The elongation behavior and the mechanical properties of the body wall in the burrowing sea cucumber *Leptosynapta clarki*

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Abstract

The movement and locomotion of an organism are governed in part by the mechanical properties of its body wall. *Leptosynapta clarki,* a burrowing sea cucumber, collected from False Bay, San Juan Island, WA, was observed to extend its body nearly three times its minimum length as measured in a laboratory setting, using time-lapse video. Observations in cryolite revealed that a combination of whole body peristalsis and tentacular crawling facilitated elongation. Individual *L. clarki* whose tentacles were removed were only able to extend twice their minimum length and could not burrow while those with intact tentacles extended 3.4 times their minimum length. This indicates that the oral tentacles are required for extensive elongation and burrowing. Tensile tests were performed parallel to the anterior-posterior axis on sections of intact, cylindrical body wall, obtained by removing the anterior and posterior ends from anesthetized animals and leaving the viscera in place, and on isolated longitudinal strips of body wall. The samples were pulled at extension rates of either 2 mm/min which is comparable to the extension rate achieved by *L. clarki* or 50 mm/min. Neither stiffness nor strength of cut strips of body wall was affected by the rate of extension. In contrast, samples of cylindrical body wall were 3 times stiffer and 4 times stronger when pulled at the faster rate. Creep tests on samples of cylindrical body wall confirmed its viscoelastic behavior. These results suggest that viscoelastic elements in the body wall of *L. clarki* might have been disrupted when it was cut longitudinally or when the internal organs were removed. Since the natural range of maximum extension and the maximum strain from mechanical testing were very similar, it appears that *L. clarki* is operating near the maximum capacity for strain of its body wall.

Introduction

The movement and locomotion of an organism are limited by its morphology and have to be within the range of the mechanical properties of its body. In the laboratory setting, a species of burrowing sea cucumber, *Leptosynapta clarki*, elongated approximately three times its contracted state, a capacity that was unexpected, considering its thin, nearly clear body wall. This elongation behavior is not well recorded in the literature although it is clear from the observation made in the field that this ability is the normal state for *L. clarki* burrowing through sediment. Although about 70% of the Earth's surface is covered by marine sediment, the behavior of most sediment-dwelling organisms is largely unknown because it is difficult to observe burrowing organisms in their natural habitat (Dorgan *et al.,* 2006).

Leptosynapta clarki are found in False Bay, San Juan Island, WA (Sewell, 1994). Unlike typical sea cucumbers, L. clarki is not a suspension feeder but a deposit feeder. L. clarki uses its ten oral tentacles to burrow into sediment and moves sediment particles into its mouth in order to digest the organic materials on the surface of sediment particles. Unlike a typical sea cucumber that has five rows of tube feet, L. clarki does not have tube feet, respiratory tree, or radial canals (Morris et al., 1980; Figure 1) which contribute to L. clarki's vermiform appearance. Hunter and Elder (1967) studied the burrowing mechanisms of Leptosynapta tenuis, but did not note the elongation behavior or the role of elongation in burrowing.

Since L. clarki lacks tube feet it elongates by using its oral tentacles and whole-body peristalsis. The peristalsis might be used to compact the walls of burrows and loosen the sediment ahead of the organism, while the oral tentacles are used to scrape sediment to the side (Hunter and Elder, 1967). As the animal burrows it might extend itself through the sediment; thus elongation might be associated with typical burrowing behavior.

The elongation of L. clarki might be limited or facilitated by the mechanical properties of its body wall. There have been extensive studies on the mechanical properties of sea cucumber body wall (Motokawa, 1984; Thurmond and Trotter, 1996; Motokawa and Tsuchi, 2003). The properties of body wall differ greatly between species and between different habitats. Actinopyga echinites, a species of sea cucumber typically found in areas exposed to strong waves had a stiffer body wall than Holothuria leucospilota another species of sea cucumber found in calmer waters (Motokawa, 1984). However, little work has been done on the body wall characteristics of any infaunal

species. In this paper I studied the elongation ability, the elongation mechanisms, and the role of the body wall in elongation.

