Investigation of a Subcutaneous Gelatinous Tissue in the Hadal Snailfish

Proximate Chemical Composition, Comparative Video Analysis, and Robotic Modelling

Keywords: Hadal, snailfish, gel, swimming mechanics, robotic modelling

Mackenzie Gerringer
University of Hawaii at Manoa
mgerring@hawaii.edu

Functional Morphology and Ecology of Fishes
Friday Harbor Labs
2014

ABSTRACT

Deep-sea fishes are known to have muscles that are higher in water content than their shallow-living relatives. In addition to this watery muscle, some deep-dwelling fishes also have a gelatinous layer either directly below the skin or around the spine. This study investigated the composition and implications of this mysterious gelatinous tissue in one of the planet's deepest-living fishes. Gel tissues from eight deep water species were analyzed for water content, ionic composition, and osmolality. Bulk protein, lipid, and carbohydrate assays were also conducted. These analyses do not support the hypotheses that this tissue plays a role in nutrient storage or buoyancy. The gelatinous layer is most obvious in the hadal snailfish *Notoliparis kermadecensis*, making it an appropriate model organism to investigate gel function. The authors propose that the gelatinous tissue that surrounds the fish's muscle may act as an energetically inexpensive method of increasing swimming efficiency. To test this hypothesis, swimming performance in the gelatinous hadal snailfish was compared to swimming performance in the tidal snailfish, *Liparis florae*, which have similar morphology, but with no subcutaneous gel. Video analyses show that *Liparis florae* swam more body lengths per second than their hadal counterparts. A robotic snailfish model was also used to analyze the impacts of the gelatinous layer on locomotory performance. The robot swam trials with tails of varying water volume. The model showed higher swimming performance with a water-filled tail than with an empty one. Data from these three analyses suggest that the gelatinous layer may aid hadal snailfish locomotion.

INTRODUCTION

Organisms in the deep-sea have developed incredible adaptations to thrive under harsh conditions, including low light, low temperatures, high pressure, and limited food availability. In some species, distinct watery tissue layers are also present (**Figure 1**). These gelatinous layers have been described in mid-water fishes, and are hypothesized to be an adaptation for buoyancy (Yancey, *et al*. 1989). Another early hypothesis is that this tissue is used for energy storage. Some also suggested that the gel could be unpalatable, thus acting as an effective predator deterrent. While this gelatinous layer has been noted in a number of species descriptions and dissection anecdotes, its composition and function have not been studied.

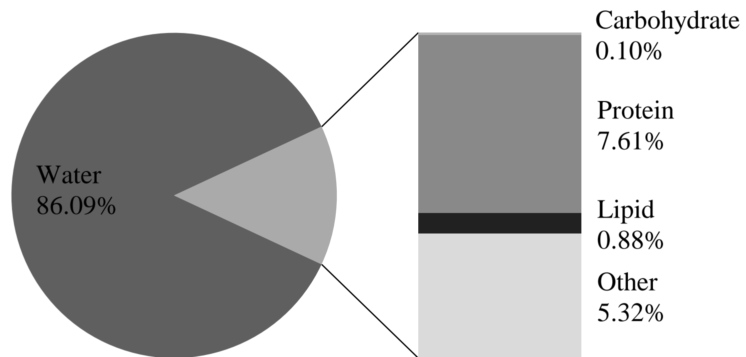

Figure 1: Average White Muscle composition for study species. Compiled from data from Drazen (2007).

This study describes the proximate chemistry of these gelatinous layers in seven benthic and benthopelagic species, to investigate hypotheses about the potential buoyancy or alternative function of this layer. Although this gelatinous layer appears in a number of deep-sea fishes, it is most obvious in the transparent hadal snailfishes, the planet's deepest living vertebrates, which have been particularly successful in the trench environment (Jamieson, 2009). The author proposes that the gel layer may provide a locomotory

advantage by increasing the volume of the tail and creating a larger, stiffer paddle than the thin caudal muscles would otherwise offer. Swimming efficiency of the hadal snailfish is compared to an intertidal snailfish that does not have gel through video analysis. Finally, a robotic snailfish with tail gel and without tail gel was used to model the potential effects of gel on swimming performance.

