Comparative biomechanics of three eel species with special attention to moray eel adaptations for knotting

An essay submitted in partial fulfillment of

the requirements for graduation from the

Honors College at the College of Charleston

with a Bachelor of Science in

Marine Biology

Holly Caudle

May 2019

Advisor: Dr. Andrew Clark

Condensed title:

Mechanics of moray eel skins

Manuscript title:

Comparative biomechanics of three eel species with special attention to moray eel adaptations for knotting

Author:

Holly Caudle

Address:

Department of Biology, College of Charleston, 66 George Street, Charleston, SC 29424, USA

Email of corresponding author:

caudlehi@g.cofc.edu

Key words: *Gymnothorax*, *Anguilla*, *Monopterus*, fish skin, material properties

Introduction:

Knotting is a difficult behavior to execute, even in elongate animals like hagfish and eels, as it requires long axis twisting coupled with high-curvature bending. These extreme body curvatures put dangerously high axial strains on the body (especially the muscles and skin) of most fishes (Clark *et al* 2016). The farther away from the body's neutral axis, the more tension that material will experience, meaning that the skin on the outer edge will face the greatest tension when the body is curved (Vogel 2013). Hagfish have been observed to tie their bodies in knots when confronted with food items that are too large to be swallowed whole (Clark and Summers 2012; Uyeno and Clark 2015; Clark *et al* 2016). In these cases, a knot is formed beginning in the tail, and it moves up the body to provide leverage against the food item so that a bite may be torn off. Similarly, various species of moray eels have been observed utilizing knot-tying to tear pieces of food or to remove prey items from small caves where they are lodged (De Sylva 1986; Miller 1987; Miller 1989; Santos and Castro 2003; Mehta *et al* 2010; Barley *et al* 2015; Malcolm 2016). While not every knotting moray species has been extensively studied, research indicates that different species use different kinds, numbers, and complexities of knots (Miller 1989; Malcolm 2016). The material properties of hagfish skins have been recently studied (Clark *et al* 2016; Patel *et al* 2018), but very little research has been done on what allows moray eels to tie knots.

Rotational feeding has been observed in various elongated fishes, including Anguillids and Synbranchids, and functions similarly to knotting in that it helps predators consume prey too large to be swallowed whole by tearing off bites (Helfman and Clark 1986; Miller 1987). Rotational feeding places less tension on the skin than knotting does, because the animal rotates along its longitudinal axis rather than creating extreme curvatures (Helfman and Clark 1986).

Since no major body curvatures are created, rotational feeding is more common than knotting (Helfman and Clark 1986; Miller 1987).

In this study, we examined the skin of purplemouth moray eels, American eels, and Asian swamp eels. Purplemouth morays have been observed tying knots, while both the other eels have been observed utilizing rotational feeding (Helfman and Clark 1986; Miller 1987). The Asian swamp eel is a phylogenetic outgroup, as it is a member of Synbranchiformes rather than Anguilliformes as the other two eels are. The primary goal of the present study is to determine if moray eels have anisotropic skin that is similar to that of hagfish, and thus might be conducive to knot tying. We expect that the moray will have skin similarly anisotropic to the Pacific hagfish, while the American eel and the Asian swamp eel will have skin that is typically anisotropic.

Materials and Methods:

Species

Data sets were gathered from skins dissected from specimens of Purplemouth moray eels *Gymnothorax vicinus* (Castelnau, 1855), Asian swamp eels *Monopterus albus* (Zuiew, 1793), and American eels *Anguilla rostrata* (Lesueur, 1821). All specimens were stored in a -30 ºC freezer at the College of Charleston (Charleston, SC) and thawed once prior to dissection, tissue preparation, and testing. Specimens of *G. vicinus* (n = 3, *TL* = 73.0-95.8 cm) and *A. rostrata* (n = 3, *TL* = 39.4-57.8 cm) were provided by the South Carolina Department of Natural Resources. We obtained specimens of *M. albus* (n = 3, *TL* = 55.9-61.0 cm) from *H & L* Asian Market (Charleston, SC).

