COMPARISON OF MECHANICAL PROPERTIES OF SKIN FROM PACIFIC HAGFISH, *EPTATRETUS STOUTII*, AND PENPOINT GUNNEL, *APODICHTHYS FLAVIDUS*

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ABSTRACT

Peak stress and stiffness of skin from two elongate fishes, *Apodichthys flavidus* and *Eptatretus stoutii,* were mechanically tested at 25mm/minute. Quasi-static tension tests to failure were conducted on skin from dorsal samples from the anterior and posterior ends. Both hagfish and gunnel skin show anisotropy in opposite trends. Hagfish have more deformation resistance longitudinally and gunnels show more resistance circumferentially. Location of samples did not have a significant difference for measurements of either property for both fish. Stiffness exhibited a significant difference for both species between the different directions.

INTRODUCTION

Skin has numerous functions, including protection from injury and pathogens and a supportive and flexible framework for the body (Frolich, 1997 and Hebrank, 1980). Layers of collagen strengthen the skin, creating a rigid support for the body. The flexibility of skin allows for specific locomotive patterns used by different organisms. Skin from different organisms will vary in strength and stiffness, along with other properties. Some organisms breathe through their skin, others have less permeable skin in order to keep from desiccating in drier climates (Rivera *et al.,* 2005). In most organisms, the skin is in strong connection to the muscle through myosepta, creating forces on the skin (Gemballa and Vogal, 2002). Pacific hagfish, *Eptatretus stoutii*, do not have these strong connections between their skin and muscle, instead, they have very loose skin and a connection along the dorsal midline (Gemballa and Vogal, 2002).

Apodichthys flavidus (Girard) and *Eptatretus stoutii* (Lockington) are two elongate fishes who swim with anguilliform motion. While they have similar locomotive patterns, their life history is very different. Pacific hagfish live in deeper waters in a fairly homogenous habitat, and penpoint gunnels are intertidal and subtidal, encountering strong wave action, rocky shores, and varying water levels (Haynes *et al*., 2009 and McInerny & Evans, 1970). Hagfish often tie themselves in knots to pull pieces of flesh from carcasses and when they need to clear slime off of their bodies. Gunnels do not tie themselves in knots, however, they do have to work through crevices between rocks in their habitat.

Past studies have looked at fish, including eels and tunas, and terrestrial vertebrates including snakes and geckos. Some species have high degrees of resistance to tensile forces while others have lower. Some are isotropic, and others are anisotropic, having less resistance in one direction or the other. With differences in the connectivity of muscle to skin between these two species along with the different life histories of these two species, the stiffness and peak stress are likely to differ.

The aims of this study were: 1) to determine whether the skin of these two elongate fishes show a difference in stiffness and peak strength based on location on the animal; 2) to determine whether the skin is anisotropic in stiffness and peak strength; 3) to run comparisons of stiffness and peak strength between the two species.

MATERIALS AND METHODS

Specimen Collection

Pacific hagfish, *Eptatretus stoutii*, were collected off the coast of Southern California in July 2011 by California Fish and Game then immediately frozen upon and kept frozen until ready for use. The hagfish (n=4, Total length 34.5-40.5cm) were thawed only once prior to use in the current experiment and the fish were then refrozen for potential use in other work on musculature and feeding. Penpoint gunnels, *Apodichthys flavidus*, were collected while seining at Jackson Beach, San Juan Island, Washington in July 2012. The pholids (n=4, Total length 28.7-35.6cm) were then kept in tanks connected to the flow-through system at the University of Washington Friday Harbor Laboratories, Friday Harbor, Washington. Gunnels were sacrificed using MS222 and were used in two studies on skin, the current study and another on frictional properties of pholid skin.

Sample Preparation and Experimental setup

Sixteen skin samples were taken from each specimen, eight anterior and eight posterior with four from the left side and four from the right. The samples were further divided between circumferential and longitudinal directions and tested for stiffness (rectangular samples, mean width=5.54mm, mean length=16.75mm) or peak stress (hourglass shaped samples, mean width=1.95mm, mean length=17.33mm) (Figure 1). Anterior samples were taken from an area at about 25% of the body length and posterior samples were taken at about 75% of the body length. Mechanical properties were measured using Synergie100 Materials Testing System (MTS) with TestWorks 4 software (MTS). In addition to skin used in the materials testing portion of this study, samples were removed and photographed under 25X magnification with a SteREO Discovery V.20 microscope (Zeiss) (Figure 2).

In order to reduce slippage, a small square of paper towel was folded over the portion of skin sample in the grips on the MTS. After finger-tightening the screws, dry ice was applied to the grips for about 10 seconds before testing the sample to failure. Samples were tested at 25mm per minute, recording at 10 hertz. The Materials Testing System produced an output of Force and Extension. From these measurements, stress and strain curves were created using width, length, and thickness of each sample (Figure 3 and 4). From the stress and strain curves, stiffness (Young's Modulus) was calculated as the maximum slope (Figure 4A) and peak stress was calculated as the maximum stress before failure (Figure 4B). Cut samples were kept wet using paper towels soaked in ringer's solution (full seawater for hagfish samples and 1/3 seawater for pholid samples).

