Pairwise modulation of jaw muscle activity in two species of elasmobranchs

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Abstract

Most studies of the function of feeding muscles in fish have implanted electromyogram electrodes unilaterally to understand the motor pattern associated with a behavior. The few studies that have implanted bilaterally have found that paired muscles may be activated asynchronously, often resulting in visible kinematic asymmetry. We investigated modulation of pairwise asynchrony (modulation in the activation patterns of left and right members of a muscle) of feeding muscles during capture and processing of two types of prey in spiny dogfish *Squalus acanthias* and little skates *Leucoraja erinacea* (Elasmobranchii). Two asynchrony indices quantified the degree to which muscles in a pair were activated out of phase (lag index, AI_{lag}) and the degree to which durations differ (duration index, AI_{dur}). Feeding behaviors for both species were compared according to these indices and total event duration using principal components analysis. Both species modulated pairwise asynchrony according to prey type, exhibiting more asynchronous motor patterns when feeding on more complex prey items (those requiring more processing); however, the motor patterns underlying this asynchrony differed between species. Dogfish process complex prey using head-shaking, which requires alternating activation of contralateral head muscles (i.e. high lag index value). In contrast, little skates process complex prey using a completely unilateral behavior in which prey is moved to one corner of the jaws and jaw muscles are activated on that side only (i.e. high duration index value). Asynchronous behaviors are not detected by unilateral implants; our data demonstrate the importance of bilateral implantation to identify fine details of feeding motor patterns, particularly those involving complex prey.

Introduction

A diverse diet suggests a range of feeding structures, a suite of behaviors, or both. As an example of the former, teeth may vary in shape (i.e. heterodont dentition) providing different functional occlusal surfaces. However, many fish species lack heterodont dentitions and therefore can only vary feeding behaviors when faced with multiple prey types. Modulation of feeding behaviors allows a generalist fish predator to diversify prey capture strategies and broaden its diet by increasing the range or size of consumed prey items (Liem, 1980; Lauder, 1981; Ferry-Graham, 1997). Many fish modulate motor patterns and kinematic behaviors according to prey type (Liem, 1978; Friel & Wainwright, 1999), prey size (Liem, 1978; Ferry-Graham, 1998), and the stage of the feeding cycle (e.g. capture or processing; Liem, 1978, 1979, 1980; Lauder & Norton, 1980; Friel & Wainwright, 1999).

Studies of modulation of muscle activity during feeding in fish have largely relied on unilateral recordings (from electrodes implanted on one side of the head only), focusing on modulation of the timing of activation of one muscle relative to another on the same side of the head (behavioral modulation) to understand the activation pattern behind a kinematic behavior (e.g. Motta *et al.*, 1997; Wilga & Motta, 1998*a,b*, 2000; Friel & Wainwright, 1999). By comparison, pairwise modulation (modulation in activation patterns of left and right members of a muscle pair), another source of functional variation, has received limited attention. Feeding studies in bony fish using bilateral implantation have found that species from two disparate groups of actinopterygians are capable of modulating the synchrony of activation of members of a muscle pair according to the stage of the feeding cycle (Liem, 1978, 1979, 1980; Lauder & Norton, 1980). In these studies, prey processing and manipulation, which involved biting behaviors in all species, elicited more pairwise asynchrony (i.e. greater delay between onset times of left and right members of a muscle pair) than did capture behaviors. This suggests that pairwise modulation may be

an important behavioral tool that increases the flexibility of the feeding apparatus for predators, and that pairwise asynchrony may be more prevalent when fish feed on more complex prey items, such as those requiring the shearing or tearing action of the teeth to rip prey. This points to a possible mechanical function for pairwise asynchrony in fish: if we assume asynchronous muscle activation causes asymmetrical jaw movements, it may result in the bilaterally asymmetric application of tooth forces of differing magnitude, timing and/or direction for reducing prey.

In this study, we investigate modulation of pairwise synchrony using bilateral electrode implantation in jaw muscles of two species of generalist elasmobranch predators feeding on two prey types. Spiny dogfish and little skates are trophic generalists that feed on a variety of prey items (dogfish: small fish, shrimp, mollusks; skates: shrimp, amphipods, polychaetes, mollusks, fish; Jones & Geen, 1977; Bowman *et al.*, 2000; Steimle *et al.*, 2000). The dietary versatility of these species implies functional versatility of the feeding system, and spiny dogfish and little skates do modulate the kinematics of capture behaviors between suction and biting (Wilga & Motta, 1998*a*; Wilga, Motta & Sanford, 2007). A previous study (Gerry *et al.*, 2008) has shown that both species employ greater asynchronous activation in response to complex prey. We believe the single metric [asynchrony index (AI)] used to quantify asynchronous behavior has limited utility in showing true differences among species and behaviors. In this study, we improve the methods of the previous work, presenting a suite of metrics for describing the axes of variation that characterize asynchronous muscle activation in feeding elasmobranchs; we expect these to be useful in studies of other taxa.

Materials and methods

Study animals

Six spiny dogfish *Squalus acanthias* (Linnaeus), (73–82 cm total length) were collected by otter trawl in Narragansett Bay, Rhode Island or off the coast of Woods Hole, MA, USA. Sharks were housed in a circular, flow-through seawater tank (3 m diameter) at 15 °C. Sharks were fed herring *Clupea harengus*, cut to one-half mouth width or squid pieces, *Loligo* spp., twice weekly to satiation.

Three little skates *Leucoraja erinacea* (Mitchill), (25–28 cm disc width) were collected by otter trawl in Narragansett Bay, Rhode Island, USA. Skates were housed in a circular, re-circulating seawater tank (1.2 m diameter) at 15 °C. Skates were fed silverside (*Menidia menidia*), squid (*Loligo* spp.) or shrimp pieces (*Penaeus* spp.) three times weekly to satiation.

