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## A soft origin for a forceful bite: motor patterns of the feeding musculature in Atlantic hagfish, *Myxine glutinosa*

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### ABSTRACT

Despite lacking jaws and substantial rigid support for feeding muscles, hagfishes can forcefully grasp and ingest chunks of flesh from their prey. When feeding, bilaterally folding dental plates protrude from the mouth, then forcefully retract. This cyclic protraction and retraction occurs in the anterior region of the hagfish feeding apparatus (HFA) and involves both a cartilaginous skeleton and a complex array of muscles that act as a hydrostat. We recorded motor patterns from the largest muscles in the HFA in six specimens of *Myxine glutinosa*: the deep protractor muscle (DPM), clavatus muscle (CM), perpendicularis muscle (PM), and tubulatus muscle (TM). Individuals normally used four gape cycles to ingest food and four gape cycles to intraorally transport food. We measured burst duration from each muscle and the onsets of kinematic events and the onsets of CM, PM, and TM bursts relative to the onset of the DPM. The DPM fired during protraction, while the CM, PM and TM fired during retraction. Our study corroborates our anatomical predictions about DPM and CM function. Activation of the circumferentially and vertically oriented fibers of the TM and PM stiffens the origin of the CM, allowing it to forcefully retract the dental plates. The progressive decrease in retractor muscle activity during gape cycles following ingestion suggests a reliance on passive properties of the musculoskeletal system for retraction.

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### 1. Introduction

Hagfishes are an evolutionarily and ecologically successful lineage of jawless fishes that have remained morphologically conserved for over 300 million years (Bardack, 1991). All extant hagfish species are benthic, opportunistic scavengers of marine vertebrates and invertebrates (Martini, 1998; Auster and Barber, 2006). The hagfish feeding apparatus (HFA) possesses dentition and cartilage but is predominantly composed of muscles. Though the feeding apparatus of all hagfish species possesses a similar arrangement of teeth, cartilages, and muscles, the proportionalities of the feeding apparatus and the total tooth number vary between species (Fernholm, 1998; Clark and Summers, 2007). Despite their jawless condition, hagfish can forcefully remove tissue from carcasses and are capable of ingesting relatively large pieces of food (Clark and Summers, 2007). Interestingly enough, these challenging tasks are

performed with a feeding apparatus almost entirely composed of soft tissue.

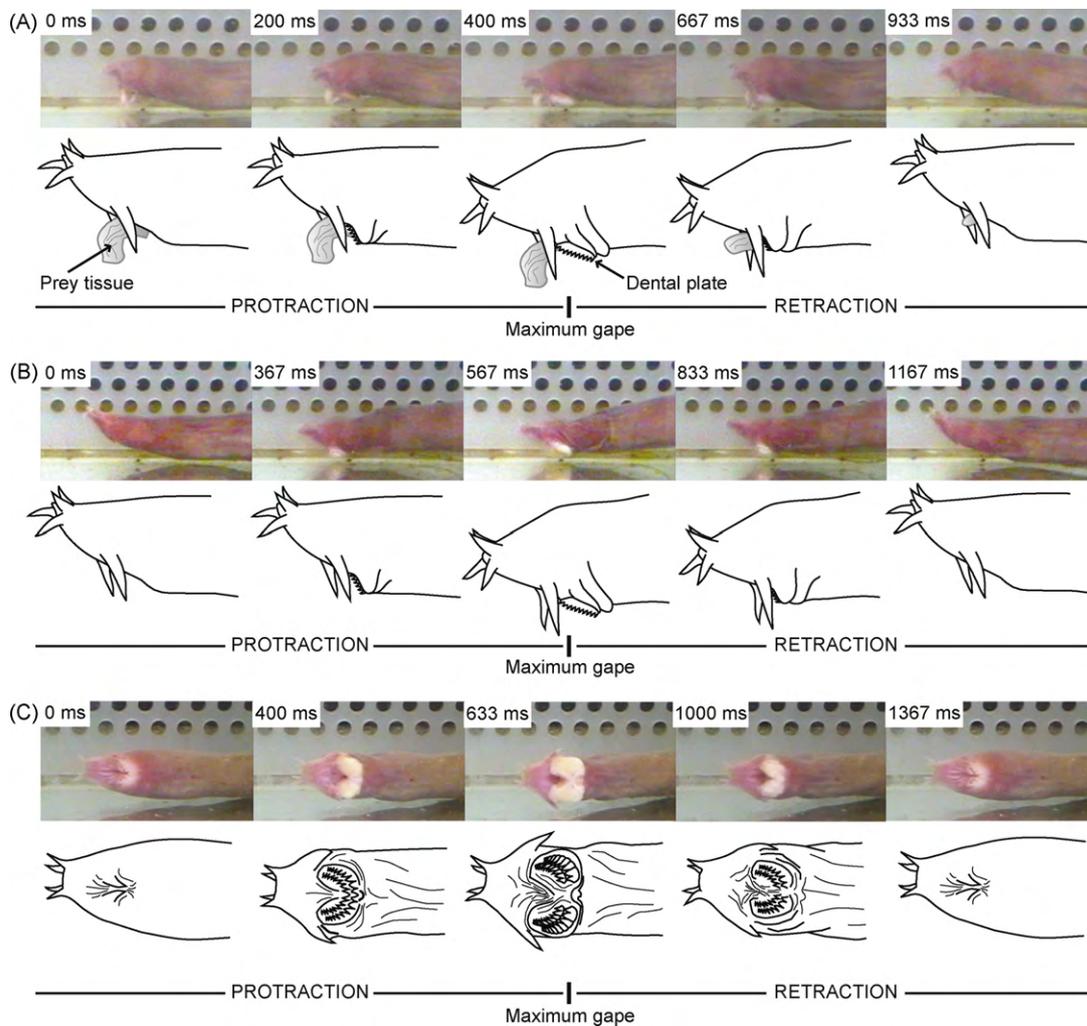
Hagfish grasp and swallow prey by repeatedly protracting and retracting a pair of bilaterally symmetric dental plates supporting two rows of posteriorly curved, keratinous teeth. Dental plate halves laterally unfold during protraction and medially fold during retraction. A feeding bout consists of a prey capture event followed by a transport event (Fig. 1). The capture event includes dental plate protraction–retraction movements used for grasping, tearing, and ingesting prey, while dental plate movements following ingestion are involved in intraorally transporting food. Despite relatively long gape cycle times, hagfishes can protract their dental plates up to 180° and retract their dental plates with forces comparable to gnathostome bite forces (Clark and Summers, 2007). Dental plate retractile forces can be amplified with knot-tying behaviors, which often occur when a specimen is struggling to remove tissue from a large carcass (Strahan, 1963; Martini, 1998). Modeling suggests that the absence of jaws and rigid support for the retractor muscles does not constrain the dynamic, forceful, and rapacious feeding behaviors of hagfish (Clark and Summers, 2007).

