

RESEARCH ARTICLE

Hagfish Houdinis: biomechanics and behavior of squeezing through small openings

Calli R. Freedman¹ and Douglas S. Fudge^{1,2,*}

ABSTRACT

Hagfishes are able to squeeze through small openings to gain entry to crevices, burrows, hagfish traps and carcasses, but little is known about how they do this, or what the limits of this ability are. The purpose of this study was to describe this ability, and to investigate possible mechanisms by which it is accomplished. We investigated the hypothesis that the passive movement of blood within a hagfish's flaccid subcutaneous sinus allows it to squeeze through narrow apertures that it would not be able to if it were turgid. To test this hypothesis, we analyzed videos of Atlantic hagfish (*Myxine glutinosa*) and Pacific hagfish (*Eptatretus stoutii*) moving through narrow apertures in the lab. We measured changes in body width as the animals moved through these openings and documented the behaviors associated with this ability. We found that hagfishes are able to pass through narrow slits that are less than one half the width of their bodies. Our results are consistent with the idea that a flaccid subcutaneous sinus allows hagfish to squeeze through narrow apertures by facilitating a rapid redistribution of venous blood. In addition, we describe nine distinct behaviors associated with this ability, including a form of non-undulatory locomotion also seen in snakes and lampreys. Our results illuminate a behavior that may be a critical component of the hagfish niche, as a result of its likely importance in feeding and avoiding predators.

KEY WORDS: Locomotion, Burrowing, Scavenger, *Myxine glutinosa*, *Eptatretus stoutii*

INTRODUCTION

Although it was long believed that hagfishes are mostly sedentary animals, it has more recently come to light that they can be active scavengers and hunters that rely on elaborate locomotor and behavioral repertoires (Zintzen et al., 2011; Lim and Winegard, 2015). Hagfishes are burrowers (Gustafson, 1935; Strahan, 1963; Fernholm, 1974) and anguilliform-mode swimmers (Adam, 1960), and they are capable of tying themselves into knots (Adam, 1960; Clark et al., 2016). They are also able to squeeze their bodies through small holes. In a study of size-selectivity of hagfish trap escape holes, Harada et al. (2007) report that inshore hagfish (*Eptatretus burgeri*) are readily able to pass through holes with perimeters equal to their girth, and are only reliably retained when their girth is more than 1.5-times larger than the hole perimeter. The purpose of the present study was to explore and describe the ability

of two species of hagfish to squeeze through tight spaces, and to test possible mechanisms by which this behavior is accomplished.

Hagfishes possess some of the highest blood volume to body mass ratios among vertebrates (Forster et al., 2001). For example, the blood volume of the Pacific hagfish (*Eptatretus stoutii*) is approximately twice that of mammals, ranging from 169 to 187 ml kg⁻¹ (McCarthy and Conte, 1966; Forster et al., 1989). Nearly 30% of this volume is contained within the venous sinus system, of which the large subcutaneous sinus (SCS) is a dominant component (Forster, 1997). The SCS almost completely surrounds the body of the hagfish and is flaccid, meaning that it has the potential to hold a larger volume of blood than it usually contains (Forster, 1997). The physiological advantage of this large blood volume remains unclear, and the functional significance of the SCS is similarly poorly understood. Osmoregulation and oxygen delivery during exercise have been discounted as possible roles of SCS blood (Forster et al., 1989), although there is some evidence in the Atlantic hagfish (*Myxine glutinosa*) that the SCS might be involved in cutaneous respiration (Lesser et al., 1997). In contrast, Clifford et al. (2016) found little evidence of cutaneous respiration in the Pacific hagfish. Davison et al. (1990) found no evidence that the SCS serves as a sink for lactate ions during exhaustive exercise, and the SCS is also unlikely to serve as a hydrostatic skeleton given its flaccid nature (Forster et al., 1989). Recent studies have demonstrated cutaneous transport of nutrients (Glover et al., 2011; Schultz et al., 2014), ammonia (Clifford et al., 2014) and trace metals (Glover et al., 2015) in Pacific hagfish, suggesting that the large blood volume held within the SCS may be involved in acid–base regulation and nutrient acquisition from the surrounding water. Whatever the function of the SCS, the occurrence of this distensible compartment surrounding the hagfish's body is likely to have implications for locomotion within confined spaces.

