

## Electromyography of the buccal musculature of octopus (*Octopus bimaculoides*): a test of the function of the muscle articulation in support and movement

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### Summary

The buccal mass musculature of the octopus (*Octopus bimaculoides*) was studied with electromyography to test the predictions of a previous morphological study in which we suggested that the muscles of the buccal mass serve as both the effectors of movement and as the joint itself, forming a new category of flexible joint termed a 'muscle articulation'. The predictions of muscle function were tested by correlating muscle electrical activity in isolated buccal masses with spontaneous beak movements. Bipolar electromyography electrodes were implanted in the various beak muscles and beak position was recorded simultaneously with an electronic movement monitor ( $N=14$ ). The results are consistent with the hypothesis that the lateral mandibular muscles produce opening movements of the beaks and provide the first definitive

explanation of the opening mechanism. The results are also consistent with the hypothesis that the superior mandibular muscle functions primarily in closing. Co-contraction of the lateral mandibular muscles and the superior mandibular muscles was also observed, suggesting that these muscles may also stabilize the beaks during movement or provide a means of controlling the location of the pivot between the beaks. This study provides an important first test of the predictions of the role of the complex musculature found in muscle articulations such as the cephalopod buccal mass.

Key words: biomechanics, Cephalopoda, electromyography, muscle articulation, *Octopus bimaculoides*.

### Introduction

#### *Joints and the muscle articulation*

Any structure in which a rigid portion, or link, is capable of movement relative to another requires a joint in between. Thus joints are mechanical elements that form the connection and allow movement between links. In providing support, these links may be loaded almost completely in compression, as in the limbs of an elephant (Biewener, 1990), or in tension as in the forelimbs of a brachiating gibbon (Bertram, 2004). Links also provide skeletal support for the attached muscles and may amplify their force, displacement or velocity.

Animal joints show great morphological diversity; however, all may be divided into two categories, sliding and flexible, based on the nature of the connection between the links (Wainwright et al., 1982). Elephant knees and gibbon elbows are examples of sliding joints, in which links are in contact and compressional forces are transmitted directly through the joint. The morphology of the articulating surfaces and joint capsule controls the range of motion and number of degrees of freedom. The movement of links connected by a flexible joint relies on the flexibility of the connecting material. But flexible material tends to buckle when loaded in compression and thus a survey of flexible joints by Wainwright et al. (Wainwright et al., 1982)

found only distal leg segments of smaller insects use this mechanism.

The joint studied here, termed a 'muscle articulation' (Uyeno and Kier, 2005), is a type of flexible joint: the muscle and connective tissues that connect the links also hold them apart. In the cephalopod buccal mass the links are represented by the two rigid beaks and the joint is composed of the connecting musculature that allows the beaks to rotate and translate relative to one another. We believe these beak motions are possible because the musculature includes muscle groups with fibers oriented in a three-dimensional arrangement known as a muscular hydrostat (Kier and Smith, 1985). In such systems, one or more orientations may function as an antagonist to the others. Uyeno and Kier suggested that, unlike other flexible joints, the cephalopod beak joint relies on a muscular hydrostatic mechanism to bear compression and create movement (see Uyeno and Kier, 2005). Here, we test the hypothesized functions of the buccal mass musculature and provide the first experimental evidence of a mechanism that can open the beaks.

#### *Cephalopod buccal mass morphology*

We studied the buccal mass of the California two-spot

octopus *Octopus bimaculoides* (Pickford/McConnaughey 1949). This spherical structure is located in a sinus formed by the base of the arms and includes an upper and a lower beak made of chitin (Hunt and Nixon, 1981) that is embedded in the mandibular muscles (Fig. 1). Each beak is a roughly U-shaped link, with one end folded over itself to form the rostral tip and the jaw angle, both of which are used in biting and shearing food, and the hood that projects beyond the jaw angle (Fig. 2). By convention, the rostra are referred to as being anterior. The upper beak is inverted relative to the lower beak. The rounded dorsal surface of the upper beak and the analogous ventral surface of the lower beak are termed crests. The left and right sides of the beaks are referred to as the lateral walls. The fold that forms the rostrum and hood of the lower beak also has enlarged dorsal extensions, termed the left and right wings. The upper beak fits within the lower beak such that the lateral walls overlap, but they do not contact each other within the joint. Only the biting surfaces (i.e. the rostra and jaw angles) contact. A 'pivot area' was described between the upper and lower beak, in which the axis of rotation was typically located, suggesting the position of the pivot is not fixed (Kear, 1994).

Five mandibular muscles (the superior, left and right lateral, anterior and posterior mandibular muscles) connect the beaks of *O. bimaculoides* to each other and to the buccal mass sheath, a connective tissue sheet that encapsulates the muscular part of the buccal mass (Uyeno and Kier, 2005) (Fig. 3). The superior mandibular muscle (Fig. 3, SMM, colored green) is a robust dorsal muscle with three divisions. This muscle originates along the crest of the upper beak and includes a central division

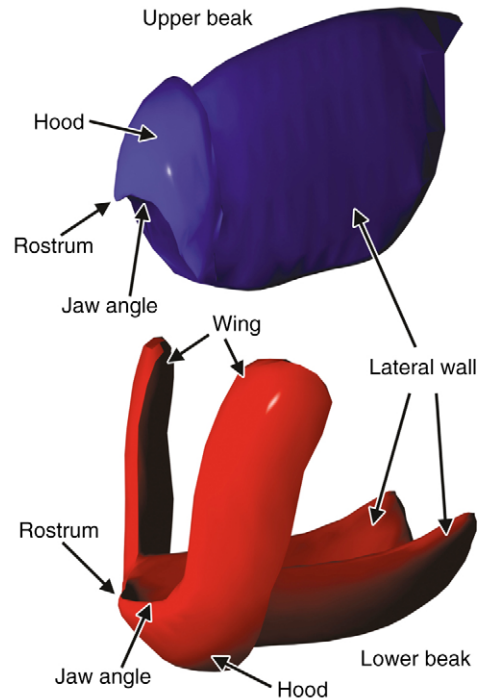


Fig. 2. Diagram of the upper (blue) and lower (red) beaks of *O. bimaculoides*. Both beaks are U-shaped in cross section and the anterior end is folded over itself to form the biting surfaces (i.e. the rostrum and the jaw angles). The upper beak has enlarged lateral walls that fit within, but do not contact the lower beak. The lower beak has enlarged wings and a reduced hood relative to the upper beak.