Data analysis

Data were analyzed using Microsoft Excel and SPSS (IBM). To reduce the effect of pseudoreplication, duplicated measurements from left and right sides of animals were averaged and used as one data point. Resulting in one measurement for each combination of location, direction, and test per animal with eight total combinations. A nested Anova was used to compare the strength and stiffness of skin between location on the animal and direction of sample. Due to small sample size, graphical analysis was used for between species comparison instead of statistical analysis.

RESULTS

Under 25x magnification, differences between the two species are evident (Figure 2). Pholid skin is covered in scales while hagfish skin lacks scales and appears very smooth. Hagfish skin also has a thick layer of fatty endodermis making up over two thirds of the skin's thickness (Figure 2C). Pholid skin had an even thickness between anterior and posterior samples, however, the thickness of hagfish skin was significantly different between anterior and posterior samples (One tailed T Test, $p<0.001$) (Figure 5).

Peak stress and stiffness did not indicate a significant difference when separated by location, anterior or posterior for either *Apodichthys flavidus* or *Eptatretus stoutii* (p values >0.2) (Figures 6 & 7). Additionally, neither *A. flavidus* nor *E.stoutii* show a difference for peak stress between circumferential and longitudinal directions (p values>0.2) (Figures 6A & 7A). Unlike peak stress, the Young's Modulus did show anisotropy when comparing direction (circumferential versus longitudinal) when calculated for *A. flavidus* ($p<0.001$) and *E. stoutii* ($p<0.05$) (Figures 6B & 7B). Between species comparisons illustrate a disparate trend with the hagfish showing higher peak stress and stiffness in the longitudinal direction and the pholid demonstrating a greater peak stress and stiffness in the circumferential direction (Figure 8).

DISCUSSION

Both *Apodichthys flavidus* and *Eptatretus stoutii* showed anisotropic stiffness. This is often observed in other species, such as the skipjack tuna, *Katsuwonus pelamis,* and the American eel, *Anguilla rostrata*. Both *A. rostrata* and *K. Palamis* show increased deformation resistance in the circumferential direction (Hebrank, 1980, Henbrank & Hebrank, 1986). *A. flavidus* follows this trend of more resistance to deformation in the circumferential direction and less resistance longitudinally, however *E. stoutii* shows an opposite trend, more resistance longitudinally than circumferentially. This may be related to movement in the animals, gunnels show more lateral movement in their sigmoidal locomotive patterns and while hagfish also have undulations in swimming, the have more ability to twist as their ability to tie themselves in knots shows. In spite of lacking myosepta, hagfish skin has tensile properties comparable to those of other fish and terrestrial vertebrates (Table 1).

Neither species showed a significant difference in stiffness or peak stress. Some species, such as the spadenose shark, *Scoliodon laticaudus*, and the common gartersnake, *Thamnophis sirtalis* do have a difference in tensile strength and stiffness between the anterior and posterior areas (Naresh, 1997 and River *et al*., 2005). The difference may be linked to different undulatory patterns. The gunnel and hagfish also did not show significant differences in peak stress between the different directions. The power of these two analyses was low and more samples are necessary to be sure the lack of significant difference is true and not only an artifact of small sample size.

A continuation of this study to include a larger number of individuals and more species would increase the depth and strength of the comparisons begun in this study. Including multiple species of agnathans, teleosts, and chondrichthyans may aid in resolving the phylogenetic relationships among fish taxa. Additionally, increasing measurements to include ventral measurements would find if there are differences in the strength and stiffness of skin between dorsal and ventral areas. Hagfish have stronger connections between skin and muscle than on the dorsal side which will likely have an impact on the mechanical properties.

