

Characterization of body knotting behavior used for escape in a diversity of hagfishes

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Abstract

Hagfishes possess flexible bodies that allow them to tie and manipulate body knots. These knots are used to remove mucous from the body, escape tight spaces, pull prey from burrows and, since they lack opposable jaws, to create leverage during feeding events. Despite its importance, knotting is poorly understood because this fast and three-dimensionally complex behavior is difficult to study both in the laboratory and in their natural benthic habitats. To consistently stimulate analyzable knotting behaviors, we developed a novel restraint device to record high-speed, biplanar video in three species of hagfish from two major taxa (Eptatretinae and Myxiniinae): *Eptatretus stoutii*, *Eptatretus springeri* and *Myxine glutinosa*. These data were analyzed to characterize knot type and kinematics of knot formation. We found that despite reduced radii of curvature and strain associated with more complex knots, all species of hagfish preferred the simplest overhand knots. While all species were physically capable of tying more complex knots, *E. stoutii* exhibited a lower 'behavioral' stiffness by tying complex knots more frequently and coiling when at rest. The hyper-flexible, hyper-redundant hagfish body may require a high level of neural input for control. However, analyses of knotting motions indicate that hagfish seem to employ only four movements (body bends, twists, surface contacts and tail insertions into body loops) but in different combinations depending on the knot being tied. Furthermore, these movements and combinations are conserved across all species tested. We hypothesize that control of knotting in hagfish may employ motor primitives to reduce neural input.

Introduction

Hagfishes are perhaps the most flexible-bodied of vertebrate taxa (Miyashita *et al.*, 2019). This is best demonstrated by their ability to tie their eel-like bodies into knots and effectively manipulate these knots for a variety of purposes. Adams (1960) noted that Atlantic hagfish (*Myxine glutinosa* Linnaeus, 1758) used the knots for removing slime from their bodies. Since then, knotting has been reported facilitating escape behavior (Strahan, 1963; Martini, 1998), to extricate prey from burrows during active predation (Zintzen *et al.*, 2011), and as an approach for handling oversized or tough food items (Clark & Summers, 2012).

Knotting facilitates macrophagy by enhancing the ability of the jawless hagfish feeding apparatus to remove portions from oversized food (Clark & Summers, 2012; Clark & Uyeno, 2019). After the tooth plates are embedded into the food item, a knot is formed at the tail and progresses toward the head. As the knot slides over and past the head, a body loop is pressed against the surface of the item and creates a stable platform against which the retractile force of the tooth plates

can be most effectively applied (Clark *et al.*, 2010; Uyeno & Clark, 2015; Clubb *et al.*, 2019). Here, the body loop and the tooth plates function like a pair of counterbalancing jaw elements that rival those in gnathostomes. Knotting enables hagfishes to exploit a greater diversity of food that might otherwise be unavailable if they were solely dependent on the rasping forces of the tooth plates (Clark & Summers, 2012).

Hagfishes are highly adapted for knotting. Their bodies are flexible and elongate, they lack vertebrae, and they acquire most of their flexural stiffness from their notochords (Long *et al.*, 2002). The axial muscles are comprised of three muscle groups (Vogel & Gemballa, 2000) complexly arranged to permit multiaxial bending and twisting (Clark *et al.*, 2016). Hagfish skins are unusually loose and permit whole-body deformations that would normally be restricted by taut skins (Clark *et al.*, 2016). The skin is baggy because it encapsulates a large subcutaneous sinus that contains 30% of the venous blood volume (Forster, 1997) and is therefore so loose that it hinders steady swimming by damping body movements (Long *et al.*, 2002). The surface characteristics of hagfish skins may also decrease resistance to knot movements. Hagfish skins lack

the scale-bearing *stratum corneum* present on the epidermal layers of most fish skins (Andrew & Hickman, 1974). Instead, the superficial layer of the hagfish epidermis is equivalent to the *stratum germinativum* and forms an ideal surface to reduce friction and binding of the knot. Finally, the lack of paired fins allows any knot formed along the body to move freely along its entire length. Oddly, while hagfish can secrete slime, we do not see them use it as a lubricant during knotting.

Physical adaptations for extreme flexibility are only useful if paired with appropriate neural control mechanisms (Nishikawa *et al.*, 2007). The continuously deformable hagfish body can be modeled as an infinite number of short links, each connected in series by joints that possess a wide range of motion and multiple degrees of freedom. Such structures are described as 'hyper-redundant' (Trivedi *et al.*, 2008; Hwang *et al.*, 2019). While hyper-redundant bodies may be flexible, their neural control can become enormously complex (Vladu *et al.*, 2012). Animals with flexible body parts may have evolved solutions to reduce this control input. For example, Sumbre *et al.* (2006) noted that, in reaching for a target, an octopus arm will localize movement to the same three relative points. While the arm could theoretically bend at any point, this stereotyped 'joint' at a fixed position represents a simplified neural control 'program' that greatly reduces the amount of control input needed. These simplified motion programs are referred to as 'motor primitives' (Mussa-Ivaldi *et al.*, 1994; Thoroughman & Shadmehr, 2000; Flash & Hochner, 2005). Hart & Giszter (2010) noted that the use of a library of modular primitives can be rearranged to create a vast array of complex behaviors. It may be reasonable to expect that hagfishes also represent a hyper-redundant system in which motor primitives play a large role in the neural control of body knotting.

Hagfish knotting mechanics are described here using terminology defined by both knot-tyers and mathematicians that specialize in the topological field of knot theory. A knot, from a structural perspective, is formed in a flexible body when segments cross other segments to form looped interlacings (Ilyutko & Manturov, 2012). Ashton *et al.* (2011) provide a beautifully functional definition: Knots that are tied in flexible bodies are compliant machines capable of organizing tensions and contact forces to bind tightly and resist unraveling in different ways depending on the structure of the knot. As this structure becomes increasingly complex, categorizing and naming knots becomes more challenging as the possible unique geometric organizations of any given knot increases with additional crossings and loops. Here, we use the standard terms, classifications and illustrations developed by knot topologists as summarized in Adams (2004). Planar knot diagrams are standardized illustrations that depict flattened knots that appear as if they were tied into a strand after which both free ends are fused together. Thus, a strand with no knot in it looks like the letter 'O' and is referred to as an 'unknot' (Ashley, 1944). More complex knots that bear more crossovers may be tied to form one or more unique knot variations. These fundamentally unique knots are known as 'knot primitives' (Adams, 2004) and are described here using the Alexander & Briggs (1927) shorthand notation that identifies its number of crossovers and a variation identifier (in subscript) (Fig. 1). For example, the

two simplest knots are the 3_1 or overhand knot with three crossovers and the 4_1 or figure-eight knot with four crossovers (Alexander & Briggs, 1927; Ashley, 1944).