METHODS

I. Proximate Chemistry

The tissues analyzed in this study were collected from the Monterey Bay Canyon, located off the coast of California, in April and October of 2009. Trawl depths ranged from 750 to 3,000 m. Gelatinous layer tissue samples were taken from 4 benthopelagic species (*Bothrocara brunneum, Careproctus melanurus, Careproctus cypselurus, Spectrunculus grandis*), 2 benthic species (flatfishes *Embassichthys bathybius, Microstomus pacificus*), and a new species of eelpout *Pachycara* n sp. (habitat uncertain). **Table 1** shows catch dates, trawl depths, standard and total lengths, mass, and sex of each specimen. Samples of the gelatinous layer were taken from behind the fish's head, from the layer directly below the skin. Tissues were kept in small vials at -80ºC in liquid nitrogen on the ship, then at -80ºC in the lab. Samples were shipped to the University of Hawaii, Manoa on dry ice. In 2011, additional gel samples were dissected from *Pachycara* n sp. for analysis. Samples were shipped on dry ice to Whitman College. Lab analyses were performed in the summer of 2011.

Gel samples from the hadal snailfish *Notoliparis kermadecensis* were collected from the Kermadec Trench in November, 2011. Dissections and freezing proceeded as with the Monterey collections. Preliminary lab analyses (water content, osmolality) were

performed in April, 2012. Additional samples were collected on the HADES K cruise to the Kermadec Trench in April of 2014. These will be used for future chemical and material property analyses.

Species	Fish ID#	Depth-Trawl #	Date	Standard Length (cm)	Total Length (cm)	Mass (g)	Sex
Bothrocara brunneum	3	1000-4	10.11.09	51	52.9	707	M
Bothrocara brunneum	3	2000-2	4.13.09	57.7	59.3	890.1	F
Bothrocara brunneum	4	2000-3	4.13.09		59.5	779.7	F
Careproctus cypselurus	1	1000-1	4.8.09		17.8	47.8	M
Careproctus melanurus	2	1000-1	4.8.09		25.6	193.2	F
Careproctus melanurus	4	750-1	10.2.09	15.5		24.47	F
Careproctus melanurus	5	750-1	10.2.09	13.7	15.2	36.46	F
Embassichthys bathybius	2	1000-1	10.1.09	32.4	37.4	627.4	M
Embassichthys bathybius	8	1000-3	10.10.09	31.7	35	735.1	F
Embassichthys bathybius	9	1000-4	10.11.09	37.5	41.4	880.3	F
Embassichthys bathybius	11	1000-4	10.11.09	30.2	33.7	537.4	
Microstomus pacificus	4	1000-1	10.1.09	46.6	50.4	1342.3	F
Microstomus pacificus	5	1000-1	10.1.09	40.3	46.2	1127.1	F
Microstomus pacificus	6	1000-1	10.1.09	43.7	48.5	1224.8	F
Pachycara n. sp.	1	3000-4	10.8.09	35.6	37.4	503.6	F
Pachycara n. sp.	3	3000-4	10.8.09	34.7	36.6	450.8	M
Pachycara n. sp.	8	3000-4	10.8.09	37.7	38.5	501.6	F
Spectrunculus grandis	3	2000-1	10.9.09	141	146	17463.3	

Table 1: Trawl and specimen information for gel samples tested. Standard length is measured from the tip of the snout to the posterior end of the hypural bone. Total length includes the caudal fin. Trawl depths are indicated in meters (compiled by Nicole Condon, University of Hawai'I, Manoa).

Buoyancy. Fresh gelatinous and white muscle tissues were placed in sea water and sink times were compared at sea shortly after capture. These tests were conducted in sea water at 5^{0}C. **Water Content**. Samples were dried at 60^{0}C for three days and remaining dry mass was compared to original tissue mass. This was done for both gel and white muscle tissues.