Tissue Preparation

Large rectangular portions of skin were removed from each specimen of *A. rostrata* and *M. albus* at about 50% *TL*, from which eight samples were fabricated for tensile testing (Fig 1A-C). Four of these eight samples were pulled along the circumferential (or hoop) axis of the body, while the remaining four were strained along the longitudinal axis. For each direction, we created two rectangular samples (25.0 mm long X 4.0 mm wide) and two dumbbell-shaped samples (25.0 mm long X 2.0 mm wide in the narrow region). The dumbbell-shaped samples were prepared by cutting triangular notches on each side of the sample at half length. Following the preparation of the samples, they were kept hydrated with KimWipes dipped in 1:3 artificial seawater. The same preparation procedures were followed for skin samples from *Gymnothorax vicinus*, however, we tested skins dissected from the parabranchial (<10% *TL*), pre-anal (50% *TL*), and post-anal regions (75% *TL*). Samples from the parabranchial region were tested to compare to the rest of the body, as this region must be able to expand to accommodate both eating and respiration.

Material Testing

Eel skin samples were subjected to quasistatic uniaxial tensile tests to failure, in which a rectangular or dumbbell-shaped sample, clamped between a stationary and actuating clamp, was stretched until it broke (Fig. 1D). All tensile tests were conducted with an Imada EMX-275 motorized vertical test stand equipped with an Imada ZP-110 Force Gauge and a Mitutoyo Digimatic height gauge (Imada, Inc., Northbrook, IL). To prevent the samples from slipping out of the clamps, we placed small rectangles of 80-grit sandpaper between the clamp and skin sample. After securing each sample, the sample's initial length (L_0 or grip separation), width, and thickness were measured using digital calipers (± 0.01 mm) (Fig. 1C). Once clamped and

dimensions measured, each sample was stretched at 25.0 mm min^{-1} and force-length data were recorded on Imada SW2X software. Using the sample's dimensions, these force-length data were converted to the stress-strain data, from which material properties were measured (Fig. 1D, E).

Stress (σ) and strain (ε) values were calculated from force (F) and distance (L) measurements. Stress was calculated as:

$$\sigma = \frac{F}{CSA}$$

in which F equaled the applied tensile force and CSA equaled the cross-sectional area perpendicular to the applied force. Strain was calculated as:

$$\varepsilon = \frac{\Delta L}{L_0}$$

where ΔL equaled the change in sample length during the testing period. J-shaped curves were typical among most tests on the eel skins. The sample's stiffness (E) equaled the slope, or the ratio of change in stress to change in strain, of the steeper linear portion of the stress-strain curve. Stiffness data were gathered from samples shaped as rectangles. From the tests on dumbbell-shaped samples, strength (= peak stress), extensibility (= peak strain), and the toughness or strain energy storage per unit volume (= area under the stress-strain curve) were measured (Fig. 1E).

Data Analysis

From each animal of a given species, we calculated the mean of each direction-specific material property (e.g. stiffness in the hoop axis). A grand mean was subsequently calculated from these individual mean data. We first used a 3X2 factorial ANOVA to assess the effects of species (all three eel species) and the direction of applied tensile loads (longitudinal and hoop) on the material properties of the skins from all eel species. A second factorial ANOVA was used for

comparing data between both species of non-muraenid eels: *A. rostrata* and *M. albus*. A third factorial ANOVA was used to assess the effects of the direction of tension and the location of on the body (parabranchial, pre-anal, and post-anal regions) of the moray eel *G. vicinus*. In all three ANOVA performed, the species, direction, and location on the body were treated as factors, and, the material properties (stiffness, strength, extensibility, and toughness) were treated as the dependent variables. Post hoc paired *t*-tests were used for comparing the material properties between longitudinal and hoop-wise directions within each species. We used $P<0.05$ as the criterion for significant differences between means in all statistical analyses.

Results:

The results from the first factorial ANOVA for comparing material properties across the skin of all species revealed a significant difference in stiffness between species ($F_{2, 17} = 32.02$; $P < 0.0001$), with *M. albus* skins being significantly stiffer than *A. rostrata*, and *G. vicinus* having the least stiff skins (Fig. 2A; Table 1). This was also confirmed by the results from the second factorial ANOVA employed for comparing stiffness between *A. rostrata* and *M. albus* ($F_{1, 11} = 17.43$; $P = 0.003$) (Fig 2A; Table 2). Results from our third factorial ANOVA showed that *G. vicinus* skins from the postanal region were significantly stiffer than the parabranchial or preanal regions ($F_{2, 17} = 12.19$; $P = 0.001$) (Fig. 3A; Table 3). Furthermore, the stiffness of the skin in *G. vicinus* increases from the parabranchial region to the postanal region (Fig. 3A). Similarly, strength of the skin differed significantly between locations ($F_{2, 17} = 4.92$; $P = 0.027$), with strongest skins from the postanal region and the weakest in the parabranchial region (Fig. 3B; Table 3).