A knotted hagfish body is exposed to tensile, compressive and shearing forces. Pieranski *et al.* (2001) used computer simulations to describe the geometry of loops within tightened knots. Assuming that tighter curvatures form pinch points that exhibit higher local forces, they were able to predict where areas of stress may occur within a given knot. In comparing the 3_1 and the 4_1 knots (Fig. 2), the more complex 4_1 knot is formed by a longer length of material, the loop radii are greater, and thus the concentration of internal stresses are lessened. We use these ideas to identify the range of knots that hagfish are capable of tying and which they may prefer. Ashton *et al.* (2011) calculated the minimum length needed of a given diameter of strand used to tie, and maximally tighten, a selection of prime knots. These calculations showed the minimum length-to-width ratios needed for the simplest prime knots to be: $3_1 = 16.37$, $4_1 = 21.05$, $5_1 = 23.61$, $5_2 = 24.74$, $6_1 = 28.36$, $6_2 = 28.52$ and $6_3 = 28.93$. While their baggy skins can obfuscate width measurements in hagfishes, a rough but consistent external measurement just posterior to the head showed that all but the most complex of these knots fall within the physiologically relevant knot morphospace for each species: *E. stoutii* had an average length-to-width ratio of 22.41 and *M. glutinosa* was relatively longer and thinner with an average ratio of 28.58. This suggests that *E. stoutii* may only be able to tie 3_1 and 4_1 knots and that *M. glutinosa* may be able to tie knots as complex as the 6_2 knot.

In this study, we investigate several aspects of body knotting behaviors in hagfish. First, we characterize the kinematics of knotting behavior using a novel technique designed to stimulate controlled and repeatable knotting behaviors that can be clearly video recorded for motion analyses. Second, we determine if the kinematics and style of knotting varies between species representing the major subfamilies that account for 90% of the diversity of extant hagfishes: Eptatretinae and Myxiniinae. Third, we analyze body knotting motions to generate hypotheses of how hagfishes control their hyper-redundant bodies.

Materials and methods

Animals

We obtained 27 total individuals across three species of hagfishes; 20 *E. stoutii* (mean total length (*TL*) and mass (*M*) = 493 mm and 162 g), three *Eptatretus springeri* (Bigelow and Schroeder, 1952; mean *TL* and *M* = 536 mm and 253 g) and four *M. glutinosa* (mean *TL* and *M* = 452 mm and 67 g). Live specimens of *E. stoutii* were provided to us by the Washington Department of Fish and Wildlife and from Olympic Coast Seafoods LLC (Port Angeles, WA, USA), *E. springeri* were provided by the Florida State University Coastal and Marine Laboratory, and *M. glutinosa* were provided by the National Marine Fisheries Services and Cape Ann Seafood Exchange (Gloucester, MA, USA). The hagfishes were housed in a flow-through system containing six opaque five-gallon

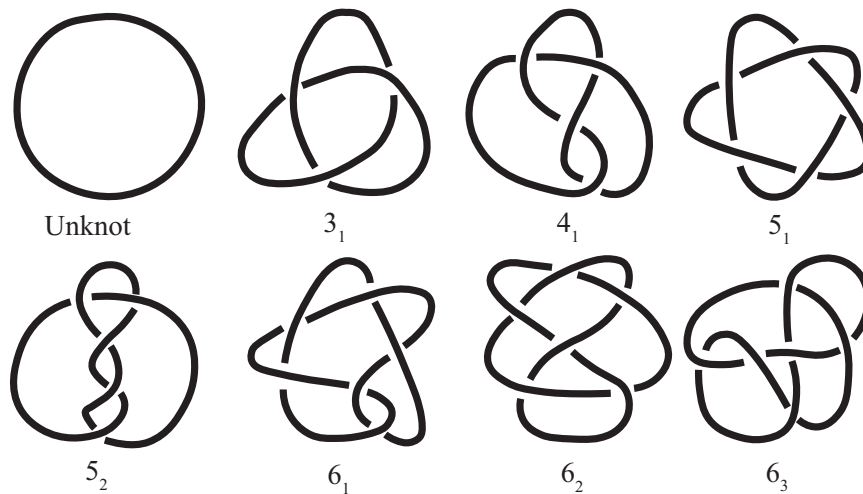


Figure 1 Organization of knots using the Alexander/Briggs shorthand notation (modified from Alexander & Briggs, 1927). Based on the length-to-width ratio of hagfish, the eight simplest knots have potential biological relevance. Knots in this classification scheme are represented as standard topological knotted loops in which the number and organization of crossovers are counted. The common names for these knots are as follows: 0 (a simple loop) = unknot, 3_1 = overhand (trefoil) knot, 4_1 = figure-eight, 5_1 = torus knot, 5_2 = three-twist knot, 6_1 = stevedore knot, 6_2 = Miller Institute knot, 6_3 knot has no commonly used name, however crochet artists may note that this knot represents the slip knot and first chain stitch of any project. Each of these knots are represented in their, more realistic, linear form in Fig. 8.