We hypothesized that when a hagfish squeezes through a tight aperture, blood collects in the sinus in the trailing portion of the body, such that the leading portion of the body can fit through. Underinflation of the SCS is a key component of this hypothesis, as a turgid SCS would be limited in its ability to accommodate blood from other parts of the body. While fluid redistribution could explain some aspects of squeezing behavior, it does not provide a mechanism by which hagfishes power their movements through tight spaces. Hagfishes are effective burrowers, and burrowing behavior has been observed both in aquaria and in the wild (Gustafson, 1935; Strahan, 1963; Fernholm, 1974; Martini and Heiser, 1989; Martini et al., 1997). Martini (1998) suggests that a series of longitudinal contractions is involved in hagfish burrowing, although the precise mechanism of burrowing in hagfish remains elusive. In the current study, we observed hagfishes under controlled laboratory conditions within a transparent arena, which allowed us to not only measure changes to their body morphology, but also to characterize the behaviors they employ while moving through narrow openings. It also allowed us to quantify the limits of this

¹Dept. of Integrative Biology, University of Guelph, Guelph, Ontario, Canada N1G 2W1. ²Schmid College of Science and Technology, Chapman University, Orange, CA 92866, USA.

*Author for correspondence (fudge@chapman.edu)

 D.S.F., 0000-0001-9569-1374

behavior for the Atlantic and Pacific hagfish. Our results show that both species employ several stereotyped behaviors in their attempts to pass through narrow openings, and that rapid redistribution of blood within the SCS occurs during these passages.

MATERIALS AND METHODS

Experimental animals

Pacific hagfish [*Eptatretus stoutii* (Lockington 1878)] (size range: 12.0–19.2 mm in width), collected from Bamfield Marine Station (Bamfield, BC, Canada) and Atlantic hagfish (*Myxine glutinosa* Linnaeus 1758) (size range: 13.0–24.0 mm in width), collected from the Shoals Marine Laboratory (Appledore Island, Kittery, ME, USA) and the Huntsman Science Centre (St Andrews, NB, Canada) were maintained at the Hagen Aqualab (Guelph, ON, Canada) in a 2000 liter tank filled with chilled artificial seawater (34‰, 12°C). The hagfishes were fed squid monthly to satiety as outlined in University of Guelph Animal Care Protocol 2519. All procedures adhered to guidelines set forth by the University of Guelph Animal Care Committee. A total of nine Atlantic and 10 Pacific hagfish were used in this study.

Experimental protocol

The original enclosure design included hole-shaped apertures of various diameters; however, the hagfish did not reliably move through these apertures, and the wall material also obstructed our view of the animal's body. During preliminary trials, we noticed the hagfish were especially interested in exploring the corners of the enclosure. To address these issues, we built a triangular Lexan enclosure (Fig. S1), which had a hinged wall that could be moved such that a slit was created at one corner. Hagfish were placed, one at a time, into the enclosure within a larger tank filled with artificial seawater (12°C, 34‰). The animals were free to swim within the enclosure, which had a volume of 1.17 m³ (0.203 m × 0.0508 m × 0.176 m). The body width of each hagfish was initially estimated using a ruler, and the width of the slit was adjusted based on the size of the hagfish being studied, starting at half the estimated body width. Hagfish typically attempted to pass voluntarily through the slit within minutes of being placed in the enclosure. Once a hagfish successfully moved through the slit, the width was decreased by approximately 0.25 mm and the hagfish was returned to the enclosure. This process was repeated until the hagfish failed to escape, with failure defined as multiple unsuccessful attempts through the slit over a period of 30 min. Animals that made no attempts to escape and/or rested inside the enclosure over a 30 min period were not considered to have failed and such trials were not included in this study. A camera was mounted on a tripod to film the setup from above. Video recordings were obtained of hagfish moving through the slits at a frame rate of 30 frames per second using a Sony Action Cam 4K (1080p; Sony, Minato-ku, Tokyo, Japan).