Electromyography and video recording

Electromyograms (EMGs) were recorded using bipolar electrodes constructed from 2–3 m lengths of 0.002-cm-diameter insulated alloy wire (California Fine Wire Co., Grover Beach, CA, USA), stripped of insulation 0.05 cm from the end, and anchored into the muscle by forming

short hooks with the exposed wires. A second 5 cm length of insulated wire was implanted alongside the first electrode to verify electrode placement in case the electrode was pulled out. Electrodes were implanted into cranial muscles that control jaw movements using 24 G hypodermic needles. All muscles were implanted bilaterally (i.e. in both members of the pair) except the coracomandibularis, which served as the reference muscle.

Sharks were anesthetized using $0.175\,\text{g}\,\text{L}^{-1}$ tricaine methanesulfonate (MS-222), then maintained on a dosage of $0.058\,\text{g}\,\text{L}^{-1}$ pumped over the gills during surgery using a re-circulating pump. EMGs were recorded from nine cranial muscles known to function during feeding (Wilga & Motta, 1998*a*): left/right anterior division of the dorsal quadrato-mandibularis (jaw adductor); left/right ventral quadrato-mandibularis (jaw adductor); left/right preorbitalis (upper jaw protractor and jaw adductor); left/right epaxialis (head elevator); coracomandibularis (lower jaw depressor) (Fig. 1).

Skates were anesthetized using $0.10\,\text{g}\,\text{L}^{-1}$ MS-222, then maintained on a dosage of $0.05\,\text{g}\,\text{L}^{-1}$ during surgery. The following muscles were recorded in skates because they are believed homologous to those recorded here in dogfish (Miyake, McEachran & Hall, 1992; Wilga & Motta, 1998*b*; Dean & Motta, 2004*b*): left/right anterior quadratomandibularis (jaw adductor); left/right posterior quadratomandibularis (jaw adductor); left/right preorbitalis lateralis (upper
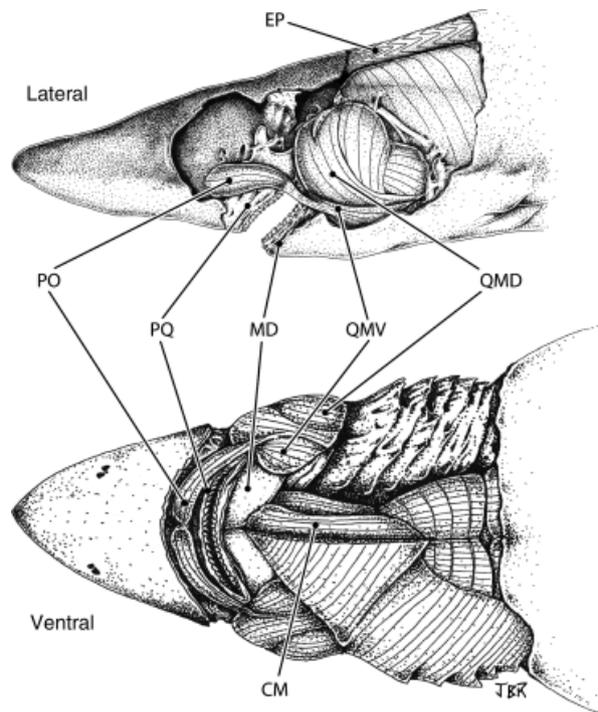
Figure 1 Left lateral and ventral view of the head of a spiny dogfish *Squalus acanthias*, with the eye removed to show implanted cranial muscles. Muscle fiber direction is indicated. QMD, anterior division of dorsal quadratomandibularis; QMV, ventral quadratomandibularis; PO, preorbitalis; EP, epaxialis; CM, coracomandibularis; PQ, palatoquadrate or upper jaw; MD, mandible or lower jaw.

jaw protractor); coracomandibularis (jaw depressor) (Fig. 2). After implantation, the electrode wires were tied to a suture loop fixed in the dorsal body skin and all wires glued together to form a single cable. Surgery lasted *c*. 30 min.

Following surgery, fish were returned to the experimental tank and monitored for normal swimming behavior. Electrode wires were inserted into a multi-pin connector joined to 3 m low-noise cables attached to 16 channel differential AC amplifiers (model 1700, A-M Systems, Sequim, WA, USA) at a gain of 10 000, bandpass 10–10 000 Hz with 60 Hz notch filter. Muscle activation was recorded simultaneously using an AD Instruments Powerlab 16SP analog to digital converter and CHARTVIEW software (AD Instruments, Colorado Springs, CO, USA). Feeding trials were initiated after normal swimming behavior was resumed, at least 1 h post-recovery.

During feeding trials, individuals were fed two functionally different prey types, chosen because we expected them to require different capture and processing behaviors (Verwaijen, Van Damme & Herrel, 2002; Dean, Bizzaro & Summers, 2007). We considered prey items smaller than one-half mouth width to be 'simple' (i.e. require less varied capture and processing motor patterns) and those larger than mouth width to be 'complex' (i.e. require more varied capture and processing motor patterns). Sharks were offered pieces of herring cut from the muscular body of the fish and scaled to one-half mouth width (simple) or half of a herring including the skeletal elements of the head or tail (complex). Skates were offered pieces of shrimp tail with the carapace

intact and either scaled to one-half mouth width and lacking the telson and uropods (simple) or larger than mouth width and including the telson and uropods (complex). We are interested in whether pairwise asynchrony is modulated according to prey type; however, it should be noted that in this design, size and complexity are potentially confounding prey variables (e.g. a 'complex' piece of shrimp is both large and has more inedible structures to remove).

