewing. These differences at the phylogenetic tip-level suggest that hyoid movements during mastication likely serve different functions across mammals (Hiemae et al. 1981). Similar differences, hinting at variation in hyoid movement, and thus its function during chewing, appear when data from early fluoroscopy and EMG studies of mammals are compared: in the omnivorous marsupial opossum (*Didelphis virginiana*), the hyoid only moves forward during slow closure of the jaw (Crompton et al. 1975), but in the carnivorous cat (*Felis domesticus*), the hyoid moves forward during both the slow and fast phases of jaw closure (Thexton 1984; Thexton and McGarrick 1994). The synchronized tongue and hyoid movements in the cat imply a function of retaining food between the tongue and palate.

Insights from studying coordination of activity in the jaw and hyoid musculature

The MAPs of hyoid protraction and retraction during chewing were more tightly coordinated in the omnivorous pig than in the herbivorous alpaca and goat, both at the level of variability in timing of peak activity within each of the hyoid strap muscles (Table 1) and at the level of overall variability in timing of peak activity of both the hyoid muscles (Fig. 3). This result would lead us to reject the hypothesis that chewing in the trophic generalist (omnivores) is characterized by less coordinated MAPs than chewing in trophic specialists (herbivores). The factors underlying this difference remain unclear but

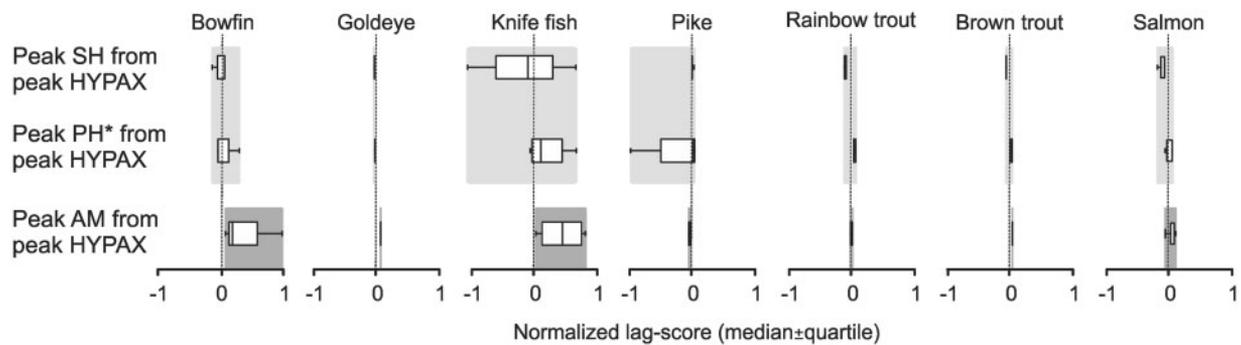


Fig. 5 Coordination of peak-timing of activity in the jaw and hyoid musculature is high during chewing in some basal bony fishes. The plot shows normalized cross-correlation lag-scores for peak-timing of activity in the hyoid retractor (SH), hyoid protractor (PH, *PIM in the knife fish) and jaw adductor (AM) muscles, relative to peak activity of the body-wall muscle-driven jaw-abductor mechanism (HYPAX). $N = 4$ chew sequences for all species. Bowfin and pike, which only process food by chewing, have more variable chewing MAPs than do goldeye, rainbow trout, brown trout or salmon (Table 1), all salmoniform (S-formes) and osteoglossomorph (O-morph) taxa, which use both raking and chewing food processing (Konow and Sanford 2008b). The high variability of MAP in the knife fish, which both chews and rakes, could be caused by the high level of behavioral modulation during feeding in this taxon (Konow et al. 2008).