Osmotic Pressure. The vapor pressure osmometer, Wescor 550, was used to determine sample osmolality. Samples were ground in a homogenizer and centrifuged at 2,000 RPM for 10 minutes. 10 microliters of the liquid was measured via vapor pressure osmometer. The 290 mmol/kg standard (based on the osmolality of plasma) was checked periodically to confirm accurate calibration. **Proximate Chemistry Sample Preparation**. A section of frozen tissue was cut and weighed to obtain about 0.1 g, with a precision of 0.0001. The section was ground in 7% perchloric acid, PCA, or 70% ethanol, to precipitate proteins. The sample was left refrigerated overnight. The microfuge tube was centrifuged for 20 minutes at 15,000 RPM at 4ºC. The supernatant was removed to a new tube for ion, osmolyte, and TMAO tests. The remaining pellet was used for protein analysis. Since ethanol was used for osmolyte and ion analysis, the sample was evaporated. The samples dissolved in PCA were titrated with 2M KOH to neutralize. The resulting precipitate was removed by collecting the supernatant from the centrifuged sample. The PCA method, which is more accurate, was not used for ion analysis, because of the required addition of potassium. The leftover ions and osmolytes were dissolved in the appropriate amount of distilled water. Before HPLC analysis, the sample was passed through a hydrophobic lipid cartridge (Spe-ed SPE cartridges, Octadecyl C18/18%, 100mg/ml, Catalog Number 12001) and bacterial film (Millex syringe filter units, Durapore PVDF, pore size 0.22 micrometers, filter diameter 13 millimeters). **Protein**. Protein content was determined with the Bicinchoninic Acid (BCA) Protein Assay (Smith, *et al.* 1985). BSA, bovine serum albumin was used as a standard. **Lipids**. Lipid contents for these samples were analyzed using the Iatroscan technique (Fraser, *et al*. 1985). This analysis was conducted by Jason Friedman at the University of Hawai'i at Manoa. **Glycosaminoglycans**. Chondroitin Sulfate

Standard (750 ug/mL and 1mol. Sodium Acetate). Alcian blue dye (0.50M sodium acetate to produce 1.4 mg/mL solution). Dye precipitated, particles in the solution led to inaccurate readings. **Carbohydrates**. Carbohydrate analysis was conducted according to the Dubois *et al.* Carbohydrate Assay protocol using phenol and sulfuric acid (Dubois, *et al.* 1956). The Devor protocol with sulfonated α-naphthol was also attempted (Devor, 1950). The protocol uses a solution of 0.400 g α-naphthol in 100 mL sulfuric acid with a glucose standard. The Beckman Coulter DU 730 spectrophotometer was used for a scanning wavelength test. Samples were scanned from 400nm to 650nm. As many of the readings appeared to be at the maximum absorbance for the machine, samples were left to fade overnight. Standards were persistently inconsistent, so the test was abandoned and the Dubois protocol carried out. **Ions**. Sodium and potassium levels were analyzed using atomic absorption. Samples (1% dilution) were dissolved in 10 milliliters of 1% nitric acid. The atomic absorption spectrophotometer, Perkin-Elmer 3030B was used. **Osmolytes** Osmolytes, compounds that help regulate a cell's osmotic pressure, were analyzed using High Performance Liquid Chromatography, HPLC (Wolff, *et al.* 1989). HPLC is designed for small, polar molecules that are pumped at high pressure through a narrow resin column in a stainless steel tube. Water, with EDTA, ethylenedinitrilotetraacetic acid, to control metal ions, was used as a solvent. 50 microliters of each sample was analyzed with individual run times of about 45 minutes. **TMAO**. Although trimethylamine oxide is also an osmolyte, its levels cannot be analyzed by HPLC, as it does not change the refractive index of water. Iron, from $FeSO_4$/EDTA solution at 50ºC, was used to reduce TMAO to TMA, a hydrophobic molecule. When picric acid was added to a solution with TMA, a yellow product formed. By measuring the fluorescence of the solution at 410 nanometers

and comparing the results to a standard, the TMAO concentration of a tissue was determined. The test is a standard fishing industry assay used to monitor spoilage. The protocol, originally from Wekkel and Barnett (1991), was modified by Kelly and Yancey (1999).

II. Comparative Video Analysis

Hadal snailfish video was collected on three cruises by baited camera on a free-falling lander (Alan Jamieson, Oceanlab). Video of *Notoliparis kermadecensis* was taken in April and May of 2014. The swimming performance of *Pseudoliparis amblystomopsis* was analyzed from video taken in 2007 in the Japan Trench (**Figure 2**). Swims were analyzed when a clear dorsal view was seen and the fish appeared to be swimming straight. Future analysis will utilize particle movement seen in the video to correct for current. In the present study, videos with low evidence of current were chosen.

As a shallow-water comparison, *Liparis florae* captured by beach seine and hand net off San Juan Island were filmed swimming (**Figure 2**). Only straight, burst swims were analyzed. Swim trials from nine individuals were analyzed, with three swims per individual. For each swim, body lengths per second were recorded. Tail beat frequency and pectoral fin strokes per tail beat were also counted. Analyses were conducted using the software ImageJ.