A Photron Fastcam 1280 PCI model high-speed video system (Photron USA Inc., San Diego, CA, USA) was used to record video images (recording rate: 125 frames s^{-1}, shutter: 1/1000 frames s^{-1}, resolution: 1280 × 256) during the skate experiments. The Photron was synchronized to the Powerlab by a data-acquisition unit (National Instruments, Austin, TX, USA). Skates cup the prey with the pectoral fins while feeding; therefore, feeding behaviors were filmed in ventral view from a 45°-angled mirror to identify feeding behaviors. Because sharks have mouths that are readily observable laterally, visual observations of dogfish feeding modes were annotated to EMG sequences.

At the termination of each experiment, the animal was euthanized by MS-222 overdose according to University of Rhode Island IACUC guidelines. Electrode positions were verified by dissection. EMGs of sharks were analyzed for simple (23 events, $n = 6$) and complex prey (biting: 11 events, $n = 5$; with head-shaking: 14 events, $n = 3$) behaviors. EMGs of skates were analyzed for simple (53 events, $n = 3$) and complex prey (101 events, $n = 3$) behaviors.

Asynchrony index (AI)

Each behavioral event was considered to begin with the onset of lower jaw depression (i.e. coracomandibularis activity = time zero) and end with the offset of the last muscle active before the next contraction of the coracomandibularis, indicating the next gape cycle. To compare across behavioral events, the onset, offset and duration (in ms) for individual muscle bursts were calculated relative to the onset of activity in the coracomandibularis for that event. In one behavior type (dogfish head-shaking; see 'Results'), the coracomandibularis was not active; thus, bursts were standardized to the right dorsal quadratomandibularis, which was active in all head-shaking events.

Behavioral events could be characterized by coracomandibularis onset followed by a series of muscle pair events (pairwise activations), except for head shaking. For each muscle pair event (i.e. activity of left and right members of a muscle pair), we qualified the following variables: the duration (D_1; ms) of activity of the first-active muscle; the duration (D_2; ms) of activity of the second-active muscle; the lag (L) between the onset of D_1 and D_2 (i.e. the onset of D_2 relative to D_1); the total event length (TE) or the elapsed time between the onset and offset of all activity for each muscle pair.

These variables contribute to two dimensionless indices to describe pairwise asynchrony; this improvement to our previously reported, single AI (Gerry *et al.*, 2008) accounts for differences in both phase relationship and duration of
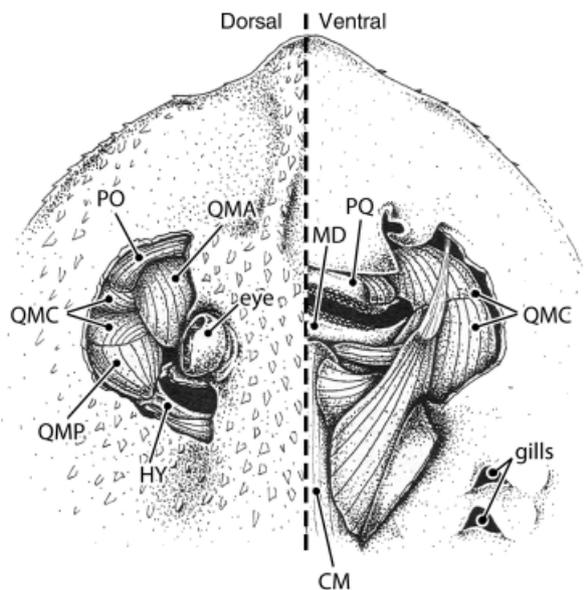
Figure 2 Dorsal and ventral view of the head of a little skate *Leucoraja erinacea* with fiber direction indicated to show implanted cranial muscles. Skin is left intact except where it overlays the muscles of interest. QMA, anterior quadratomanibularis; QMP, posterior quadratomandibularis; PO, preorbitalis lateralis; QMC, quadratomandibularis complex; HY, hyomandibula; CM, coracomandibularis; PQ, palatoquadrate or upper jaw; MD, mandible or lower jaw.

the two muscle bursts. The duration index,

$$AI_{dur} = \left| \frac{(D_1 - D_2)}{(D_1 + D_2)} \right|$$

increases as the bursts become more different in their durations, ranging from bursts of equal duration (0.00) to the extreme condition of unilateral activation (1.00), where the second muscle is never active (duration = 0.0 ms; Fig. 3).