Both *M. albus* and *A. rostrata* had anisotropic skins, by which the stiffness was significantly higher in the circumferential direction than the longitudinal direction (Fig. 2A; Table 4). Skins of *A. rostrata* were significantly stiffer ($P = 0.020$) in the circumferential direction ($E_{circ} = 273.04 \pm 33.84$ MPa) than in the longitudinal direction ($E_{long} = 96.25 \pm 12.37$ MPa), specifically nearly three times as stiff. *M. albus* skins were also significantly stiffer ($P = 0.048$) in the circumferential axis than in the longitudinal axis, with the circumferential axis being over twice as stiff as the longitudinal axis (Table 4). On the other hand, the skins of *G. vicinus* were isotropic, being equally as stiff in both the circumferential and longitudinal directions. Skins of *G. vicinus* were isotropic in the parabranchial, preanal, and postanal regions of the body (Table 3). However, across all eel species, there were no significant differences in strength, nor were there significant interactions between direction and species (Table 1). The paired *t*-tests revealed that there was no significant difference in strength between directions for any of the species (Fig. 2B; Table 4). Toughness was not significantly different between species ($F_{2, 17} = 1.32$; $P = 0.304$) (Table 1), nor were there significant differences between directions in any species (Fig. 2D; Table 4). We also found no differences in toughness in *G. vicinus* skins between the locations or direction of applied tension (Tables 3 and 4).

Extensibility significantly differed between all eel species ($F_{2, 17} = 16.58$; $P = 0.0003$), with *A. rostrata* having the most extensible skin in the longitudinal direction and *G. vicinus* having the most extensible skin in the circumferential direction (Fig. 2C; Table 1). The factorial ANOVA used for comparing the non-muraenids also showed that *A. rostrata* skin was significantly more extensible than *M. albus* ($F_{1, 11} = 34.44$; $P = 0.0003$) (Table 2). Results from post hoc paired *t*-tests showed that the longitudinal direction was significantly more extensible than the circumferential direction only for *A. rostrata* ($P = 0.0186$) and *M. albus* ($P = 0.0084$),

while there was no significant difference for *G. vicinus* (Fig. 2C; Table 4). The ANOVA showed no significant difference in the extensibilities of *G. vicinus* skins across locations on the body (Table 3), however paired *t*-tests showed that postanal skin was significantly more extensible in the circumferential direction than the longitudinal direction ($P = 0.044$) (Fig. 3C; Table 4). Nonetheless, the skins of G. *vicinus* demonstrate an increase in longitudinally-directed extensibility but retained similar peak strains in the circumferential direction across locations (Fig. 3C).

Skin thickness between the three species was significantly different. Both *A. rostrata* and *G. vicinus* had significantly thicker skin than *M. albus*, but that *A. rostrata* skin was not significantly different than *G. vicinus* skin ($F_{2, 95} = 56.46$; $P = <0.001$) (Fig. 4A, Table 4). Results also showed that the parabranchial region of *G. vicinus* had significantly thicker skin than the rest of the body ($F_{2, 71} = 34.43$; $P = <0.001$) (Fig. 4B, Table 4).

Discussion:

Material Properties of Eel Skins:

G. vicinus skins were isotropic like the skins from the hagfish genus *Myxine* (Patel *et al* 2018), and remain isotropic throughout the body (Figure 3A). *Eptatretus* is a genus of hagfish that employs a greater diversity of knots than *Myxine* (Haney, 2017), however, the skins of *Eptatretus* are twice as stiff in the longitudinal direction and twice as extensible in the circumferential direction (Clark *et al* 2016). *G. vicinus* and *Myxine* have isotropy indices of 1.1 and approximately 1.0, respectively, while *Eptatretus stoutii* has an isotropy index of approximately 0.5. These data indicate that having a lower isotropy index is preferable for knot-tying. While *G. vicinus* skins are significantly less stiff than those of *A. rostrata* and *M. albus*

(Fig 2A; Table 1), they are much stiffer and stronger than the skins of *E. stoutii* (Clark *et al* 2016). *G. vicinus* stiffnesses fall within the range of shark skin stiffnesses reported in Creager and Porter (2018). However, *G. vicinus* skins are stiffer and stronger than the tight-fitting skins from the penpoint gunnel *Apodichthys flavidus* and the sea lamprey *Petromyzon marinus* (Clark *et al* 2016).