TABLES AND FIGURES

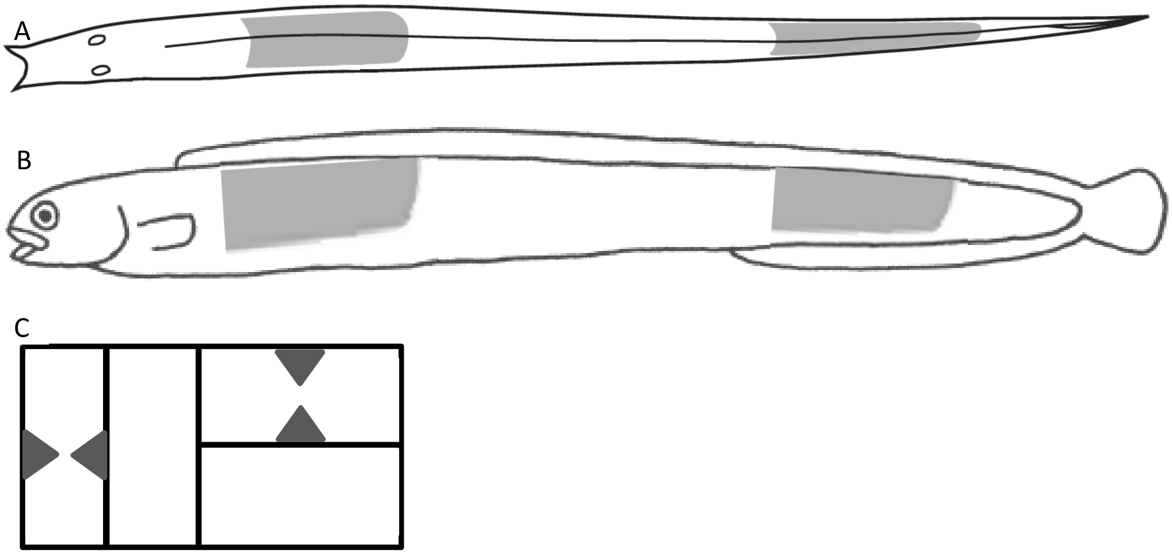


Figure 1. Layout of location of samples taken from specimens of *E. stoutii* (A) and *A. flavidus* (B). Samples were also taken from the right side of *A. flavidus*. Samples were taken circumferentially and longitudinally (C).

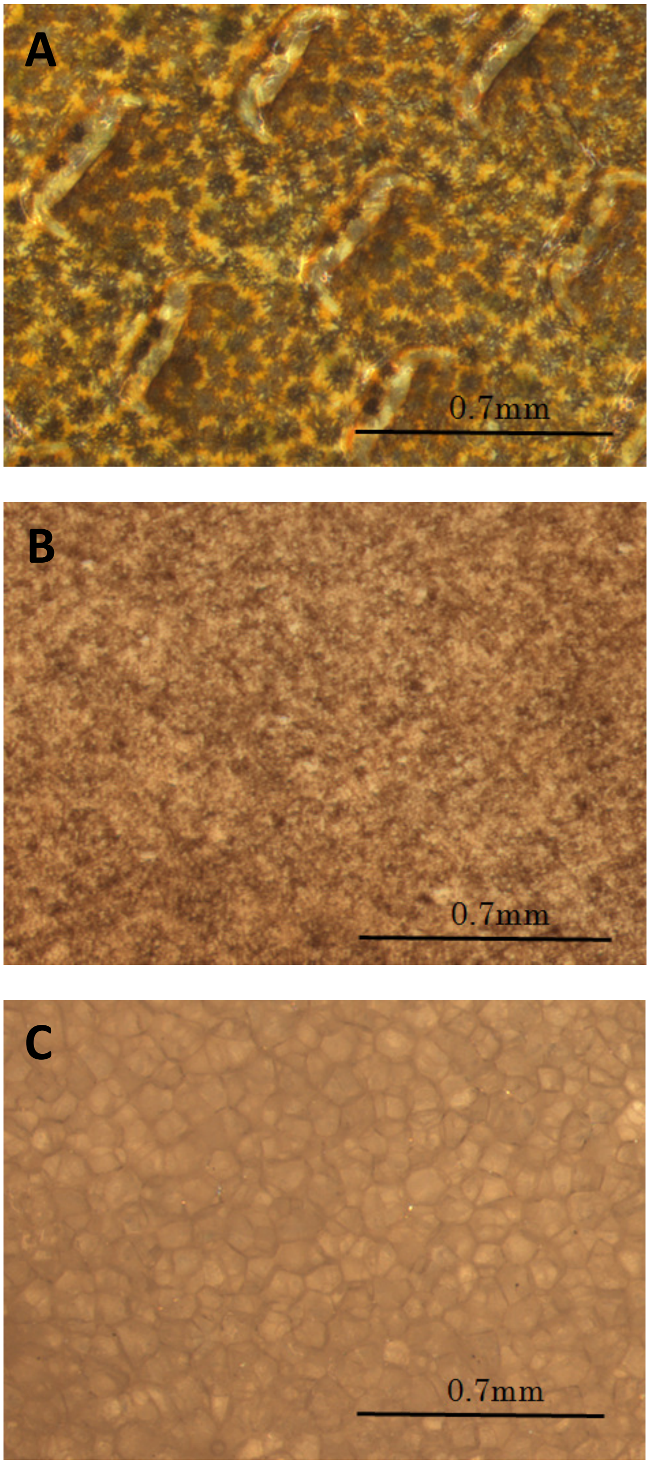
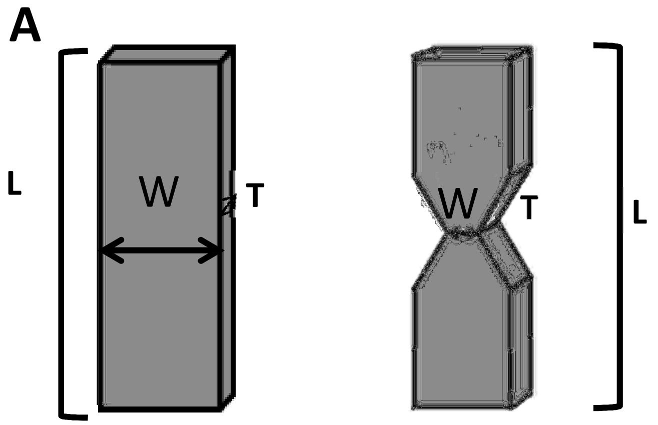