The lag index,

$$AI_{lag} = \frac{L}{0.5 \times (D_1 + D_2)}$$

is the ratio of lag length to the average burst duration. This index begins at 0.0 for bursts with no lag (i.e. bursts that begin simultaneously) and increases without limit as bursts become more out of phase (i.e. as L increases). An illustration of index values for different burst and lag lengths is shown in Fig. 3. Both indices are normalized to the combined durations of antimere activity (i.e. $D_1 + D_2$ is in the denominator of both indices) and therefore are inherently related; however, they are separable parameters because
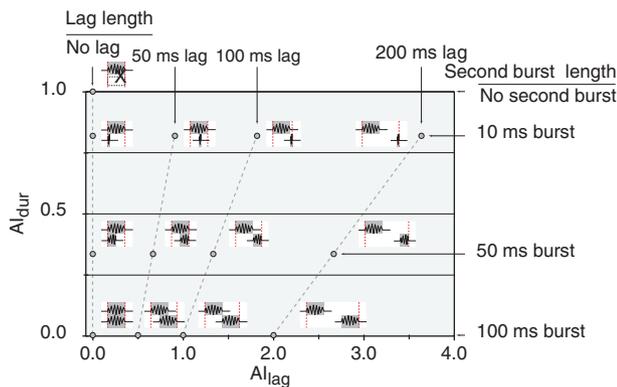
Figure 3 Diagram of the effect of muscle burst synchrony and duration on the two asynchrony indices used in this study: duration number (AI_{dur}, describing relative difference in burst durations) and lag number (AI_{lag}, describing the lag between the onsets of the two muscle bursts). Values of these indices can change independent of one another and so each point on the graph represents a particular combination of AI_{dur} (*y*-axis) and AI_{lag} (*x*-axis); the lag between the two bursts increases to the right of the figure (i.e. high AI_{lag}) and the duration of the second burst decreases toward the top of the figure (i.e. high AI_{dur}), reaching a maximum of $AI_{dur} = 1.0$ when only one muscle is active (i.e. when the bursts are as dissimilar as possible in length). In the particular events depicted, the first-active muscle is always active for 100 ms; lag and second-active burst duration values are indicated in milliseconds at the top and right of the graph, respectively. The illustrations above each graph provide a pictorial representation of each hypothetical pairwise event; muscle bursts are illustrated as shaded bars with the total event time (TE) represented by the interval between red dashed lines. For example, the muscles in the pairwise event described by $AI_{dur} = 0.82/AI_{lag} = 0.00$ have similar onsets, but one of the muscles is active for 10 ms, making the total event 100 ms. The muscles in the pairwise event described by $AI_{dur} = 0.00/AI_{lag} = 2.00$ have similar durations but the second burst begins 200 ms after the first.

they can change independently of one another (e.g. pairs with the same AI_{lag} can have different AI_{dur} values; Fig. 3). Both indices are calculated relative to burst durations and absolute measures of time are lost; to account for this, TE were also compared for all events.

We are interested in whether asynchrony as a whole increases in relation to food type. To this end, AI_{lag}, AI_{dur}, and TE were calculated from all muscle pair-burst instances. In other words, a skate feeding trial where the anterior quadratomandibularis, posterior quadratomandibularis and preorbitalis were all active generated three pair-burst events, for each of which all three asynchrony variables were calculated. All recorded pair-burst events were compared in the subsequent analysis to provide an understanding of the overall dynamics of feeding events and examine whether, from a pairwise perspective, these exhibit some predictable changes with prey type. The variability in muscle activation patterns according to behavior/prey type and the loss of electrode attachment in some types of events resulted in inadequate statistical power to investigate differences by specific muscle group (e.g. whether skate preorbitalis asynchrony changes with more complex prey).

Statistics

Principal components analysis (PCA) was used to investigate sources of variation among dogfish and skate feeding behaviors according to the three variables above (AI_{dur}, AI_{lag}, TE). Before the PCA, data from each pair of variables (i.e. AI_{dur} vs. AI_{lag}, etc.) were plotted against one another in Draftsman's plots to verify lack of intervariable correlations. Because units differed among the three variables, data were analyzed in the PCA using a correlation matrix with Multivariate Statistical Package software (MVSP; version 3.13, Kovach Computing Services, Wales, UK).

Kaiser's rule determined the number of interpretable axes (Legendre & Legendre, 1983); the third axis explained $< 33.3\%$ of variation and was ignored. Component loadings of the different behaviors observed were compared along PC1 and PC2 using one-way ANOVAs followed by Tukey *post hoc* tests to determine whether simple and complex prey elicited different behaviors and whether these responses were characterized by differences in the three variables. We compared simple and complex prey events for both species and also including those dogfish events that involved a distinctive head-shaking behavior (see 'Results'). Behavioral modulation is indicated by statistically different responses to different stimuli (Sanford, 2001); since the AIs were represented on both axes (see 'Results'), we considered a significant difference in behaviors (e.g. skate feeding on simple vs. complex prey) along either axis as evidence of pairwise modulation. To gain an overall picture of how feeding activation patterns change with prey 'complexity,' feeding events were recorded in their entirety (i.e. from the first ingestion of prey to the last obvious feeding movement) and therefore included instances of capture and processing behaviors, as well as suction and biting feeding modes. As we focus here on broad-scale, prey-associated changes in overall asynchrony and due to the potential

loss of statistical power in sub-dividing our data with additional factors, we compared only between species and between prey type behaviors.

Results

Principal components (PCs) 1 and 2 were interpretable and accounted for 74.26% (PC1 = 39.77%, PC2 = 34.50%) of total variation. PC1 showed a divided effect for the three variables, with TE (0.578) and AI_{lag} (0.734) loading strongly and positively and AI_{dur} (−0.356) loading negatively and contributing comparatively less to the variation along that axis (Fig. 4). By contrast, PC2 was strongly positively polarized, with TE (0.576) and AI_{dur} (0.815) contributing significantly. The component loadings for the variables (Fig. 4) indicate how strongly their influences align with the principal axes themselves, but also designate unique portions of the resultant graph where each variable dominates (Fig. 4 inset). Data clustered in the upper left (at *c.* −30° on the graph) represent muscle pair events where the two bursts differed considerably in duration (high duration index values; i.e. unilateral biting); those in the upper right (*c.* +40°) represent events with long total event durations (high total event value); and those in the lower right (*c.* +95°) correspond to events where the two bursts were separated by a long lag phase (high lag index value).