The anisotropy observed in *A. rostrata* and *M. albus* is typical of other teleosts and sharks (Hebrank and Hebrank 1986; Naresh *et al* 1997; Clark *et al* 2016). It was hypothesized that many of these anisotropic skins functioned as external tendons, and those skins had anisotropy characterized as the circumferential direction of skin was twice as stiff as the longitudinal direction (Wainwright *et al* 1978; Hebrank 1980). This pattern of anisotropy is the same pattern observable in the walls of pressurized cylinders as it allows for expansion and contraction without kinking or breaking. Some fish skins have not been hypothesized to act as an external tendon, but these skins still display anisotropy with the circumferential direction being significantly stiffer, but less than twice as stiff, as the longitudinal direction (Wainwright *et al* 1978; Hebrank and Hebrank 1986). *A. rostrata* and *M. albus* both exhibit isotropy indices (ratio of circumferential stress to longitudinal stress) of >2.0, meaning that these skins were more than twice as stiff in the circumferential axis. Specifically, *A. rostrata* had an index of 2.8 and *M. albus* had one of 2.3. It is interesting to note how much stiffer *A. rostrata* skins were in the circumferential direction than the longitudinal direction.

The parabranchial region of *G. vicinus* has an extensibility of over 40% its original length in the longitudinal direction, a number that is very similar to the extensibility of *Eptatretus stoutii* (Clark *et al* 2016), and is also comparable to the extensibility of *Diodon holocanthus*, a balloon fish (Brainerd 1994). *D. holocanthus* skins become taut at 40% original length, while the

total extensibility is closer to 50% (Brainerd 1994). Highly extensible skin in the parabranchial region would be beneficial for moray eels as the throat must accommodate large prey items as well as the activity of pharyngeal jaws (Helfman and Clark 1986; Mehta and Wainwright 2007). Extensible skin around the throat would be beneficial to other eels as well, such as *A. rostrata*, which has been known to consume large pieces of food (Helfman and Clark 1986). The other regions of *G. vicinus* are more extensible than the skins of *Lepomis gibbosus* and *Monacanthus ciliatus*, which have extensibilities of around 10% and 15%, respectively (Brainerd 1994). The other eel species tested in this study also generally had more extensible skin than either *L. gibbosus* or *M. ciliatus*, suggesting the possibility that elongated fish have more extensible skin. More extensible skin could be beneficial to elongated fish because anguilliform swimming involves more skin bending than carangiform or subcarangiform swimming (Hebrank 1980). Highly extensible skin is also beneficial to moray eels because it allows more deformation; i.e. it is more amenable to the curvatures required for knotting.

The skins of *G. vicinus* demonstrate a rostrocaudal gradient in every material property tested. Stiffness values increase from head to tail, as does strength and toughness. Conversely, extensibility values decrease from head to tail in the longitudinal direction but stay relatively constant in the circumferential direction. Similar rostrocaudal gradients are seen in other ray-finned fishes, where stiffness increases down the body (Kenaley *et al* 2018). This stiffness gradient has been hypothesized to function as a force transmitter during swimming (Kenaley *et al* 2018). Rostrocaudal gradients have also been noted in shark skins: of the body, the postanal region consistently had the stiffest skin (Creager and Porter 2018). However, shark skin from the head was found to be the stiffest, differing from the parabranchial region of moray eels (Creager

and Porter 2018). Having more compliant skin anterior to the anus is beneficial as it allows the gut to expand with food.

Morphology of* G. vicinus *skin:

G. vicinus skins were found to be significantly thicker than those of *M. albus*, but they had no significant difference with *A. rostrata*. One explanation for this is that *M. albus* is the phylogenetic outgroup in this study; it is possible that Anguilliformes as an order has thicker skin than the Synbranchiformes. Since *G. vicinus* skins were not significantly thicker than those of *A. rostrata*, it cannot be concluded that thick skins are an adaptation for knot-tying. Fishelson (1996) suggested that thicker skins are an adaptation for eels that live in and frequently come in contact with abrasive environments. In our study, we discovered that the parabranchial skin of *G. vicinus* is significantly thicker than the skin covering the rest of the body. This particular finding contrasts with another study that found very little variation in the thickness of the skins from different regions of the body (Fishelson 1996).