Table 2 Patterns of onset of activity in the jaw and hyoid musculature of gnathostomes during chewing

Taxon	HP	HR	JA	JD*	References
Lungfish (<i>Lepidosiren paradoxa</i>)	4	2	3	1	Bemis and Lauder 1986
Bichir (<i>Polypterus senegalus</i>)	2	1	3	1	Lauder 1980
Gar (<i>Lepisosteus oculatus</i>)	2	1	3	1	Lauder 1980
Bowfin (<i>Amia calva</i>)	2	1	2	1	Konow and Sanford 2008b
Bony-tongues (Osteoglossomorpha)	2	1	2	1	Konow and Sanford 2008b Konow et al. 2008
Pike (Esociformes) (<i>Esox americanus</i>)	2	1	2	1	Konow and Sanford 2008b
Salmonids (Salmoniformes)	2	1	2	1	Konow and Sanford 2008b Konow et al. 2008
Lizards (Lepidosauria)	1	2	3	2	Herrel et al. 2001
Opossum (<i>Didelphis virginiana</i>)	1	2	3	1	Crompton et al. 1975
Tenrec (<i>Tenrec ecaudatus</i>)	1	2	3	2	Oron and Crompton, 1985
Pig (<i>Sus scrofa</i>)	1	3	4	2	Present study; Kayalioglu et al. 2007
Alpaca (<i>Lama pacos</i>)	1	2	4	3	Present study
Goat (<i>Capra hircus</i>)	4	3	2	1	Present study
Rat (<i>Rattus norvegicus</i>)	1	3	2	n/a	Westneat and Hall 1988; Weijs and Dantuma 1975
Rabbit (<i>Oryctolagus cuniculus</i>)	1	3	2	1	Liu et al. 1993; Naganuma et al. 2001
Cat (<i>Felis domesticus</i>)	2	3	4	1	Hiiemae et al. 1981 Thexton and McGarrick 1994
Human (<i>Homo sapiens</i>)	1	2	3	1	Palmer et al. 1992

Notes. In basal bony fishes, including the lungfish (*Lepidosiren*) (light and medium grey), the hyoid retractor muscle (HR) fires before the hyoid protractor (HP) or the jaw adductor (JA). In amniotes, including lizards and mammals (dark grey), the hyoid protractor typically fires simultaneously with the jaw adductor (JD) muscle, but prior to the hyoid retractor.

*Hypaxial and epaxial trunk musculature in fishes (see text).

we hypothesize that they could include different material properties of the bolus, with different associated requirements of rearranging the bolus during chewing, and rumination in the herbivorous artiodactyls. There may be as of yet unrecognized differences in morphology of the hyoid suspension, and

the clear differences in length of the hyoid strap muscles, due to neck elongation in the herbivorous artiodactyls (Fig. 2) might place different functional requirements, on the variance in activity patterns of the protracting and retracting strap muscles of the hyoid.

In contrast, when evaluating differences in the MAP of mandible movement, as is traditionally done in studies of chewing in mammals, we found that the MAP of chewing in the pig is less coordinated than in the herbivores. This finding alone would lead us to accept the hypothesis that the level of coordination in chewing MAP is related to trophic status. This finding corresponds well with earlier findings of pigs lacking a precise post-canine occlusion and using bilateral grinding with frequent reversals of the masticatory power-stroke between chews (Herring 1976; Brainerd et al. 2010). In contrast, goats and alpacas have precisely fitting selenodont teeth used in the shearing of fibrous foods, which necessarily will constrain variability in jaw movements during the power stroke of the chewing cycle. If the power stroke of the chewing cycle is more variable in pigs, then there is likely a concomitant change in the variability of jaw abduction, like in primates (Ross et al. 2010). Indeed, the variable activity-pattern in the anterior digastric muscle (Fig. 3) seems to support this idea. In contrast, the constrained power stroke in goats and alpacas as a result of their occlusal morphology should result in relatively minor changes in the overall durations of the phases of jaw abduction and adduction during chewing, producing a more stereotyped chewing cycle. This notion is supported by ontogenetic data from goats, alpacas and rabbits showing that changes in the duration of the chewing cycle and changes in occlusion may be related, and that tooth wear during development may create facets that facilitate shearing (Weijs et al. 1989; Stover and Williams 2011). Together, our data demonstrate differences in the variability of hyoid versus jaw MAPs.