Methods

Collection and observation

Leptosynapta clarki were collected by hand from False Bay, San Juan Island (between N 48° 29.152' W 123° 04.085' and N 28° 29.139' W 123° 04.090 measured with Garmin GPS76) and kept in their native sediment in a sea table with flowing seawater. In order to visualize the body elongation of *L. clarki,* three to four healthy *L. clarki* were placed in a 6cm by 6cm square Petri dish and videotaped for 2 hours at a rate of one frame per second. Before *L. clarki* were placed in the dish, each individual was prodded gently with a pair of blunt tweezers for 10 times to ensure that the individual was contracted to its minimum length. In total, sixty-five *L. clarki* were video taped as they elongated. From these videotapes, the length of each sea cucumber was measured every 15 minutes in actual time by measuring the length between the anus and the base of oral tentacles using ImageJ. Maximum and minimum lengths were recorded. I calculated maximum relative extension that expresses an individual's ability to extend by dividing the maximum length achieved in two hours by the initial minimum length. The wet weight of each individual was measured without blotting on the towel before or after filming because there was a high correlation between wet weight without blotting on the towel and with blotting. In order to make sure that the difference of weight was not caused by water inside the organism, each organism was prodded gently and slowly with tweezers 10 times before weighing, picked up with a blunt tweezers, gently tapped on the side of the Petri dish, and weighed.

To visualize how *L. clarki* burrow, five individuals were placed one at a time in a 2 X 8 X 10 cm clear plastic box filled with cryolite sediment and were video taped. Cryolite is a mineral that has a reflective index of 1.34, so when it is submerged in water it is nearly clear (Josephson and Flessa, 1972). To enhance the quality of videos, the plastic box with cryolite and *L. clarki* was lighted from the back with a light box. Thus it was possible to videotape *L. clarki* burrowing into nearly clear sediment and to make a detailed examination of how it uses its tentacles and peristalsis.

In order to determine to what degree elongation and burrowing were facilitated by either peristalsis or the movement of oral tentacles, *L. clarki* with tentacles and without tentacles were examined. Twenty-two sea cucumbers were anesthetized with 3% magnesium chloride for three hours. For half of the animals, all the tentacles were removed using iridectomy scissors. The other half of the animals were used as a control and were handled similarly but their tentacles were left intact. Any *L. clarki* with defects other than missing tentacles were discarded. *L. clarki* were given 12 hours of recovery period in a glass bowl on the sea table with flowing seawater. Three of these animals at a time were then placed onto a 6cm by 6cm Petri dish and videotaped for two hours at the rate of one frame per second. After this experiment, organisms were moved to a cup of their native sediment with diameter of 10 cm and height of 12 cm. *L. clarki* with or without tentacles were gently placed on the surface of sediment and videotaped for two more hours on time-laps in order to determine whether the presence of oral tentacles affects burrowing capacity of *L. clarki*.

Material testing

Sample preparation Individual *L. clarki* were anesthetized with 3% (isotonic) magnesium chloride for three hours (Sewell, 1994). The test sample was prepared in two ways. For the first test sample, cylinders of body wall were prepared by cutting off the anterior and posterior ends of *L. clarki* without removing the viscera and gonads. For the second test sample, strips of body wall were prepared by carefully cutting the cylinders of body wall along an ambulacrum with iridectomy scissors. The viscera and gonads were removed from the body wall. Both types of samples were kept in the magnesium chloride solution at 12ºC until tested. All the samples were tested within 24 hours of dissection.

Tensile Test The body wall of *L. clarki* was gripped in a tensiometer with each end wrapped in thick layers of Scott Single-Fold towels. In order to test if the body wall had a viscoelastic property, I tested mechanical properties under two rates of extension. The samples were pulled either at the rate of 2 mm/min or 50mm/min with MTS Synergie 100 with 10N load cell until the sample failed. 2 mm/min represented the natural rate of extension of *L. clarki* as seen from the time lapse video tapes, while 50 mm/min represented a fast rate of pulling that is likely to exceed anything *L.* experiences in nature. The samples were kept hydrated by application of drops of 3% magnesium chloride with a plastic pipette during testing. The temperature of 3% magnesium chloride was maintained to be between 8 to 14 ºC. The droplets were applied at least every minute until the drop of solution traveled down through the sample. Stress and strain were recorded at the frequency of 1Hz when pulled at 2mm/min and of 100Hz when pulled at 50mm/min. Strain was defined to be the change in length divided by the original length. Stress (MPa) was defined to be load divided by the cross sectional area of the sample. Stiffness was calculated by choosing two points that represented the longest linear

portion of individual stress and strain graphs. Maximum stress, strain at maximum stress, and maximum strain were noted. Data were excluded if the sample broke at the grip for all the measurements except for stiffness.