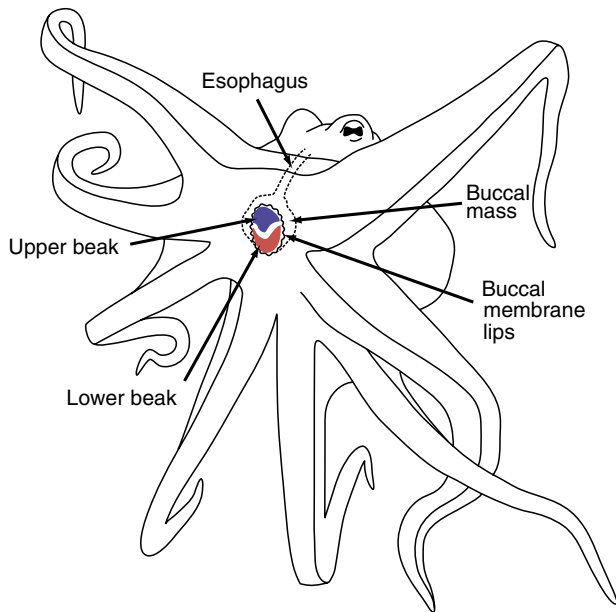


Fig. 1. An oral view of *O. bimaculoides* in a vertical hanging position. The buccal mass is located in a sinus formed by the base of the arms and includes an upper (blue) and lower (red) chitinous beak embedded in the mandibular muscles. Also shown are the esophagus and the buccal membrane lips, a ring of tissue that seals the sinus and covers the beaks.

and left and right divisions that extend anteroventrally to insert on the enlarged wings of the lower beak. These robust left and right divisions constitute the bulk of the superior muscle and include fibers oriented parallel to the line from origin to insertion.

The lateral mandibular muscles (Fig. 3, left and right LMMs, colored purple) are robust, cylindrical, and symmetrically paired muscles originating on a large area of the left and right lateral walls of the upper beak. The muscle extends laterally and has a somewhat smaller insertion on the buccal mass sheath. Three different orientations of muscle fibers are observed. The first group of fibers originates on the lateral walls of the upper beak and extends parallel to the long axis of the muscle to insert on the buccal mass sheath. The other two groups of muscle fibers are perpendicular to the orientation of the first as well as to each other, one group oriented dorsoventrally and the other anteroposteriorly.

The anterior mandibular muscle (Fig. 3, AMM, colored yellow) is relatively thin and originates on the anterior portion of the lower beak crest and overlying buccal mass sheath and follows the curve of the crest dorsally to insert on the lateral walls of the upper beak just below the level of the upper beak crest. Its muscle fibers follow a direct course from origin to insertion. The posterior mandibular muscle (Fig. 3, PMM,

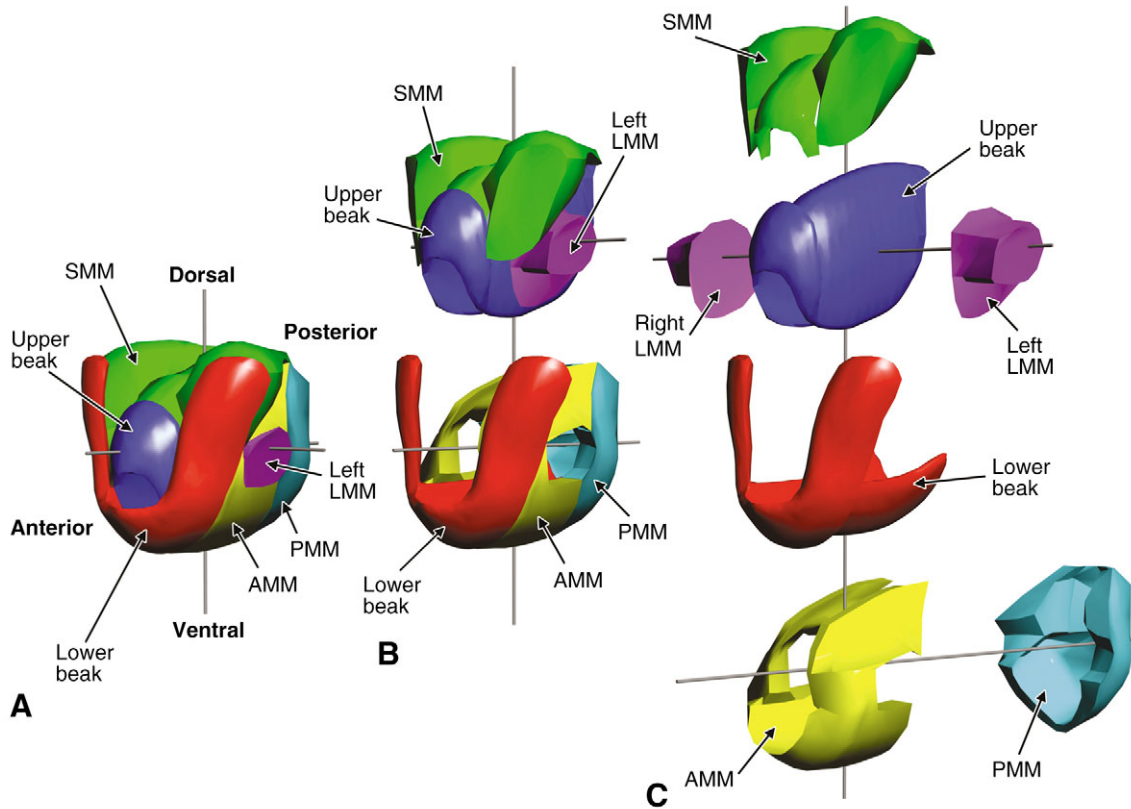


Fig. 3. Schematic diagram showing the beaks and musculature of *Octopus bimaculoides*. (A) The beaks and musculature of a complete buccal mass. (B) The upper beak and lower beak separated to show the underlying muscles. (C) The beaks and musculature separated to show the shape of the beaks and the shape of the individual muscles. AMM, anterior mandibular muscle (yellow); LMM, lateral mandibular muscle (purple); PMM, posterior mandibular muscle (blue); SMM, superior mandibular muscle (green).

colored light blue) is the smallest and thinnest of the mandibular muscles. It is a thin sheet of muscle that originates on the posterior region of the lower beak crest and extends directly to an insertion on the lateral walls of the upper beak below its crest. As the posterior edges of the trough-like beaks are open, the posterior mandibular muscle, along with the overlying buccal mass sheath, forms the posterior wall of the buccal cavity and serves to contain and secure the buccal complex within the buccal cavity. This buccal complex includes the radula, lateral buccal palps, salivary papilla, salivary glands and radular support system (Nixon and Young, 2003).

The sequence of activation of these muscles is controlled by the inferior buccal ganglion (Fig. 4), which receives input from the superior buccal lobe of the brain through the paired interbuccal connectives (Young, 1965; Young, 1971). Boyle et al. (Boyle et al., 1979b) noted that the inferior buccal ganglion functions as a central pattern generator that is probably modulated by sensory feedback from the musculature and the brain. After severing the interbuccal connectives and excising the buccal mass, it performs biting movements that are similar to *in vivo* beak movements with respect to the position of the upper beak relative to the lower one throughout each bite cycle. Boyle et al. (Boyle et al., 1979b) first described this bite cycle,

and Kear (Kear, 1994) later modified the description (Fig. 5). Our observations agree with those of Kear (Kear, 1994) and so we use her terminology here. There are five phases during which the upper beak is: (A) closed in its resting position; (B) opening; (C) fully opened; (D) closing; (E) closed with the upper beak rostrum retracted behind the lower beak rostrum.