Differences in coordination of chewing MAPs across jaw-bearing vertebrates

Variance in the pair-wise coordination of peak activity in the jaw and hyoid musculature during chewing in basal bony fishes is generally lower than in artiodactyl mammals, often by an order of magnitude (Table 1). Among fishes, taxa that have chewing MAPs characterized by exceptionally low levels of variance are those that also utilize raking to reduce their food. Apparently, the inclusion of additional processing mechanisms in the feeding repertoire impacts the variability of the ancestral behavior (see Konow and Sanford 2008b; Konow et al. 2008).

We found some support for our hypothesis that the robust bony suspension of the fish hyoid would be powered by a less variable MAP than would the muscular sling that suspends the hyoid in mammals.

However, our data did not support the idea that the gracile jaw suspension of fishes, involving fewer degrees of freedom in architecture of the jaw muscles, results in more variable actuation of the jaw than in mammals, which have a robust suspension of the jaw and a more complex jaw adductor musculature. The latter may be due to constraints placed on the rhythmicity of oral jaw movements by the aquatic medium (see Gintof et al. 2010).

A shift in chewing MAP following the fish-tetrapod split

Our meta-analysis of timing of activity-onset in EMG data for the jaw and hyoid muscles during chewing reveals differences in the hyoid protraction-retraction sequence between anamniotes (bony fishes) and amniotes (lepidosaurs and mammals). During chewing, basal aquatic-feeding gnathostomes first activate their hyoid retractor, and then their hyoid protractor, while the jaw adductor is activated along with the hyoid protractor (cf. Lauder 1980; Konow and Sanford 2008b; Konow et al., 2008). On the contrary, in amniotes, the hyoid protractor is activated first, then the jaw is closed, and finally the hyoid retractor is activated (Crompton et al. 1975; Westneat and Hall 1992; Liu et al. 1993). If these divergent patterns of onset of activity in the hyoid musculature were to be considered purely in the context of concentric (shortening) muscle contraction, then the observed MAPs would translate into loop motion patterns of the hyoid during chewing with opposite directions in anamniotes and amniotes. Additionally, the pattern of hyoid motion in amniotes is shifted in timing relative to the jaw abduction-adduction cycle.

However, inferences about the pattern of hyoid motion based exclusively on onset patterns of EMG activity are complicated by the fact that hyoid strap muscles often undergo complex patterns of contraction. For instance, the sternohyoid often lengthens during feeding in a number of taxa, e.g. during suction feeding in Largemouth bass (Carroll 2004), some catfishes (Van Wassenbergh et al. 2005) and Rainbow trout (Konow and Sanford 2008b), but also among amniotes, such as during food capture and chewing in agamid lizards (Herrel et al. 2008), during chewing in *Didelphis* (Crompton et al. 1975) and during swallowing in the pig (Konow et al. 2010). This places a priority on future studies involving EMG recordings of hyoid muscle activity synchronized with visualization of hyoid motion patterns, for instance using XROMM (Brainerd et al. 2010).

The identical patterns of onset of activity in hyoid muscles during chewing in basal bony fishes, notably including the lungfish, strongly suggest that the MAP-shift must have occurred around the aquatic-terrestrial transition in a stem-tetrapod taxon. We propose that studies of the robustness of the hyoid skeleton might help pin-point the taxon-level at which the shift in MAP occurred. Shifts in MAP, which we define as a reorganization of EMG in two or more muscles driving a discrete behavior, are to be expected at major transitions (Alfaro and Herrel 2001; Wainwright 2002). Yet, such shifts have very rarely been quantified (Grubich 2000; Alfaro et al. 2001; Wainwright 2002; Konow and Sanford 2008b). Instead, MAPs are enigmatic in that they are conserved in deep phylogeny (Wainwright et al. 1989; Wainwright 2002) while labile at higher levels (Lauder and Shaffer 1988; Smith 1994). There are several possible explanations why shifts in MAP rarely are detected. Previous studies may have been biased from: (1) insufficiently broad sampling across phylogeny; (2) inadvertent focus on taxa with an extraordinarily high degree of conservation of MAP; (3) insensitivity in conventional time-series analyses to subtle changes in coordination of MAPs, and specifically for chewing behavior; and (4) a lack of comparative studies of coordination between the EMGs of the jaw and hyoid muscles. Another alternative, of course, is that conservation of MAPs in deep phylogeny is a true physiological phenomenon.