Creep Test In order to confirm the viscoelastic properties of the body wall, creep tests were performed. The body wall of *L. clarki* was gripped onto a tensiometer with each end wrapped in thick layers of tissue paper. The samples were pulled at the rate of 5 mm/s until it reached the strain of 0.8 and were held at that extension for 300 seconds while the change in stress was recorded.

Results

Leptosynapta clarki elongated their bodies to an average of 2.85 times their minimum length within two hours (Figure 2). Maximum relative extension, their ability to extend beyond their contracted length, decreased as weight increased (Figure 3). Weight of *L. clarki* ranged from 2.30g to 0.06g, maximum relative extension from 1.57 to 4.94, and the minimum length from 5.1 mm to 34.2 mm.

 L. clarki without tentacles elongated significantly less than the ones with tentacles (Wilcoxon / Kruskal-Wallis Tests; $z = 3.5491$, $p = 0.0004$; Figure 4). None of *L. clarki* without tentacles burrowed into the sediment, while all *L. clarki* with intact tentacles successfully burrowed into the sediment. *L. clarki* successfully burrowed into cryolite and the use of the oral tentacles and peristalsis were observed in the video (Figure 5).

There was not significant difference between stiffness of strips of *L. clarki* body wall pulled at 2mm/min and at 50mm (Student's t-test; $p = 0.9870$). Eleven strips pulled at 2mm/min had stiffness of 0.0204 MPa ± 0.00897. Four strips pulled at 50 mm/min had

stiffness of 0.0206 MPa ± 0.00865. Typical curves from tensile test of strips of body are shown in Figure 6. In contrast the response of the cylinder samples pulled at 2mm/min and 50mm/min had dramatically different shapes, the cylinders pulled at 50mm/min displayed a much greater stiffness and strength than any of the other modes of pulling. Six cylinders pulled at 2mm/min had stiffness of 0.0140 MPa ± 0.00816, while seven cylinders pulled at 50mm/min had stiffness of 0.0730 MPa ± 0.0480 (Student's t-test; $p = 0.0006$). The average maximum stress from cylinders pulled at 2mm/min was 0.0117 MPa ± 0.0065 and at the average maximum stress from cylinders pulled at 50mm/min was 0.0396MPa ± 0.0219. Creep test performed on cylinder body wall showed that load decreased to nearly zero within 2 seconds when the body wall was held at a constant strain (Figure 7). The average maximum strain of cylinders pulled at 2mm/min was 1.787 ± 0.253.

Discussion

On the average, *Leptosynapta clarki* elongated 2.85 times their contracted body length. This maybe a conservative value since these *L. clarki* were observed on plastic surfaces, while in their natural habitat *L. clarki* is likely to be able to anchor itself onto the sediment using mucus and the surface of its body wall and thus be able to generate more force as it extends. The tentacles had a critical role in elongation and burrowing. When the tentacles were removed, *L. clarki* could only elongate to 2 times its smallest body length and was unable to burrow into sediment. However, this does not mean that peristalsis does not play a role in elongation. The increase in length from the minimal posture up to two times the body length was achieved by peristalsis.

Peristalsis was suggested to have a role in enlarging and stabilizing the burrow (Hunter and Elder, 1967). Polychaetes use either peristalsis or undulatory movement to cause crack propagation in the sediment, which enables worms to burrow more efficiently (Dorgan *et al.,* 2006). As seen in Figure 5.D, *L. clarki* exhibited strong concentration of peristalsis on its anterior end and the movement of sediment was observed associated with this peristaltic movement. It is possible that peristalsis in burrowing sea cumbers not only stabilized the burrow but also caused crack propagation in the sediment and facilitated burrowing of *L. clarki* (Dorgan *et al.,*2006).