The HFA is subdivided into two morphologically discrete regions: a hard component and a soft component (Fig. 2). The hard component, which includes the dental plate, underlying basal plate, and protractor musculature, is best modeled as a fixed pul-

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**Fig. 1.** Video images and illustrations of hagfish feeding behaviors. (A) Left lateral view of a hagfish gape cycle during a capture event, (B) left lateral view of a hagfish gape cycle during transport, (C) ventral view of a hagfish gape cycle during transport. The time at each event is indicated in the upper left corner of each video image.

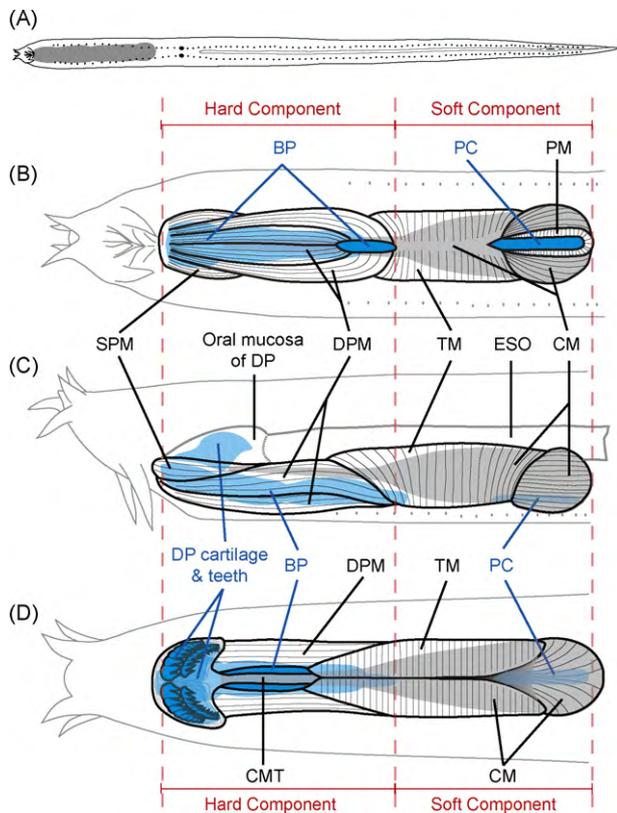
ley (Clark and Summers, 2007). The basal plate supports dental plate movement and is the origin for the deep protractor muscle (DPM) and superficial protractor muscle (SPM) (Fig. 2B and C), which are hypothesized to drive protraction and unfolding of the dental plates, respectively (Cole, 1907; Dawson, 1963).

The soft component is comprised of three muscles: the clavatus muscle (CM) (also known as the retractor mandibuli (Ayers and Jackson, 1901) or longitudinalis linguae (Cole, 1907)), the perpendicularis muscle (PM), and the tubulatus muscle (TM) (also known as the constrictor muscui mandibuli (Ayers and Jackson, 1901) or copulo-copularis (Cole, 1907)) (Fig. 2). Though the anatomy of the CM presents strong evidence for its function as a dental plate retractor, the functions of the TM and PM are unclear. The smallest muscle, the PM, possesses vertically oriented fibers packed within the mid-sagittal plane of the posterior third of the CM (Figs. 2B and 3A). Overlying the PM are the longitudinally arranged fibers of the CM. The anterior two thirds of the CM are tightly enveloped within an overlying array of circular fibers of the TM (Fig. 3A). Within this region of overlap, the CM progressively tapers in the anterior direction where it connects to the long, narrow retractor tendon at the anterior border of the TM (Fig. 3A). The dense packing and three-dimensional fiber orientation of the CM, PM, and TM make the soft component of the HFA a muscular hydrostat (see Kier and Smith, 1985).

As with all muscular hydrostats, the soft component retains a constant volume of incompressible fluid (myoplasm), therefore

activity from a muscle will cause an overall change in the shape of the muscular hydrostat. This deformation is essential to movement (Kier and Smith, 1985; Vogel, 2003). Predicted shape changes in the muscular hydrostat during dental plate protraction and retraction can be useful in generating hypotheses on PM and TM function (Fig. 3B). When dental plates are protracted, the CM elongates in the anterior direction and simultaneously becomes narrower. As the anterior part of the CM projects forward beyond the array of TM fibers, a portion of the CM initially overlapping with the anterior fibers of the PM projects forward, within the array of TM fibers. However, the PM wedges the posterior CM and prevents it from being completely enveloped by the TM during protraction. As a result, the CM–TM region becomes shorter and wider, while the CM–PM region becomes less bulbous (Fig. 3B). Considering that protractor muscles are relatively weak and that activity in the TM and PM may preclude passive anterior elongation of the CM, we hypothesize that none of the muscles in the muscular hydrostat are active during protraction (Fig. 3B).

When dental plates retract, the CM shortens and widens as its anterior portion returns within the array of the anterior TM fibers and its posterior portion is projected backwards beyond the array of the posterior TM fibers (Fig. 3B). During retraction, the CM–TM region becomes narrower and elongates in the posterior direction and the CM–PM region becomes more bulbous. Considering the CM's capacity for generating large magnitudes of force and the softness of its origin, we hypothesize that all muscles in the hydrostat



**Fig. 2.** The feeding apparatus of the Atlantic hagfish, *Myxine glutinosa* and its two major subdivisions. Rigid tissue (cartilage and teeth) is colored blue, with faded shades of blue indicating the portions of tissue beneath softer tissue. (A) Ventral view of a specimen of *M. glutinosa*. Shaded area indicates the location of the feeding apparatus. (B) Ventral view, (C) lateral view, and (D) dorsal view of the feeding apparatus and its position in the head. Blue text indicates rigid tissue: BP, basal plate; DP, dental plate; PC, perpendiculus cartilage. Black text indicates soft tissue: CM, clavatus muscle; CMT, clavatus muscle tendon; DPM, deep protractor muscle; ESO, esophagus; PM, perpendicularis muscle; SPM, superficial protractor muscle; TM, tubulatus muscle. The CM and CMT are shaded for reference.

(CM, PM, and TM) will fire during retraction (Fig. 3B). As the CM retracts the dental plates, its origin would be stiffened by simultaneous activity from the PM and TM. As the TM fires, it squeezes the anterior portion of the CM, restoring the CM to its retracted position and preventing the CM from shifting forward as it pulls the dental plates. Activity from the PM and its connection with the perpendiculus cartilage would fix the position of the posterior CM and prevent the CM from shifting forward during retraction. PM activity would also help pull the posterior part of the CM beyond the array of the posterior TM fibers and thus restore the bulbous shape of the CM–PM region (Fig. 3B).