The thicknesses measured in this study fall within the range of thicknesses expected from Fishelson (1996), as do the total lengths of the animals they were collected from. Larger moray individuals have thicker skin, which could be an adaptation against predation as well as to protect from the abrasive environments occupied by moray eels (Fishelson 1996). Similarly, *E. stoutii* was found to have little variation in skin thickness when the head and tail regions were examined (Clark *et al* 2016). In the case of *G. vicinus*, having thicker skin around the face and throat could be to protect sensitive organs, such as the gills, from any defenses employed by the prey items, as well as from the abrasive materials making up hiding places of prey (Fishelson 1996; Mehta 2008).

While *G. vicinus* has isotropic skin like *Myxine* does, *G. vicinus* has actually been observed tying more kinds of knots than *Myxine* has (Malcolm 2016; Haney 2017). This indicates isotropic skins are not limiting factors in the diversity of knotting movements in morays. During gross dissections, we observed that *G. vicinus* skin was looser than the skins of both *A. rostrata* and *M. albus*. We define looseness as the presence of a space between the inner surface of the skin and the outer surface of the body core. Hagfishes possess a prominent subcutaneous sinus positioned between the skin and core that could contain up to 30% of the venous blood volume (Chapman 1963; Forster 1997; Clark *et al* 2016). *G. vicinus* has a loose-fitting skin in contrast to the tight-fitting skins of other fishes, including *A. rostrata* and *M. albus*, however the volume of the space between the core and skin is smaller than the subcutaneous sinuses in *Eptatretus* and *Myxine*. The very loose skin is likely an important air to their knot-tying abilitites, as it reduces the tension and shear placed on the skin while the body is undergoing the extreme curvatures required for knotting (Clark *et al* 2016).

Much of the characteristics of *G. vicinus* skin that have been examined thus far bear more similarities to the properties previously examined in hagfish skins (Clark *et al* 2016; Patel *et al* 2018) meaning that they are similar to the properties of hagfish that are hypothesized to allow knotting (Clark *et al* 2016). Loose skins are hypothesized to be particularly beneficial for knot-tying as they experience less strain than taut skins do (Clark *et al* 2016). Loose skins are also more compliant in the circumferential direction, which also aids the knotting process. Research done on the loose skins of hagfish has also indicated that it is more difficult to puncture, despite the lack of scales (Boggett *et al* 2017). Puncture resistance could also be very beneficial for moray eels in part because of the abrasive environments they live in, but also as protection against prey defenses. Some morays have been observed predating on other, smaller species of

morays, which can twist around to bite the attacker. More puncture resistant skin would protect the predator, as well as the prey species.

Future Research into Moray Eels:

Research done on moray eels has indicated that durophagous feeding habits have evolved independently in multiple genera, as have the specific head and jaw structures required for durophagy (Reece *et al* 2010). It would be interesting to determine if morays have differentiated cranial skin based on what kinds of prey individual species are adapted to consume. For example, durophagous species might have thicker skin that is more difficult to puncture as protection against crustaceans. Morays have also been found to have differentiated olfactory rosettes depending on whether that species hunts via sight or smell (Fishelson 1995). A follow up study could then examine if there were any differences in the visual systems between morays that hunt via sight or smell. Studies have succeeded in isolating ciguatoxins from moray eel skins in areas with endemic ciguatera (Lewis *et al* 1991). This is particularly interesting as it means that moray eels have toxic skin in certain areas of the world. Moray eels in the Red Sea have been observed hunting cooperatively with groupers, a behavior that indicates significant communication between the two reef predators (Bshary *et al* 2006).

Future research pertaining to knotting in moray eels should examine the differences between observed knotting techniques and material properties of the skins. Malcolm (2016) found that within the genus *Gymnothorax*, different species used different numbers of knots. Similarly, *Myxine* hagfishes use fewer knots than *Epatretus* hagfishes, which is reflected in the properties of their skins (Patel *et al* 2018). Studying the skins of *G. prasinus* and *G. prionodon* (Malcolm 2016) would determine if knotting ability within a single genus was at all correlated

with material properties. Additionally, the knotting habits of *G. vicinus* should be studied to provide a third species for the previous study.

Literature Cited:

Barley SC, RS Mehta, JJ Meeuwig, and MG Meekan (2015) To knot or not? Novel feeding behaviours in moray eels. Mar. Biodiv. DOI: 10.1007/s12526-015-0404-y.