The stress-strain curve obtained by pulling strips of body wall at different rates did not differ greatly. In all cases the body wall extended with relatively little force. The lack of difference in material properties at different rates of these samples suggested that the body wall was not viscoelastic. However the stiffness of cylinders of body wall pulled at 50 mm/min was higher than the stiffness when pulled at 2mm/min. The effect of the rate of extension on mechanical properties indicated that the sea cucumber body wall in cylinder form was indeed viscoelastic.

There are at least two possible explanations for these different responses. First, it is possible that there were viscoelastic elements within the body wall that were disrupted when cylinders were cut open. The second possibility is that the viscera and gonads of *L. clarki* provided a viscoelastic response that was removed when the viscera and gonads were removed. This viscoelastic behavior of the body wall enables *L. clarki* to lengthen with lower energy. If *L. clarki* operated at faster speed, as in 50mm/min, the organism would need larger force to elongate. Since *L. clarki* is moving so slowly, it can elongate without spending much energy on extending its body wall (the body wall offers little

resistance at this rate of elongation). Studies on epifaunal species of sea cucumbers have shown that the dermis of some species has viscoelastic properties (Thurmond and Trotter, 1996) but in those cases, the extreme elongation was not a natural part of the animals' behavior.

Another interesting result from the tensile tests was the relationship between strain obtained from the test and the natural range of extension of the animals obtained from the videos. The maximum length of the whole body divided by its minimum length, can be considered as a sort of natural strain (e.g. change in length divided by the original length). Since the average maximum extension was 2.85 times of the minimum body length, the average maximum strain that *L. clarki* naturally experienced would be 1.85. Interestingly, strain of 1.85 is very close to the average maximum strain calculated from cylinders of body wall pulled at 2mm/min (Figure 8). This result showed that *L. clarki* might be operating near the maximum of the physiological limit of the body wall.