The power of evolutionary comparative analyses grows as a function of increased coverage of taxa (Harvey and Pagel 1991). The present study is limited in this regard due to the considerable difficulties involved with generating phylogenetic depth in analyses of complex physiological measurements for comparative evolutionary studies. These difficulties are reflected by the fact that synchronously recorded EMG data from jaw and hyoid muscles during chewing only exist for five taxa from four divergent orders, out of the 29 species from nine orders already represented in the FEED database (Wall et al. 2011). Our study has added primary data from three artiodactyls to this sample, and showed that cross-correlation analysis is a useful methodological complement to conventional time-series analyses of EMG. More importantly, by exploring jaw and hyoid MAP in unison and across deep phylogeny, we reveal what potentially is a rich history of variation in form and function at diverse phylogenetic levels. Our results suggest that collection of additional data could lead to identification of the taxon-level at which the MAP-shift occurred.

Acknowledgments

Thanks to Gregory Kurtz for help with MATLAB scripts, to Shaina Holman, JoAnna Sidote, and Jillian Davis for animal wrangling, to Harold Heatwole for stylistic changes, and to Rui Diogo and one anonymous referee for their insightful suggestions, which greatly improved the paper. We appreciate that NESCent, the steering committee of the FEED working-group and the Society for Integrative and Comparative Biology made this symposium possible.

Funding

National Science Foundation IOB-0520855 (to S.H.W.), National Institute of Health DC-03604 (to R.Z.G.), National Science Foundation IOB-0444891, and DBI-0420440 (to C.P.J.S.).

References

- Aerts P, Osse JWM, Verraes W. 1987. Model of jaw depression during feeding in *Astatotilapia elegans* teleostei cichlidae mechanisms for energy storage and triggering. *J Morph* 194:85–109.
- Agarsson I, May-Collado LJ. 2008. The phylogeny of Cetartiodactyla: the importance of dense taxon sampling, missing data, and the remarkable promise of cytochrome b to provide reliable species-level phylogenies. *Mol Phylogenet Evol* 48:964–85.
- Alfaro ME, Herrel A. 2001. Introduction: major issues of feeding motor control in vertebrates. *Am Zool* 41:1243–7.
- Alfaro ME, Janovetz J, Westneat MW. 2001. Motor control across trophic strategies: muscle activity of biting and suction feeding fishes. *Am Zool* 41:1266–79.
- Alfaro ME, Santini F, Brock C, Alamillo H, Dornburg A, Rabosky DL, Carnevale G, Harmon LJ. 2009. Nine exceptional radiations plus high turnover explain species diversity in jawed vertebrates. *Proc Natl Acad Sci USA* 106:13410–4.
- Anapol F. 1988. Morphological and videofluorographic study of the hyoid apparatus and its function in the rabbit (*Oryctolagus cuniculus*). *J Morph* 195:141–57.
- Anderson PSL. 2008. Cranial muscle homology across modern gnathostomes. *Biol J Linn Soc* 94:195–216.
- Basmajian J, Stecko G. 1962. A new bipolar electrode for electromyography. *J Appl Phys* 17:849.
- Bemis WE, Lauder GV. 1986. Morphology and function of the feeding apparatus of the lungfish, *Lepidosiren paradoxa* (Dipnoi). *J Morph* 187:81–108.
- Brainerd EL, Baier DB, Gatesy SM, Hedrick TL, Metzger KA, Gilbert SL, Crisco JJ. 2010. X-ray reconstruction of moving morphology (XROMM): precision, accuracy and applications in comparative biomechanics research. *J Exp Zool A* 313:262–79.

