Differences in the Contractile Properties of the Circular Mantle Muscles of Loliginid Squid (*Loligo pealei* and *Lolliguncula brevis*) and Cuttlefish (*Sepia officinalis*)

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Abstract

We measured the contractile properties of the obliquely striated muscles that power escape jet behavior in two loliginid squids (*Lolliguncula brevis* and *Loligo pealei*) and the European cuttlefish (*Sepia officinalis*). Little is known about the mechanical properties of these muscles, particularly if there is variation across species. *L. brevis* had a very broad length-tension (LT) relationship and was able to produce higher relative forces over a wider range of muscle lengths. *L. pealei* showed a very narrow LT relationship and *S. officinalis* was intermediate between the two squids. *L. pealei* produced a peak isometric stress of 60.84±11.93 mN/mm2 (N=7) at a stimulus frequency of 400 Hz. *L. brevis* generated a mean stress of 63.54±48.5 mN/mm2 (N=2) at approximately 325 Hz though this is an underestimate due to a low outlier. Twitch:tetanus ratio did not vary much across species but latent period was significantly different for all three species. *L. brevis* muscle had a higher mean unloaded shortening velocity of 7.17±1.2 L/s (N=6), while *L. pealei* and *S. officinalis* had velocities of 4.88±0.34 L/s (N=10) and 4.77±0.69 L/s (N=11), respectively. Passive tension tests indicated that at small strains, *L. pealei* muscle was significantly stiffer than *L. brevis* muscle (*P*<0.001). At larger strains, the resilience and maximum stiffness for each species were similar. Differences in the contractile properties among the species we examined may not be explained solely by variations in the dimensions of the thick filaments and sarcomeres. The next step is to examine further morphological and biochemical differences that may underlie the mechanical differences we report.

Introduction

- **Squid and cuttlefish can move through water by utilizing jet-propulsion**
- **Jetting behavior can be modulated by innervation of different muscles in the mantle**
- **Ultrastructure and arrangement of the muscle fibers strongly influence the contractile properties of the mantle muscles in order to allow for the great degree of modulation**
- **Differences in structure of circular mantle muscles have been investigated in different size classes of *Sepioteuthis lessoniana* (Thompson and Kier, 2006) indicating that there may be a trade-off between muscle structure and performance**
- **Not much is known, however, about the relationship between form and function in either cephalopods or obliquely striated muscles**
- **This study examines the contractile properties of obliquely striated, circular mantle muscle fibers of two loliginid squid and one cuttlefish species, each of which seems to exhibit different locomotor behaviors (Figure 1).**
- **My research is the first of many steps aimed at further correlating variations in form with differences in function of the muscles used for jet propulsion in muscular cephalopods.**

Figure 1. Three experimental species and lifestyle/locomotor characteristics

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- **Semi-benthic, neutrally buoyant**
- **Use camouflage to hide in benthic habitats**
- **Pursue pelagic or epibenthic prey visually**
- **Primarily swims with undulating fins**

Photo by I. Bartol

- **Pelagic, negatively buoyant short-finned squid**
- **Slow swimmer, often uses fins**
- **Utilizes complex, euryhaline habitat**

- **Pelagic, negatively buoyant long-finned squid**
- **Fast swimmer**
- **Offshore, open-water habitat**
- **Uses jet often**

Materials and Methods

- **Gross dissection and tissue preparation (Figure 2)**
- **Determine stimulus current that elicits maximum response**
- **Find length (L_0)at which muscle produces peak isometric tension**
- **Perform slack-step tests to discover maximum unloaded shortening velocity (V_{max})**
- **Investigate the stimulus frequency-force relationship to find the maximum isometric force (F_{max}) at a maximum stimulus frequency**
- **Intermittent isometric force tests to assess tissue health; experiments terminated once there is a 10% decrease from initial F_{max}**

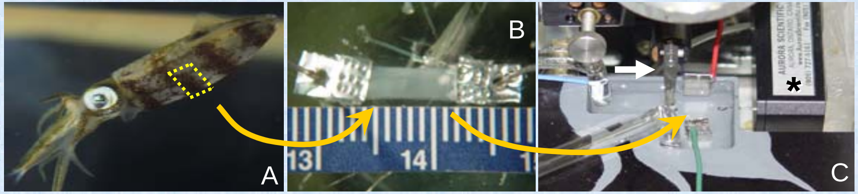

Figure 2. Schematic of muscle tissue preparation. Ventral portion of the mantle is dissected out (A), trimmed to contain only CMP (anaerobic) fibers and attached to foil clips (B), and then placed in a muscle bath (C) with lever arm (white arrowhead), force transducer (*), and electrodes. Muscle bath has a flow-through, artificial seawater system and is maintained at 20ºC.