#### *Hypothesized functions of buccal mass muscles*

The goal of this study was to understand the functioning of a muscle articulation by examining how the muscles of the octopus buccal mass open, close and otherwise move the beaks. In particular, previous studies were unable to identify both the muscle and the mechanism responsible for opening the beaks during the biting cycle. Analysis of this crucial part of the biting cycle was therefore a major focus of this study. We summarize below our hypotheses of the role of the various muscles in opening and in other beak movements and describe experimental tests for each.

#### *The superior mandibular muscle*

We hypothesize that the superior mandibular muscle (Fig. 3, SMM, colored green) is responsible for closing the beaks. Contraction of fibers in the left and right divisions connecting the crest of the upper beak and the wings of the lower beak is

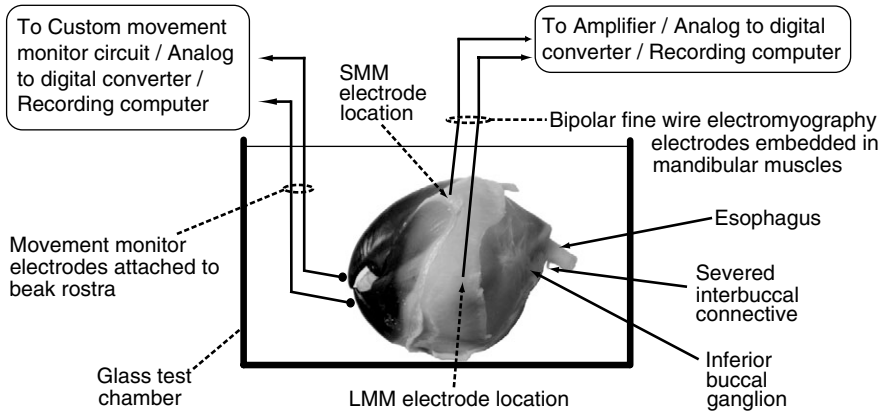


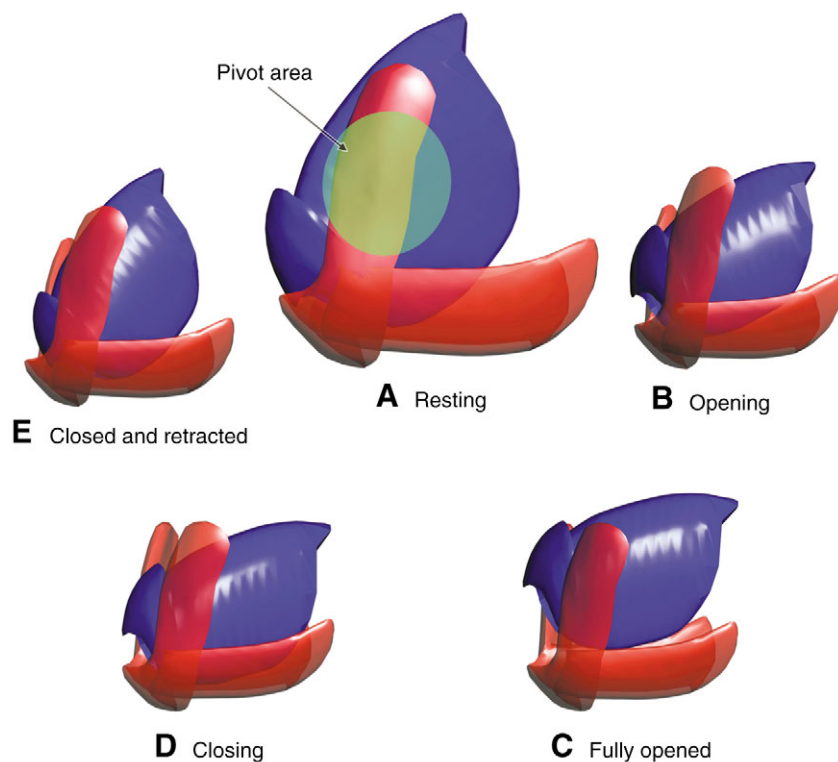
Fig. 4. Schematic diagram of the isolated buccal mass preparation showing electromyography electrode locations in the musculature, and movement monitor electrode locations on the beak rostra. Also shown are the esophagus, the ligated interbuccal connectives and the approximate location of the inferior buccal ganglion.

predicted to bring the beaks, and especially the rostra, closer together in a closing motion (Fig. 6A). As these lateral divisions of the superior mandibular muscle are robust muscles with a relatively large cross sectional area, we hypothesize that they provide most of the closing force.

Previous studies have provided some evidence for the role of the superior mandibular muscle in closing. Boyle et al. clamped the lower beak of *Octopus vulgaris* and attached a strain gauge to the upper beak with a thread (Boyle et al., 1979a; Boyle et al., 1979b). This approach allowed beak movements to be monitored, although with some mechanical loading from the apparatus. Fine wire electromyography was used to record mandibular muscle electrical activity. Recordings were made during both spontaneous bite cycles as well as evoked bites, in which the ligatured interbuccal

connectives were stimulated electrically. Boyle et al. concluded that the superior mandibular muscle was active while the beaks were held closed (Boyle et al., 1979a; Boyle et al., 1979b). They also suggested that the superior mandibular muscle was responsible for beak retraction.

In a later study of the buccal masses of a variety of coleoid cephalopods, Kear also clamped the lower beak and attached a strain gauge to the upper beak (Kear, 1994). The superior mandibular muscle was directly stimulated electrically at five locations along the central division. Stimulation resulted in closing movements at every point tested, but unlike the findings reported by Boyle et al., no retraction was observed (Boyle et al., 1979b). In this study, we observed the effect of superior mandibular muscle contraction on the movement of the upper beak without mechanical loading.



*The lateral mandibular muscles*

We hypothesize that the paired lateral mandibular muscles (Fig. 3, left and right LMMs colored purple) generate force for opening movements and also help create a dynamic pivot around which beak movements occur (Uyeno and Kier, 2005). The cylindrical lateral mandibular muscle (Fig. 6), consists of a densely packed three dimensional array of muscle. Since it is essentially constant in volume, we hypothesize that contraction of fibers that are parallel to the long axis (Fig. 6,

Fig. 5. Schematic diagram illustrating the movement of the upper beak (opaque blue) relative to the lower beak (semi-transparent red) during the bite cycle [description follows Kear (Kear, 1994) and is redrawn from Uyeno and Kier (Uyeno and Kier, 2005)]. (A) Closed in its resting position (beaks relatively enlarged to illustrate pivot area); (B) opening; (C) fully opened; (D) closing; (E) closed with the upper beak rostrum retracted behind the lower beak rostrum. The axis around which the beaks pivot was observed to be located anywhere in the pivot area indicated in A (resting).