Spiny dogfish simple and complex prey events (without head-shaking) did not differ from each other along either PC axis ($P > 0.05$, Table 1), however head-shaking events showed significantly greater values than both other behaviors along PC1 ($P < 0.001$, Table 1). Skate simple and complex events differed along PC2, with complex events exhibiting greater values ($P < 0.001$, Table 1). In general, dogfish events differed from skate events ($P < 0.001$; i.e. skate simple events differed from all dogfish events along both PCs; Table 1). Specifically, although skate and dogfish complex prey events (including head-shaking) exhibit some overlap, they largely occupy opposite ends of the parameter space, indicating that when feeding on more complex prey, skates tend toward unilateral biting (high duration index values) and dogfish toward head-shaking events where contralateral cranial muscles are activated more out of phase (high lag index values) (Fig. 4, Table 1). We note, however, that the level and type of 'complexity' for designated 'complex' food items may differ for the two predators and so other factors may drive this difference.

Behavioral descriptions

Although our analysis focused primarily on pairwise modulation according to prey type, we also summarize the observed behaviors' bilateral motor patterns to promote future work and facilitate comparisons with previous studies.

Spiny dogfish

Spiny dogfish modulated mode according to prey type: suction was used to capture and process simple prey items,
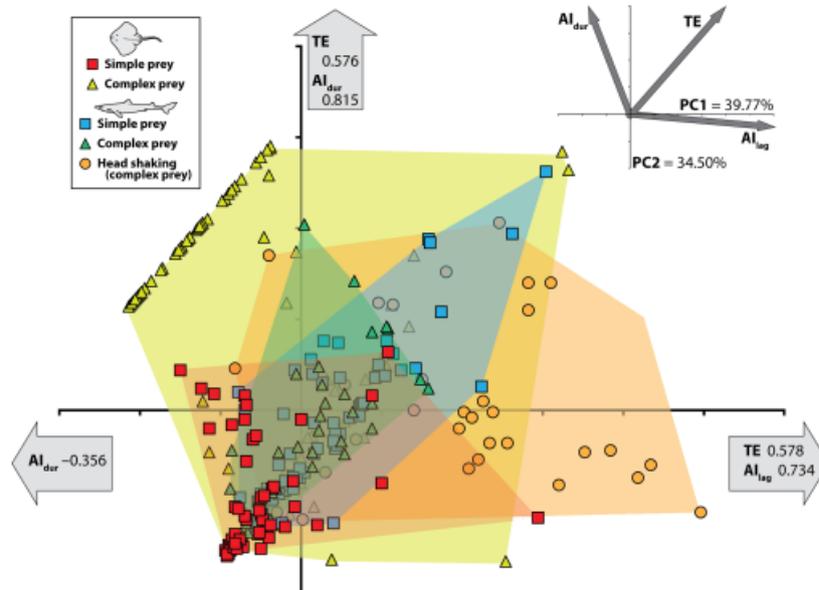
Figure 4 Plot of principal components 1 and 2 generated by the PCA, accounting for 39.77 and 34.50% of variation, respectively. Data for the two species are color-coded as indicated by the legend. Strong component loadings for the three included variables (AI_{dur}, AI_{lag}, TE) are listed in the large gray arrows adjacent to each axis, with the arrows' directions indicating directionality of loading (e.g. AI_{dur} loads negatively on PC1 and strongly and positively on PC2). The resultant loading vectors for the three variables are represented in the inset in the upper right, effectively partitioning the parameter space into three regions, each dominated by a single variable. For example, data in the upper left-hand portion of the graph (e.g. skate complex prey) are characterized by a high duration number, whereas data in the lower right-hand portion (e.g. dogfish head shaking) exhibit high lag number.

Table 1 One-way analysis of variances (ANOVAs) of pairwise comparisons from principal components analysis (PCA)

	Dogfish simple	Dogfish complex	Dogfish head-shake	Skate simple	Skate complex
PC1: AI_{lag} 0.734, AI_{dur} −0.356, TE 0.578					
Dogfish simple	1.000				
Dogfish complex	0.992	1.000			
Dogfish head-shake	<0.001	<0.001	1.000		
Skate simple	<0.001	0.022	<0.001	1.000	
Skate complex	<0.001	<0.001	<0.001	0.789	1.000
PC2: AI_{lag} −0.059, AI_{dur} 0.815, TE 0.576					
Dogfish simple	1.000				
Dogfish complex	1.000	1.000			
Dogfish head-shake	0.715	0.889	1.000		
Skate simple	0.002	0.010	<0.001	1.000	
Skate complex	<0.001	0.001	0.040	<0.001	1.000

Significance at $P<0.05$.

AI, asynchrony index; TE, total event length.

but complex prey were captured by biting and processed by a combination of biting and head-shaking. Suction (simple prey events: $AI_{dur} = 0.115$; $AI_{lag} = 0.137$; TE = 210 ms; Table 2) and biting (complex prey events, no head-shaking: $AI_{dur} = 0.217$; $AI_{lag} = 0.285$; TE = 172 ms; Table 2) were characterized by roughly similar motor patterns for the muscles observed: the onset of coracomandibularis and epaxialis muscle activity, followed by activation of the three jaw adductor muscles (preorbitalis, dorsal and ventral quadratomandibularis) (e.g. Fig 5, 'simple prey' and 'complex prey – biting'). These temporal muscle activation patterns are similar to those shown previously (Wilga & Motta, 1998a). Both biting and suction behaviors are comparatively synchronous (lag durations are <30% of the average burst duration = $AI_{lag} < 0.30$) and the difference in duration between the two bursts is <22% of the combined duration ($D_1 + D_2$) (i.e. $AI_{dur} < 0.22$) (Fig. 4). By comparison, the distinctive head-shaking behavior used by dogfish to process complex prey was the most asynchronous observed for this species ($P<0.001$, Table 1). Mean AI_{dur} values were only slightly larger than those for complex prey (0.264), however, mean AI_{lag} values were roughly four times larger (1.197), indicating that events involved a long lag period between bursts, on average 120% of the average burst duration.