Figure 2. Dissecting microscope images of pholid skin (A), the outer side of hagfish skin (B), and the underside of hagfish skin, showing the fatty subdermis layer (C), all images taken at 25X.



B

$$\text{Stress} = \frac{\text{F}}{\text{CSA}}$$

$$\text{Strain} = \frac{\Delta L}{Lo}$$

Figure 3. Measurements and calculations for stiffness and strength. Width and thickness were taken for each sample and length was measured as the grip separation once the sample was in place on the Materials Testing System (A). Strain was calculated using Force (N) divided by the cross-sectional area (Width x Thickness =CSA). Stress was calculated as the change in length (ΔL) divided by the initial length (L_o) (B).

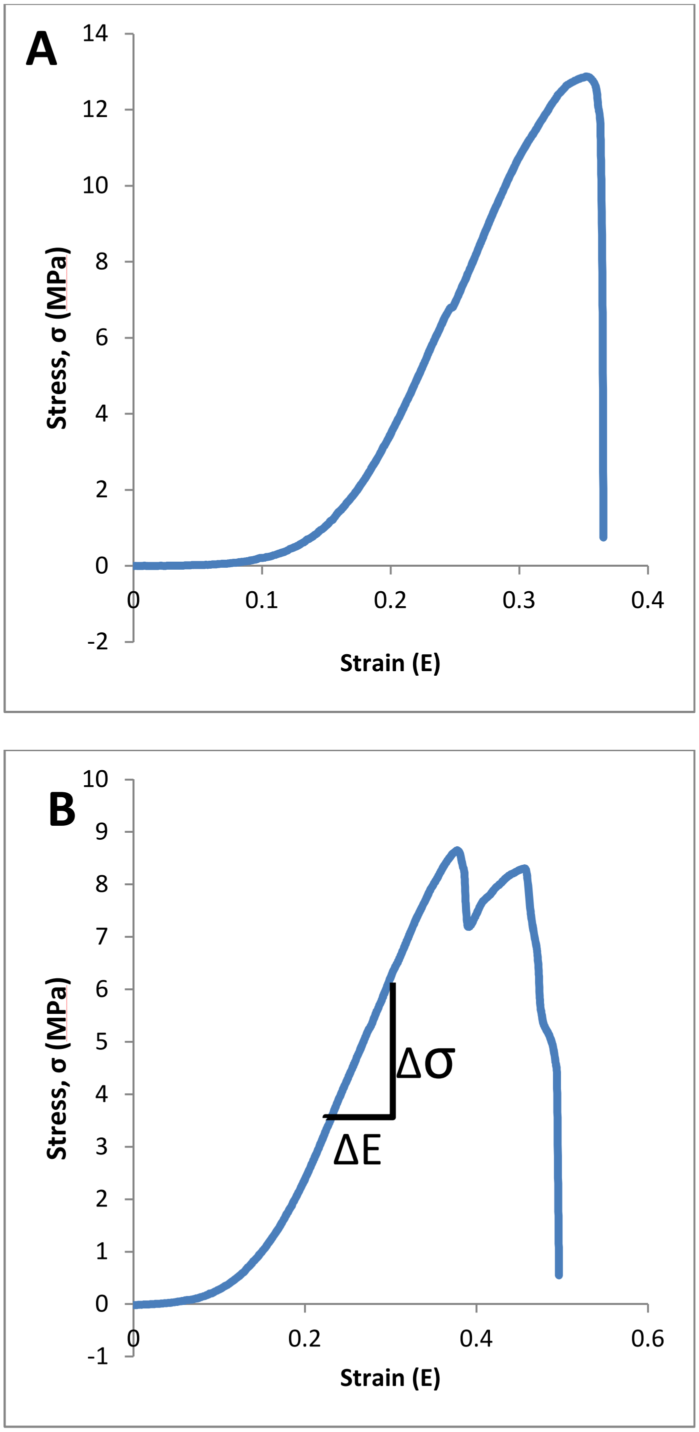


Figure 4. Stress and strain curves used to calculate Peak Stress (A) and Young's Modulus (B).