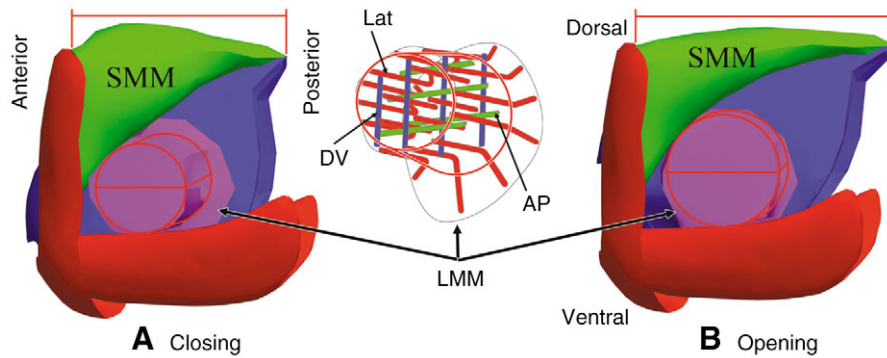


Fig. 6. A left side schematic diagram of the hypothesized muscle action during (A) closing and (B) opening states of the superior (SMM, green) and lateral (LMM, purple) mandibular muscles showing dimensional changes seen in the experimental preparation (lower beak, red; upper beak, blue). The cylindrical lateral mandibular muscle is illustrated in the central diagram with the three fiber orientations: Lat, lateral fibers (red); AP, anterior–posterior fibers (green); DV, dorsal–ventral fibers (blue).

the red lateral fibers of the central diagram) causes the muscle to become shorter and increase in diameter and circumference. This action may push the upper beak away from the lower one because the muscle originates on the lateral wall of the upper beak and passes over the lateral wall edges of the lower beak. The lateral mandibular muscles are the only sizeable muscle groups that include muscle fiber orientations capable of generating the force required to open the beaks. The definitive test of this hypothesis is whether the beaks can open in the absence of lateral mandibular muscle activity.

The muscle fiber arrangement within the lateral mandibular muscles suggests that they may also serve as a dynamic pivot for the beaks. The fibers that are parallel to the long axis of the lateral mandibular muscles (Fig. 6, red lateral fibers of the central diagram) may elongate the other fiber orientations [the dorsoventral (Fig. 6, blue fibers in the central diagram) and anteroposterior muscle fibers (Fig. 6, green fibers in the central diagram)] that are arranged perpendicularly to the long axis. As the co-contraction of the perpendicular dorsal–ventral and anterior–posterior fiber orientations will elongate the parallel lateral fibers, the three muscle orientations may serve as antagonists of one another, controlling the shape and stiffness of the lateral mandibular muscle in the manner of many muscular hydrostats. Thus, it is important to note the potential dual function of the lateral mandibular muscles; they may generate force not only for beak opening, but may also be activated with other muscles to stabilize and control the hinge axis or pivot around which the two beaks rotate. This would allow, for instance, the superior mandibular muscle to contract and modulate the angle between the two rostra, instead of simply bringing the two beaks closer together. If the lateral mandibular muscles aid in forming a dynamic pivot for the beaks, they may show activity not only during opening movements but during other beak movements as well.

The hypothesized functions of the lateral mandibular muscle described above differ from previous proposals, in part because they incorporate additional morphological information (Uyeno and Kier, 2005). Boyle et al. observed electrical activity only during closing movements and were unable to record from muscle locations that were active during opening (Boyle et al., 1979a). They therefore considered opening to be a passive movement resulting from the flexion of the lateral walls of the

upper beak (Boyle et al., 1979b). Kier simultaneously stimulated the buccal mass near the location of the left and right lateral mandibular muscles and found that this opened the beaks (Kier, 1994). Stimulation of only one side resulted in lateral movement of the upper beak. Interpretation of electrical stimulation experiments is difficult because it is unclear which of the muscles in the lateral portion of the buccal mass were stimulated. Indeed, the inferior mandibular ganglion itself may have been stimulated.

Kear described the lateral mandibular muscles as originating on the lateral wall of the upper beak and extending both to the ventral side of superior mandibular muscle and to the lateral walls of the lower beak (Kear, 1994). She suggested that contraction of these fibers pulled the posterior edges of the lateral walls of the upper and lower beaks together. Although Kear did not identify the pivot mechanism she identified the location of the axis of rotation as being between the lateral mandibular muscles and beak rostra (Kear, 1994). She concluded that contractions drawing together the posterior portions of the beaks would lever the rostra apart using the pivot area as a fulcrum. The fibers described by Kear (Kear, 1994) as connecting the upper and lower beak lateral walls were not observed in the lateral mandibular muscles of *Octopus bimaculoides*, or the other species investigated by Uyeno and Kier (Uyeno and Kier, 2005), so this mechanism cannot function in these species.

Kear (Kear, 1994) also noted the outward flexion, described by Boyle et al. (Boyle et al., 1979b), of the upper beak lateral walls. Could this outward flexing cause a shape change in the beaks that would result in opening of the rostra? Presumably this would occur by the flexing of the crest in a way that levers the upper beak rostrum dorsally. This mechanism predicts areas of flexibility of the upper beak itself that can cause shape change. We tested for this possibility as well.

#### *The anterior and posterior mandibular muscles*

Located anterior to both the lateral mandibular muscles (Fig. 3, LMM, colored purple) and the general pivot area around which the beaks rotate, the anterior mandibular muscle (Fig. 3, AMM, colored yellow) possesses fibers that connect the upper and lower beaks and is thus hypothesized to function in their closing. The location of the posterior mandibular muscle (Fig. 3, PMM, colored light blue) is opposite to that of

the anterior mandibular muscle: it is located posterior to both the lateral mandibular muscles and the beak pivot area. As the posterior mandibular muscle fibers directly connect the two beaks, it is likely that their contraction brings the posterior edges of the beaks together. Because it is positioned posterior to the pivot area, if the pivot area is actively forming a fulcrum, the posterior mandibular muscles may help in beak opening. However, if the pivot area is inactive, then the contraction of the posterior mandibular muscle fibers may simply contribute to the overall closing of the beaks. Given the relatively small cross-sectional areas of these two muscles (Uyeno and Kier, 2005), the forces generated by the anterior and posterior mandibular muscles may be relatively lower than those generated by the superior and lateral mandibular muscles.

### Materials and methods

Fourteen adult *Octopus bimaculoides* (Pickford/McConnaughey 1949) (91–254 g wet mass), obtained from Aquatic Research Consultants, Inc. (San Pedro, CA, USA) were lightly anaesthetized using 2–3% ethanol in seawater for 2.5–7 min until arm activity ceased (O'Dor et al., 1990). After the specimen was relaxed, the brain was bisected, the interbuccal connectives were severed, and the buccal mass was removed and placed in a glass test chamber containing artificial seawater (NaCl, 470 mmol l<sup>-1</sup>; KCl, 10 mmol l<sup>-1</sup>; CaCl<sub>2</sub>·6H<sub>2</sub>O, 60 mmol l<sup>-1</sup>; MgCl<sub>2</sub>·6H<sub>2</sub>O, 50 mmol l<sup>-1</sup>; glucose, 20 mmol l<sup>-1</sup>; Hepes, 10 mmol l<sup>-1</sup>; adjusted to pH 7.8 with 2.0 mol l<sup>-1</sup> NaOH) (Milligan et al., 1997) chilled to 17°C. During the experiment, the buccal mass was allowed to rest, unrestricted and ventral surface down, on the bottom to minimize mechanical loading of the joint. Bipolar fine wire electrodes were implanted in the muscles and a pair of silver/silver chloride electrodes was glued to the upper and lower beak rostra and wired to a custom movement monitor circuit (Fig. 4).