Boggett, S, JL Stiles, AP Summers, and DS Fudge (2017) Flaccid skin protects hagfishes from shark bites. Jour. Roy. Soc. Inter. 14: 20170765.

Böhlke EB, JE McCosker, and JE Böhlke (1989) Family Muraenidae. Pp 104-206 in E. Böhlke, ed. Fishes of the western north Atlantic: orders Anguilliformes and Saccopharyngiformes. Vol. 1. Sears Foundation for Marine Research, New Haven, CT.

Brainerd EL (1994) Pufferfish inflation: functional morphology of postcranial structures in *Diodon holocanthus* (Tetraodontiformes). Jour. Morph. 220: 243-261.

Bshary R, A Hohner, K Ait-el-Djoudi, and H Fricke (2006) Interspecific communicative and coordinated hunting between groupers and giant moray eels in the Red Sea. PLoS Biol. DOI: 10.1371/journal.pbio.0040431.

Clark AJ and AP Summers (2012) Ontogenetic scaling of the morphology and biomechanics of the feeding apparatus in the Pacific hagfish *Epatretus stoutii*. J. Fish Biol. 80:86-99

Clark AJ, CH Crawford, BD King, AM Demas, and TA Uyeno (2016) Material properties of hagfish skin, with insights into knotting behaviors. Biol. Bull. 230:243-256

Creager SB and ME Porter (2018) Stiff and tough: a comparative study on the tensile properties of shark skin. Jour. Zool. 126: 154-163.

De Sylva, PD (1986) The gulper eel and its knotty problem. Sea Frontiers 32: 104-108.

Fishelson, L (1995) Comparative morphology and cytology of the olfactory organs in moray eels with remarks on their foraging behavior. Anat. Rec. 243: 403-412.

Fishelson, L (1996) Skin morphology and cytology in marine eels adapted to different lifestyles. Anatom. Rec. 246: 15-29.

Fujii, R (1968) Fine structure of the collagenous lamella underlying the epidermis of the goby, *Chasmichthys gulosus*. Annot. Zool. Jpn. 41: 95-106.

Haney, WA (2017) Characterization of body knotting behavior in hagfish. Master's Thesis, Dept. of Biol. of Coll. of Arts and Sci., Valdosta States University.

Hebrank, MR (1980) Mechanical properties and locomotor functions of eel skin. Biol. Bull. 158: 58-68.

Hebrank, MR and JH Hebrank (1986) The mechanics of fish skin: Lack of an "external tendon" role in two teleosts. Biol. Bull. 171: 236-247.

Helman GS and JB Clark (1986) Rotational feeding: overcoming gape-limited foraging in Anguillid eels. Copeia 1986: 679-685.

Kenaley, CP, A Sanin, J Ackerman, J Yoo, and A Alberts (2018) Skin stiffness in ray-finned fishes: Contrasting material properties between species and body regions. Jour. Morph. DOI: 10.1002/jmor.20877.

Lewis, RJ, M Sellin, MA Poli, RS Norton, JK MacLeod, and MM Sheil (1991) Purification and characterization of ciguatoxins from moray eel (*Lycodontis javanicus*, Muraenidae). Toxicon. 29: 1115-1127.

Malcolm HA (2016) A moray's many knots: knot tying behaviour around bait in two species of *Gymnothorax* moray eel. Environ. Biol. Fish. DOI: 10.1007s10641-016-0535-4.

Mehta RS and PC Wainwright (2007) Raptorial jaws in the throat help moray eels swallow large prey. Nature 449: 79-82.

Mehta RS (2008) Ecomorphology of the moray bite: relationship between dietary extremes and morphological diversity. Phys. Bioch. Zool. 82: 90-103.

Mehta RS, AB Ward, ME Alfaro, and PC Wainwright (2010) Elongation of the body in eels. Int. Comp. Biol. 50: 1091-1105.

Miller, TJ (1987) Knotting: a previously undescribed feeding behaviour in Muraenid eels. Copeia 1987: 1055-1057.

Miller, TJ (1989) Feeding behavior of *Echidna nebulosi*, *Enchelycore pardalis*, and *Gymnomuraena zebra* (Teleostei: Muraenidae). Copeia 1989: 662-672.

Naresh MD, V Arumugam, and R Sanjeevi (1997) Mechanical behaviour of shark skin. Jour. Biosci. 22: 431-437.

Pabst, DA (1996) Springs in swimming animals. Amer. Zool. 36: 723-735.