Figure 2. Still images from video of *Liparis florae* (left) and *Pseudoliparis amblystomopsis* (right). Japan Trench footage courtesy of Alan Jamieson, Oceanlab, University of Aberdeen.

III. Robotic Model

The robotic model ANTON was designed after the Kermadec Trench snailfish, *Notoliparis kermadecensis*. The plastic (PLA) body and fins were 3D printed (ORION HB #58744) based on a model constructed from photogrammetry models of freshly captured specimens collected on the HADES Cruise in April and May of 2014 (Model – MeshMixer, Slicing – Cura, 3D Printing – Repetier Host. The swimming code was programmed onto an Arduino Nano. Tail beat frequency was chosen to match that found through video analysis of the hadal snailfish. The robot was powered by 9V battery and swam using a Servo motor connected to two piano wires that rotated the tail region (**Figure**). To simulate white muscle, a silicone rubber mold was cast for the tail. Swim trials were conducted with empty and full tail "skin". Water represented the gelatinous tissue. Video from the robot's swim trials was taken and body lengths per second were compared between trials.

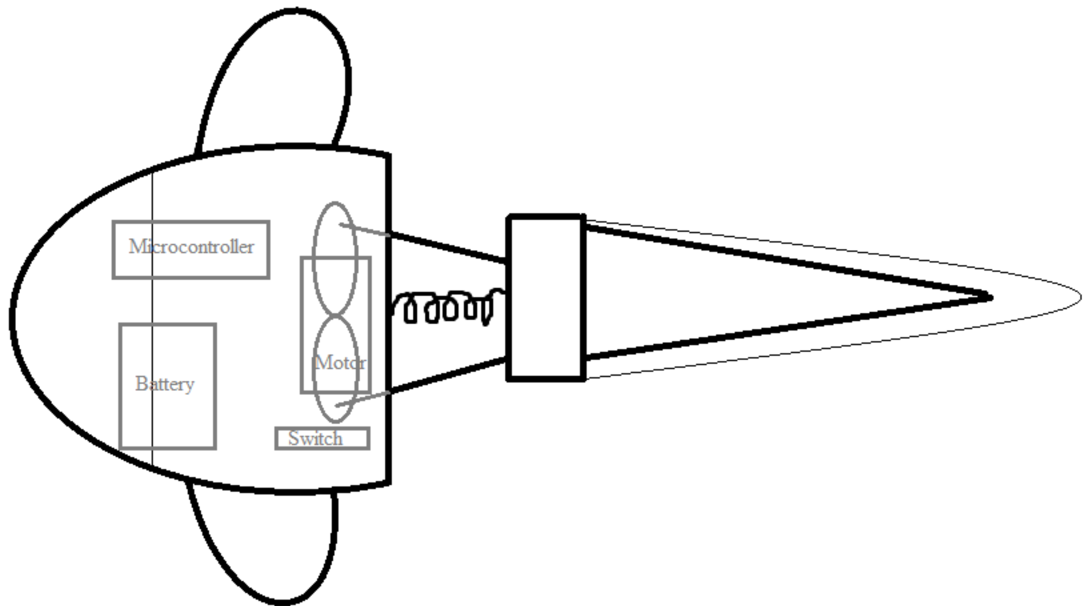

Figure 3. Schematic of robotic hadal snailfish model. Microcontroller (Arduino Nano), Motor (Tower Pro TM, Micro Servo 9g, SG90)), Battery (Duracell, 9V). Connections soldered using (Aoyue Int968A). Tail muscle is a cast silicone rubber (Ecoflex R 00-10) with a volume-adjustable skin (latex condom, Trojan Magnum). Additional materials used include hot glue, a spring, piano wire, a bottle cap, marine epoxy, electrical tape, and miscellaneous hardware as ballast.

RESULTS

I. Proximate Chemistry

Most fish tissues have an average water content of fish tissues ranges from 60 to 80% (Randall, 1997). Higher water content of white muscle in some deep water fish has been described (Drazen, 2007). **Figure 4** illustrates the average white muscle composition of the fish analyzed in this study (except for the new *Pachycara* species). We found that the gel tissue had much higher content at 94.9-98.7%.