Video analyses

All videos were reviewed at 0.25× speed and/or frame by frame using VLC media player (VLC v.2.2.1 Terry Pratchett Weatherwax, VideoLAN). Video frames were analyzed using ImageJ software (v.1.49v, National Institutes of Health) to measure the width of the body on either side of the slit. The resting width of each hagfish was measured across the widest part of the body before the animal entered the slit. Body width was measured at a distance of 1.0 cm on either side of the slit. This distance was close enough to the slit to detect possible accumulation of fluid on the trailing side, and far enough away that constriction of the body by the slit did not dominate the measurement. For three selected trials, an obvious

marking on the skin was used to follow changes in body width as the animal moved through the slit. The distance from the marking to the slit was also measured in each frame.

In order to determine whether fluid is redistributed while an animal rests, we analyzed a video of an Atlantic hagfish with its head through a tight slit that appeared to be temporarily stuck. Stills from this video were analyzed using ImageJ software to quantify head area over a period of 180 s; changes in the color of the head were quantified by measuring the mean gray value of the pixels making up the image of the head. A total of 19 frames were used in the volume analysis, however, only nine of these were used in the color analysis due to changes in the level of illumination that interfered with reliable measurement.

Behavior analyses

Upon careful review of the videos, a behavior key was constructed to describe the various behaviors associated with movement through the slit (Table S1). Once this key was completed, each video was reviewed again and each trial transcribed into a string of letter codes with time stamps. For this analysis, only those behaviors that occurred while the animal was actively attempting to move through the slit were considered, while behaviors such as exploration of, and/or resting within the enclosure were not. It should be noted that all suitable trials were used in the analysis of behavioral data, and that multiple trials of the same animal (at various slit widths) were included. A total of 20 videos of nine Atlantic hagfish and 28 videos of 10 Pacific hagfish were used in these analyses.

To make a fair comparison of the behaviors used by the two species, we compared the behavioral frequencies from the last successful trial for each animal. This approach assumes that the slit width for that trial was close to the minimum width that the animal could traverse. To quantify behavior frequency, we counted the number of times each of nine behaviors was initiated throughout a trial. We then divided this by the total number of behaviors initiated throughout the trial to correct for individual differences in activity. Of the nine Atlantic hagfish, we had a suitable video of a last successful trial for only seven.

Data analysis

Simple linear regression was used to quantify the relationship between minimum slit width and body width for both Pacific and Atlantic hagfish. An analysis of covariance (ANCOVA) was performed to test whether this relationship differed significantly between Atlantic and Pacific species. All analyses were performed using SPSS Statistics 23 (IBM Corporation, 2015) and Microsoft Excel (v.15.13.3, Microsoft, 2015). A significance level of 0.05 was used in all analyses.

RESULTS

Body width

Body width was analyzed in detail for three hagfishes (two Atlantic and one Pacific), and still images from these trials demonstrate the accumulation of fluid on the trailing side over time (Fig. 1A and Fig. 2A). For these animals, body width, measured at the same point on the body, steadily decreased as this point approached the slit, and steadily increased as it emerged on the other side, but did not recover the same width it had previously been on the trailing side (Fig. 1B and Fig. 2B). For an Atlantic hagfish moving head first through a 4.6 mm slit, the body was 10.8 mm wide at a distance of 9.6 mm on the trailing side, and only 7.5 mm at a distance of 9.1 mm on the leading side, recovering to only 69% of its former width (Fig. 1B). For a Pacific hagfish moving head first through a 7.0 mm slit, the