Though previous observers of the anatomy have consistently hypothesized that the PM activity fixes the CM in position during retraction, there have been conflicting hypotheses regarding the function of the TM (Müller, 1839; Fürbringer, 1875; Cole, 1907; Dawson, 1963). Variable hypotheses for TM function may have resulted from differing models for the CM–TM region of overlap. For example, if it were assumed that the CM possesses a uniform cross-sectional area throughout its length within the TM, regional activity in the posterior part of the TM could assist in protraction and regional activity in the anterior part of the TM could assist in retraction (Fig. 3C). This “two-cylinder” model for the CM–TM region could justify a hypothesis for the TM as a protractor and/or retractor. However, a “cone-cylinder” model for the CM–TM region more accurately represents the anatomy, in which the CM has a progressively increasing cross-sectional area in the posterior direction (Fig. 3C). The cone-cylinder model demonstrates that an activated

TM, regardless of the region of activation, can only drive the CM to the posterior direction, as the increasing mass of TM fibers in the anterior portion of the CM–TM region precludes movement of the CM in the anterior direction (Fig. 3C). Our hypothesis for TM function can be justified with the cone-cylinder model.

Our experimental objectives were to use synchronized video and electromyography (EMG) to determine activity patterns in the feeding musculature of foraging Atlantic hagfish, *Myxine glutinosa* (Linnaeus). We also quantified differences in kinematic and EMG profiles between capture and transport events. We investigated motor patterns of the DPM, CM, PM, and TM because they are the largest muscles in the HFA. Studying muscle activity in the soft component of the HFA presents an opportunity to describe the biomechanics of a muscular hydrostat and to determine how large magnitudes of force are produced and transmitted from a soft origin.

## 2. Materials and methods

### 2.1. Animal care

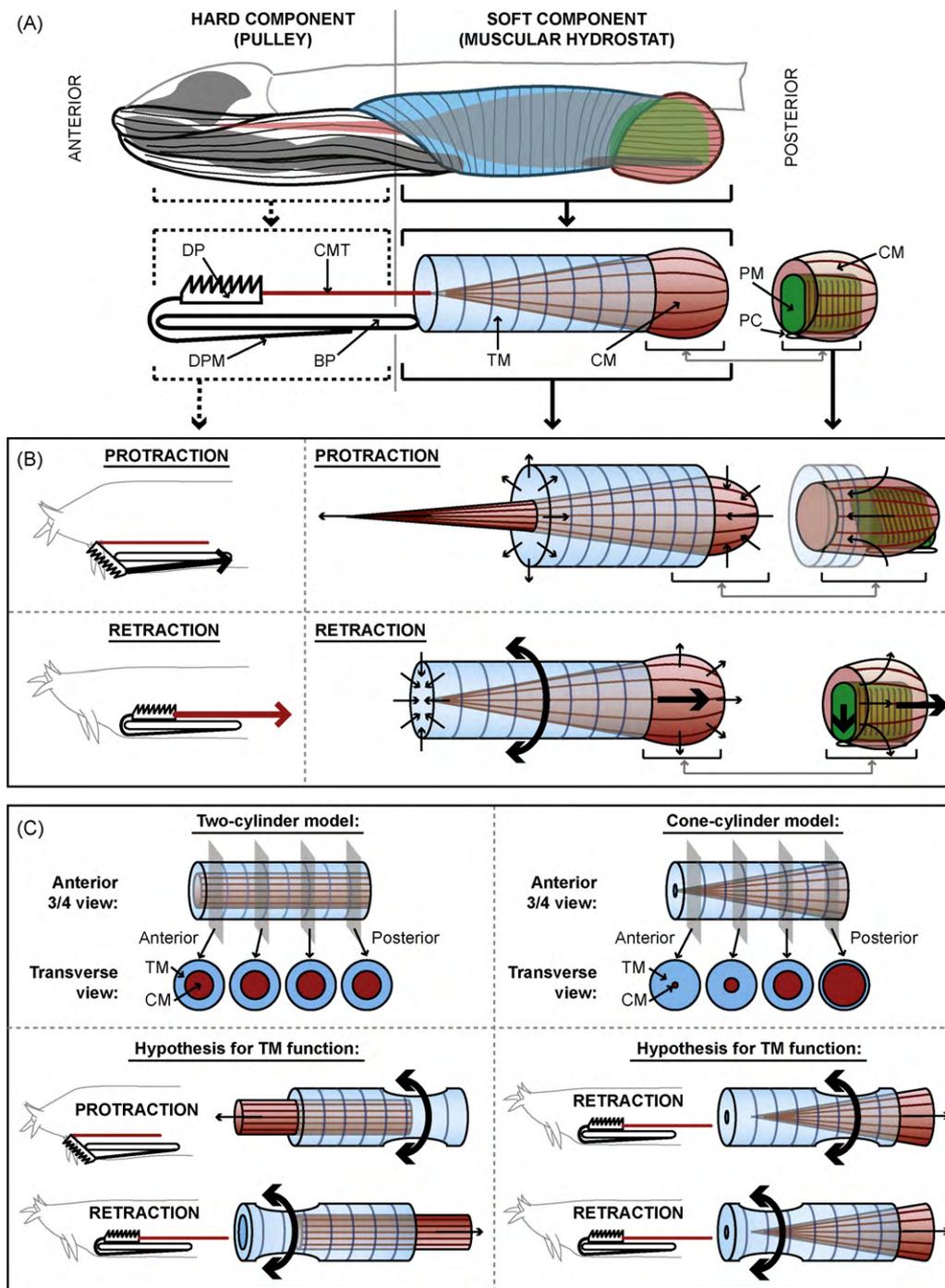
Atlantic hagfish (*M. glutinosa*) were shipped to the University of California, Irvine from Mount Desert Island Biological Laboratories (Salisbury Cove, Maine, USA). Animals were originally collected from depths of 25–60 m off of Cape Cod, Massachusetts. At UC Irvine, the animals were housed in aquaria with recirculating artificial saltwater maintained at approximately 10 °C and 34 ppt (Gustafson, 1935). Animals were housed in an aquarium (58.42 cm × 33.02 cm × 33.02 cm) with shades and cover to block ambient light and to prevent escape. Experimental specimens were filmed feeding in a smaller aquarium (50.80 cm × 25.4 cm × 31.75 cm) under moderate lighting from one 15 W fluorescent lamp. Individuals in the smaller aquarium were filmed in an area (17.78 cm × 30.48 cm × 33.20 cm) bordered with perforated white plastic sheets. *M. glutinosa* were selected for the present study over the more robust and local Pacific hagfish (*Eptatretus stoutii*) because they feed more readily in glass aquaria, are more tolerant to light, and because they respond better to EMG surgery (percutaneous electrode implantation). For maintenance, animals were fed small pieces of squid once every 3 weeks.