The molecular and structural mechanisms behind this extensive elongation ability and viscoelasticity of *L. clarki* are still unknown. In order to examine viscoelastic elements in *Leptosynapta clarki* and the role of different fibers in the body wall*, visualization of collagenous fibrils and microfibrils using transmission electron microscopy (Thurmond and Trotter, 1996), staining (Elder, 1973), and polarized light microscopy will be done in the future. Other environmental factors might affect the mechanical properties of body wall, such as temperature, exposure to sunlight, and oxygen concentration. Further understanding and investigation of the natural history of *Leptosynapta clarki* would lead to more profound interpretation of mechanical properties of its body wall and its elongation behavior.
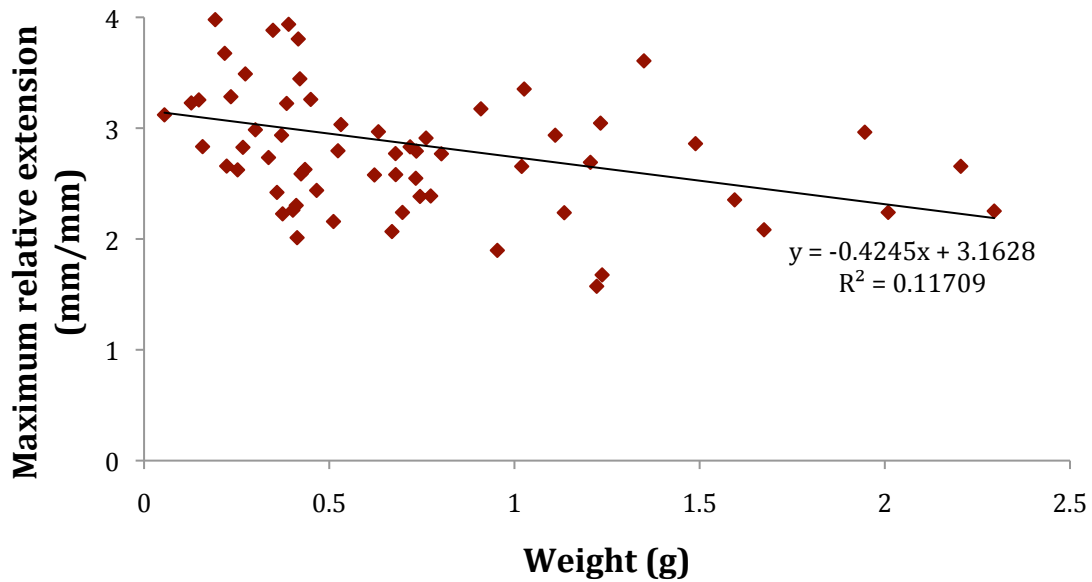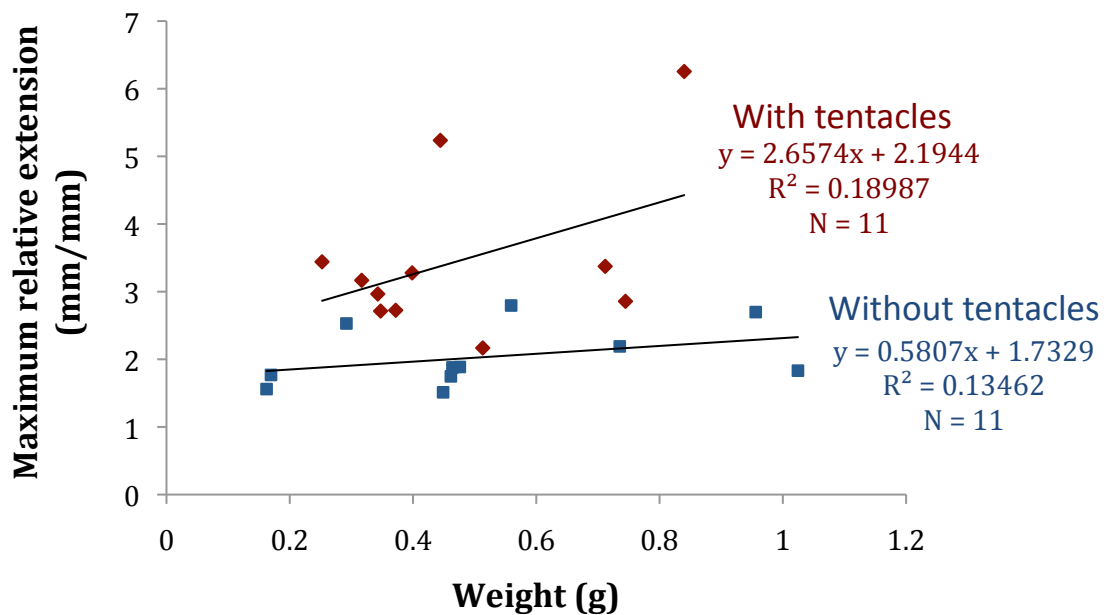
Figures
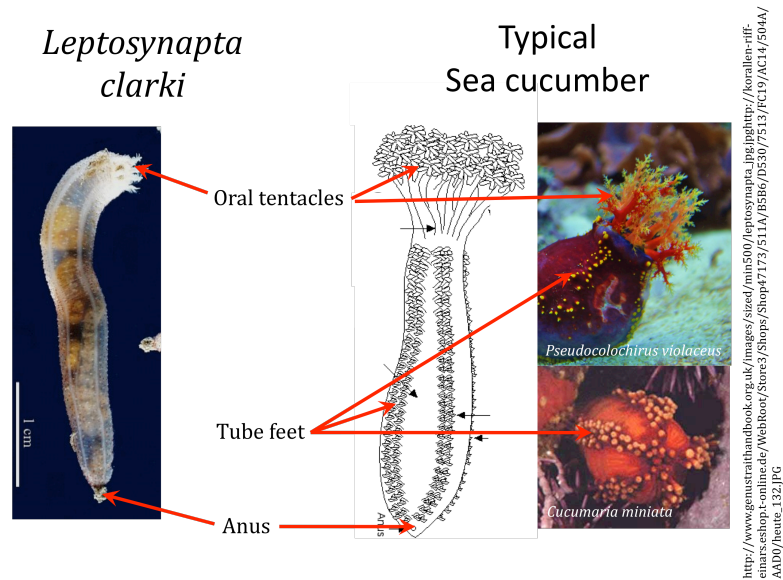
Figure 1 Anatomy of a typical sea cucumber and that of *Leptosynapta clarki.*
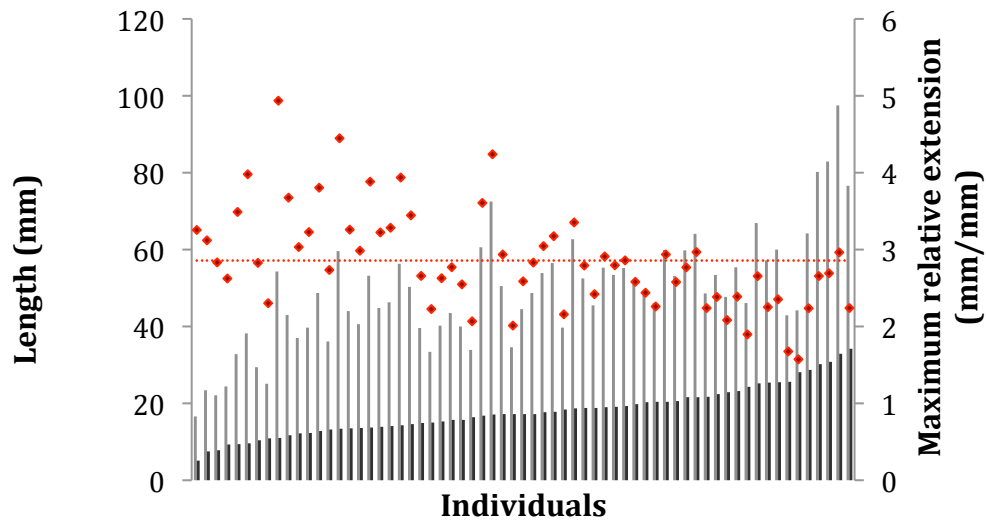
Figure 2 Elongation ability of *Leptosynapta clarki*. Results from 65 individuals were aligned by their minimum length, indicated with black bars. Gray bars indicate maximum length, and red dots indicate maximum relative extension, maximum length divided by minimum length. Red dotted line shows the average maximum relative extension, 2.85.