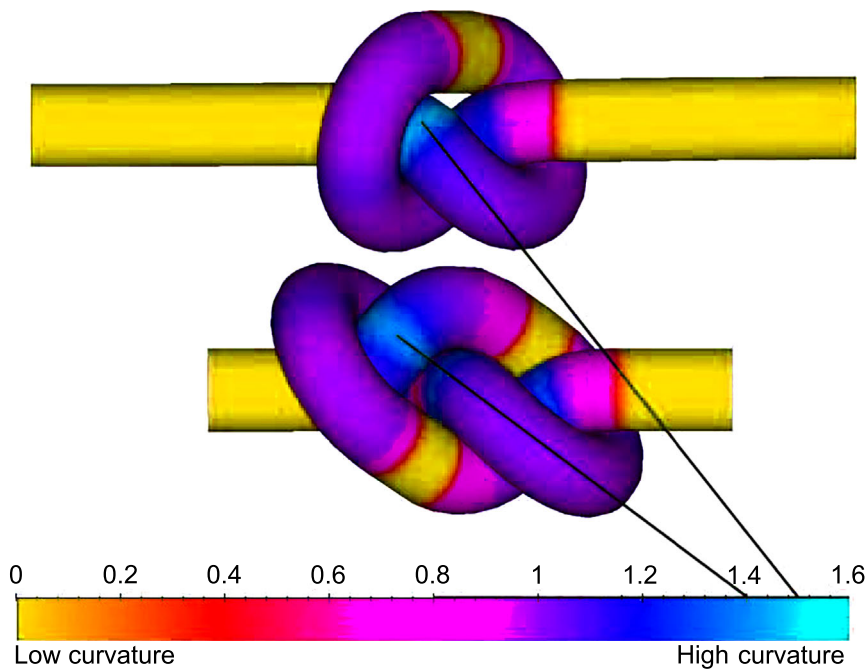


Figure 2 Simulations of tightened 3_1 overhand (top) and 4_1 figure-eight (bottom) knots. The amount of curvature is color coded such that regions of highest curvature appear blue and the lowest curving deformations are in yellow. (Modified [Daltonized for red/green color blindness] with permission from Pieranski et al., 2001). Areas of high curvature may act as force concentrators that result in more stress on the system or hagfish body at these points. Note that the overhand knot results in higher maximal curvatures than does the figure-eight knot. If a knot slides down the body of a hagfish, that relative portion of the knot is where the pressure remains.

buckets, each containing five to seven animals. Salinity was maintained at standard seawater levels (35 ppt), temperature was maintained at 13°C, and animals were kept in the dark

except when feeding or handling during experiments. Each hagfish was marked for identification using an RFID microchip inserted just below the skin in the tail region (Halo Scanners,

St. Saviour, Guernsey). We fed the hagfishes *ad libitum* and found that they fed voraciously for the first three months, consuming chunks (2–3 cm³) of mullet weekly. After this initial period, feeding behavior slowed down and we limited feedings to once per month to prevent rotting food from fouling the tank filters.

Experiments

We first attempted to induce hagfishes to knot by presenting them with firmly tethered food. However, we found that they attacked the food item with seemingly random body orientation, knotting speed and knot types. Because these variables were difficult to control, we opted to elicit an escape, rather than a feeding, stimulus to reliably stimulate and record knotting behaviors. To elicit this escape knotting behavior, in which initial orientation could be controlled, we developed a custom restraint device (Fig. 3). This device consisted of an acrylic plate that fit vertically within a 75.7 L fish tank. The plate bore a central hole into which a 29.5 mm length of PVC pipe (inner diameter = 57 mm, outer diameter = 60 mm) was press-fit in a horizontal orientation. A latex rubber membrane was made by cutting a balloon around its equator; we used the half of the balloon that included the 'neck' (Fig. 3). We used balloons of different sized necks to match the diameter of the hagfish to hold the animal tightly enough such that it could not simply swim out of the membrane and yet loosely enough that disruption in circulation or discomfort due to compression did not occur. The membrane was stretched around one end of the PVC pipe and held in place by a 45 mm nitrile O-ring that fit into a corresponding race that was lathe cut into the outside of the PVC pipe.

Anesthetized specimens of hagfish were safely inserted into, and yet firmly secured within, the neck of the membrane and their efforts to escape were recorded multiple times per individual. Hagfishes were lightly sedated in a solution of 4 mL of 20 mg sodium benzoate dissolved in 400 mL of 100% ethanol mixed with 2 L of saltwater (modified from Christiansen *et al.*, 2013) until the hagfish was unresponsive to tail pinching (approximately 10–15 min). After anaesthetization, the head of the sedated hagfish was gently placed inside the membrane's neck with the use of a goat bander (castrator) that widely stretching the neck in four directions simultaneously. The hagfish was placed such that its head anterior to the gills was held by the neck of the membrane to prevent undue mechanical or respiratory stress on the animal. The dorsal aspect of the head was oriented upwards, and the body curved such that the tail hung downwards. As the hagfish recovered from anaesthetization, its first behavior was to extricate itself. This behavior was recorded using high-speed biplanar videography. This animal use protocol was approved by Valdosta State University's Institutional Animal Care and Use Committee (Protocol AUP-00054-2013).

Videography

We used two synchronized cameras (Phantom Miro 320, Wayne, NJ, USA; fitted with Zeiss Macro-Planar T* 50 mm f/

2 lens): One was pointed at a mirror beneath the tank that was angled at 45° for a ventral view and the second camera was directed at the side of the tank for a lateral view, resulting in biplanar video (Fig. 3). For most trials, we videotaped specimens at 200 frames per second, however we ranged the sampling rates from 100–300 fps to account for variation in escape times. We used 100 fps for recording the slower knotting events of *M. glutinosa* and 200–300 fps for *E. stoutii*.

Knot characterization

To characterize knots formed by hagfishes, we used the video analysis program Tracker (Brown, 2008; Fig. 4) to measure five geometric parameters.

First, the angle of the cross-point (XO angle, Fig. 4a) is defined as the angle between two ends of the looped body at the point where the parts overlap. If the posterior portion of the body crosses over the anterior portion at right angles, we recorded this crossover angle as 90°. However, crossovers may be acute (i.e. resembling the pink breast cancer public awareness ribbon), in which the angle between the caudal and cranial ends measures less than 90°. Crossovers may also be obtuse in which the loop is more complete and the caudal end points away from the cranial end. Theoretically, to measure a crossover angle, one would measure the angle between the cranial and caudal ends at the crossover point. However, in practice, when analyzing video of animals knotting, we encountered two complications. First, one of the two camera angles may be clearer. As such, we analyzed lateral and ventral views together and selected the view that offered the most planar view of the crossover angle. Second, portions of the body often contorted to obscure the crossover angle. When this occurred, we visualized a tangent line that ran through the crossover point and used it to measure the reciprocal angle formed between a cranial or caudal end and the portion of body from the loop. We were then able to estimate the crossover angle by subtracting the reciprocal angle from 180° (Fig. 5).