### 2.2. Morphology

Specimens of *M. glutinosa* were dissected to observe the muscles involved in feeding and to produce maps for electrode implantation. Slime pores and distance from the midline were usually satisfactory indices for points of electrode implantation. We investigated motor patterns from four muscles: the deep protractor muscle (DPM), clavatus muscle (CM), tubulatus muscle (TM), and perpendicularis muscle (PM). These are the largest muscles in the hagfish feeding apparatus and experimental animals responded well to four or fewer implanted electrodes. Specimens with more than four implanted electrodes showed discomfort and usually refused to eat.

### 2.3. Videography

Individual animals were selected based on their willingness to feed. Following surgery, animals were shifted to the filming tank and were offered small rectangular portions (1.0 cm × 2.0 cm × 0.25 cm) of squid mantle wall. Each piece of squid was loosely secured to a plastic cable tie, then positioned directly in front of, and usually touching, the animal’s mouth. Feeding behaviors were recorded with a digital video camera positioned in front of the glass aquarium. Image sequences were analyzed at



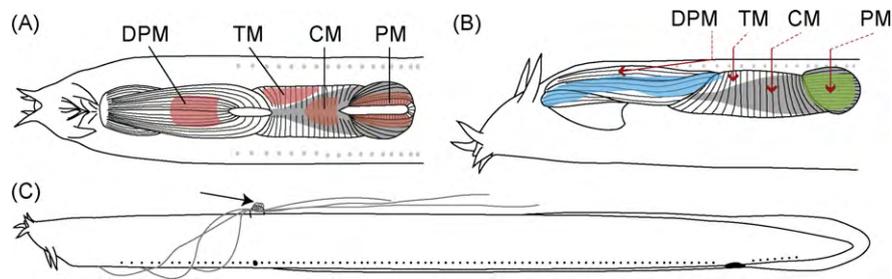
**Fig. 3.** Models of the hard and soft components of the hagfish feeding apparatus (HFA) and their use in predicting the function of the perpendicularis muscle (PM) and tubulatus muscle (TM). (A) Left lateral view of the HFA and its subdivisions with models of the subdivisions below. (B) The two regions of the muscular hydrostat and their patterns of deformation during protraction and retraction of the dental plates. Thick arrows indicate active muscles. Thin arrows indicate deformation patterns during protraction and retraction. (C) Two-cylinder and cone-cylinder models for the CM-TM region with predicted TM activity patterns. Thick arrows indicate activated TM and thin arrows indicate the resulting passive movement of the CM. *Abbreviations:* BP, basal plate; CMT, clavatus muscle tendon; DP, dental plate; DPM, deep protractor muscle.

30 frames per second, which was appropriate for studying the relatively slow gape cycles of hagfish (Clark and Summers, 2007). Data from unsuccessful feeding bouts, which resulted from rejection of food after attempting ingestion or transport, or failure to initially gasp food, were excluded from the present study.

#### 2.4. Kinematics

We simultaneously collected EMG and dental plate kinematic data from six *M. glutinosa* (total length = 33.5–57.5 cm). We ana-

lyzed 3–6 feeding bouts from each specimen, with 5–10 min intervals between feeding bouts. Kinematic time variables included gape cycle time (GCT), time to maximum gape (TMG), and dental plate retraction time (DPRT). Data were recorded from videos that showed time variables clearly. GCT was defined as the time required for a hagfish to fully protract and retract its dental plate. TMG was the elapsed time for the dental plates to be maximally protracted from a retracted position. DPRT was the elapsed time for dental plate retraction from a maximally protracted position.



**Fig. 4.** Methods for implanting the electrodes into the feeding musculature. (A) Ventral view of a hagfish specimen with red shaded areas indicating sites of electrode implantation for each muscle. (B) Lateral view of a hagfish specimen with red arrows indicating the approximate location and angle of electrode implantation. Specimens were positioned upside-down during surgery. Note that the angle at which the DPM was implanted relative to the skin was very acute in comparison to the angles at which the CM, PM, and TM were implanted. This acute angle of implantation was necessary for avoiding contact with the underlying basal plate (blue shading). The PM is indicated with green shading. (C) Left lateral view of a hagfish showing implanted electrodes and how they were secured to the loop of suture (black arrow). *Abbreviations:* CM, clavatus muscle; DPM, deep protractor muscle; PM, perpendicularis muscle; TM, tubulatus muscle.

Angular kinematic variables included maximum protraction angle (MPA) and head depression angle (HDA). These angles were measured from still-frames of hagfish individuals in lateral view with Image J software (NIH, Bethesda, MD, USA). We defined MPA as the angle between the anterior tip of the maximally protracted dental plate, the mouth opening, and the anterior tip of the snout. HDA was the angle between the anterior tip of the snout before the onset of head depression, the pivoting point of the head, and the anterior tip of the snout at the completion of head depression.

### 2.5. Electromyography

Approximately 0.75 m of 50.8  $\mu\text{m}$  diameter bipolar electrodes (CA Fine Wire Co., Grover Beach, CA, USA) were used for EMG recordings. Approximately 0.75–1.0 mm of insulation was stripped from one end of each electrode and the exposed wires were bent backwards to form a hook. Individual hagfish were anesthetized with 100 mg of tricaine methanesulfonate (MS 222), buffered with 200–250 mg of sodium bicarbonate ( $\text{NaHCO}_3$ ), in 1 l of 10–13  $^{\circ}\text{C}$  artificial seawater for approximately 40 min. A small portion of the skin on the dorsal surface of each specimen was pierced for tying a 0.5–0.75 cm diameter loop of suture, from which electrodes were secured. From the ventral surface of each animal, electrodes were implanted in the DPM, CM, TM, and PM via percutaneous injection with a 25-gauge hypodermic needle (Fig. 4). To reduce tension and potential entangling caused by movement, electrodes were looped twice around the suture loop, then super-glued together with the suture loop to the skin. Surgery for each animal lasted approximately 30 min. After surgery, the animal was placed in the filming tank and usually required 40 min to recover.

During recovery time, the distal ends of electrodes were attached to a terminal block, which in turn was connected to a four channel differential amplifier with 0.5 m long BMP cables (Model 1700; A-M Systems, Inc., Carlsborg, WA, USA). The analog input signals were amplified 100 $\times$ , band pass filtered at 20–1000 Hz, and notch filtered at 60 Hz. Amplified analog signals from the electrodes were digitized with a 14-bit, 48 kb/s low-cost multifunction DAQ (Model NI-USB 6009; National Instruments, Austin, TX, USA). EMG signals were monitored, recorded at 2 kHz, and filtered in LabView 7.1 (National Instruments, Austin, TX, USA). EMG signals from all muscles were displayed on LabView's graphic user interface.

To synchronize kinematic profiles with muscle activity patterns, an LED was positioned in the corner of the video camera's field of view and connected to the DAQ as an analog input. Voltage signals from the LED and EMG electrodes were simultaneously digitized and recorded. A voltage spike in the display indicated when the LED was turned on. The onset of the LED corresponded to the onset of the voltage spike. Time difference between the onset of the LED

and the onset of a kinematic event was determined from the image sequences of the video data, while the time difference between a burst onset and the onset of the LED was determined from the voltage signal data.