The electromyography electrodes were fabricated from Teflon-insulated, half annealed, single stranded stainless steel wire with a bare diameter of 75 µm (A-M Systems, Inc., Carlsborg, WA, USA). Approximately 0.5 mm of insulation was removed from the staggered electrode tips (Basmajian and Stecko, 1962). The inner sharp edge of a hypodermic needle was chamfered (Loeb and Gans, 1986) to prevent damaging the electrode wire. The electrode tips were inserted into the end of the needle and the remaining electrode wire was then folded over the chamfered edge of the needle tip. The needle was then used to insert the electrodes in the muscle of interest and then withdrawn (Parker, 1968), leaving the hooked electrodes embedded in the tissue.

The electrodes were implanted into the left and right divisions of the superior mandibular muscle and the left and right lateral mandibular muscles (Fig. 4). We were unable to reliably implant electrodes into the anterior or posterior mandibular muscles because they were too thin. The signals from four sets of electrodes were fed to an A-M Systems, Inc. Model 1700 four channel differential AC amplifier and digitized at 5 kHz per channel using a Powerlab 4/20 (AD

Instruments, Inc., Colorado Springs, CO, USA) analog to digital conversion unit. The electrodes were dissected out at the end of each experiment to confirm placement. In two of the preparations, the upper and lower beaks were dissected so as to completely free them from their surrounding mandibular muscles. This was done in order to assess the potential for beak openings based on flexure of the lateral walls. The interbuccal connective and areas adjacent to the electrodes were also electrically stimulated (2.5 V at 60 Hz for 2.5 s) in five of the healthiest preparations (Boyle et al., 1979a).

Beak movements were monitored by a custom movement monitoring circuit (Uyeno and Hsiao, 2006) designed to measure the resistance between two silver/silver chloride ball electrodes affixed with cyanoacrylate glue to the rostra (Fig. 4). The circuit converted the resistance into an amplified voltage output signal that was fed to a Powerlab 4/25 analog to digital conversion unit. The calibration of the circuit allowed linear distance changes between the electrodes and hence the beaks to be recorded. These data and the electromyographical data were simultaneously recorded on a computer hard drive.

The electromyographical data were analyzed using a routine written for Matlab 7 (MathWorks, Natick, MA, USA). The data were DC adjusted to set the mean to zero, rectified (full wave) and then smoothed using a lowpass, second order Butterworth filter with a time constant of 79.5 ms. A Fast Fourier Transform (FFT) frequency domain plot was used to confirm that the cutoff frequency associated with 79.5 ms retained enough resolution to display all pertinent frequencies. Movements were correlated with the electromyographical activity. Onset of activity was calculated using a Matlab 7 routine that automatically determined the standard deviation of the rectified electromyogram signal during a 1 s steady state period prior to a muscle activation. Muscle activation onset time was defined as the time at which a threshold of 2.5 standard deviations was reached. These automatic onset events were visually confirmed and then correlated with movement monitor activity.

### Results

#### *Observations of the general bite cycle*

The buccal mass preparations of *O. bimaculoides* survived for between 14 and 110 min after being removed. Three phases of beak movement were observed. The first phase, which lasted approximately one quarter of the preparation lifetime, was characterized by strong cycles of biting with a periodicity of 15–20 s (Fig. 7). The typical cycle involved prolonged full opening punctuated by rapid closing and reopening. With age, the time between rapid closings increased and became more variable until it reached 500 s or more, or until no bite cycles occurred. As the bite cycle slowed, the beaks gradually did not open as fully, decreasing to less than half opened. The second phase, which lasted approximately one half of the lifetime of the preparation, was characterized by a simplification of mandibular muscle activation patterns. This resulted in independent activation of the lateral and superior mandibular muscles that could be correlated with beak movements. Only

large openings and closings appeared to have continued from the autonomous biting cycle in the first phase, as smaller modulatory and positional movements are not present in the second phase. In this phase, the upper beak translated dorsally in an opening movement (moving from position A to C in Fig. 5) while the closing movement was abbreviated (moving directly from position C to E in Fig. 5): the rotational component of the upper beak closing movement was eliminated, reducing the closure to a combined closing and retraction movement. The third phase of the preparation occurred as the beaks closed and remained in the closed and retracted position. Although no spontaneous movements were observed, movements could be elicited in this stage by direct electrical stimulation until the preparation died.

Although perhaps more representative of *in vivo* function, analysis of the muscle activation patterns during the first cyclical biting phase (Fig. 8) is difficult because both the superior and lateral mandibular muscles were simultaneously active in almost all phases of the cycle. In the second phase, however, the pattern included only sequential opening, closing and retraction of the beaks and these frequent movements could be correlated with isolated activity of the left and right lateral mandibular muscles and of the lateral divisions of the superior mandibular muscle. The following is a summary of the muscle activity and correlated beak movements observed in the second stage.

#### *Muscle activity correlated with movements*

The electromyographical activities of the lateral and the superior mandibular muscles correlated with beak movements are described below and summarized in Table 1.

#### *Beak opening*

The lateral mandibular muscles were active in 100% of all openings. The lateral mandibular muscles were the only muscles active during 72.6% of beak openings and were co-active with the superior mandibular muscle in 27.4% of all

openings. Fig. 9 shows an example of three bursts of activity from the left lateral mandibular muscle correlated with three brief beak openings from a half-opened gape. The activity of the left superior mandibular muscle does not show any obvious correlation with beak movements.

#### *Beak closing*

The superior mandibular muscle was active either by itself or coactive with the lateral mandibular muscle during 73.3% of all closing motions. The superior mandibular muscle was the only muscle active during 42.2% of all closings and was coactive with the lateral mandibular muscles during 31.1% of all closings. The lateral mandibular muscles were the only muscles active during 18.0% of all closings. In 8.7% of closings neither the superior nor lateral mandibular muscles were recorded as being active. Fig. 10 shows two bursts of superior mandibular muscle activity that correlate with beak closing movements. The largest superior mandibular muscle activity occurs during contact between the upper and lower beaks.

#### *Muscle activity without correlated beak movement*

In 29.1% of all muscle activations the superior and lateral mandibular muscles were observed to be active in the absence of beak movement. Both showed activity in the absence of beak movements in 18.0% of all muscle activations. The lateral mandibular muscles were active by themselves in 3.8% of the cases and the superior mandibular muscle was active by itself in 7.3% of cases.