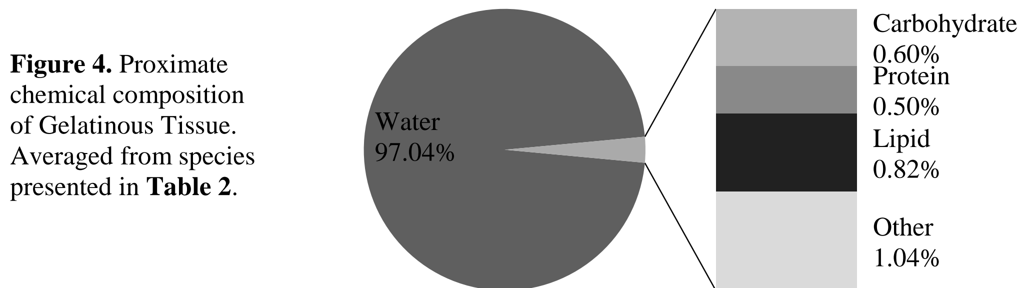

Figure 4. Proximate chemical composition of Gelatinous Tissue. Averaged from species presented in **Table 2**.

Table 2 provides a complete summary of the proximate chemistry data collected by this study. Osmolality and high sodium and low potassium levels indicate that the gel tissue has a low cell content. Shallow fish muscles have a normal sodium to potassium ratio of 0.15 (Drazen, 2009), while these results indicate a reversed ratio of 24 ± 7. It would therefore be expected that the tissues would resemble plasma osmolarity, in deep-sea fishes up to 500mOsm (Gillett *et al.*, 1997). The potassium contents seem similar to that of seawater (9.96 mmol/liter K^+). Sodium levels were significantly lower than that of seawater (470.2 mmol/liter Na^+). Tissues with lower salinity than seawater will provide buoyancy, as water density increases with salinity (Randall, 1997).

Sample Code	Depth (m)	mm K	mm Na	TMAO (mM)	% Water	% Protein	% Carb	% Dry Wt	% Lipid	Osmolality (mOsm)
Cm4	750	7.84	188.8	7.25	98.37	0.106	0.999	1.63		
Cm5	750	8.17	154	5.73	98.22	0.06		1.78		
Bb3a	1000			6.58	97.69	0.396		2.31		
Cc1	1000	8.5	158.3	5.56	97.86	0.23	0.5069	2.14	0.15	
Cm2	1000	9.4	128.2	4.55	98.73	0.462		1.27	0.2	
Eb11a	1000			3.71	95.99	0.373		4.01		
Eb11b	1000	8.32	216.8	6.41	96.34	0.263	0.7586	3.66	0.88	358
Eb2	1000	10.24	159.2	3.37	95.16	0.258	0.5564	4.84	3.59	
Eb8	1000	4.92	191.1	4.05	98.06	0.231	0.361	1.94		383.5
Eb9	1000				97.83	0.115		2.17	0.26	
Eb9	1000	5.47	182.1	1.01	98.43		0.3553	1.57		388
Mp4	1000	11.84	188.1	1.85	95.8	0.172	0.3504	4.2	1.73	311.5
Mp5	1000	7.67	192.6	14	95.65	2.835	0.7459	4.35	0.89	
Mp6	1000	5.47	182.1	2.87	97.87	0.302	0.5239	2.13	0.28	
Bb3b	2000	8.35	190.6	3.88	98.31	0.385	0.5841	1.69	0.14	385
Bb4	2000	10.11	201.1	4.38	96.65	0.341		3.35	0.41	
Sg3	2000	12.79	205.1	8.6	96.54	0.627	1.252	3.46		355
Pb1	3000	6.54	188.1	25.8	95.43	0.88		4.57	1.39	
Pb3	3000	5.8	186.1	28.5	94.85	0.739		5.15	1.22	
Pb8	3000	6.68	212.2	23.61	97.04	0.343	0.3756	2.96		467

Table 2: Proximate Chemistry Results by Depth. Fish codes indicate species and sample number.

II. Comparative Video Analysis

Both the hadal snailfish and the tidal snailfish swam with "s-form" tail beats with a maximum of two nodes. **Figure 5** shows the comparative swimming performance of the types of snailfish. The shallow water snailfish, which has a higher muscle volume in the tail and lacks gel, swam faster than their deeper-living relatives. *Liparis florae* also took more pectoral fin strokes per tail beat than the hadal fishes.