Individual animals were euthanized with an overdose of MS 222 once data collection was completed. Following euthanasia, animals were measured for TL and mass, then dissected to confirm electrode placement. To determine the percentage of hard tissue comprising the HFA, we recorded the mass of the intact feeding apparatus and its hard tissue mass (dental plate mass + basal plate mass + perpendicularis cartilage mass).

Filtering of digitized EMG signals was performed in LabView 7.1. A threshold peak detection method was used to determine burst onset and offset (Hodges and Bui, 1996). EMG signals were root mean square rectified, then plotted to determine a threshold value. Threshold values were greater than or equal to 120% noise levels. After rectifying and threshold filtering, a bar plot was made, which displayed portions of the original signal that were above or equal to the threshold value. Burst onsets and offsets were determined from the bar plot. Burst duration equaled the difference between burst offsets and onsets. EMG signals from all muscles were analyzed for burst duration and burst presence. Onsets of kinematic time variables and the burst onsets of the CM, TM, and PM were determined relative to the onset of the DPM, which served as the reference muscle.

### 2.6. Statistical analyses

Quantitative analyses of the feeding behavior and muscle activity were performed on 3–6 feeding bouts per specimen. Each feeding bout included all gape cycles involved in the capture and transport of a piece of squid. Mean values (gape cycle time, time to maximum gape, dental plate retraction time, maximum protraction angle, head depression angle, burst duration (of all muscles), onsets of the TM, CM, and PM relative to the onset of the DPM) were determined from each specimen, then a grand mean was calculated using the means of all specimens. A one-way analysis of variance (ANOVA) was used to compare the grand means of the kinematic time variables, burst durations of all muscles, and relative onsets of the CM, TM, and PM between capture feeding events and transport feeding events, with feeding events representing independent groups and the EMG and kinematic data representing the test variables. A two-way ANOVA was used to examine the effect of differential muscle activity patterns (see Section 3.2) on dental plate retraction times during transport, with motor patterns as fixed factors and retraction times as dependent variables. All statistical analyses were performed in SYSTAT 12.0 (Systat Software Inc., San Jose, CA, USA) and the criterion for significance was  $P < 0.05$ .

**Table 1**  
Durations of dental plate movements from capture and transport feeding events in *Myxine glutinosa*. Angular kinematic variables were measured from transport feeding events only. Values are means  $\pm$  S.E.M. *N* indicates the number of specimens and gape cycles, respectively. *P*-Values were determined using one-way ANOVA.

Kinematic variable	Capture	Sample size	Transport	Sample size	<i>P</i> -Value
Gape cycle time (ms)	1040 $\pm$ 59	<i>N</i> = 6, 79	986 $\pm$ 37	<i>N</i> = 6, 75	0.451
Time to maximum gape (ms)	388 $\pm$ 13	<i>N</i> = 6, 79	408 $\pm$ 7	<i>N</i> = 6, 75	0.215
Dental plate retraction time (ms)	633 $\pm$ 49	<i>N</i> = 6, 79	539 $\pm$ 32	<i>N</i> = 6, 75	0.137
Maximum protraction angle (°)	N/A	N/A	180 $\pm$ 0.70	<i>N</i> = 5, 25	N/A
Head depression angle (°)	N/A	N/A	12 $\pm$ 0.70	<i>N</i> = 5, 25	N/A

**Table 2**  
Motor patterns of the feeding musculature of *Myxine glutinosa* during capture and transport feeding events. Values indicating burst duration and onsets are means  $\pm$  S.E.M. *N* indicates the number of specimens and gape cycles. Burst presence is the number of times when bursts were present during the analyzed gape cycles (%). *P*-Values were determined using one-way ANOVA. All onsets are relative to the onset of the DPM. CM, clavatus muscle; DPM, deep protractor muscle; PM, perpendicularis muscle; TM, tubulatus muscle.

Muscle	Capture	Sample size	Transport	Sample size	<i>P</i> -Value
DPM duration (ms)	354 $\pm$ 25	<i>N</i> = 6, 53	374 $\pm$ 67	<i>N</i> = 6, 43	0.788
DPM burst presence (%)	100	<i>N</i> = 6, 53	100	<i>N</i> = 6, 53	N/A
CM onset (ms)	488 $\pm$ 67	<i>N</i> = 5, 42	505 $\pm$ 25	<i>N</i> = 5, 34	0.822
CM burst duration (ms)	431 $\pm$ 35	<i>N</i> = 5, 42	273 $\pm$ 22	<i>N</i> = 5, 34	0.005
CM burst presence (%)	100	<i>N</i> = 5, 42	64	<i>N</i> = 5, 34	N/A
TM onset (ms)	431 $\pm$ 31	<i>N</i> = 6, 56	465 $\pm$ 26	<i>N</i> = 6, 36	0.413
TM burst duration (ms)	363 $\pm$ 47	<i>N</i> = 6, 56	346 $\pm$ 22	<i>N</i> = 6, 36	0.743
TM burst presence (%)	100	<i>N</i> = 6, 56	80	<i>N</i> = 6, 36	N/A
PM onset (ms)	421 $\pm$ 29	<i>N</i> = 3, 15	486 $\pm$ 21	<i>N</i> = 3, 15	0.144
PM burst duration (ms)	363 $\pm$ 26	<i>N</i> = 3, 15	226 $\pm$ 41	<i>N</i> = 3, 15	0.06
PM burst presence (%)	100	<i>N</i> = 3, 15	64	<i>N</i> = 3, 15	N/A

### 3. Results

#### 3.1. Feeding behavior and kinematics

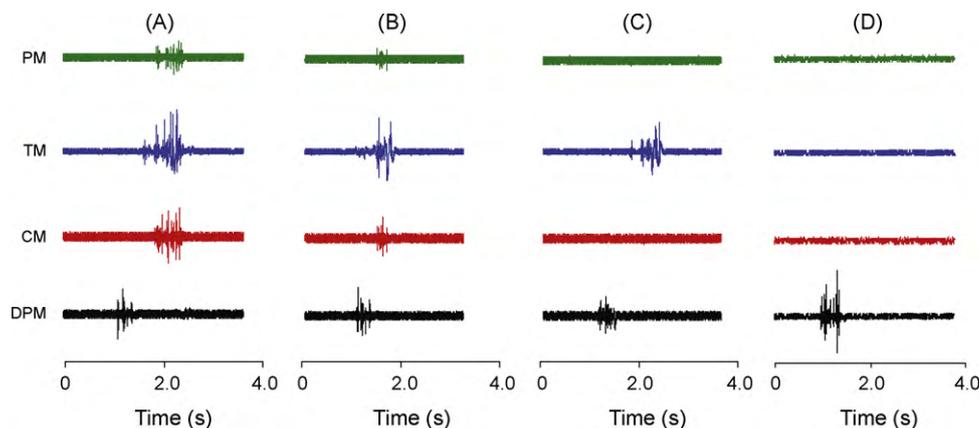
EMG electrode implantation did not impair the ability of *M. glutinosa* to successfully capture and transport food. Each hagfish normally used four gape cycles to grasp and ingest prey (capture event) and four additional gape cycles to intraorally transport prey (transport event). Successful feeding bouts lasted approximately 30 s.