During head shaking, the shark orients the prey in the center of the mouth and swings the head laterally from side to side to tear it (this study; Wilga & Motta, 1998a). This is a rapid, dynamic behavior, and one preorbitalis electrode was consistently displaced; thus, the pairwise contribution of this muscle to this behavior cannot be assessed. Head-shaking begins with synchronous activation of the paired epaxialis muscles followed by a pattern of cyclical asynchronous pairwise activation where both quadratomandibularis muscles on one side of the head are activated simultaneously and simultaneously with or just following the contralateral epaxial muscle (Group 1). This was followed by contralateral activation relative to Group 1 of all three muscle pairs (Group 2) and then reactivation of Group 1 (Fig. 5). For example, if the left dorsal and ventral quadratomandibularis

and right epaxialis muscles were activated first (Group 1) this would be followed by activation of the right dorsal and ventral quadratomandibularis and left epaxialis muscles (Group 2) and then reactivation of Group 1. Often, activation of Groups 1 and 2 overlapped slightly, when Group 2 onset slightly preceded Group 1 offset (Fig. 5).

There were no obvious patterns in total event length for subsequent burst pairs in a head-shaking event (i.e. burst pair events did not get longer or shorter during the head-shaking event). However, in most observed events, each subsequent burst showed a decrease in duration and lag numbers, indicating that bursts were becoming increasingly similar in length and more synchronous as food was reduced. This cyclical asynchronous pairwise activation was not observed in skates (i.e. skates do not reactivate the first-active member of a muscle pair after a bilaterally asynchronous event).

Little skates

Skates used suction and biting behaviors for simple and complex prey, unlike dogfish, where only suction is used to capture simple prey. Simple prey elicited behavior that was synchronous compared with skate complex prey events: index values indicate that lag was only 24% of average burst duration ($AI_{lag} = 0.236$) and the difference in duration between the two bursts is <17% of the combined duration ($AI_{dur} = 0.169$). In general, simple prey events were characterized by coracomandibularis activity slightly preceding simultaneous bilateral activation of the jaw adductor muscles (Fig. 5).

In contrast, skate complex prey feeding was distinguished by extreme asynchronous activity, in particular, high duration index values. On average, the difference in duration between the two bursts was greater than for any other behavior observed (nearly 60% of the combined duration; $AI_{dur} = 0.582$; $P<0.001$). This high value was driven largely by regular unilateral activity (i.e. $AI_{dur} = 1.00$) where complex prey was pushed to one corner of the mouth and the
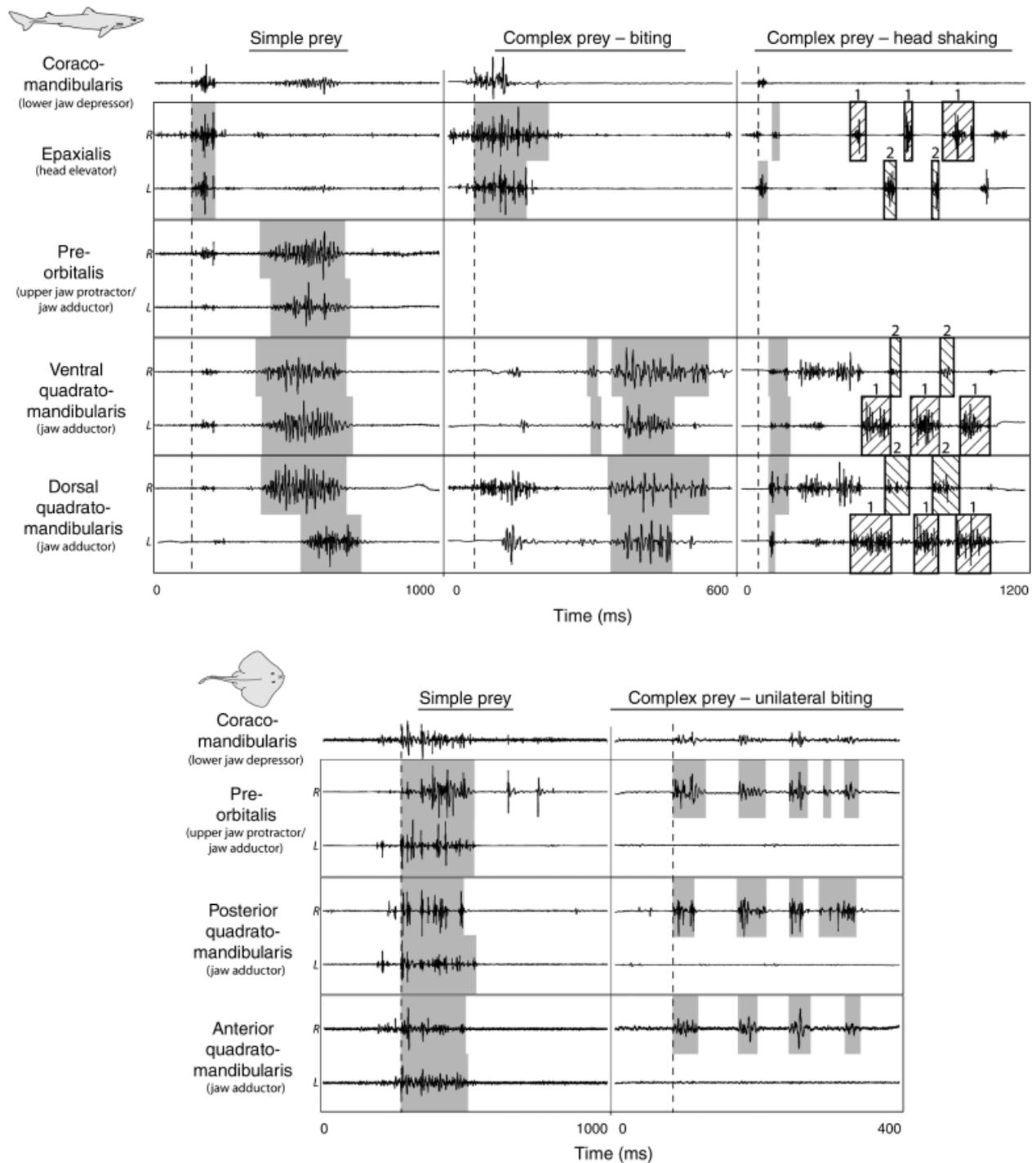
Figure 5 Representative electromyograms of a spiny dogfish capture of simple prey, biting of complex prey and head-shaking of complex prey and of a little skate capture of simple prey and unilateral biting of complex prey. Muscle pairs are indicated by L (left) or R (right). QMD, dorsal quadratomandibularis; QMV, ventral quadratomandibularis, QMA anterior quadratomandibularis; QMP, posterior quadratomandibularis; PO, preorbitalis; EP, epaxialis; CM, coracomandibularis. Shaded areas bound the activity of synchronous muscle pairs. Dashed lines indicate onset of CM activity.