- Bramble DM, Wake DB. 1985. Feeding mechanisms in lower tetrapods. In: Hildebrand M, Bramble DM, Liem KF, Wake DB, editors. *Functional Vertebrate Morphology*. Cambridge: Belknap Press. p. 230–61.
- Carroll AM. 2004. Muscle activation and strain during suction feeding in the largemouth bass *Micropterus salmoides*. *J Exp Biol* 207:983–91.
- Crompton AW, Cook P, Hiiemae K, Thexton AJ. 1975. Movement of the hyoid apparatus during chewing. *Nature* 258:69–70.
- Diogo R, Abdala V, Lonergan N, Wood BA. 2008. From fish to modern humans - comparative anatomy, homologies and evolution of the head and neck musculature. *J Anat* 213:391–424.
- Edgeworth FH. 1935. *The cranial muscles of vertebrates*. London: Cambridge University Press.
- Franks HA, German RZ, Crompton AW, Hiiemae KM. 1985. Mechanism of intra-oral transport in a herbivore, the hyrax (*Procavia syriacus*). *Arch Oral Biol* 30:539–44.
- Gintof C, Konow N, Ross CF, Sanford CPJ. 2010. Rhythmic chewing with oral jaws in teleost fishes: a comparison with amniotes. *J Exp Biol* 213:1868–75.
- Grubich JR. 2000. Crushing motor patterns in drum (Teleostei: Sciaenidae): functional novelties associated with molluscivory. *J Exp Biol* 203:3161–76.
- Harvey PH, Pagel MD. 1991. *The comparative method in evolutionary biology*. Oxford: Oxford University Press.
- Herrel A, Cleuren J, Vree F. 1996. Kinematics of feeding in the lizard *Agama stellio*. *J Exp Biol* 199:1727–42.
- Herrel A, Cleuren J, Vree F. 1997. Quantitative analysis of jaw and hyolingual muscle activity during feeding in the lizard *Agama stellio*. *J Exp Biol* 200:101–15.
- Herrel A, Meyers JJ, Nishikawa KC, Vree FD. 2001. The evolution of feeding motor patterns in lizards: modulatory complexity and possible constraints. *Am Zool* 41:1311–20.
- Herrel A, Schaerlaeken V, Ross CF, Meyers J, Nishikawa K, Abdala V, Manzano A, Aerts P. 2008. Electromyography and the evolution of motor control: limitations and insights. *Integr Comp Biol* 48:261–71.
- Herrel A, Verstappen M, De Vree F. 1999. Modulatory complexity of the feeding repertoire in scincid lizards. *J Comp Phys A* 184:501–8.
- Herring SW. 1976. The dynamics of mastication in pigs. *Arch Oral Biol* 21:473–80.
- Herring SW. 1993. Functional morphology of mammalian mastication. *Am Zool* 33:289–99.
- Hiiemae KM. 1976. Masticatory movements in primitive mammals. In: Anderson DJ, Matthews B, editors. *Mastication*. Bristol: John Wright and Sons, Ltd. p. 105–18.
- Hiiemae KM. 1978. Mammalian mastication: a review of the activity of the jaw muscles and the movements they produce in chewing. In: Butler P, Joysey K, editors. *Studies on the Development, Structure and Function of Teeth*. London: Academic Press. p. 359–98.