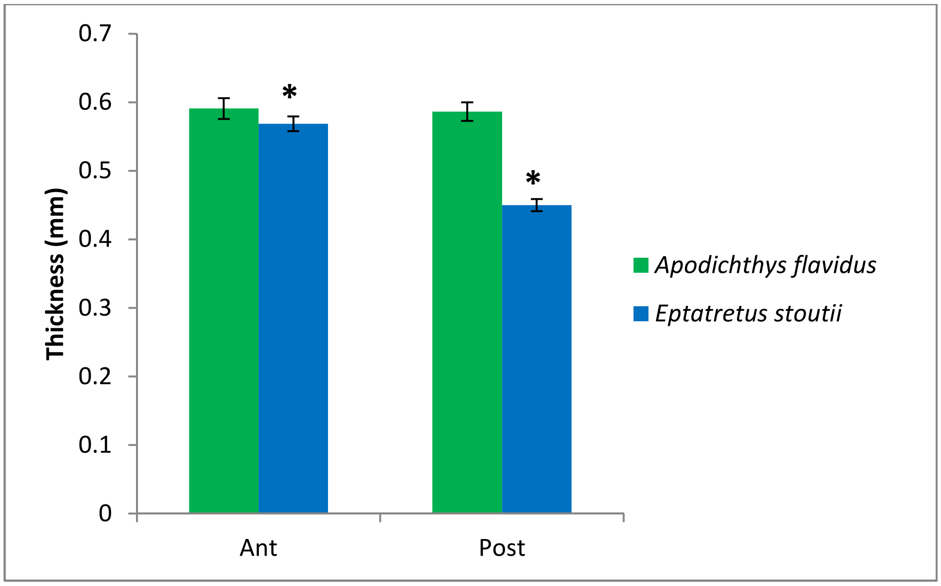


Figure 5. Skin thickness in *A. flavidus* was not significantly different between posterior and anterior samples (p>0.3). *E. stoutii* skin was significantly different in thickness between anterior and posterior (p<0.001).

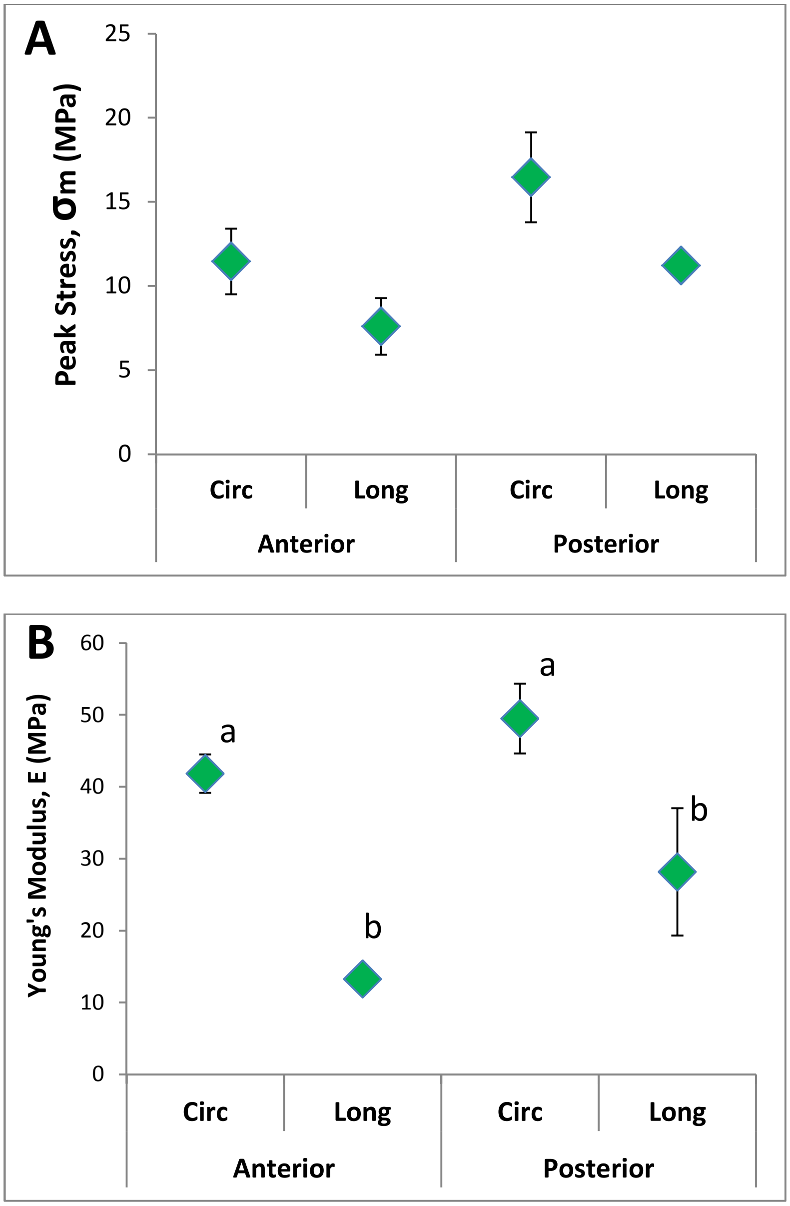


Figure 6. Comparisons of peak stress (A) and Young's Modulus (B) for *Apodichthys flavidus* (n=4 for each measurement). Peak Stress was not significantly different for location (F=1.739, p>0.3) or direction (F=2.963, p>0.05) (A). Young's Modulus was also not significantly different for location (F=0.331, p>0.6) however, direction did show a significant difference (F=15.382, p<0.001) (B).