Figure 5b also presents body proportion comparisons from capture data. The hadal snailfish had a longer tail relative to total body size than the tidal snailfish. Although it could not be quantified in these dissections, anecdotally, the smaller hadal snailfish seemed to have proportionally less gel.

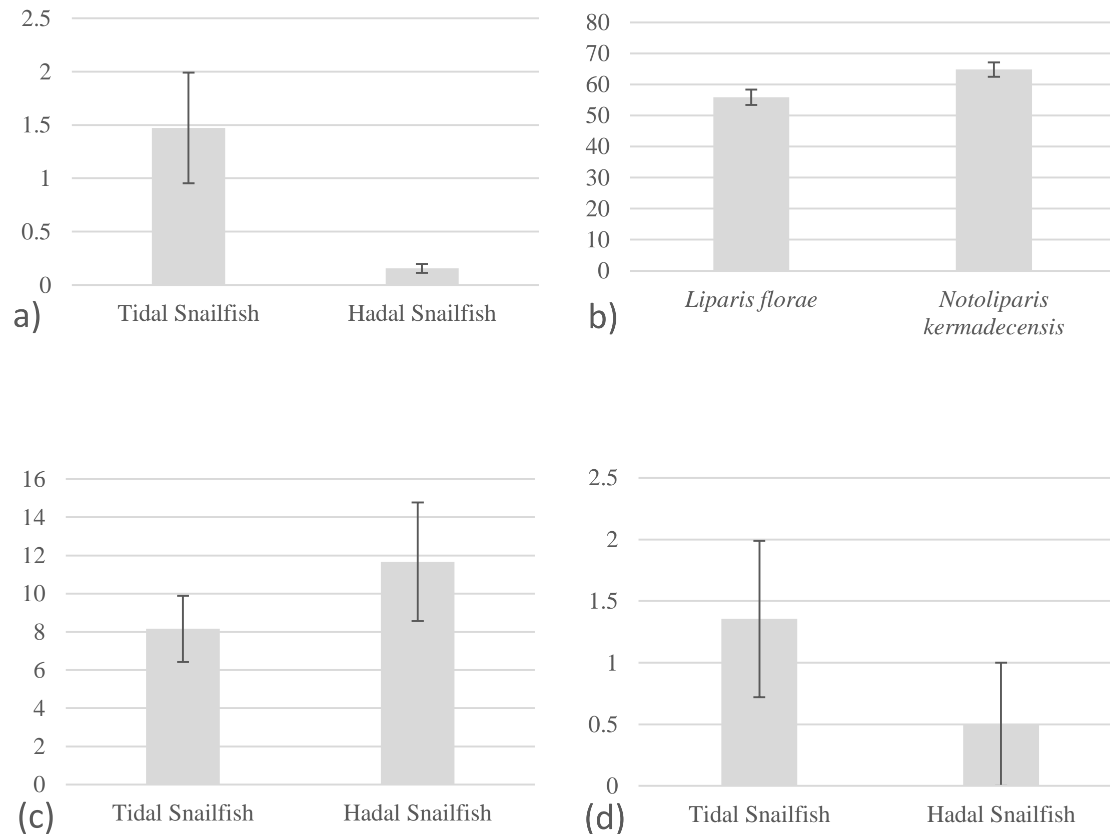

Figure 5. a) Average Body Lengths per Second. **b)** Percent Caudal Region/Total Length. Captured *Notoliparis kermadecensis* averaged caudal region that was 55.85 +/- 2.47% of the total length of the animal (n=41), while *Liparis florae* had an average 64.8 +/- 2.32% total length (n=5). Caudal region was measured from beginning of anal fin to end of tail. **c)** Average percent wave amplitude/body length. **d)** Average pectoral fin strokes made per tail beat. Sections a), b), and c) show data from the tidal snailfish *Liparis florae* (n=9) and the hadal snailfishes *Notoliparis kermadecensis* and *Pseudoliparis amblystomopsis* (n=4).

III. Robotic Model

Swimming performance clearly varied between the empty and full tail. With the water-filled tail (simulating gel), the robot swam forward at an average rate of 26.78 mm/second (Standard Deviation = 0.15 mm/sec). With the skin emptied around the silicone rubber tail, progress was much slower, on average 7.78 mm/second (Standard Deviation = 2.59 mm/sec). In parts of the no gel swim trials, the robot barely swam forward. **Figure 6** illustrates this difference in swimming performance.