#### *Observations of beak activity during direct nerve and muscle stimulation*

Electrical stimulation of the beak musculature and nerves was also attempted in order to explore the functional role of the musculature in beak movements. Stimulation of the left or right divisions of the superior mandibular muscle results in asymmetrical beak closing. For example, if the right division is stimulated, the upper beak rostrum closes to the right of the lower beak rostrum. Likewise, stimulation of either the left or right lateral mandibular muscle results in an asymmetrical opening movement. For instance, stimulation of the left lateral mandibular muscle results in opening between the left sides of the upper

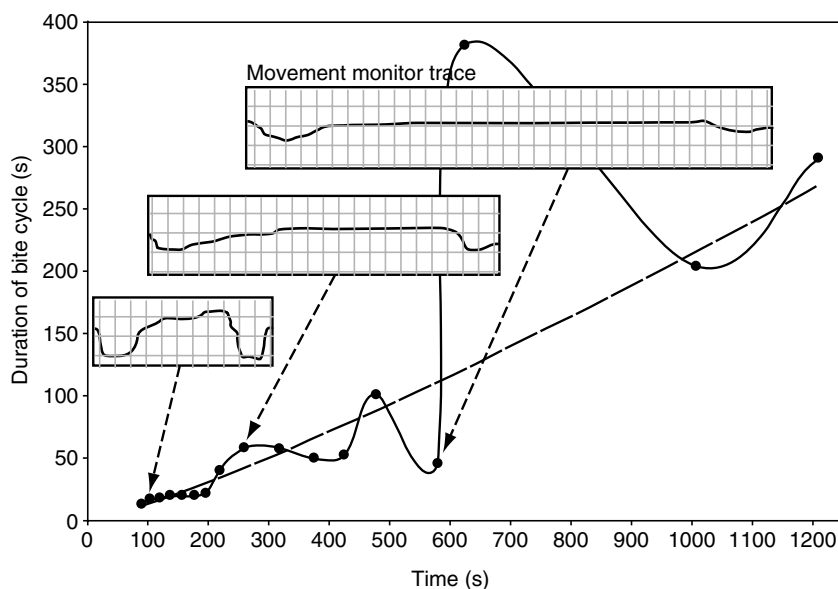


Fig. 7. Graph of duration of the bite cycle as a buccal mass preparation ages. The movement monitor traces on the insets represent the relative distance between the upper and lower beaks with respect to time. A rise in the inset traces indicate opening. Although the cyclical beak movements became smaller and the duration became longer as time progressed, the beak was capable of powerful stochastic bites up to the end of the life of the preparation (in this case 2100 s).

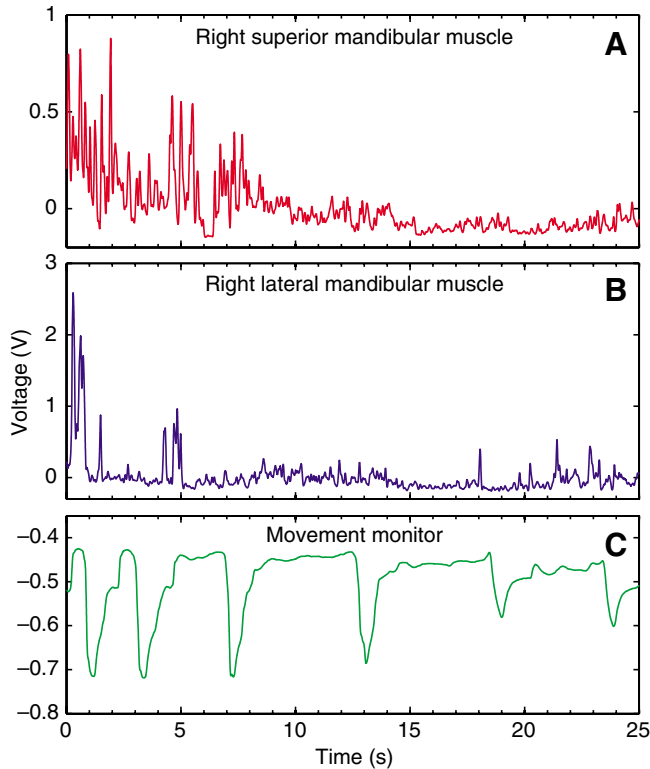


Fig. 8. Plots of rectified, averaged electromyograms of the right superior mandibular muscle (A) and the right lateral mandibular muscle (B) correlated with the output of the monitor of beak position (C) during the cyclical biting of a freshly excised beak preparation. The muscle activation patterns are complex and difficult to correlate with beak movements.

and lower beaks, but little change on the right. This causes the upper beak to rotate around the anterior–posterior axis, rolling the upper beak by as much as 30° relative to the lower beak. Stimulation of the interbuccal connectives, the neural pathway that connects the brain to the inferior buccal ganglion, elicits a nearly complete bite cycle, in which only the retraction phase seemed to be diminished. The vigor and completeness of the bite cycle decreased as the preparation aged.

*Observations of beak movements*

It is possible to observe beak movements in an isolated buccal mass of *O. bimaculoides* because portions of both the lower and upper beak are visible. The rostrum, angle and hood of the upper and lower beaks are exposed, and the enlarged

lateral wings of the lower beak are also visible (e.g. Fig. 4). During biting movements, the lower beak remains stationary relative to the buccal mass, regardless of the direction of movement of the upper beak or whether the buccal mass is resting on its side, dorsal surface or ventral surface. The upper beak shows five degrees of freedom of movement relative to the lower beak: (1) rotation about the dorsal–ventral axis or yaw; (2) rotation about the anterior–posterior axis, or roll; (3) rotation about the left–right axis, or pitch; (4) translations along the dorsal–ventral axis; and (5) translations along the anterior–posterior axis. Side to side translations were not observed, but were approximated by a combination of yaw and roll movements. This diverse array of beak movements would be impossible with a simple hinge joint between the two beaks. The musculature that serves as the joint thus allows shearing between the beaks along multiple axes. In addition, it provides for an axis of rotation that can be repositioned within the pivot area, a zone that includes most of the lateral mandibular muscles and an area dorsal and anterior to them.

*Flexibility of the freshly dissected beak*

The stiffest areas of *O. bimaculoides* beaks are a dark, opaque brown/black color. Less stiff areas are a lighter shade of brown and the most flexible and thinnest areas are tan colored or transparent. The only flexible areas of the freshly dissected upper beak are the lateral walls, which are capable of flexing outward. Maximal outward flexing results in a 30–40% increase in distance between the lower edges of the upper beak lateral walls. The flexible areas of the lower beaks include the tips of the lateral wings and the posterior edges of the lateral wall. There is a sharp demarcation approximately half way between the jaw angle and the tip of the lateral wing where the wing becomes lighter in color, more flexible and thinner. A more graded demarcation exists near the posterior tips of the lateral walls of the lower beak. Neither the upper nor the lower beaks are capable of significant longitudinal bending, perhaps due in part to their U-shaped cross-section and consequent large second moment of area. Thus, the rostra and crests do not move relative to each other.