Second, the normalized loop radius (NLR, Fig. 4a) was calculated by fitting a circle within the tightest area of a loop and then measuring its radius. Half the body width was added to this radial measurement so that the result indicated the tightest radius measured to the center of the body. We calculated the NLR by dividing this radius from the body center line by the specimen's body width. This was done to generate a relative measurement that we could compare across different species and body sizes.

Third, the cross-point overhang (XP overhang, Fig. 4a) was defined as the amount of the caudal end that extended past the hagfish's body at a cross-point before it wrapped around the body. To progress in tying any knot, the portion of tail known as the XP overhang must begin to wrap around the body. The portion of the body that extends from the wrap must bend to continue to form the knot, the type of which depends on the angle of this bend.

The fourth parameter is the angle formed by the body that extends from the wrapped portion, or the wrapping cross-point

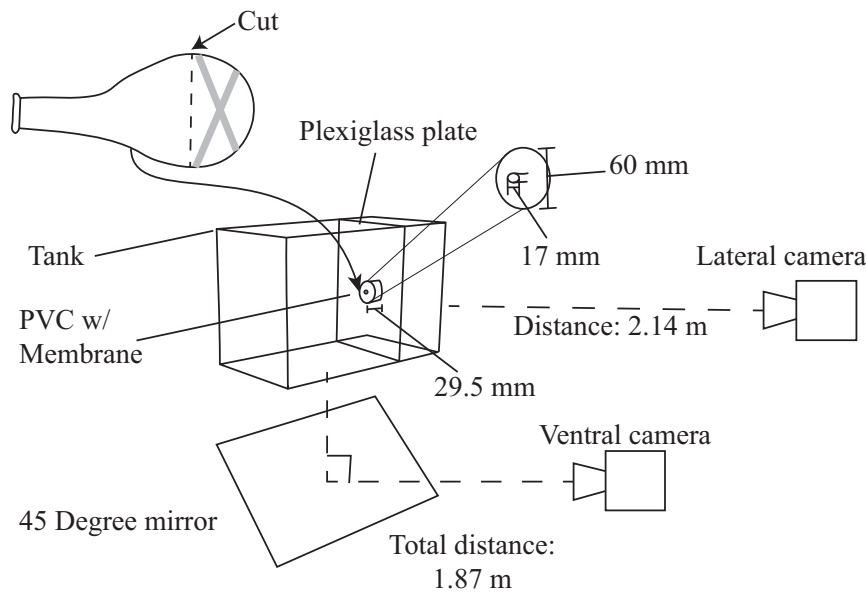


Figure 3 Experimental setup used to record biplanar high-speed video of a restrained hagfish. The system is composed of an aquarium arena fitted with a custom hagfish restraint device and a pair of synchronized cameras; one positioned to capture a lateral view and one aimed at a 45-degree mirror to capture a ventral view. The hagfish was fitted with a membrane that was fitted over a holder lathed out of a section of PVC pipe. To make the membrane, we cut the neck of a toy balloon along the dotted line as shown. We disposed of the right half and kept the half with the rolled 'neck'.

angle (WXP angle, Fig. 4b). Regardless of the type of knot formed, its completion must include the insertion of the tail into a loop of a certain size.

Our fifth parameter is the tail-insertion point (TIP, Fig. 4c). This is represented by the width of the loop measured between the inner edge of the inserted tail and the opposite edge of the loop. Theoretical the tightest loop would have a TIP equal to zero.

Statistics

To determine if differences in knotting kinematics (geometric variables) existed for any given knot type as tied by different species, and if differences existed between knot types within each species, we performed statistical analyses using SPSS (IBM, Armonk, NY, USA). Each geometric parameter was tested for normality using the Shapiro–Wilk test. If the data were normally distributed, we then compared species and knot types for each geometric parameter using an ANOVA. For each univariate comparison, the variance was tested using Levine's test of equality of error variance. If equal variance was assumed, then the Tukey *post hoc* analysis was performed. If equal variance could not be assumed, then we employed a Games–Howell *post-hoc* analysis. If the data were not normally distributed, then we performed the nonparametric Kruskal–Wallis test to account for lack of normality.

Results

We recorded and analyzed 100 escape events in which a hagfish freed itself from the membrane. We obtained 84 events

from *E. stoutii* (20 individuals), six from *E. springeri* (three individuals) and ten from *M. glutinosa* (four individuals). Hagfish tied three types of knots and used two other methods to escape the restraint device. The three knot types were the 3_1 (overhand knot, Figs. 1, 6b,c and 7), 4_1 (figure-eight knot, Figs. 1, 6a and 7) and the 5_2 (three-twist knot, Figs. 1 and 7). The two escape methods that did not involve knots included a 'loop-leverage' movement, in which a loop from an unformed knot (or a 0, 'unknot') is pressed against the surface of the membrane and used as leverage to escape. The last escape method category included all other observed behaviors including shaking out of the membrane and undulatory swimming backward or forward through the membrane and failure to escape within 2 min of the first knotting attempt. Table 1 lists these escape behaviors performed by all species.

Crossover angle (XP angle), normalized loop radius (NLR) and crossover overhang (XP overhang) were statistically similar between different types of knots. Only the tail-wrap angle (WXP angle) was statistically different ($P < 0.0001$) between all knot types. Using a Tukey *post hoc* analysis, we resolved which knots exhibited WXP angles that were different from each other: overhand knots were statistically different from both figure-eight knots and three-twist knots ($P < 0.0001$), while figure-eight and three-twist knots were not significantly different from each other ($P = 0.168$). Since there were not enough tail-insertion point (TIP) measurements for three-twist knots, only overhand and figure-eight knots were compared and were found to be statistically different ($P < 0.0001$). Since variance of TIP measurements between knot types could not be assumed to be equal, we performed an independent samples