Figure 3 As *L. clarki* become larger, their ability to extend (as represented by maximum relative extension) decreases..

Figure 4 The relationship between the degree of extension and weight in sea cucumbers without tentacles and with intact tentacles. Red symbols indicate maximum relative extension from sea cucumbers with tentacles and blue symbols indicate maximum relative extension from sea cucumbers without tentacles.
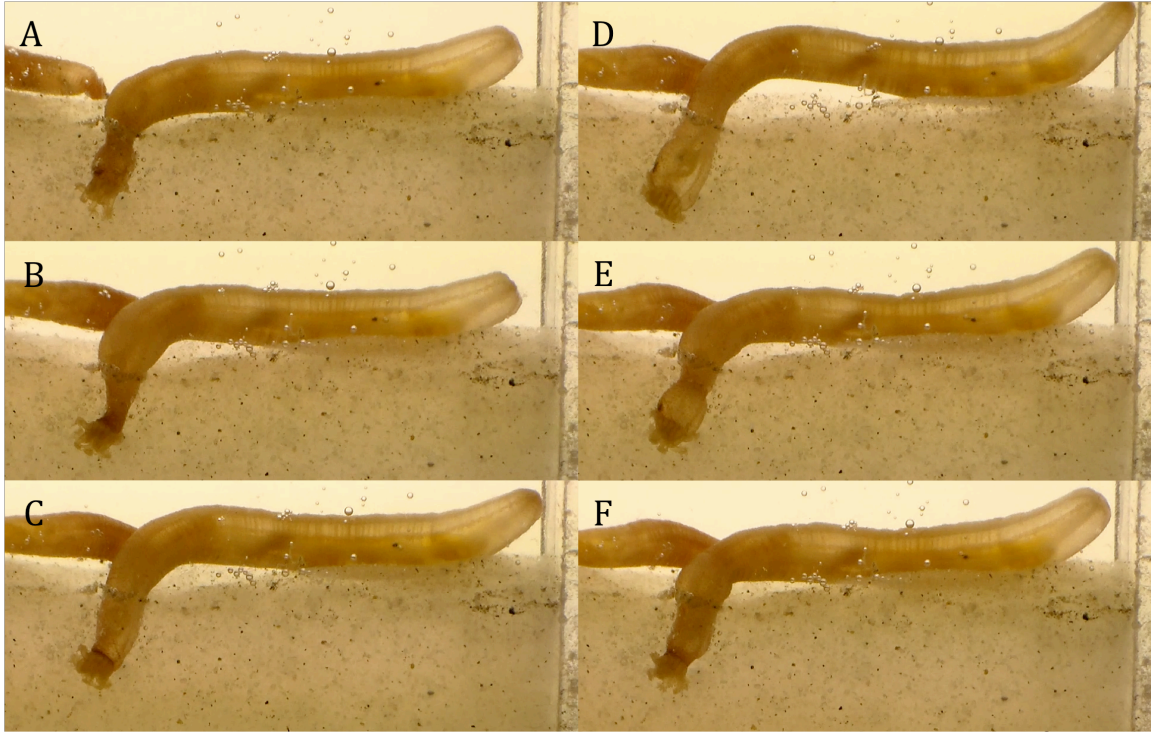
Figure 5 *Leptosynapta clarki* burrowing into cryolite. (A) use of oral tentacles to move sediment to the side of the developing burrow (B) initiating of peristalsis (C) continuing of peristalsis (D) peristalsis at its peak with the concentration at its anterior end (E) relaxation and decline of peristaltic wave and (F) resumption of movement of oral tentacles.