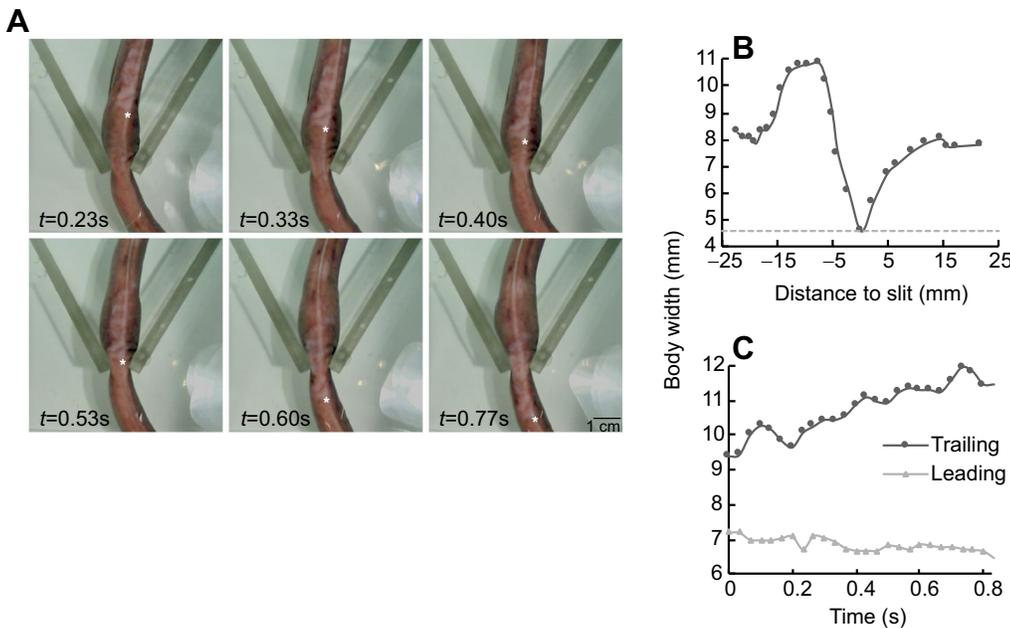


Fig. 1. Changes in body width as an Atlantic hagfish (*Myxine glutinosa*) moves head first through a 4.6 mm slit. (A) Still frames illustrating the movement of a hagfish through the slit. Note the bulge on the trailing side of the slit as the body passes through. The asterisk marks the point on the body where body width was measured in B, which was ~85% down the body from the head. (B) Body width measured at the point indicated by the asterisk in A. Negative distances indicate how far this point on the animal is on the trailing side of the slit (i.e. inside the enclosure) and positive distances indicate the body has emerged on the leading side. The dashed line indicates the width of the slit. (C) Body width at the trailing and leading sides of the slit. The data in B and C were obtained from still frames of the trial. For C, the body was measured at a distance of 1.0 cm from the slit on both the leading and trailing sides in each frame.

body was 12.9 mm wide at a distance of 9.0 mm on the trailing side, and only 10.7 mm at a distance of 9.0 mm on the leading side, recovering to only 83% of its former width (data not shown). For an Atlantic hagfish that moved tail first through a 5.3 mm slit, the body was 10.4 mm wide at a distance of 9.7 mm on the trailing side, and only 9.6 mm at a distance of 9.7 mm on the leading side, recovering to only 92% of its former width (Fig. 2B). The overall trend in body width on the trailing and leading side (measured at a distance of one cm from the slit in each frame) was also consistent. Body width increased in width on the trailing side, and slightly decreased in width on the leading side over time (Figs 1C and 2C).

For one Atlantic hagfish that moved partway through the slit and appeared to get stuck, the leading region of the body increased steadily in size after the animal stopped moving, with the surface area

increasing from 249 to 300 mm² (Fig. 3). The color of this leading region also changed from pink to a deep purple as it increased in size (Fig. 3). These changes almost certainly were due to a net flow of blood from the trailing to the leading side of the hagfish's body.

Linear regression analysis revealed a positive relationship between minimum slit width and body width for Atlantic hagfish ($r=0.92$, $N=7$, $P=0.003$) and Pacific hagfish ($r=0.89$, $N=10$, $P=0.0005$) (Fig. 4). The line of best fit for Pacific hagfish appeared to be elevated relative to the line for Atlantic fish, suggesting that Atlantic hagfish are better at getting through narrow openings for a given body size, but ANCOVA analysis revealed that the difference in elevation is not significant ($F_{1,14}=3.9$, $P_{1,14}=0.068$). The slope of the relationship between minimum slit width and body width for both species together was 0.281.