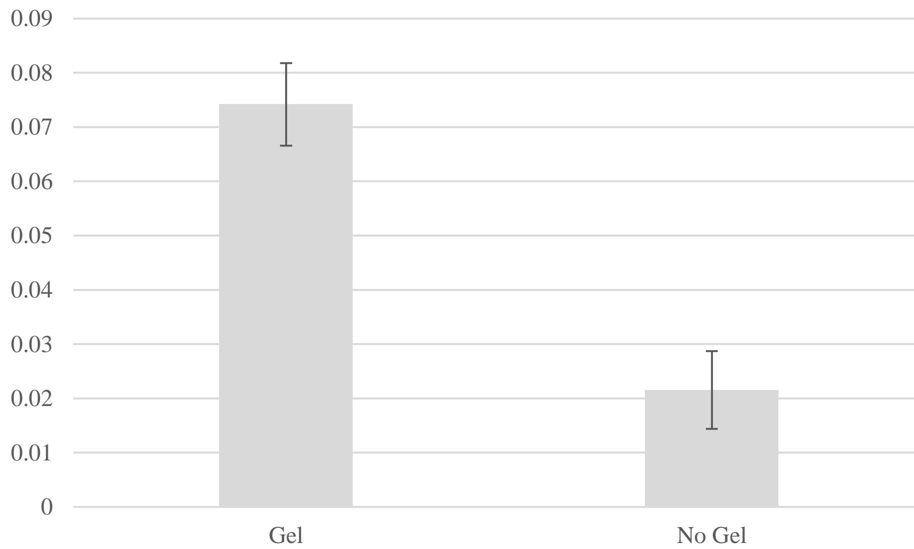

Figure 6. Body Lengths per Second in Swim Trials. With gel, ANTON swims at approximately half of the velocity of the hadal snailfish measured in this study (0.157 body lengths per second, standard deviation = 0.0417).

DISCUSSION

This study represents the first look at the composition and function of subcutaneous gelatinous tissue, using the hadal snailfish as a model organism. Proximate chemical analysis of gelatinous tissue in seven benthic and benthopelagic species showed high water content and low protein, lipid, and carbohydrate content in comparison to white muscle. In concert with the ionic compositions and osmolalities of these tissues, these data suggest

that the layer is predominately extracellular fluid. The results of this study refute the energy storage hypothesis, as gelatinous tissue contained such a low nutritive content.

Video analyses provide an initial characterization of hadal snailfish swimming mechanics. The hadal snailfish are slower than their shallow relatives, a trend seen across a number of deep-radiating taxa. Future investigations of gel function should consider the volume and position of gelatinous tissue in fish from different depths, habitats, and life stages. Material properties of the gel should also be analyzed under hadal temperatures and pressures. It is likely that the gel is stiffer at hadal conditions, and could therefore play an even greater role in hadal snailfish swimming. The robotic model data suggest that the presence of the gelatinous layer enhances swimming performance in the hadal snailfish. As the gel is mostly water, it is energetically inexpensive to form, but the benefit to locomotory capacity is significant.

ACKNOWLEDGEMENTS

I would like to thank, Adam Summers, Alan Jamieson, Amy Scott-Murray, Thom Linley, the Friday Harbor Labs Fish Class 2014, Stacy Farina, the captains and crew of the R/V Kaharoa, R/V Thompson, R/V Centennial, R/V Sonne, R/V Hakuho Maru, Jeff Drazen, Nicole Condon, Jason Friedman, Carrie Laxson, Stephanie Crofts, Misty Paig-Tran, Ashley Rowden, Matteo Ichino, and Tim Shank for making this research possible through their contributions. Support for this project came from NSF, the Total Foundation, and the Stephen and Ruth Wainwright Endowment.