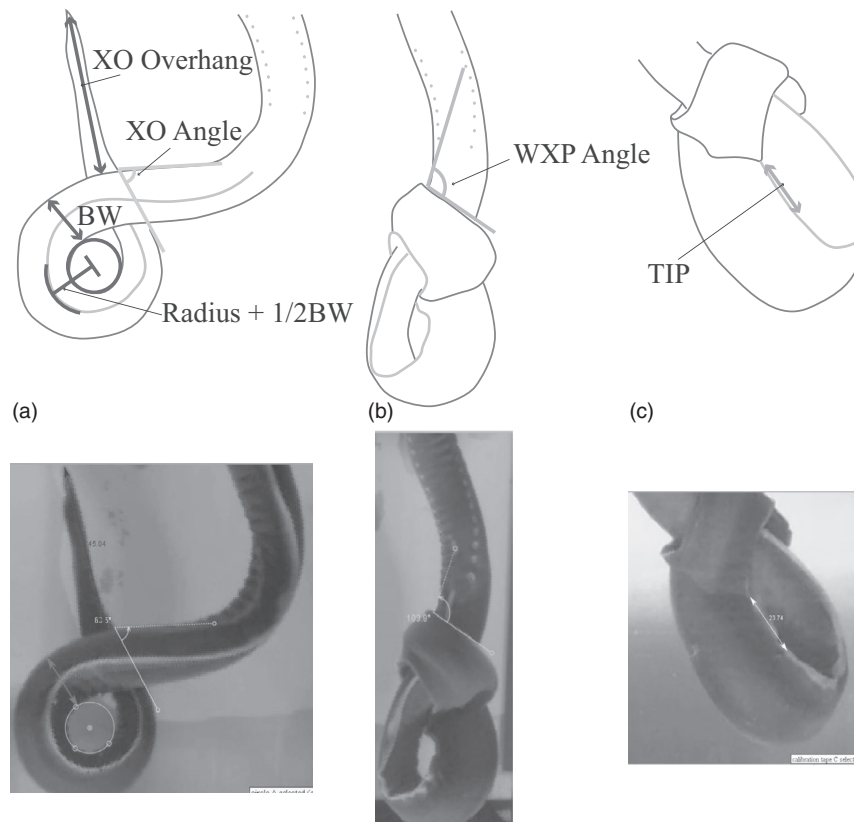


Figure 4 Geometric parameters that were measured to characterize knot formation. The ventral midline is indicated as a gray line. (a) We measured the crossover (XO) overhang as the length of tail projecting beyond the crossover point before the tail began its next movement (usually wrapping around the body). The crossover angle (XO angle), is the angle formed by the portions of body and tail entering the loop. We measured the radius of curvature of the tightest portion of the loop as the distance from the center of the loop to the center of the body (radius + $\frac{1}{2}$ body width). We divided this body loop radius by the body width (BW) so that loops made by animals of different sizes could be compared. (b) The wrapping cross-point angle (WXP Angle) was measured as the angle of the wrapping portion of the tail made relative to the anterior portion of the body being wrapped. An angle greater than 90° indicated that the tail was being inserted into the loop (as shown). An angle less than 90° indicated that the tail may wrap around the anterior portion of the body (not observed). The gray dots represent the ventrolateral gill pores. (c) The tail-insertion point (TIP) was measured as the distance between the inner edge of the loop and the tail as it was being inserted.

Kruskal–Wallis test. When comparing all three species at the $\alpha = 0.05$ confidence level, all measurements were statistically the same.

We observed that hagfishes used four distinct motions to create any of the knots that they tie (Fig. 8). The first motion, when a hagfish bends the body laterally, either to the left or right, was termed a body bend (Bend). The second motion, a 90° body twist (Twist), occurred when a caudal portion of the body twisted along its longitudinal axis. This twist could also be left or right handed, but was always the opposite of the previous motion. The third motion was a lateral body surface contact (Touch) used to complete a loop. In successful knots, its handedness was always the same as the preceding body twist. The fourth motion was an insertion (Insert) of the tail into the loop and was employed to complete the knot.

We observed that the formation of any given knot type could be decomposed into a specific combination of sequence and number of these four motions (listed in Table 2). While

hagfish can theoretically form at least eight types of knots, some were never employed. To hypothesize the sequence of movements required to tie these unused knots, we noted our own movements while tying knots into ribbons (Fig. 7). For knots used by hagfish, we documented the sequence of movements by analyzing frames of video from recorded events. While hagfish exhibited superfluous motions before and after the formation of a knot, the order of motions during the knotting process always followed those listed in Table 2 for every successful event.

In comparing the motion sequences for knots tied by hagfish, we have identified two rules that govern knot formation. First, since our hagfishes were restrained at their cranial ends, we found that knot creation always began in the caudal region and propagated cranially until terminated. Termination either occurred because of a successful escape, in which the head was pulled through a body loop and the knot unraveled, or, if a knot failed to form, a loop leverage movement was executed.

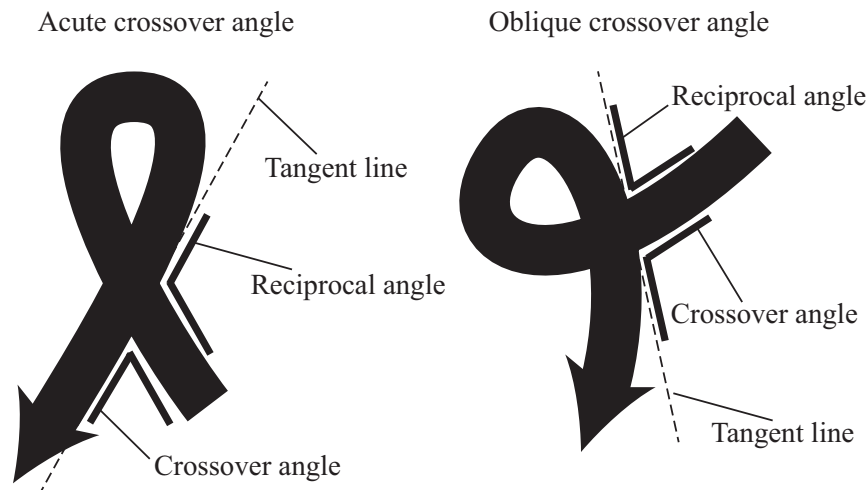


Figure 5 Measuring acute and oblique crossover angles (XOA) formed by hagfishes. Crossover angles were measured from the lateral or ventral camera view that offered the most planar view of the body loop and crossover angle. If the crossover angle was obscured and not directly measured, we then used a visualized tangent line and reciprocal angle measurement to estimate the acute or oblique crossover angle.