Results from the kinematic analyses are included in Table 1. Gape cycle time (GCT), time to maximum gape (TMG), and dental plate retraction time (DPRT) were similar during both capture and transport events. Gape cycle times during capture and transport events were approximately 1 s, with protraction of the dental plates occurring during the initial 35–40% of the gape cycle. Individuals attained 180° gape angles and depressed their heads by 12° at the time of peak gape. The onset of dental plate retraction immediately followed peak gape, however, dental plate movement was

very slow within the initial 25–50% of the retraction period (DPRT). Velocities increased when the dental plates began to fold medially and rotate about the leading edge of the basal plate as the plates entered the mouth.

#### 3.2. Muscle activity patterns

Results from the EMG analyses are shown in Table 2. DPM activity only occurred during dental plate protraction and CM, TM, and PM activity only occurred during retraction. The DPM fired in 100% of the protraction events during prey capture and intraoral transport (Table 2). CM, TM, and PM activity occurred in 100% of the retraction events during the capture phase (Table 2 and Fig. 5A). However, when transporting food, the TM was active in 80% of the gape cycles, while the CM and PM fired in 64% of the gape cycles (Table 2). On average, all three retractor muscles fired during the first two of four transport gape cycles (Fig. 5B). The TM was the only active retractor muscle in the third gape cycle and retractor muscle activity was absent during the final gape cycle (Fig. 5C and D).

**Fig. 5.** Electromyograms of four feeding muscles from an individual *Myxine glutinosa* performing one gape cycle. (A) EMGs from one gape cycle during a capture event. (B) EMGs from one gape cycle during a transport event in which the dental plates experience active tension during retraction. (C) EMGs from one gape cycle during a transport event in which the dental plates experience moderate tension during retraction. (D) EMGs from one gape cycle during a transport event in which the dental plates experience passive tension during retraction. Abbreviations: CM, clavatus muscle; DPM, deep protractor muscle; PM, perpendicularis muscle; TM, tubulatus muscle.

Despite the variation in muscle activity patterns, mean dental plate retraction times were similar in all gape cycles following ingestion ( $P=0.204$ , two-way ANOVA).

Mean burst durations of the DPM, PM, and TM were similar during capture and transport events (Table 2 and Fig. 6). However, CM bursts significantly decreased in duration from  $431 \pm 31$  ms (mean  $\pm$  S.E.M.) during capture events to  $273 \pm 22$  ms (mean  $\pm$  S.E.M.) during transport events (Table 2 and Fig. 6).

Consistent activity patterns of the DPM during hagfish feeding rendered it an appropriate reference for determining the relative onsets and durations of the retractor muscles and kinematic time variables. The onset of the DPM preceded the onset of dental plate protraction and its offset occurred prior to the time of peak gape (= offset of dental plate protraction) during both capture and transport (Fig. 6).

During dental plate retraction for both capture and transport events, the onsets of the PM and TM always preceded the onset of the CM (Fig. 6). The onset of dental plate retraction preceded the onsets of all retractor muscles during both capture and transport (Fig. 6). All retractor muscles stopped firing prior to the offset of

dental plate retraction in capture and transport, after the dental plates rotated over the anterior edge of the basal plate.

#### 4. Discussion

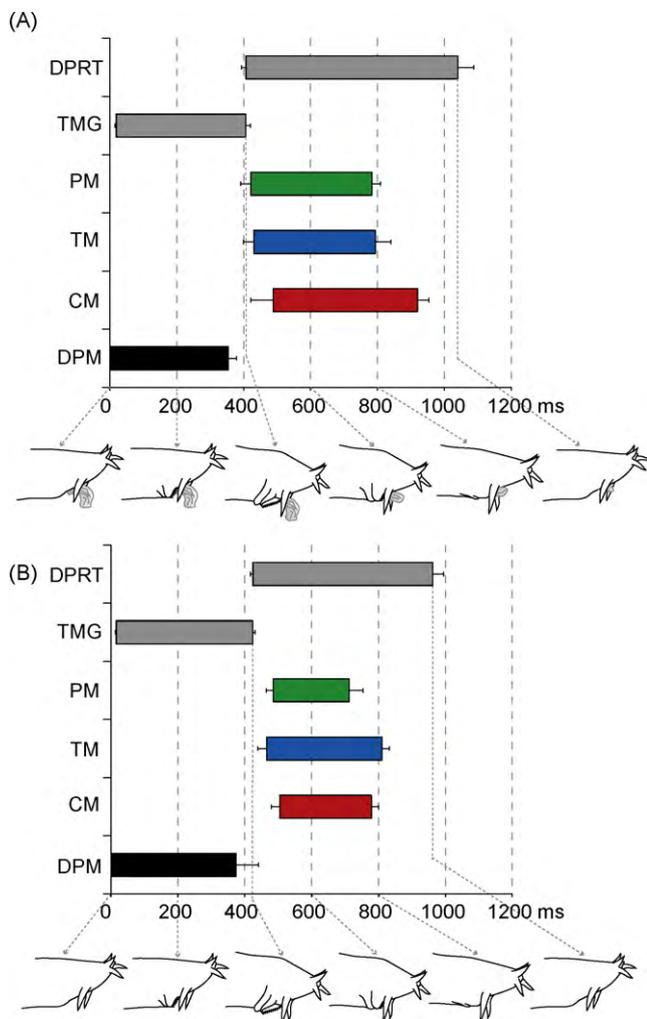
##### 4.1. Muscle function and previous hypotheses

Our results are consistent with the hypothesis that force produced by the simultaneous firing of the TM and PM stabilizes and helps transmit the retractile force from the CM to the dental plates. In the soft component of the hagfish feeding apparatus (HFA), the CM is the direct retractor of the dental plates and the TM and PM support retraction by stiffening the origin of the active CM. The TM and PM were inactive during dental plate protraction and therefore support the cone–cylinder model (Fig. 3D). Activation of the CM did not occur without preliminary and overlapping firing from the PM and TM. When activated, the posterior body of the CM is anchored by the active vertical fibers of the PM while activity from the circumferential TM fibers secures the anterior body. Synergism from these three muscles is a necessity for grasping and ingesting prey; however, this pattern of muscle activity is altered when prey is intraorally transported.