Patel, RG, EBL Kennedy, BL Clubb, TA Uyeno, and AJ Clark (2018) Comparative biomechanics of diverse hagfish skins. Int. Comp. Biol. 58: E174-E174.

Reece, JS, BW Bowen, DG Smith, and A Larson (2010) Molecular phylogenetics of moray eels (Muraenidae) demonstrates multiple origins of a shell-crushing jaw (*Gymnomuraena, Echidna*) and multiple colonizations of the Atlantic Ocean. Molec. Phylo. Evol. 57: 829-835.

Santos FB and RMC Castro (2003) Activity, habitat utilization, feeding behaviour, and diet of the sand moray *Gymnothorax occelatus* (Anguilliformes, Muraenidae) in the south western Atlantic. Biota Neotropica 3: 1-7.

Uyeno TA, and AJ Clark (2015) Muscle articulations: flexible jaw joints made of soft tissues. Int. and Comp. Biol. 55:193-204

Vogel, S (2013) Comparative biomechanics: life's physical world. Princeton University Press, Princeton, New Jersey.

Wainwright, SA, F Vosburgh, and JH Hebrank (1978) Shark skin: Function in locomotion. Science 202: 747-749.

Zintzen V, CD Roberts, MJ Anderson, AL Stewart, CD Struthers, and ES Harvey (2011) Hagfish predatory behaviour and slime defence mechanism. Sci. Rep. 1: 1-6.

Table 1: Summary of factorial ANOVA results comparing material properties from the skins of all species examined in this study: *A. rostrata*, *M. albus*, and *G. vicinus* (preanal region only).

	Stiffness (MPa)		**Strength (MPa)**		**Extensibility**		**Toughness* (MJm^{-3})**	
	$F_{2, 17}$	***P***	$F_{2, 17}$	***P***	$F_{2, 17}$	***P***	$F_{2, 17}$	***P***
Species	32.018	**<0.001**	1.838	0.201	16.576	**<0.001**	1.317	0.304
Direction	48.105	**<0.001**	14.409	**0.003**	15.231	**0.002**	2.814	0.119
Sp. Vs Dir.	10.567	**0.002**	1.691	0.225	2.115	0.163	0.256	0.778

Significant *P*-values ($P<0.05$) are bolded. Data sets were gathered from three animals per species. Dir., direction. Sp., species.

Table 2: Factorial ANOVA results on the material properties of the non-muraenid eels, *A. rostrata* and *M. albus*.

	Stiffness (MPa)		**Strength (MPa)**		**Extensibility**		**Toughness* (MJm^{-3})**	
	$F_{1, 11}$	***P***	$F_{1, 11}$	***P***	$F_{1, 11}$	***P***	$F_{1, 11}$	***P***
Species	17.425	**0.003**	0.919	0.364	34.438	**<0.001**	0.176	0.686
Direction	59.697	**<0.001**	29.261	**<0.001**	34.908	**<0.001**	4.771	0.604
Sp. Vs Dir.	0.671	0.436	0.070	0.798	2.650	0.142	0.108	0.751

Significant *P*-values ($P<0.05$) are bolded. Dir., direction. Sp., species.

Table 3: Summary of ANOVA results comparing material properties from the parabranchial, preanal, and postanal regions of *G. vicinus*.

	Stiffness (MPa)		**Strength (MPa)**		**Extensibility**		**Toughness* (MJm^{-3})**	
	$F_{2, 17}$	***P***	$F_{2, 17}$	***P***	$F_{2, 17}$	***P***	$F_{2, 17}$	***P***
Location	12.194	**0.001**	4.916	**0.028**	1.585	0.245	2.163	0.158
Direction	0.004	0.954	0.387	0.545	2.325	0.153	0.303	0.592
Loc. Vs Dir.	0.646	0.541	0.021	0.979	10.747	**0.002**	0.545	0.593

Significant *P*-values ($P<0.05$) are bolded. Dir., direction. Sp., species.

Table 4: Single factor ANOVA results for skin thickness.

All Species		***G. vicinus* Locations**	
$F_{2, 95}$	***P***	$F_{2, 71}$	***P***
56.461	**<0.001**	34.432	**<0.001**

Significant *P*-values ($P<0.05$) are bolded.

Table 5: Material properties of skin from *A. rostrata*, *M. albus*, and the three locations on *G. vicinus*, and results from paired *t*-tests comparing mean data between body axes (direction) within each species.