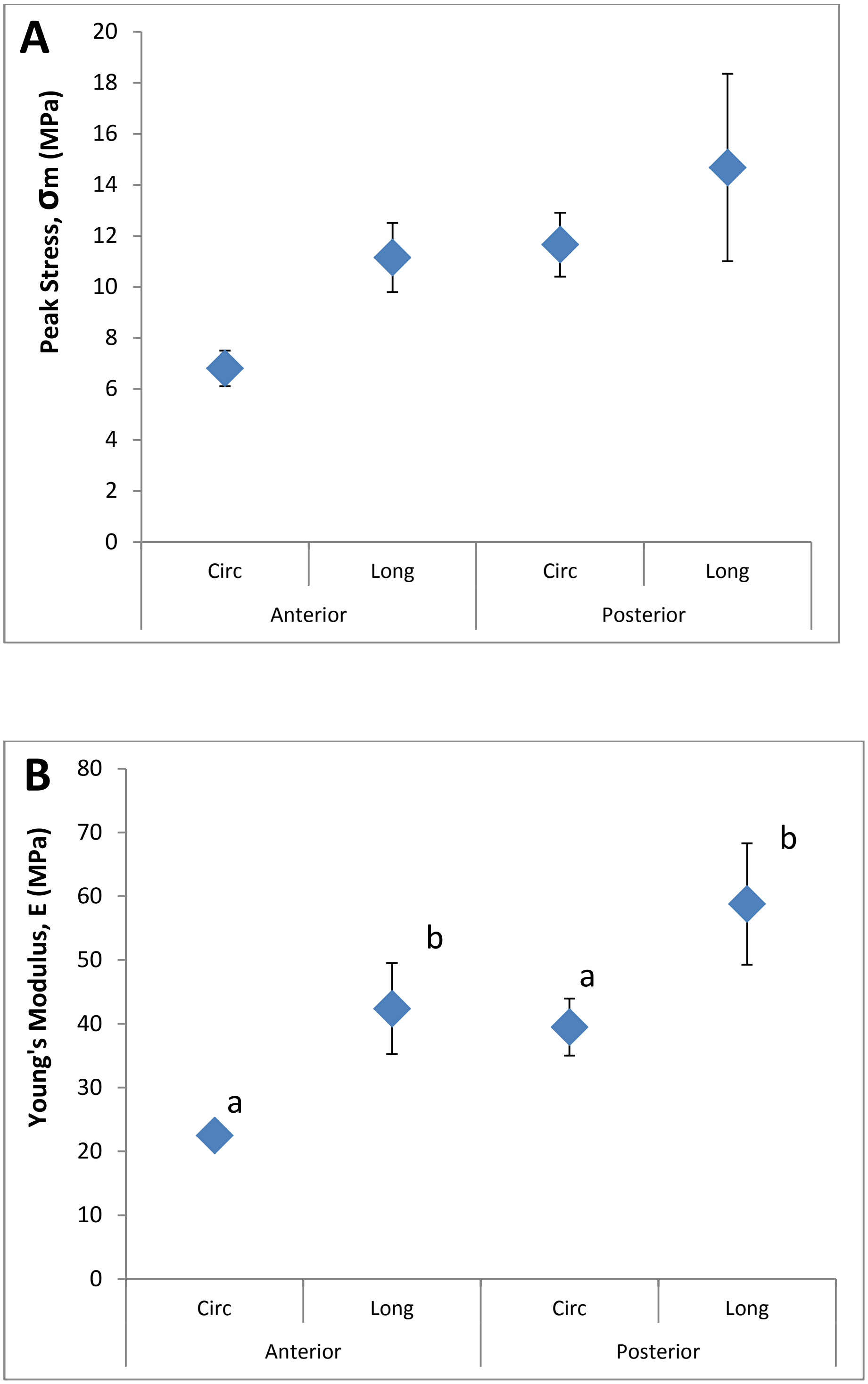


Figure 7. Comparisons of peak stress (A) and Young's Modulus (B) for *Eptatretus stoutii* (n=4 for each measurement). Peak Stress was not significantly different for location (F=2.510, p>0.2) or direction (F= F=1.611, p>0.2) (A). Young's Modulus was also not significantly different for location (F=1.454, p>0.3) however, direction did show a significant difference (F=4.688, p<0.05) (B).