**Discussion**

Kear (Kear, 1994) noted that the most conclusive evidence for the role of the various muscles in beak movements requires *in vivo* intramuscular electromyography records during

Table 1. *Mandibular muscle activity correlated with movement of the beaks*

	Mandibular muscles				Total (%)
	Superior (%)	Lateral (%)	Both active (%)	No activity (%)	
Openings	51 (27.4)	186 (100)	51 (27.4)	0 (0%)	186 (38.0)
Closings	118 (73.3)	79 (49.1)	50 (31.1)	14 (8.7)	161 (32.9)
No movement	124 (86.7)	107 (74.8)	88 (61.5)		143 (29.1)
				Total	490 (100)



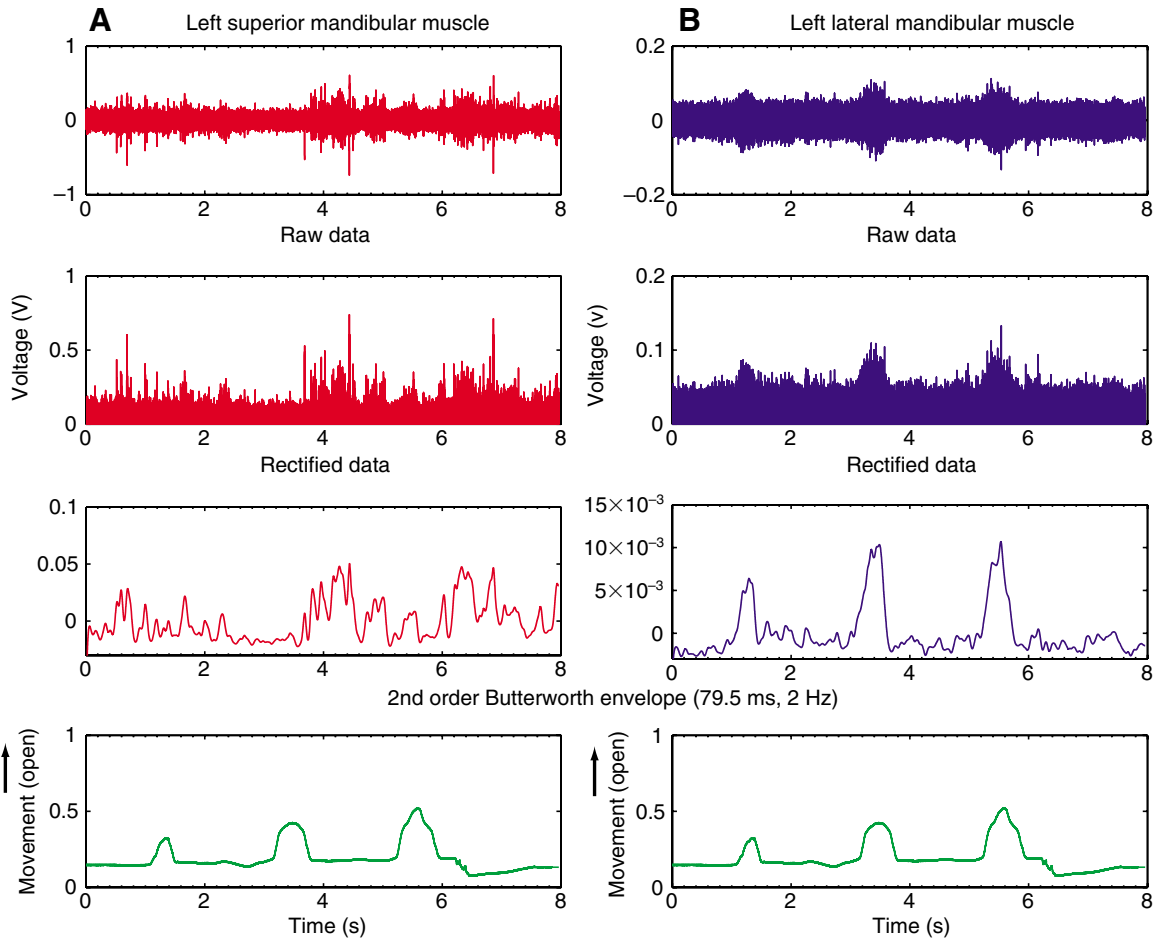


Fig. 9. Plots of the raw electromyograms (top) with rectified and then averaged signals plotted successively below from the left superior mandibular muscle (A) and left lateral mandibular muscle (B). The bottom traces are the correlated output from the monitor of beak position. Note that beak opening is correlated with activity of the left lateral mandibular muscle.

feeding. Although *in vivo* experiments are indeed preferable, they present substantial experimental difficulties because implanting electrodes requires invasive surgery close to the brain, electrode placement cannot be assured because the buccal mass is encapsulated within the base of the arm musculature, and post-operative movements of the octopus often dislodge embedded electrodes (Kear, 1994).

The excised buccal mass preparation represents a useful alternative approach. Boyle et al. (Boyle et al., 1979a) found the autonomous biting movements of the excised beak to be similar to normal beak movements. They suggest that the bite cycle is under the control of a central pattern generator in the inferior mandibular ganglion, which is part of the excised preparation, and thus the similarity to *in vivo* movements is less surprising (Boyle et al., 1979a). The biting cycles of the initial phase of the preparation are similar to those observed *in vivo*, but they are difficult to analyze because of the complexity of movement. Although this complexity is probably more representative of the full range of muscle function in the intact animal, analysis of the less complex, discrete movements found in the second phase is more instructive because the opening,

closing, and retracting movements are similar to those of the normal bite cycle and isolated muscle activity can be correlated with specific beak motions. We did not observe this second phase of activity in buccal mass preparations of the other coleoids studied [the Atlantic brief squid *Lolliguncula brevis* (Blainville 1823) or the cuttlefish *Sepia officinalis* Linnaeus 1758] perhaps because these movements are more apparent in species (such as *Octopus bimaculoides*) in which excised buccal mass preparations have a longer life span.

#### *Assessment of functional hypotheses for the mandibular muscles*

##### *The superior mandibular muscle*

The superior mandibular muscle is active during the majority of the beak closings and during all of the rapid closings in which the beaks quickly clamp together. This, together with a previous analysis that showed that this muscle contains the largest number of muscle fibers in an orientation that could effect this motion (Uyeno and Kier, 2005), suggest that the superior mandibular muscle is the prime force generator in beak closure. This muscle is also co-active with the lateral