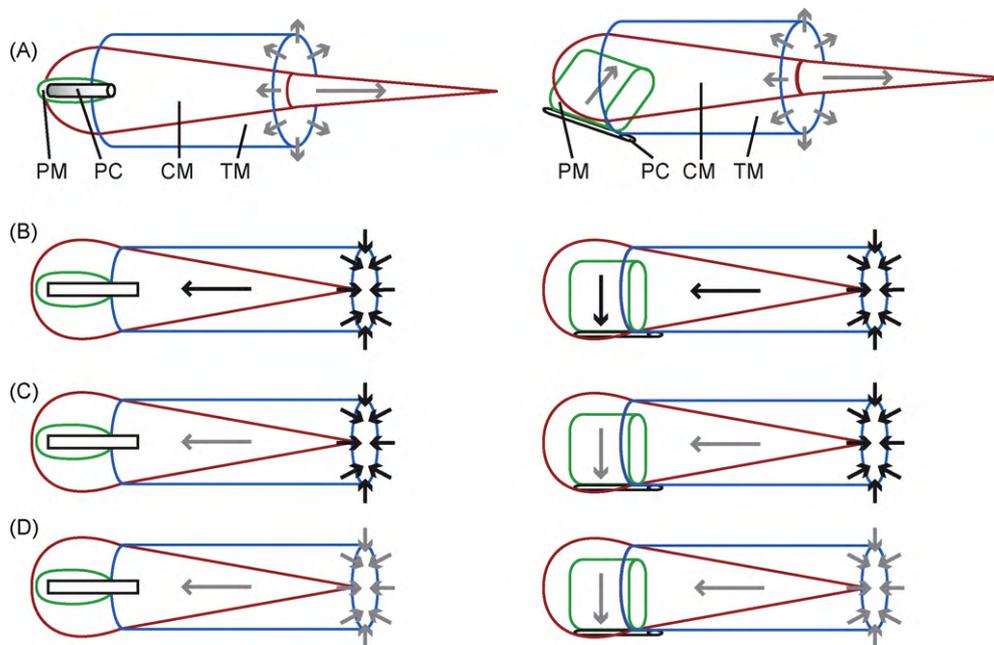
Dental plate retraction during transport can be active (involving activity from all three retractor muscles, Figs. 5B and 7B), moderately active (with TM activity alone, Figs. 5C and 7C), or passive (with no retractor muscle activity, Figs. 5D and 7D). A transition from active retraction to passive modes of retraction was typical over the course of multiple transport events. After ingestion, the CM and PM were less involved in retracting the dental plates (Table 2 and Figs. 5 and 6). When intraorally transporting food, the burst presence from both muscles declined and there was a significant decrease in burst duration of the CM (Table 2 and Fig. 6B). Perhaps less force is required to retract the dental plates during intraoral transport. As demonstrated in the cone–cylinder model, the arrangement of CM mass relative to TM mass throughout the length of the soft component of the HFA may account for the occurrence of moderately active and passive retraction of the dental plates. Pressure generated by the active circumferential fibers of the TM may restore a stretched CM to its retracted position in the absence of CM and PM activity (Figs. 3C and 7C). Also, the absence of activity from any retractor muscle during non-feeding behaviors suggests that the bulk of TM mass localized in the anterior region of the soft HFA, near the origin of the retractor tendon, may suffice for keeping the dental plates and CM in retracted positions.

Rigid tissue, cartilage and teeth, account for only 15–20% of the feeding apparatus mass and most of this rigid tissue is localized in the hard component of the HFA, where protraction occurs. Protraction of the dental plates normally comprises the initial 35–40% of the gape cycle and involves bilateral unfolding of the dental plates coupled with the unveiling of oral mucosa to expose the teeth prior to contact with prey. Hagfishes rely on the protractor muscles to attain remarkably wide gapes. Considering the burst presence of the DPM and its relatively limited force production, we can assume that the superficial protractor muscle (SPM) would also fire in every gape cycle involving maximum protraction. We did not study the activity patterns in the SPM because of its small size and because specimens normally refused to eat when multiple electrodes were implanted in the protractor muscles. Although the protractor muscles are substantially weaker than the retractors, protraction of the dental plates is a relatively rapid and dynamically complex process. This dynamic complexity is probably tied with the anterior locality of hard tissue in the HFA.

The motor patterns we encountered in the feeding musculature of *M. glutinosa* corroborate anatomical predictions about the functional roles played by the CM and DPM (Cole, 1907; Dawson, 1963). Though there has been a consensus for the PM activating



**Fig. 6.** Summary of muscle activity and dental plate kinematic time variables of *Myxine glutinosa* during (A) capture and (B) transport events. Each box indicates mean duration of the burst or kinematic event with the left and right edges of each box representing mean onset and offset, respectively. Error bars on the right edge of the boxes are the standard errors of the duration and error bars on the left edge are the standard errors of the onset. 0 ms marks the onset of the deep protractor muscle (DPM). Abbreviations: CM, clavatus muscle; DPRT, dental plate retraction time; PM, perpendicularis muscle; TM, tubulatus muscle; TMG, time to maximum gape.



**Fig. 7.** Schematic representations of the deformation in the soft component of the hagfish feeding apparatus (HFA) during dental plate protraction and retraction. Left and right columns represent the soft HFA in ventral and right lateral views, respectively. Black arrows indicate forces produced by active muscles and gray arrows are forces from passive muscles. (A) Deformation in the soft HFA during dental plate protraction. (B) Deformation during dental plate retraction with active tension (bursts from all retractor muscles). (C) Deformation during retraction with moderate tension (bursts from the TM only). (D) Deformation during retraction with passive tension (no muscle activity). *Abbreviations:* CM, clavatus muscle; PC, perpendicularis cartilage; PM, perpendicularis muscle; TM, tubulatus muscle.

during retraction, there have been conflicting hypotheses regarding the function of the TM (Müller, 1839; Fürbringer, 1875; Cole, 1907; Dawson, 1963). While Müller (1839) was undecided about its function, Fürbringer (1875) predicted that the TM was a protractor. Cole (1907) acknowledged the coned shape of the CM but suggested that the TM was passive during feeding and active upon the cessation of feeding in order to maintain the retracted state of the CM. Our results of TM function in *M. glutinosa* support the hypotheses of Dawson (1963), in which the TM and CM fire simultaneously, especially during capture events, which presumably involves more forceful retraction of the dental plates.