muscles on only that side of the head were activated for several bite cycles (Fig. 5). This behavior was used in processing and began with activation of the coracomandibularis, synchronous with activation of the jaw adductor

muscles on one side only, followed by repeated activation of the muscles on that side with no contralateral activation. For example, when the prey was positioned in the right corner of the gape the coracomandibularis was activated

simultaneously with the right anterior and posterior quadratomandibularis and right preorbitalis (Fig. 5). Repeated bites on the prey were characterized by cyclical activation of working-side muscles with no activation of contralateral musculature. Skate complex prey feeding represented the only behavior in this study with a completely unilateral motor pattern without contralateral activation. This observed unilateral pattern was not due to implantation or measurement error as bilateral behavior was recorded before and following unilateral events.

Discussion

Spiny dogfish and little skates modulate pairwise asynchrony of the jaw adductors when feeding on different prey types or processing prey in distinct manners (e.g. head-shaking). Although bilateral implantation of *all* feeding muscles would provide the most comprehensive understanding of the timing of muscle activity and which muscles are driving kinematic events, this is often impractical, as there is typically a limit to the number of electrodes experimenters can implant into an animal (i.e. due to a limited number of recording channels or the animal's unwillingness to feed). Most studies on the feeding motor patterns of fish have used unilateral implantation to determine the muscles important to vertebrate feeding (e.g. Motta *et al.*, 1997; Wilga & Motta, 1998*a,b*, 2000; Wilga *et al.*, 2001). However, whereas unilateral implantation may allow recording from a greater number of muscles and therefore provide a broader picture of which muscles are driving kinematic events in a feeding behavior, bilateral implantation can detect contralaterally differential muscular activity.

Pairwise modulation can vary with feeding behavior, type of food presented, and phylogenetic history (this study; Liem, 1978, 1979, 1980; Lauder & Norton, 1980; Gerry *et al.*, 2008). The greater the degree of asynchrony (e.g. head-shaking, Wilga & Motta, 1998*a*; Gerry *et al.*, 2008; or unilateral biting, Summers, 2000), the more bilateral implantation will provide detailed estimations of variability and/or timing. Bilateral studies separate out these variations, which may be inaccurately estimated in those unilateral implantation studies that consider the *average* of the activation patterns they record in one member of a muscle pair as representative of the bilateral condition. The potential pitfalls of this assumption are highlighted by the extremes in our dataset. At one extreme of our observed parameter space, behaviors that exhibit low lag and high

duration index values (e.g. skate unilateral biting, Fig. 4; Summers, 2000) might not be detected at all by unilaterally implanted EMGs if muscle activity only occurs on the non-implanted side of the head. Therefore there would be a danger of missing the activation pattern entirely. In contrast, for behaviors that exhibit high-lag and low-duration index values (e.g. dogfish head shaking; Fig. 4), unilateral implantation would misrepresent behavioral onset/offset and overestimate variability. This may explain why studies using unilateral implantation typically have larger variation within individuals than among individuals or behaviors, because they encompass the variation of both motor patterns. The majority of previous data recorded from unilateral EMGs of fish (e.g. Wilga & Motta, 1998*a,b*; Motta *et al.*, 2008) have focused on comparatively rapid suction behaviors on comparatively small prey items, therefore we suppose unilateral and bilateral studies would likely have reported similar mean onsets. However we believe the sequence of activation between muscle pairs of species that use the comparatively slower behaviors of biting or ram feeding almost exclusively (see compilation table of reported timings in Clark & Summers, 2007) will exhibit asynchronous effects.

Bilateral implantation has the potential to provide greater resolution in detecting differential timing of pairwise activity in muscles, particularly for studies of processing behaviors (i.e. those involving complex prey). In this study, simple prey events were synchronous ($AI_{dur} < 0.22$; $AI_{lag} < 0.30$) when compared with behaviors exhibited in response to complex prey. Such structurally complex food items require processing – either a reduction in size or the removal of indigestible material (Summers, 2000; Dean, Wilga & Summers, 2005; Dean *et al.*, 2007) – represented in our study by unilateral biting and head-shaking behaviors. In the predators studied here, complex prey processing behaviors exhibited three- to eight-fold increases in asynchrony (lag or duration index) relative to other behaviors (Table 2).