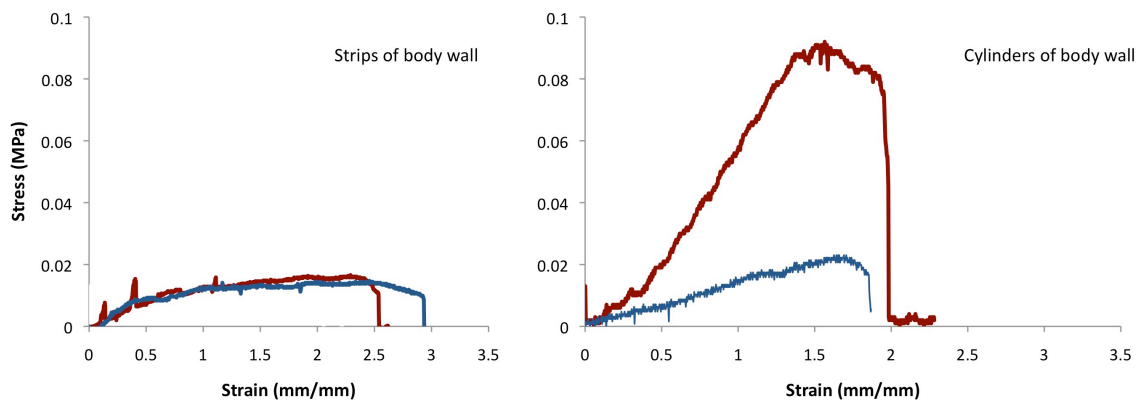
Figure 6 Representative stress-strain curve among 10 samples. Stress-strain graph on the left was from strips of body wall and on the right was from cylinders of body wall. Red lines indicate results from pulling at the rate of 50mm/min and blue lines indicate the results from pulling at 2mm/min.
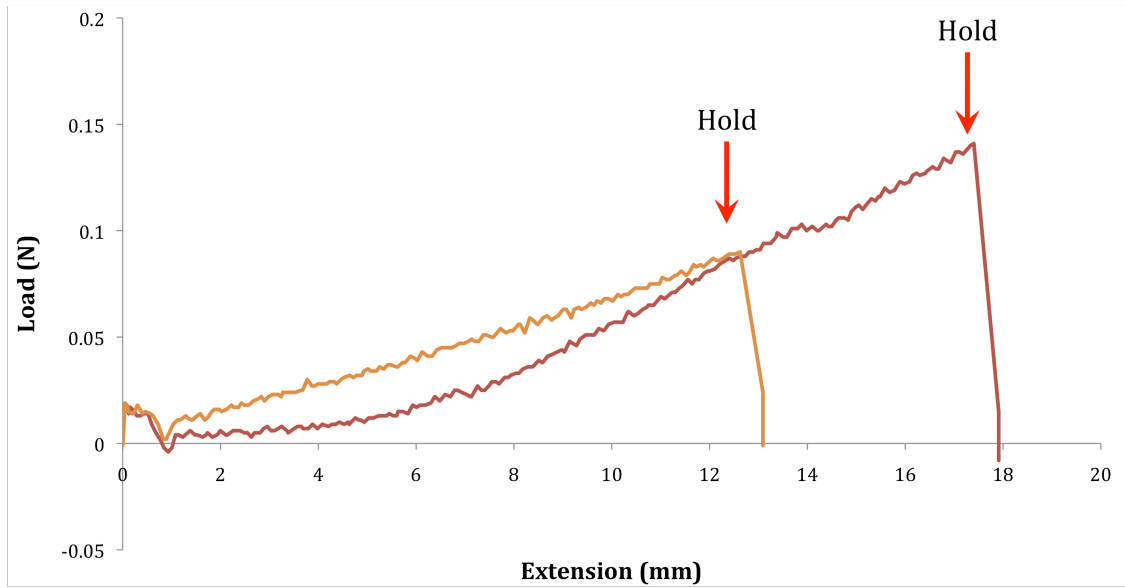
Figure 7 The result of creep test on two samples of cylindrical body wall. The load decreased when the body walls were held at a constant extension.
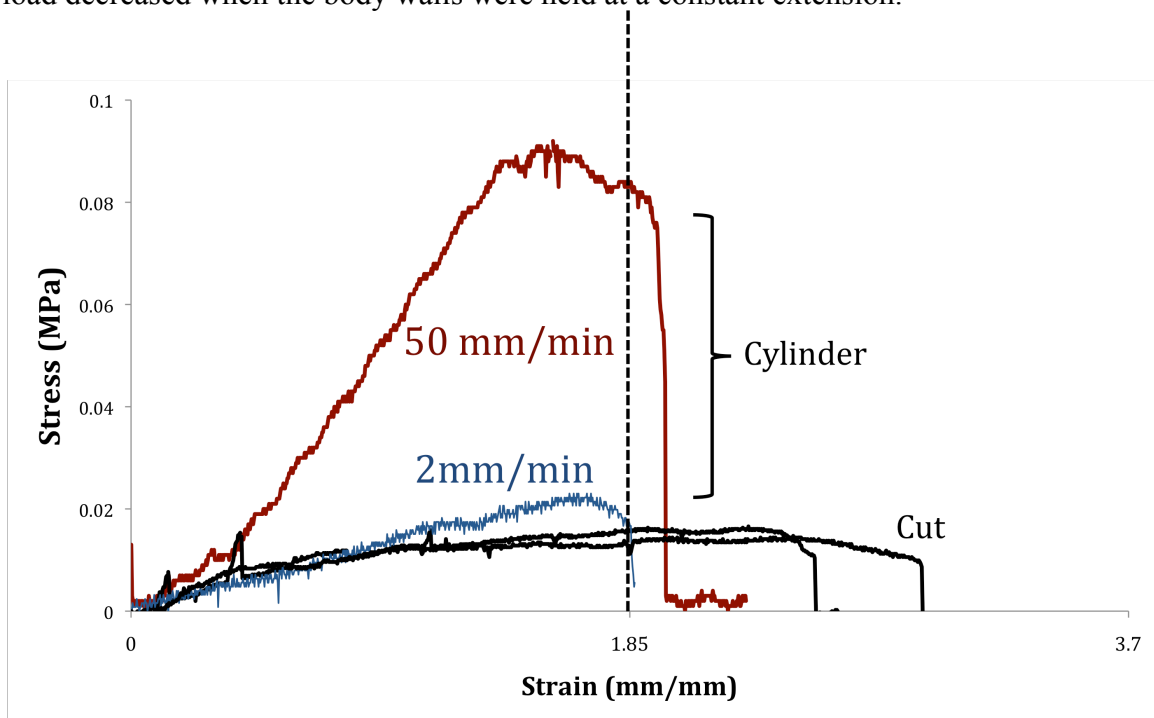
Figure 8 The natural range of extension in *Leptosynapta clarki* in terms of strain is noted with vertical dotted line over the representative stress-strain curves obtained from tensile tests on different *L. clarki* body wall samples.

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