- Hiiemae KH, Thexton A, McGarrick J, Crompton AW. 1981. The movement of the cat hyoid during feeding. *Arch Oral Biol* 26:65–81.
- Ishiguro NB, Miya M, Nishida M. 2003. Basal euteleostean relationships: a mitogenomic perspective on the phylogenetic reality of the “Protacanthopterygii”. *Mol Phylogen Evol* 27:476–88.
- Kayalioglu M, Shcherbaty V, Seifi A, Liu ZJ. 2007. Roles of intrinsic and extrinsic tongue muscles in feeding: electromyographic study in pigs. *Arch Oral Biol* 52:786–96.
- Klingener D. 1972. *Laboratory anatomy of the mink*. 2nd Edition. Dubuque, Iowa: Wm. C. Brown Co.
- Konow N, Camp AL, Sanford CPJ. 2008. Congruence between muscle activity and kinematics in a convergently derived prey-processing behavior. *Integr Comp Biol* 48:246–60.
- Konow N, Sanford CPJ. 2008a. Biomechanics of a convergently derived prey-processing mechanism in fishes: evidence from comparative tongue bite apparatus morphology and raking kinematics. *J Exp Biol* 211:3378–91.
- Konow N, Sanford CPJ. 2008b. Is a convergently derived muscle-activity pattern driving novel raking behaviours in teleost fishes? *J Exp Biol* 211:989–99.
- Konow N, Thexton A, Crompton AW, German RZ. 2010. Regional differences in length change and electromyographic heterogeneity in sternohyoid muscle during infant mammalian swallowing. *J Appl Physiol* 109:439–48.
- Langenbach GEJ, van Eijden TMGJ. 2001. Mammalian Feeding Motor Patterns. *Am Zool* 41:1338–51.
- Lauder G V. 1979. Feeding mechanics in primitive teleosts and in the halecomorph fish *Amia calva*. *J Zool London* 187:543–78.
- Lauder GV. 1980. Evolution of the feeding mechanism in primitive actinopterygian fishes: a functional anatomical analysis of *Polypterus*, *Lepisosteus*, and *Amia*. *J Morph* 163:283–317.
- Lauder GV, Shaffer HB. 1988. Ontogeny of functional design in tiger salamanders (*Ambystoma tigrinum*) - are motor patterns conserved during major morphological transformations. *J Morph* 197:249–68.
- Liem KF. 1978. Modulatory multiplicity in the functional repertoire of the feeding mechanism in cichlid fishes. *J Morph* 158:323–60.
- Liu ZJ, Masuda Y, Inoue T, Fuchihata H, Sumida A, Takada K, Morimoto T. 1993. Coordination of cortically induced rhythmic jaw and tongue movements in the rabbit. *J Neurophysiol* 69:569–84.
- Loeb GE, Yee WJ, Pratt CA, Chanaud CM, Richmond FJR. 1987. Cross-correlation of EMG reveals widespread synchronization of motor units during some slow movements in intact cats. *J Neurosci Methods* 21:239–49.
- Miller ME, Evans HE, Christensen GC. 1979. *Miller's Anatomy of the Dog*. Philadelphia: Saunders.
- Muller M. 1987. Optimization principles applied to the mechanism of neurocranium levation and mouth bottom