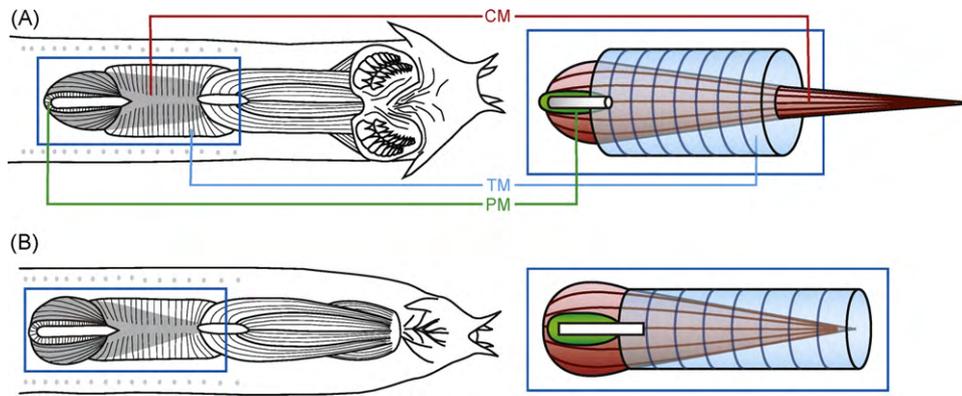
#### 4.2. Form and function of the soft component of the hagfish feeding apparatus

The soft component of the HFA is a cylindrical muscular hydrostat consisting of muscle fibers with circular, bipennate (semi-longitudinal), and vertical orientations. With the exception of the vertically oriented fibers characteristic of the PM, the soft HFA is morphologically similar to the dental plate retractor musculature of lampreys (Lanzing, 1958; Hardisty and Potter, 1971; Yalden, 1985). Unlike protrusible muscular hydrostatic systems (e.g., tongues and tentacles), the soft HFA is only involved in retracting the dental plates and is incapable of performing highly complex movements (e.g., twisting and bending). Instead, the soft posterior HFA produces and transmits large magnitudes of force with little deformation for stabilizing the relatively simple yet forceful anterior–posterior movements of the dental plates. Dental plate retractor muscles possess functionalities similar to those of cephalopod buccal mass muscles, which are muscular hydrostats classified as muscle articulations that play both stabilizing and actuating roles to control beak movements (Uyeno and Kier, 2007).

Manipulating the dental plates of recently euthanized specimens of *M. glutinosa*, with the HFA exposed, can provide a picture of the shape changes in the soft HFA during feeding (Fig. 8). Overall, there were only small changes in the length and width of the

muscular hydrostat when manipulating the dental plates, however, the patterns of shape change met our predictions. During protraction, the CM–TM region became slightly shorter and wider and the CM–PM region became less bulbous (Fig. 8A). During retraction, the CM–TM region became longer and narrower and the CM–PM region became more bulbous (Fig. 8B). Retraction of protracted dental plates in manipulated specimens was normally passive but could also be induced by gently pressing down on the anterior TM. These anatomical manipulations suggest that the soft component of the HFA is a relatively inflexible muscular hydrostat that deforms minimally during feeding.

Muscular hydrostats undergoing minimal deformation with activated musculature tend to stiffen (Kier and Smith, 1985). Cylindrical muscular hydrostats working against heavy loads or a highly resistive medium typically undergo less deformation than predicted by the geometric relationship between the radius and length of isovolumetric cylinders (Chapman, 1950; Chiel et al., 1992). These characteristics seem suited to the natural circumstances in which hagfishes feed. We speculate that the arrangement of retractor muscle masses, the fiber architecture of the CM, and the relatively smaller magnitudes of force produced by the protractor muscles preclude a high degree of length and width changes in the soft HFA. A system resisting major dimensional changes provides favorable conditions for generating and transmitting high magnitudes of force. Assuming that mean sarcomere lengths of hagfish feeding muscles fall within the conserved range of conventional vertebrate sarcomeres, overstretching the fibers of the CM, TM, and PM during protraction would constrain retractile forces (Gordon et al., 1966). However, some vertebrate muscles (e.g., chameleon tongue retractors) circumvent this constraint with highly derived ultrastructural morphologies that permit adequate force production at extreme lengths (Rice, 1973; Herrel et al., 2001, 2002). A curtailed range of movement in the soft component of the HFA appears to be a tradeoff for the capacity to produce high magnitudes of force. Bending is circumvented or minimized as a result of the central location of the CM, as longitudinal muscle fibers tend to



**Fig. 8.** Probable deformation in the muscular hydrostat portion of the hagfish feeding apparatus during feeding. This pattern of deformation was observed when manipulating the dental plates of euthanized specimens. (A) Left: ventral view of the feeding apparatus with protracted dental plates. The clavatus muscle has been shaded for reference. Right: schematic of the muscular hydrostat in the protracted position. (B) Left: ventral view of the feeding apparatus with retracted dental plates. Right: schematic of the muscular hydrostat in the retracted position. *Abbreviations:* CM, clavatus muscle; PM, perpendicularis muscle; TM, tubulatus muscle.

be located peripherally in muscular hydrostats that perform bending. Also, the absence of oblique or helically arranged fibers in the soft HFA precludes torsional movements (Kier and Smith, 1985).

#### 4.3. Elastic properties of the hagfish feeding apparatus

A plausible initiator for dental plate retraction is elastic recoil in the stretched retractor tendon, retractor muscles, and other connective tissues. As in rigid musculoskeletal systems, elastic recoil occurs in softer, protrusible muscular hydrostatic systems (Van Leeuwen et al., 2000). This reliance on elastic recoil may help explain the fatigue resistance of the hagfish feeding system. When hagfishes feed on large carcasses in the wild, they are known for engorging themselves, and even continue feeding after the first bits of ingested food exit the anus (Baldwin et al., 1991). Natural hagfish feeding behaviors usually occur in hypoxic conditions (e.g., within the body cavity of a carcass) and involve cyclic protraction and retraction of the dental plates that persists for several minutes. The retractor muscles, particularly the TM and CM, are composed of thick fibers that can produce a lot of force but should fatigue relatively rapidly due to their dependence on anaerobic glycolysis (Baldwin et al., 1991). Although Baldwin et al. (1991) have shown that the retractor muscles can sustain extraordinarily high lactate concentrations, hagfishes would benefit from using passive mechanisms to retract the dental plates. Our data show reduced burst presence and duration from the retractors can be explained by a reliance on elastic recoil of the connective tissue of the oropharynx and the musculoskeletal system.

## 5. Conclusions

We investigated activity patterns in the major feeding muscles of the jawless feeding apparatus of *M. glutinosa*. The hagfish feeding apparatus is composed of a hard and a soft component, which are comprised of muscles functioning as dental plate protractors and retractors, respectively. There appears to be a tradeoff between force and speed within the two regions of the HFA. The retractor muscle fibers are arranged as a muscular hydrostat that produces force from a soft origin. Motor patterns and the arrangement of muscle masses demonstrate that the clavatus muscle can forcefully retract the dental plates when it is fixed by activated vertical and circumferential fibers from the perpendicularis and tubulatus muscles. We presume the soft component undergoes minimal deformation during cyclic protraction and retraction of the dental plates and the progressive shift from active to passive dental plate retraction during intraoral transport events can be attributed

to the passive recoil of the oropharynx and musculoskeletal system. Furthermore, this system may be an insightful model for the engineering of soft-bodied hydrostatic robots that could negotiate various tasks in unstructured and delicate environments generally inaccessible to rigid-bodied robots (Trivedi et al., 2008).

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