We propose that the requirements for prey processing drive asynchrony, as the most asynchronous behaviors were elicited by complex prey and during processing, and as head-shaking cycles tended to become more synchronous as prey were reduced. Lacking a muscular tongue or pharyngeal jaws to process ingested prey, cartilaginous fish apparently rely on the inertia of the prey item or pressure drag forces imparted on the prey from high-speed water flow – the result of head-shaking in sharks like spiny dogfish or coordinated

Table 2 Index and total event values for feeding events by species and behavior

Variable	AI_{lag}	AI_{dur}	TE
Dogfish simple	0.137 ± 0.009	0.115 ± 0.010	210.034 ± 5.372
Dogfish complex	0.285 ± 0.032	0.217 ± 0.016	172.182 ± 8.824
Dogfish head-shake	1.197 ± 0.053	0.264 ± 0.014	211.500 ± 6.335
Skate simple	0.236 ± 0.007	0.169 ± 0.003	62.778 ± 1.357
Skate complex	0.163 ± 0.003	0.581 ± 0.004	136.579 ± 1.088

Values show means ± SEM.
AI, asynchrony index; TE, total event length.

circulation of water currents within the oral cavity in batoids and specialized suction feeding sharks (Motta *et al.*, 2002; Dean *et al.*, 2005; Higham, Day & Wainwright, 2006; Wilga *et al.*, 2007). In both methods, unilateral control of the jaws anchors the prey during processing, while hydrodynamic or inertial forces on the prey against the teeth dismantle it. The dominant dismembering force would depend on prey size. In head-shaking, the teeth are sliced through the prey either by motion of the head creating drag forces that push it against the cutting edges of teeth (smaller prey items) or via the prey's inertia resisting the predator's head movements (larger prey items) (Wilga & Motta, 1998*a*; Ramsay & Wilga, 2007). In contrast, we believe that when skates are not tearing pieces from prey, they rely largely on drag forces for processing, stripping away unwanted material by passing oscillating water flows from the buccal cavity over prey items gripped at one corner of the mouth.

The fundamental differences in processing behavior between these species are evident in the feeding anatomy. The mechanism of head-shaking can be explained by muscle asynchrony and tooth shape. Dogfish dentition exhibits two functional regions, with each tooth bearing an oblique, medially-oriented slicing blade that terminates in short, sharp and laterally-directed gripping cusp (Cappetta, 1987; Ramsay & Wilga, 2007). During head-shaking, epaxialis muscles are contracted in concert with the contralateral jaw adductors, 'pinching' the medially directed cutting edges against the prey. As a result, the drag/inertial forces on the prey are resisted by the cutting blades. When dogfish position prey at the symphysis, as was often observed in this study, the mirror-image orientation of parasymphyseal teeth allows the dentition to function as a cutting edge into the prey when the head is swung in either direction. This mechanism is clearly effective: spiny dogfish can bisect oversized piscine prey with only two or three shakes of the head (Jones & Geen, 1977; Wilga & Motta, 1998*a*).

In contrast to those of dogfish, the piercing tooth cusps of little skates are oriented lingually; head-shaking motor patterns with high lag numbers (as in dogfish) would be ineffective. Instead, prey is positioned at one corner of the jaws and working side jaw adductors are activated repeatedly, pinching the prey and creating a balancing-side channel through which water can move during hydrodynamic reduction of the prey item. Anchoring of the prey by working side teeth could assist in retaining edible parts of the prey (Summers, 2000) while moderate pressures created in the buccal and pharyngeal cavities (A. Stoehr & C. Wilga, unpubl. data) remove any unwanted material.

The effectiveness and flexibility of the skate processing mechanism (i.e. the ability to produce a water jet for processing) hinges on decoupling of the left and right sides of the jaws as well as of the anterior and posterior visceral arches. The euhyostylic jaw suspension of batoids has more independent functional units than that of sharks. The two hyomandibular cartilages are not adjoined by ventromedial hyoid elements and may exhibit non-coordinated movements. Further, if the symphysis is unfused, the two halves of the jaw may exhibit a similar degree of independence

(Dean & Motta, 2004*a,b*). The left and right sides of the visceral arches are less constrained to move together. In addition to this sagittal decoupling in both the mandibular and hyoid arches, these arches are not linked to the branchial arches, as in sharks where the ceratobranchials articulate with the basihyal. This dissociation may allow independent movement of the jaws and the pharynx (Dean *et al.*, 2005, 2007). Unilateral grasping of the prey in the jaws can therefore coincide with coordinated water movement actuated by the buccopharynx.

Pairwise asynchrony has been noted in a variety of tetrapods, and in mammalian mastication experimental studies where bilateral implantation is the norm (e.g. Cundall, Lorenz-Elwood & Groves, 1987; Lieberman & Crompton, 2000; Williams *et al.*, 2007 and references therein; Herrel *et al.*, 2008). Our findings combined with the scant bilateral data from bony fish (Liem, 1978, 1979, 1980; Lauder & Norton, 1980) suggest pairwise modulation is basal in the vertebrate clade and also likely widespread among fish. These data also delineate conditions under which we might expect asynchrony in feeding events: for example, asynchronous activation is likely prevalent in processing behaviors on complex prey whereas synchronous activation is typical of capture behavior of simple prey. We would also expect more synchronous activity in rapid behaviors that tend to exhibit less modulation, such as suction feeding, and particularly in teleosts, where the often narrow and compressiform buccal morphology implies little advantage to decoupling the left and right sides of the head. We hope this study will encourage investigation – through bilateral EMG implantation – of feeding pattern modulation in fish, particularly for behaviors involving processing.

Acknowledgments

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