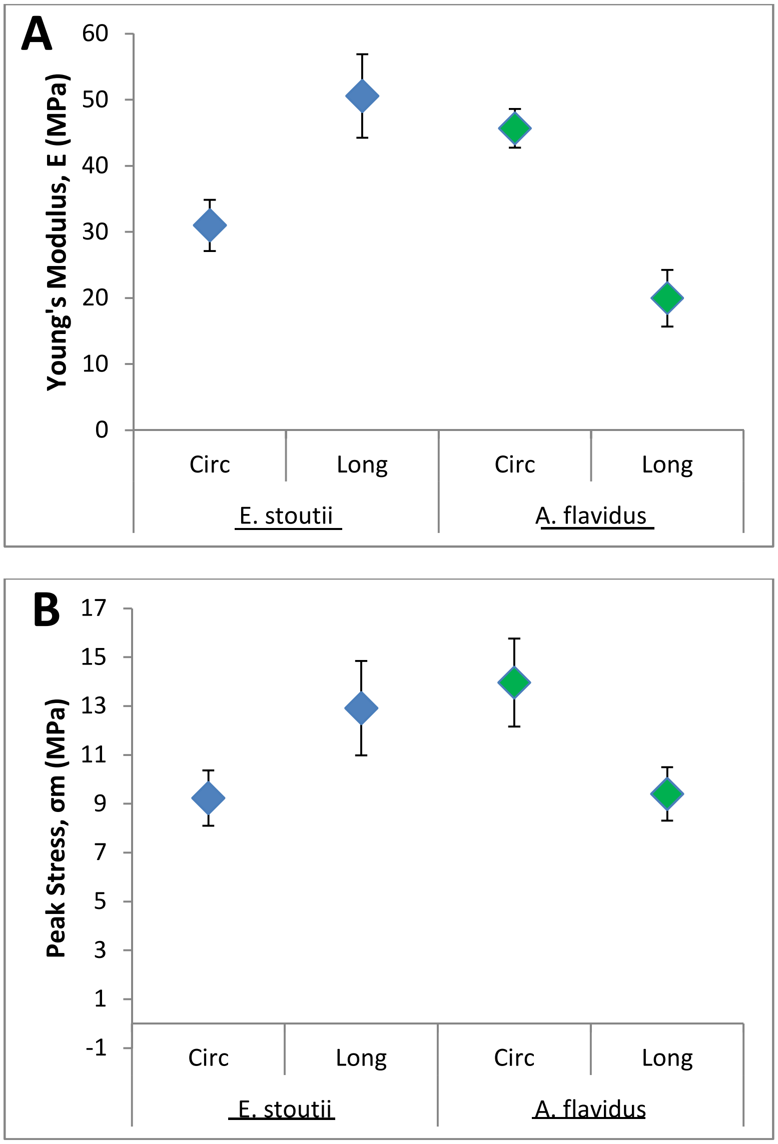


Figure 8. Young's Modulus (A) and Peak Stress (B) for each species by direction. Posterior and anterior results were averaged for this analysis.

Table 1. Mechanical properties of skin from fish and terrestrial vertebrates, current study in bold.

Species	Stiffness (MPa)	Strength (MPa)	Direction	Location	Source
Leiostomus xanthurus	16.4	N/A	Circumferential	Lateral	Hebrank & Hebrank, 1986
Leiostomus xanthurus	2.41	N/A	Longitudinal	Lateral	Hebrank & Hebrank, 1986
Katsuwonus pelamis	60.2	N/A	Circumferential	Lateral	Hebrank & Hebrank, 1986
Katsuwonus pelamis	6.92	N/A	Longitudinal	Lateral	Hebrank & Hebrank, 1986
Anguilla rostrata	14.7	N/A	Circumferential	Lateral	Hebrank, 1980
Anguilla rostrata	3.54	N/A	Longitudinal	Lateral	Hebrank, 1980
Scoliodon laticaudus	N/A	32	Circumferential	Posterior	Naresh et al., 1997
Scoliodon laticaudus	N/A	24	Longitudinal	Posterior	Naresh et al., 1997
Scoliodon laticaudus	N/A	22	Circumferential	Anterior	Naresh et al., 1997
Scoliodon laticaudus	N/A	14	Longitudinal	Anterior	Naresh et al., 1997
Teratoscincus scincus	2.78	0.53	Longitudinal	Dorsal	Bauer et al., 1989
Teratoscincus scincus	2.4	0.61	Circumferential	Dorsal	Bauer et al., 193
Teratoscincus scincus	1.38	0.43	Longitudinal	Ventral	Bauer et al., 1993
Teratoscincus scincus	2.42	0.62	Circumferential	Ventral	Bauer et al., 1993
Nerodia f. pictiventris	41.4	28.91	Longitudinal	Ventral	Jayne, 1988
Nerodia f. pictiventris	194.2	8.84	Longitudinal	Dorsal	Jayne, 1988
Ahaetulla prasina	82.7	13.03	Longitudinal	Ventral	Jayne, 1988
Ahaetulla prasina	60.6	55.85	Longitudinal	Dorsal	Jayne, 1988
Laticauda colubrina	255	82.95	Longitudinal	Ventral	Jayne, 1988
Laticauda colubrina	584.5	7.91	Longitudinal	Dorsal	Jayne, 1988
Enhydrina schistosa	24.3	18.44	Longitudinal	Ventral	Jayne, 1988
Enhydrina schistosa	21.9	28.05	Longitudinal	Dorsal	Jayne, 1988
Hydrophis melanosoma	152	27.91	Longitudinal	Ventral	Jayne, 1988
Hydrophis melanosoma	204.3	12.68	Longitudinal	Dorsal	Jayne, 1988
Acrochordus granulatus	50.4	6.53	Longitudinal	Ventral	Jayne, 1988
Acrochordus granulatus	25.3	5	Longitudinal	Dorsal	Jayne, 1988
Eptatretus stoutii	24.62	13.61	Circumferential	Anterior	Demas & Clark, unpublished data
Eptatretus stoutii	28.65	20.02	Circumferential	Posterior	Demas & Clark, unpublished data
Eptatretus stoutii	27.82	16.18	Circumferential	Ventral	Demas & Clark, unpublished data
Eptatretus stoutii	38.82	16.78	Longitudinal	Anterior	Demas & Clark, unpublished data
Eptatretus stoutii	61.83	26.71	Longitudinal	Posterior	Demas & Clark,