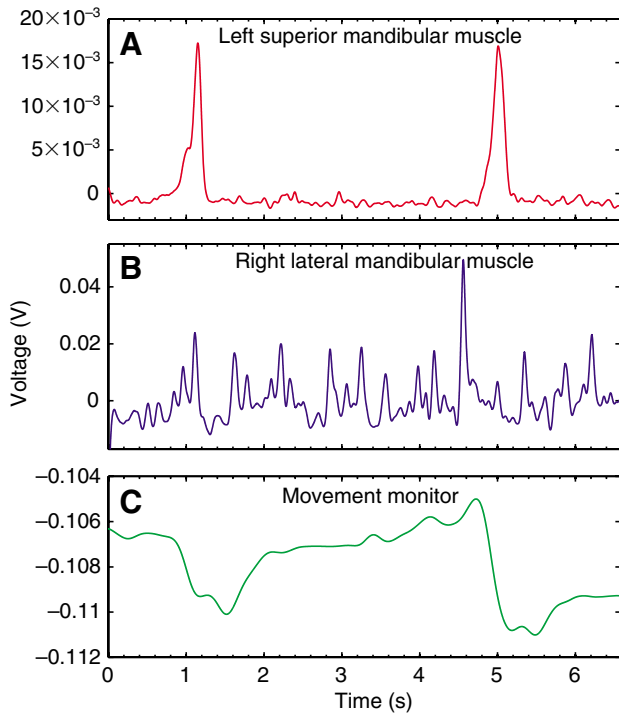


Fig. 10. Plots of rectified, averaged electromyograms of the left superior mandibular muscle (A) and the right lateral mandibular muscle (B) correlated with the output of the monitor of beak position (C). Note that beak closing is correlated with superior mandibular muscle activity.

mandibular muscle in 27% of openings, suggesting also that it may either stabilize the beaks or modulate the movements produced by other beak muscles.

#### *The lateral mandibular muscles*

No beak openings occurred in the absence of lateral mandibular muscle activity. The lateral mandibular muscles were identified previously as the only muscles that have a fiber arrangement that could produce opening force and they are likely to be the major beak opening muscles (Uyeno and Kier, 2005). The competing hypothesis does not seem to be valid: contraction of the lateral mandibular muscles probably does not elevate the upper beak by flexing the lateral walls. No shape change that results in the movement of the upper beak was observed in the freshly dissected beak. We did observe flexing of the lateral walls in the buccal mass preparation, but we agree with Kear's assessment that this flexion probably accommodates the movement of the palps and radula/odontophore complex (Kear, 1994).

#### *Mandibular muscles as a dynamic hinge*

In addition to producing the force required to open the beaks, we hypothesized previously that the lateral mandibular muscles may also form a pivot for other beak motions. Our experimental results are consistent with this hypothesis, but do not provide

a definitive test. If the lateral mandibular muscles are only involved in opening the beaks, activity would be observed only during these motions. We also observed them to be active, often in concert with the superior mandibular muscle, during closing and during phases without motion. These data suggest that the lateral mandibular muscles may modulate the effects of superior mandibular muscle contraction during the production of complex beak movements, but we cannot determine from our data whether they are simply stabilizing beak movements or if they are serving a more dynamic role in altering the location of the pivot between the two beaks. A definitive test of this hypothesis will require more precise three-dimensional kinematics in conjunction with finer scale sampling of electrical activity from the musculature.

#### *The anterior and posterior mandibular muscles*

We were unable to record from either the anterior or the posterior mandibular muscles because the muscle layers were too thin for our electrodes and the connective tissue sheath surrounding the buccal mass complicated electrode placement. Although we were not able to test the functional predictions for these muscles it is likely that these muscles produce less force than the superior and lateral mandibular muscles because of their small cross sectional areas (Uyeno and Kier, 2005). Based on the fiber arrangement, the anterior mandibular muscle may retract the upper beak (Uyeno and Kier, 2005). The posterior mandibular muscle may maintain tonus of the posterior buccal wall and perhaps, in conjunction with the lateral mandibular muscles, open the beak (Kear, 1994). Tests of these predictions will require a novel experimental approach.

#### *Summary of the opening and closing movement*

##### *Closing*

The superior mandibular muscles were active during the majority of beak closures, especially the rapid ones of large amplitude. In approximately one quarter of the cases, in which closing was slower and of smaller amplitude, activity was observed only in the lateral mandibular muscle or no activity was seen in either the lateral or superior mandibular muscles. This suggests three possibilities: the anterior or posterior mandibular muscles may be able to close the beak; elasticity of the buccal sheath may close the beaks; or movement of structures within the buccal cavity, such as the bolsters or the radula/odontophore complex may close the beak.

Our conclusions are in general agreement with the data provided by previous studies. Boyle et al. (Boyle et al., 1979a) correlated the activity of the superior mandibular muscle with both closing and retraction movements. Kear (Kear, 1994) observed closing movements without retraction in response to stimulation of the superior mandibular muscle. She attributed the retraction to either an artifact of the experimental setup used by Boyle et al. or the activity of the inferior mandibular muscle. Uyeno and Kier (Uyeno and Kier, 2005) redescribed anterior portions of the inferior mandibular muscle as the anterior mandibular muscle. The anterior mandibular muscle may indeed be active in retraction, but as described above, we were

unable to implant electrodes in this muscle to explore its function.

### Opening

We observed the lateral mandibular muscles to be active during every beak opening and thus conclude that their activity is required for this movement. In addition, during opening movements, we observed the axis of rotation of the upper beak relative to the lower beak varied and could be located over a rather large area that includes the lateral mandibular muscles (Fig. 5). These results differ in some respects from those of previous studies. Boyle et al. (Boyle et al., 1979a) were unable to correlate any muscular activity to beak opening movements and proposed a passive mechanism. We are uncertain why Boyle et al. (Boyle et al., 1979a) did not observe muscle activity during opening, but Kear (Kear, 1994) suggested that the buccal mass sheath may have insulating properties that hampered recordings. Kear (Kear, 1994) stimulated the center of the lateral mandibular muscles and found this produced the strongest opening movements. Her results are thus in general agreement with our observations, although she suggested a different opening mechanism (see above).

### Conclusions and future directions

The cephalopod buccal mass is a flexible joint in which the lateral mandibular muscle functions as a muscular hydrostat, providing force for opening of the beaks. The superior mandibular muscle probably produces the majority of the closing force. Co-contraction of the superior mandibular muscle and lateral mandibular muscles may stabilize beak movements and might also provide a means of actively controlling the position of the hinge between the beaks. The upper and lower beaks are connected by the lateral mandibular muscles so that they bear any reactive forces generated by the bite and thus they replace the function of the contacting surfaces of articulating skeletal elements. These three functions, a pivot, an antagonistic muscle, and the element that bears compressive and shear forces, are all provided by soft tissue and represent the key functional characteristic of the muscle articulation. Such an arrangement may allow a larger range of motion and greater number of degrees of freedom than a more conventional articulated joint. In the case of the buccal mass, five degrees of freedom were identified (anterior–posterior and dorsal–ventral translations as well as rotations in all three orthogonal planes). A potential trade-off for the gain of this flexibility may be the increased complexity of neuromuscular control that is required to produce this diversity of movement.

Muscle articulation joints may be a more common biomechanical feature in invertebrates than previously recognized. The eversible jaws of marine polychaetes and the hooks of interstitial turbellarians are currently under

investigation and appear to share many characteristics with the buccal mass of cephalopods. Perhaps the diversity and complexity of motion that are allowed by a muscle articulation provide important advantages in feeding and manipulation. These characteristics may also make them useful models for engineers designing biologically inspired artificial joint mechanisms.

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