- depression in bony fishes (Halecostomi). *J Theor Biol* 126:343–68.
- Naganuma K, Inoue M, Yamamura K, Hanada K, Yamada Y. 2001. Tongue and jaw muscle activities during chewing and swallowing in freely behaving rabbits. *Brain Res* 915:185–94.
- Oron U, Crompton AW. 1985. A cineradiographic and electromyographic study of mastication in *Tenrec ecaudatus*. *J Morphol* 185:155–82.
- Palmer JB, Rudin NJ, Lara G, Crompton AW. 1992. Coordination of mastication and swallowing. *Dysphagia* 7:187–200.
- Pérez LM, Toledo N, De Iuliis G, Bargo MS, Vizcaíno SF. 2010. Morphology and function of the hyoid apparatus of fossil xenarthrans (Mammalia), Vol. 271. Wiley Subscription Services, Inc, A Wiley. Company. p. 1119–33.
- Price SA, Bininda-Emonds OR, Gittleman JL. 2005. A complete phylogeny of the whales, dolphins and even-toed hoofed mammals (Cetartiodactyla). *Biol Rev Camb Philos Soc* 80:445–73.
- Reilly SM, Lauder GV. 1990. The evolution of tetrapod feeding behavior: kinematic homologies in prey transport. *Evolution* 44:1542–57.
- Reilly SM, McBrayer LD, White TD. 2001. Prey processing in amniotes: biomechanical and behavioral patterns of food reduction. *Comp Biochem and Physiol A Mol Integr* 128:397–415.
- Ross CF, Baden AL, Georgi J, Herrel A, Metzger KA, Reed DA, Schaerlaeken V, Wolff MS. 2010. Chewing variation in lepidosaurs and primates. *J Exp Biol* 213:572–84.
- Ross CF, Eckhardt A, Herrel A, Hylander WL, Metzger KA, Schaerlaeken V, Washington RL, Williams SH. 2007. Modulation of intra-oral processing in mammals and lepidosaurs. *Integr Comp Biol* 47:118–36.
- Sanford CPJ, Lauder GV. 1990. Kinematics of the tongue-bite apparatus in osteoglossomorph fishes. *J Exp Biol* 154:137–62.
- Schwenk K, Throckmorton GS. 1989. Functional and evolutionary morphology of lingual feeding in squamate reptiles - phylogenetics and kinematics. *J Zool* 219:153–75.
- Smith KK. 1984. The use of the tongue and hyoid apparatus during feeding in lizards (*Ctenosaura similis* and *Tupinambis nigropunctatus*). *J Zool* 202:115–43.
- Smith KK. 1986. Morphology and function of the tongue and hyoid apparatus in *Varanus* (varanidae, lacertilia). *J Morph* 187:261–87.
- Smith KK. 1994. Are neuromotor systems conserved in evolution? *Brain Behav Evol* 43:293–305.
- So KKJ, Wainwright PC, Bennett AF. 1992. Kinematics of prey processing in *Chamaeleo jacksonii* - conservation of function with morphological specialization. *J Zool* 226:47–64.
- Stover KK, Williams SH. 2011. Intraspecific scaling of chewing cycle duration in three species of domestic ungulates. *J Exp Biol* 214:104–12.
- Takada Y, Izumi M, Gotoh K. 2009. Comparative anatomy of the hyoid apparatus of carnivores. *Mammal Study* 34:213–8.
- Thexton AJ. 1984. Jaw, tongue and hyoid movement—a question of synchrony? Discussion paper. *J R Soc Med* 77:1010–9.
- Thexton AJ. 1996. A randomisation method for discriminating between signal and noise recordings of rhythmic electromyographic activity. *J Neurosci Methods* 66:93–8.
- Thexton AJ, McGarrick JD. 1994. The electromyographic activities of jaw and hyoid musculature in different ingestive behaviours in the cat. *Arch Oral Biol* 39:599–612.
- Van Wassenbergh S, Herrel A, Adriaens D, Aerts P. 2005. A test of mouth-opening and hyoid-depression mechanisms during prey capture in a catfish using high-speed cineradiography. *J Exp Biol* 208:4627–39.
- Wainwright PC. 2002. The evolution of feeding motor patterns in vertebrates. *Curr Opin Neurobiol* 12:691–5.
- Wainwright PC, Mehta RS, Higham TE. 2008. Stereotypy, flexibility and coordination: key concepts in behavioral functional morphology. *J Exp Biol* 211:3523–8.
- Wainwright PC, Sanford CPJ, Reilly SM, Lauder GV. 1989. Evolution of motor patterns aquatic feeding in salamanders and ray-finned fishes. *Brain Behav Evol* 34:329–41.
- Wall et al. 2011. Feeding Experiments End-user Database (<http://www.feedexp.org/>).
- Weijs WA, Dantuma R. 1975. Electromyography and mechanics of mastication in the albino rat. *J Morph* 146:1–33.
- Weijs WA, Brugman P, Grimbergen CA. 1989. Jaw movements and muscle activity during mastication in growing rabbits. *Anat Rec* 224:407–16.
- Weijs WA, Muhl ZF. 1987. The effects of digastric muscle tenotomy on jaw opening in the rabbit. *Arch Oral Biol* 32:347–53.
- Westneat MW. 1990. Feeding mechanics of teleost fishes (Labridae, Perciformes): a test of four-bar linkage models. *J Morph* 205:269–96.
- Westneat MW, Hall WG. 1992. Ontogeny of feeding motor patterns in infant rats: an electromyographic analysis of suckling and chewing. *Behav Neurosci* 106:539–54.
- Wilga CD, Wainwright SA, Motta PJ. 2000. Evolution of jaw depression mechanics in aquatic vertebrates: insights from Chondrichthyes. *Biol J Linn Soc* 71:165–85.
- Williams SH, Stover KK, Davis JS, Montuelle SJ. Mandibular corpus bone strains during mastication in goats (*Capra hircus*): a comparison of ingestive and rumination chewing. *Arch Oral B*. In press (doi:10.1016/j.archoralbio.2011.02.014).
- Wren TAL, Do KP, Rethlefsen SA, Healy B. 2006. Cross-correlation as a method for comparing dynamic electromyography signals during gait. *J Biomech* 39:2714–8.