Results

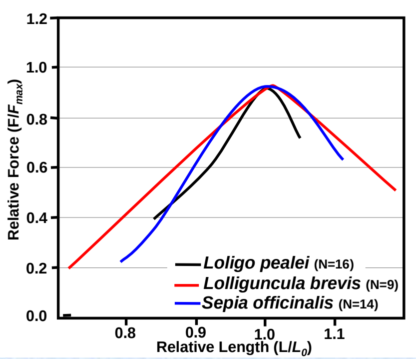

Figure 3: Length-Tension Curves. Relative Force plotted against Relative Length. Note that although the Relative Length scales are identical, the *L. pealei* curve is over a short range of lengths and has a sharp peak. The *L. brevis* length-tension curve is broader and has a flatter peak. The length-tension curve for *S. officinalis* is more intermediate.

Species	F_{max}, mN/mm²	V_{max}, L/s
L. pealei	**60.84±11.93 (N=10)**	**4.88 ± 0.34 L/s (N=10)**
L. brevis	**63.54±48.5 (N=2)**	**7.17 ±1.20 L/s (N=6) ***
S. officinalis	**No cross-sectional area**	**4.77 ± 0.69 L/s (N=11)**

Table 1: Summary of Active Contractile Properties. All values are ±S.E.M. Though there are no significant differences in any of the columns, the sample sizes for *L. brevis* and *S. officinalis* are small; larger sample sizes may elucidate significant relationship (*e.g.- V_{max} P=0.072 for *L. brevis*). Peak tetanus frequency for F_{max} was 400 Hz in *L. pealei,* 325±25Hz in *L. brevis*, and 350 Hz in *S. officinalis*.

Figure 4. Representative Passive Tension and Recovery Curve. The blue line shows the increase in stress (mN/mm²) on the muscle as it is extended past L_0. The red line shows the decline in stress as the tissue is returned to its starting length. The difference between the area under the initial passive tension curve and the subsequent recovery indicates its resilience. The teal line shows the force dropping while length remains constant.

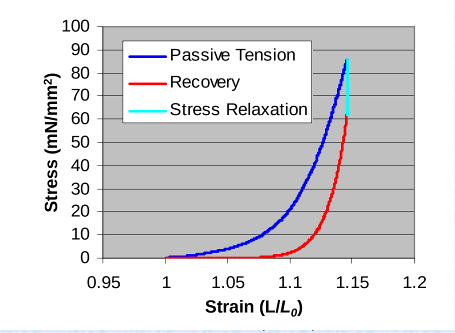

Table 2 Summary of Passive Tension Properties. All data are expressed as means ± S.E.M. Stiffness values are expressed in megapascals (MPa or 1000 mN/mm²). The maximum stiffness is the slope of the tangent to the steepest portion of the curve (right side of the curves). The linear region of the curve is proportional to the series elastic component of the tissue (left side of the curves). This was the only significantly different aspect of passive tension between *L. pealei* and *L. brevis* (*P*<0.001,*).

Species	% Resilience	Maximum Stiffness (MPa)	Stiffness of Linear Region of Curve (MPa)
***L. pealei* (*N*=8)**	**54.2 ±4.94%**	**2.13±0.28**	**0.46±0.11***
***L. brevis* (*N*=4)**	**41.2±2.93%**	**1.99±0.85**	**0.085±0.027***

Discussion

- **Variation in contractile properties of circular mantle muscle exists among cephalopods with different jetting behaviors; larger sample sizes may reveal more significant relationships, particularly for F_{max} and V_{max}**
- **The flatter, broader *L. brevis* length-tension curve suggests that the brief squid may be able to sustain near-peak forces over a wider range of lengths relative to *S. officinalis* and both of these species more so than *L. pealei,* which produces peak forces over a short range of muscle lengths**
- ***L. brevis* contracts with higher peak forces and at a higher unloaded shortening velocity than the other two species; this indicates that myofilament arrangement alone may not explain performance differences**
- **Though resilience and maximum stiffness of the tissues are similar for the loliginids, the stiffness that may be linked to series elastic component is different between the two squids**
- **Future Directions:**
  - **Complete tests with *S. officinalis* and more *L. brevis* specimens**
  - **Measure thick filament lengths for all species**
  - **Test for different isoforms of myosin ATPase**
  - **Investigate possible ontogenetic differences within each species**
  - **Compare contractile properties bewteen SMR and CMP circular muscle fibers**
  - **Examine Work and Power output of the circular muscles**

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