REFERENCES

Ball, J.N., D.M. Ensor. 1965. "Effect of Prolactin on Plasma Sodium in the Teleost, *Poecilia Latipinna*." *Journal of Endocrinology*. 32: 269-270.**Barbosa, I., S. Garcia, V. Barbier-Chassefiere, J,P. Caruelle, I. Martelly, D. Papy-Garcia, 2003.** "Improved and simple micro assay for sulfated glycosaminoglycans quantification in biological extracts and its use in skin and muscle tissue studies." *Glycobiology*. 13: 647-653. **Clarke, A., L.J. Holmes, D. Gore. 1992.** "Proximate and elemental composition of gelatinous zooplankton from the Southern Ocean." *Journal of Experimental Marine Biology and Ecology*. 155: 55-68. **Devor, A.W. 1950.** "Carbohydrate Tests Using Sulfonated alpha-Naphthol." *American Chemical Society*. 72: 2008-2012. **Doyle, T.K., J.D.R. Houghton, R. McDevitt, J. Davenport, G.C. Hays. 2007.** "The energy density of jellyfish: Estimates from bomb-calorimetry and proximate-composition." *Journal of Experimental Marine Biology and Ecology*. 343: 239-252. **Drazen, J.C. 2007.** "Depth related trends in proximate composition of demersal fishes in the eatern North Pacific." *Deep-Sea Research Part I*. 54: 203-219. **DuBois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers, F. Smith. 1956.** "Colorimetric Method for Determination of Sugars and Related Substances." *Analytical Chemistry*. 28.3: 350-356. **Gillett, M.B., J.R. Suko, F.O. Santoso, and P.H. Yancey. 1997.** "Elevated Levels of Trimethylamine Oxide in Muscles of Deep-Sea Gadiform Teleosts: A High-Pressure Adaptation?." *Journal of Experimental Zoology*. 279: 386-391. **Gold, E.W. 1979.** "A Simple Spectrophotometric Method for Estimating Glycosaminoglycan Concentrations." *Analytical Biochemistry*. 99: 183-188. **Herr, J.E., T.M. Winegard, M.J. O'Donnell, P.H. Yancey, D.S. Fudge. 2010.** "Stabilization and swelling of hagfish slime mucin vesicles." *Journal of Experimental Biology*. 213: 1092-1099. **Herring, P. 2002.** *The Biology of the Deep Ocean*. New York: Oxford University Press. **Hunter, J.H., J.L. Butler, C. Kimbrell, E.A. Lynn. 1990.** "Bathymetric patterns in size, age, sexual maturity, water content, and caloric density of dover sole, *Microstomas pacificus*." *CalCOFl Rep*. 31: 132-144. **Hyndman, K.A., and D.H. Evans. 2009.** "Short-Term Low-Salinity Tolerance by the Longhorn Sculpin, *Myoxocephalus octodecimspinosus*." *Journal of Experimental Zoology*. 311: 45-56. **Jamieson, A.J., T. Fujii, M. Solan, A.K. Matsumoto, P.M. Bagley, and I.G. Priede. 2009.** “Liparid and macrourid fishes of the hadal zone: *in situ* observations of activity and feeding behavior.” *Proceedings of the Royal Society B*. 276: 1037-1045. **Kelly, R.H., P.H. Yancey, 1999.** “High contents of trimethylamine oxide correlating with depth in deep-sea teleost fishes, skates, and decapods crustaceans. *Biol.Bull*, 19618-25. **Lucas, C.H. 1994.** "Biochemical composition of *Aurelia aurita* in relation to age and sexual maturity." *Journal of Experimental Marine Biology and Ecology*. 183: 179-192. **Prosser, L., W. MacKay, K. Kato. 1970.** "Osmotic and Ionic Concentrations in Some Alaskan Fish and Goldfish from

Different Temperatures." *Physiological Zoology*. 43.2: 81-89. **Randall, D.J., and A.P. Farrell. 1997.** *Deep-Sea Fishes*. San Diego, CA: Academic Press. **Samerotte, A.L., J.C. Drazen, G.L. Brand, B.A. Seibel, P.H. Yancey. 2007.** "Correlation of Trimethylamine Oxide and Habitat Depth within and among Species of Teleost Fish: An Analysis of Causation." *Physiological and Biochemical Zoology*. 80.2: 197-208. **Wekkel J. C., H. Barnett. 1991.** New method for analysis of trimethlyamine oxide using ferrous sulfate and EDTA. *Journal of Food Science*, *56*, 132-138. **Wolff, S. D., P.H. Yancey, T.S. Stanton, R.S. Balaban. 1989.** "A simple HPLC method for quantitating major organic solutes of renal medulla." *American Journal of Physiology*. 256:5. **Yancey, P. H., A.L. Fyfe-Johnson, R.H. Kelly, V.P. Walker., & M.T. Aunon. 2001.** Trimethylamine oxide counteracts effects of hydrostatic pressure on proteins of deep-sea teleosts. *Journal of Experimental Zoology*, *289*, 172-176.