Table 1 Observational counts of escape behaviors employed by each species of hagfish

Type of Knot	Total	<i>Eptatretus stoutii</i> ($n = 20$, events = 84)	<i>E. springeri</i> ($n = 3$, events = 6)	<i>Myxine glutinosa</i> ($n = 4$, events = 10)
Loop-Leverage (0_1)	11	10	1	0
Overhand (3_1)	45	37	3	5
Figure-Eight (4_1)	33	31	2	0
Three-Twist (5_2)	4	4	0	0
Other	7	2	0	5

This happened when the tail did not insert into a loop, resulting in a failed knot. As a result, the loop propagated cranially until it contacted the membrane and pressed against it for leverage. Second, Bends and Twist/Touch sequences alternated between sides. For example, if a left Twist/Touch sequence followed a left Bend or another left Twist/Touch sequence (i.e. fail to alternate between sides), it always resulted in an incomplete knot and necessitated a restarting of the knotting process.

Discussion

Describing knotting behaviors

We have described the diversity of knots that hagfish employ by using a novel restraint system that may be of use in investigating other aquatic, body knotting vertebrates, such as sea snakes and moray eels. We opted to stimulate escape knotting behaviors, rather than feeding knotting behaviors, because knotting during unrestrained bouts of feeding use highly variable movements that we could not analyze or compare. Using escape as the stimulus led to consistent knotting behaviors in which we could control the initial orientation of the knot and, because we knew where the knot would be formed, we could readily predetermine camera region of interest. The result was

clear high-speed video of knotting events that was used for 3D kinematic analysis.

We hypothesized that hagfishes would (a) prefer more complex knots, due to the reduced internal stresses at the pinch points and (b) the most complex knot they would be able to tie is the 6_2 knot, based on average length-to-width ratio. These hypotheses were not supported; while a 5_2 knot was tied by *E. stoutii*, simpler knots were favored; the most common knot tied by both species was the simpler 3_1 knot, similar to the behavior of moray eels in the genus *Gymnothorax* moray eels (Malcolm, 2016). Behaviorally, *E. stoutii* was the species that knotted most readily, and therefore, from which most of our recordings were made. This species tied 3_1 knots and 4_1 knots in similar numbers ($3_1 = 44\%$ to $4_1 = 37\%$). This led us to hypothesize that perhaps the difference between tying a 3_1 knot and a more complex knot is simply dependent on initial body conditions. For example, if the initial touch formed an acute crossover angle, then a figure-eight was normally formed. However, if an oblique crossover angle was initially formed, then an overhand knot was usually tied. However, an alternate hypothesis is that naturally behaving hagfish may be able to select knots based on their mechanical functions. Pickwell (1971) noted such selectivity in another slender-bodied aquatic vertebrate, the pelagic yellow-bellied sea snake (*Hydrophis* (formerly *Pelamis*) *platurus*, Linnaeus, 1776), used 4_1 knots

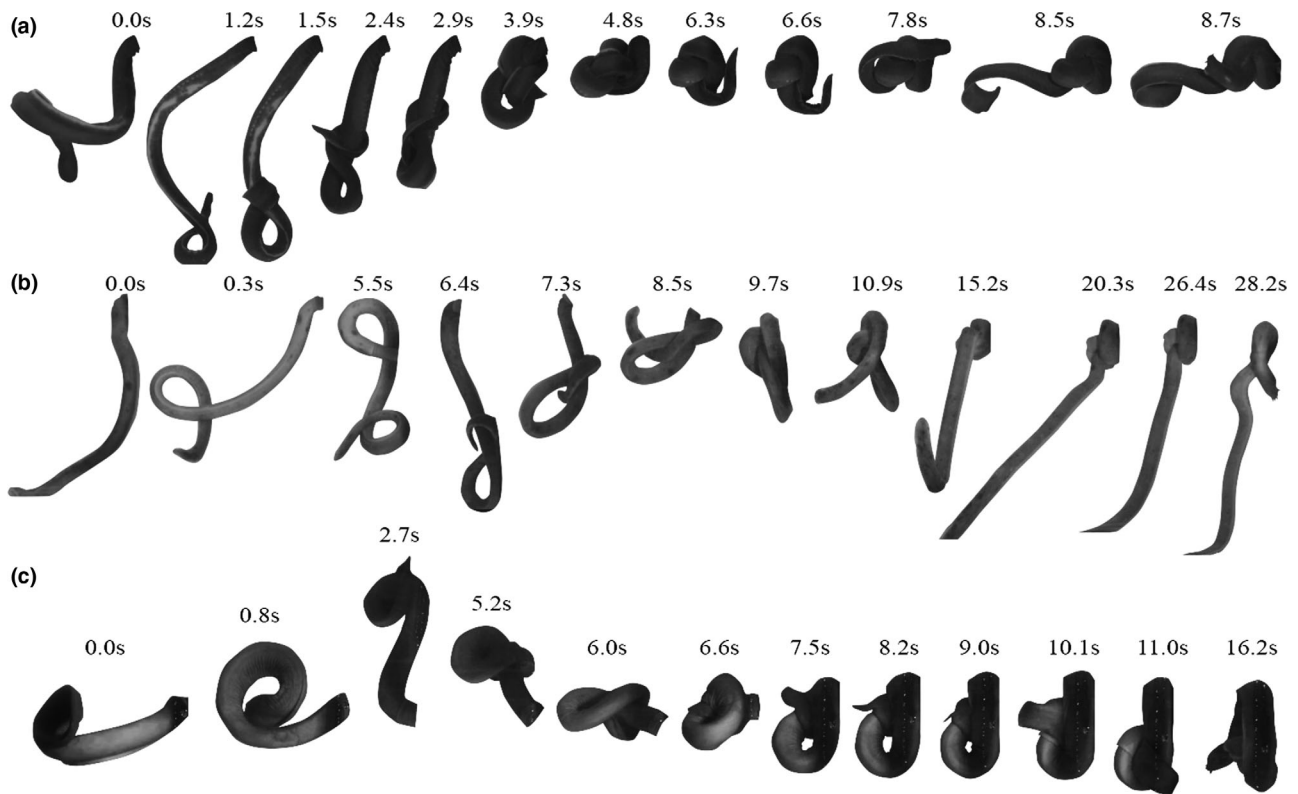


Figure 6 Representative video image sequences of knotting behaviors in three species of hagfish. The background and restraint device were removed from each video image and the time at each event is included above each image. (a) Image sequence of a specimen of *Eptatretus stoutii* creating a figure-eight (4_1) knot and using the knot to free itself from the restraint device. (b) A specimen of *Myxine glutinosa* and (c) a specimen of *E. springeri* employing overhand (3_1) knots for extrication. Note the significantly longer elapsed time during the knotting event in *Myxine* compared to both species of *Eptatretus*.