	Stiffness (MPa)			**Strength (MPa)**			**Extensibility**			**Toughness* (MJm^{-3})**		
	Circ.	**Long.**	**P-value**	**Circ.**	**Long.**	**P-value**	**Circ.**	**Long.**	**P-value**	**Circ.**	**Long.**	**P-value**
A. rostrata	273.04 (± 33.84)	96.2568 (± 12.37)	**0.020**	76.66 (± 9.80)	42.37 (± 4.16)	0.130	0.209 (± 0.015)	0.365 (± 0.037)	**0.018**	5.36 (± 1.19)	3.57 (± 0.41)	0.316
M. albus	400.85 (± 29.07)	182.13 (± 21.85	**0.048**	84.82 (± 7.42)	46.99 (± 3.07)	0.069	0.121 (± 0.004)	0.210 (± 0.011)	**0.008**	4.83 (± 0.66)	3.50 (± 0.09)	0.176
***G. vicinus* (para)**	69.92 (± 16.71)	58.34 (± 11.23)	0.428	37.24 (± 12.61)	33.63 (± 6.12)	0.721	0.271 (± 0.021)	0.447 (± 0.021)	0.055	4.51 (± 1.52)	5.24 (± 0.83)	0.491
***G. vicinus* (pre)**	105.96 (± 26.93)	95.15 (± 9.29)	0.616	53.69 (± 11.62)	45.11 (± 10.22)	0.748	0.310 (± 0.041)	0.346 (± 0.036)	0.709	5.81 (± 0.89)	5.27 (± 1.20)	0.833
***G. vicinus* (post)**	132.50 (± 2.99)	152.57 (± 13.60)	0.274	76.67 (± 17.64)	70.19 (± 8.96)	0.468	0.357 (± 0.017)	0.256 (± 0.020)	**0.044**	8.37 (± 1.59)	6.46 (± 0.95)	0.108

Values for the material properties along circumferential (circ.) and longitudinal (long.) axes are shown as the mean ± SEM. Significant *P*-values ($P<0.05$) are bolded.

Figure Legends:

Figure 1: Procedures for collecting, fabricating, measuring, and testing skin samples. (A) Diagrams of Muraenid eel (top) and non-Muraenid eel (bottom). The gray rectangles represent locations of skin samples collected for testing. (B) Diagrams of the fabrication of skin samples. Each location yielded four circumferential samples and four longitudinal samples. Half of the samples for each direction were dumbbell shaped, while the other half were rectangular. Triangular notches were cut out from rectangular samples at half-length to form dumbbell samples. (C) This image shows how the samples were measured to calculate the cross-sectional area (CSA). (D) Schematic diagram of a uniaxial tensile test, using a dumbbell sample. This image also shows how we obtained the L_0 measurement. (E) Sample stress-strain curve obtained from data collected during testing. Stiffness was calculated as the slope of the linear portion of the curve. Strain energy storage per unit volume (toughness) was the area underneath the curve from dumbbell samples, while the maximum stress and strain values of dumbbell samples gave strength and extensibility, respectively.

Figure 2: Material properties of the skins of three species of eels: *A. rostrata*, *M. albus*, and *G. vicinus*. The properties measured include (A) Stiffness, (B) Strength, (C) Extensibility, and (D) Toughness. All graphs show mean values and the error bars represent the SEM. Asterisks indicate significant differences between directions.

Figure 3: Material properties of skins collected for the parabranchial, preanal, and postanal regions of *G. vicinus*. The material properties measured include (A) stiffness, (B) strength, (C) extensibility, and (D) Toughness. Note the rostrocaudal gradient in the stiffness, strength, and

toughness of the skins. Also worth noting is the high extensibility in longitudinally strained skin samples in the parabranchial regions of the body. All data are means and error bar values are SEM. Asterisks indicate a significant difference.

Figure 4: Thickness of eel skins. (A) Thickness of the skins of *A. rostrata*, *M. albus*, and *G. vicinus* (preanal region only). *A. rostrata* skins are significantly thicker than *M. albus*, as indicated by the letter "a." *M. albus* skins are also significantly thinner than those of *G. vicinus*, shown by the letter "b." (B) Thickness measurements for *G. vicinus* regions. Error bars are SEM, and letters indicate a significant difference between species or regions. Parabranchial skin was significantly thicker than both preanal and postanal skins, as marked by the letters "c" and "d," respectively.