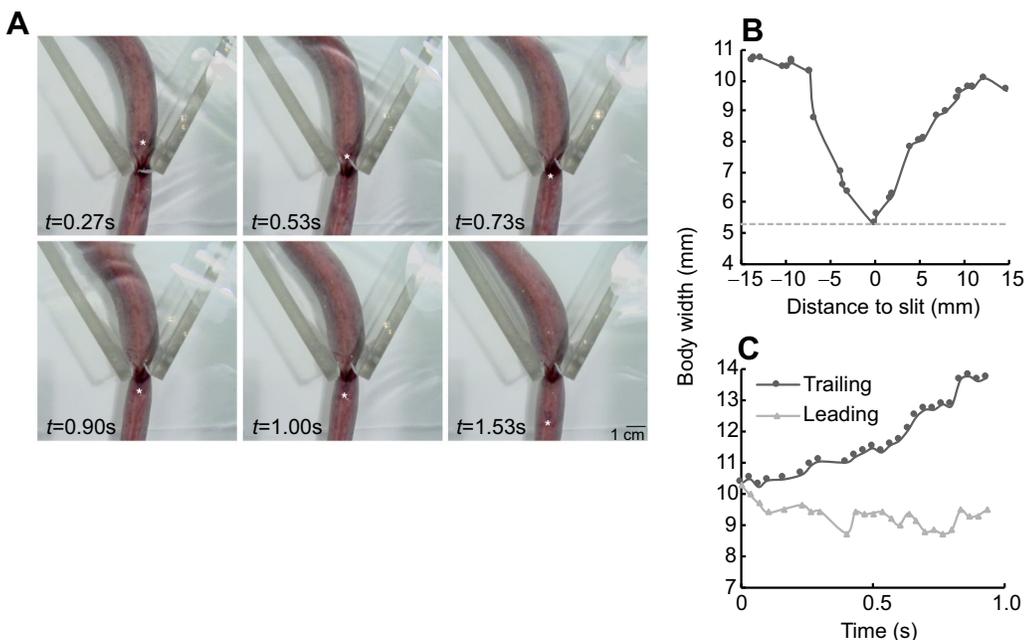


Fig. 2. Changes in body width as an Atlantic hagfish moves tail first through a 5.3 mm slit. (A) Still frames illustrating the movement of a hagfish through the slit. The asterisk marks the point on the body where body width was measured in B, which was ~25% down the body from the head. (B) Body width measured at the point indicated by the asterisk in A. Negative distances indicate how far this point on the animal is on the trailing side of the slit (i.e. inside the enclosure) and positive distances indicate the body has emerged on the leading side. The dashed line indicates the width of the slit. (C) Body width at the trailing and leading sides of the slit. The data in B and C were obtained from still frames of the trial. For C, the body was measured at a distance of 1.0 cm from the slit on both the leading and trailing sides in each frame.

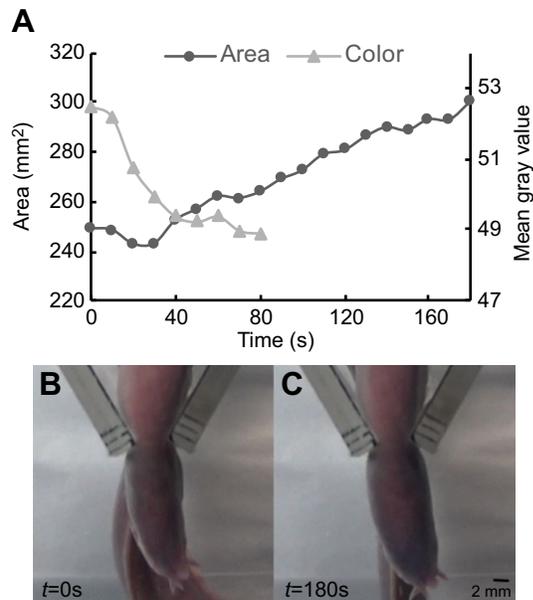


Fig. 3. Changes in head area and color over time in a resting Atlantic hagfish that had previously squeezed its head through a narrow slit. The increase in size and change in color are most likely due to the accumulation of blood in the head. (A) Color change was quantified using the mean gray value (MGV) of the pixels making up the image of the head, where 0=black and 255=white. (B) A still frame at 0 s and (C) a still frame at 180 s illustrating the enlargement and change in color of the head.