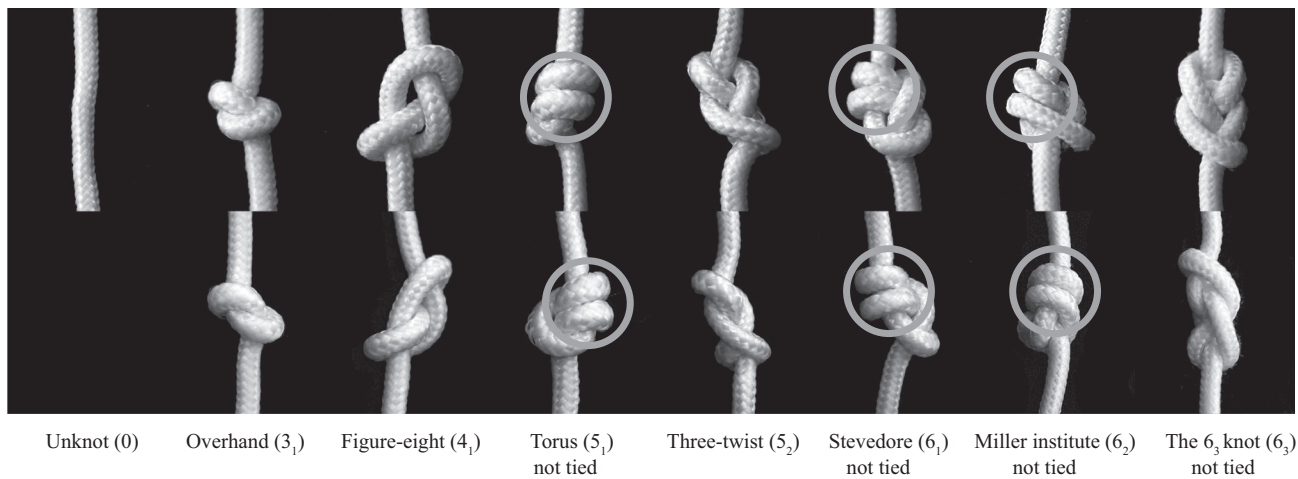


Figure 7 Tight knots in rope closely resemble body conformations in knotting hagfish. Shown here are primitive knots 0 to 6_3 . The knots with circles (5_1 , 6_1 and 6_2) indicate knots that require consecutive rotations of the tail around the body in the same direction, whereas hagfish alternate direction of bending and twisting motions. Furthermore, these resultant double loops, known as ‘frappings’, may compress the body within the loops to an unacceptable degree.

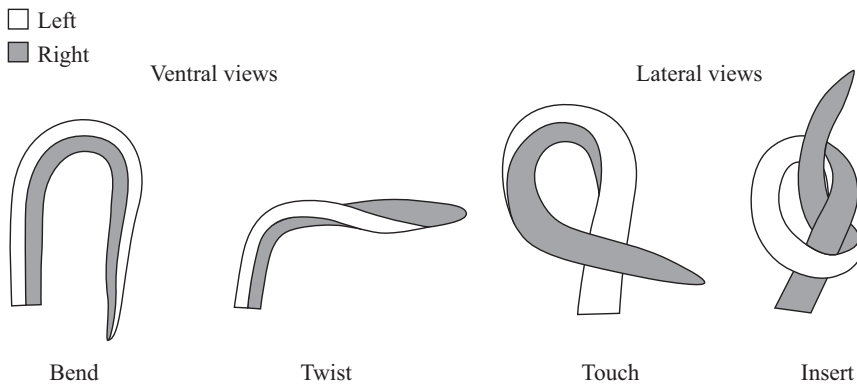


Figure 8 Hagfish employ four distinct motions that are used in different combinations and numbers to create all the knots observed. All images depict views in which the head is oriented downwards, and the lateral body sides are color coded (left = white, right = gray). Bend = The body is bent laterally to the left or right. Twist = The body generates torque to twist along the longitudinal axis by approximately 90°. This allows same sides of the body to contact during a touch. Touch = Two sides of the hagfish body make contact and thereby create a loop. Insert = After a touch, the tail tends to follow the contours of the body so that it inserts into a loop and completes the knot.

Table 2 A list of knots that may be tied by hagfish given their aspect ratio, whether they are used during escapes, and the sequence of the four distinct motions used in their tying

Type of Knot	Used by Hagfish?	Motions
Loop-Leverage (0)	Y	Bend (R), Twist (L), Touch (L)
Overhand (3 ₁)	Y	Bend (R), Twist (L), Touch (L), Insert
Figure-Eight (4 ₁)	Y	Bend (R), Twist (L), Touch (L), Twist (R), Touch (R), Insert
Torus (5 ₁)	N	Bend (R), Twist (L), Touch (L), Insert, Touch (L), Insert
Three-Twist (5 ₂)	Y	Bend (R), Twist (L), Touch (L), Twist (R), Touch (R), Insert, Twist (L), Touch (L), Insert
Stevedore’s (6 ₁)	N	Bend (R), Twist (L), Touch (L), Bend (L), Touch (L), Twist (R), Touch (R), Insert
The Miller Institute (6 ₂)	N	Bend (R), Twist (L), Touch (R), Insert, Touch (R & L), Bend (R), Touch (R), Insert
The 6 ₃ Knot (6 ₃)	N	Bend (R), Twist (L), Touch (L), Insert, Bend (R), Insert, Bend (R), Insert

The sequences of knots not employed by hagfish were estimated based on knots we tied in ribbons.