					unpublished data
Eptatretus stoutii	28.77	20.52	Longitudinal	Ventral	Demas & Clark, unpublished data
Apodichthys flavidus	**13.27**	**7.60**	**Longitudinal**	**Anterior**	**Current Study**
Apodichthys flavidus	**26.66**	**11.20**	**Longitudinal**	**Posterior**	**Current Study**
Apodichthys flavidus	**41.84**	**11.46**	**Circumferential**	**Anterior**	**Current Study**
Apodichthys flavidus	**49.49**	**16.46**	**Circumferential**	**Posterior**	**Current Study**
Eptatretus stoutii	**22.48**	**6.81**	**Circumferential**	**Anterior**	**Current Study**
Eptatretus stoutii	**39.49**	**11.65**	**Circumferential**	**Posterior**	**Current Study**
Eptatretus stoutii	**42.38**	**11.15**	**Longitudinal**	**Anterior**	**Current Study**
Eptatretus stoutii	**58.78**	**14.68**	**Longitudinal**	**Posterior**	**Current Study**

LITERATURE CITED

Bauer, A. M., A. P. Russell, and R. E. Shadwick, 1989. Mechanical properties and morphological correlates of fragile skin in gekkonid lizards. *J. Exp. Biol*. **145,** 79-102.

Bauer, A. M., A. P. Russell, and R. E. Shadwick, 1993. Skin mechanics and morphology of the gecko *Tetratoscincus scincus. Amphibia-Reptilia*. **14,** 321-331. (1993).

Frolich, L. M. 1997. The role of the skin in the origin of amniotes: permeability barrier, protective covering and mechanical support. In *Amniote Origins: Completing the Transition to Land* (ed. S. S. Sumida and K. L. M. Martin), pp. 327-352. San Diego: Academic Press.

Gemballa, S., and P. Bartsch. 2002. Architecture of the integument in lower teleostomes: Functional morphology and Evolutionary implications. J. Morph. 253, 290–309.

Haynes, T. B., E. Phillips-Mentzos, and D. E. Facey. 2009. A Comparison of the Hyposaline Tolerances of Black Prickleback (*Xiphister atropurpureus*) and Penpoint Gunnel (*Apodichthys flavidus*). Northwest Science, 83(4):361-366.

Hebrank, M.R. 1980. Mechanical properties and locomotor functions of eel skin. Biol. Bull. 158, 58–68.

Hebrank, M.R. and J. H. Hebrank.1986. The mechanics of fish skin. Lack of an “External Tendon” role in two teleosts. Biol. Bull. 171, 236–247.

Jayne, B. C. 1988. Mechanical behaviour of snake skin. *J. Zool., Lond*. **145,** 79-102. (1988).

Naresh, M. D., V. Arumugam, and R. Sanjeevi. 1997. Mechanical behaviour of shark skin. *J. Biosci*. **22,** 431-437.

McInernry, J. E., and D. O. Evans. 1970. Habitat characteristics of the Pacific Hagfish, *Polistotrema stouti. J. Fisheries Research Board of Canada.* 27(5):966-969.

Rivera, G., A. H. Savitzky, and J. A. Hinkley. 2005. Mechanical properties of the integument of the common gartersnake, *Thamnophis sirtalis* (Serpentes: Colubridae). J. of Experimental Biol. 208: 2913-2922.