Bend = a lateral bend to the left or right of body (Initial bends may be to either side, but we note them as to the right for knot comparisons), *Twist* = a left or right-handed longitudinal twist, *Touch* = Contact between left or right surfaces of the body (hagfish typically use a twist so that same sides touch each other), *Insert* = the insertion of the tail into a loop of the body.

when escaping and 3₁ knots when shedding skin. It may be that the decreased internal stresses at pinch points in the 4₁ knot lends itself to easier knot manipulation and escape, while higher knot stresses in the 3₁ knot lend itself to stretching and shedding skin. It is thus conceivable that hagfish may also use different knots, with their varying levels of internal stresses and loop configurations, for their specific sliding, jamming, unknotting and other properties. Further observations of hagfishes in a diversity of knotting situations are needed to test this hypothesis.

Despite having a suitable length-to-width ratio, we have never seen a hagfish tie a 5₁, a 6₁, or a 6₂ knot. In trying to understand why, we noted a similarity; they all form a double loop around a portion of the body (see circled features in Fig. 8). Ashley (1944) uses the nautical term ‘frapping turns’ and defines them as loops being used to lash together and

tighten parts of a knot (e.g., the 13 loops of a hangman’s noose). Because frapping turns completely encircle the body, it may pose a risk of damage by pinching off the subcutaneous sinus into two hydraulically isolated compartments or by placing too much pressure on the body core. A second possibility for the lack of 5₁, 6₁ and 6₂ knots is that they are the only ones within the biologically relevant knot morphospace that violate the alternation of handedness rule that hagfish seem to follow. This is because sequential ipsilateral movements are needed to form multiple wraps of the tail around the body.

In summary, we have described four distinct motions that hagfish use to tie knots. Different knots are a result of different sequences and numbers of these motions. Furthermore, bends and body twists and resultant contacts can be either left- or right-handed and hagfish always alternate between them. Knots used by hagfish (3₁, 4₁, 5₂) are tied using alternating handed

motions. All other knots introduced in Fig. 1 (e.g., 5_1 , 6_1 , 6_2 & 6_3) are avoided by hagfish because they either require a sequence of same-handed motions that result in either frapping turns or failure of the knot due to unraveling or are outside of the knotting morphospace that is set by body length-to-width ratio.

Comparative biology of knotting

Only three aquatic taxa of vertebrates have been observed tying body knots: sea snakes, moray eels and hagfishes. Among sea snakes (Family: Elapidae), four species have been observed exhibiting knotting behaviors: *Acrochordus granulatus* (Schneider, 1799), *Enhydrina schistosa* (Daudin, 1803), *Laticauda semifasciata* (Reinwardt in Schlegel, 1837) and *H. platurus*. These snakes employed overhand knots to aid in skin shedding, to clear ectoparasites, and to transport food items through their digestive system; however, figure-eight knots were more often used during escape maneuvers (Mays & Nickerson, 1968; Pickwell, 1971; Voris *et al.*, 1978). Among moray eels (Family: Muraenidae), nine species belonging to three genera have been reported to employ knotting: five species of *Gymnothorax*, three species of *Echidna* and one species of *Enchelycore*. Crevice dwelling moray eels feed on large food items and use the adaptations of raptorial pharyngeal jaws (Mehta & Wainwright, 2008) and knotting (Miller, 1989) for reducing large food items and for subduing and constricting prey. Moray eels also use their body knots as leverage to remove prey from crevices and to conceal food items from other swarming eels (Barley *et al.*, 2016; Malcolm, 2016). In comparison to sea snakes and moray eels, Eptatretinine hagfishes may be the taxon that most readily employs knotting behaviors.

Among the hagfishes studied here, we found a positive correlation between coiling behavior and knot complexity. When resting, *E. stoutii* have been observed winding themselves into a loose coil of two or three loops at the bottom of the seafloor (Strahan, 1963; Martini, 1998; Miyashita & Palmer, 2014); these were also the hagfish that tied the most complex knots. Resting *M. glutinosa* prefer to lie at the bottom with their bodies straight and *E. springeri* seemed to adopt an intermediate body curvature. While we have many more observations of *E. stoutii*, neither *M. glutinosa* nor *E. springeri* were seen tying the most complex knots. Despite a lack of obvious morphological differences between these species (all exhibited roughly similar length-to-width ratios, loose skins and core muscle morphologies that would not seem to limit body coiling), we considered that the correlation may be the result of differences in overall body flexibility. This may not be the case however, as the normalized loop radius (NLR) that we used as a gauge of how tightly the hagfish tied knots (i.e. flexibility) was not statistically different among the species. This suggests that while all species may possess similar mechanical flexibility, they each may exhibit a different behavioral stiffness, as observed with different resting orientations. Further biomechanical tests to assess overall body stiffness, such as those performed on American eel by Long (1998), on hagfish by Long *et al.* (2002), and lamprey by Tytell *et al.* (2010), need to be performed comparatively, in both

Myxinines and Eptatretines, to assess the relative contributions of body mechanics and behavioral control.

Possible neural control mechanisms

The neuromechanics (*sensu* Nishikawa *et al.*, 2007) of knot formation in hagfish is poorly understood. This is because both the mechanics and neural control of deformable tissue structures are not well-studied. Movements, such as those used in swimming (Summers & Koob, 2002) and axial body morphology and musculature (Nursall, 1956; Vogel & Gemballa, 2000) have been investigated, yet no hypotheses of body muscle activation patterns exist. Because our study suggests that knotting behaviors may be the result of simpler, underlying movements, hagfish may rely on a control simplification mechanism that is similar to the ‘motor primitives’ (*sensu* Hart & Giszter, 2010; Giszter, 2015) proposed for reaching behaviors in flexible octopus arms (Yekutieli *et al.*, 2005; Sumbre *et al.*, 2006). In our study, knot characteristics were statistically similar regardless of the type, and, for any given knot, the movements could be decomposed into identical sequences of the four basic motions (i.e. body ends, body twists, surface contacts and tail insertions). While further studies are needed, these results may indicate that motions are stereotyped and that there is no simultaneous control of every point along the body.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Statistical tests and their significance levels used to determine if differences in knotting geometry variables existed for various knot types as outlined in the Materials and methods section.