Behavior

A total of nine distinct behaviors were found to be associated with movement through the slit (Table S1; see Movies 1 and 2 for examples of some of these behaviors). Many of these behaviors appeared to serve very specific purposes and were observed predictably. Head-bending, for example, was only ever observed while the animal was forcing its head through the slit, and involved a bend of almost 180 deg in the dorsal or ventral direction. This behavior was consistently seen in all animals, regardless of slit width, and was commonly accompanied by eversion of the tooth plates. Loops served multiple purposes. The wide looping behavior

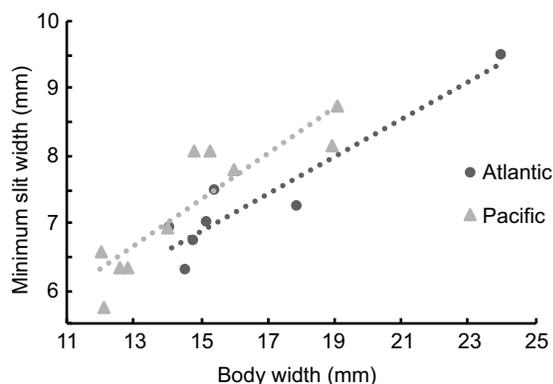


Fig. 4. Body diameter versus minimum slit width for Atlantic and Pacific hagfish. Slit limit, defined here as the smallest possible slit each animal could pass through successfully, was measured by challenging hagfish with progressively narrower slits until they could no longer pass through. Linear regression indicated that slit limit was significantly correlated with body diameter in both species (Atlantic: $r=0.92$, $n=7$, $P=0.03$; Pacific: $r=0.89$, $n=10$, $P=0.0005$), but did not differ significantly between the two species after controlling for the effect of body width using ANCOVA ($F_{1,14}=3.9$, $P_{1,14}=0.068$).

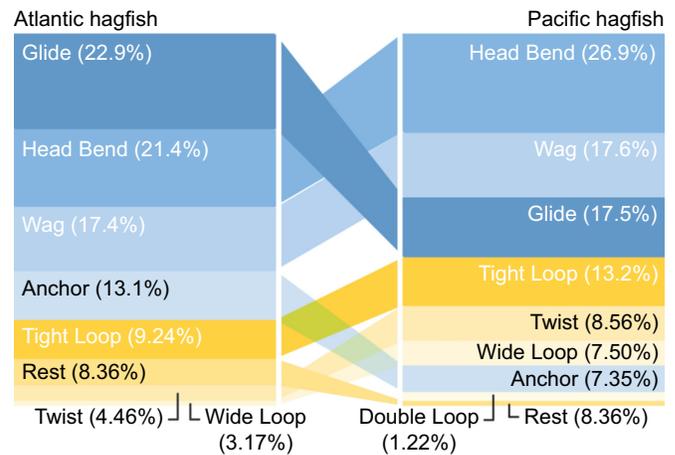


Fig. 5. Behavior profiles of Atlantic and Pacific hagfish. Box height denotes the average frequency of each behavior, calculated as a percentage of the total number of behaviors displayed. Data were collected from videos of the last successful trial of seven Atlantic and ten Pacific hagfishes and were calculated from a total of 132 behaviors for Atlantic hagfish and 206 for Pacific hagfish.

was always associated with pulling the leading portion back into the enclosure, and a tight loop was sometimes used for this purpose as well. In many cases though, the formation of a tight loop was followed by successful escape. The double loop, a variation on the tight loop, was rarely seen, but appeared to serve the same purpose as the single tight loop. In both species, the glide, head bend and wag were the most frequent behaviors observed (Fig. 5).

Multiple individuals displayed similar sequences of behaviors. Of these, the most common was a head bend (H) followed by a brief period of gliding (G). This pattern was observed at a relatively high frequency in both species (34 times in Atlantic hagfish and 56 times in Pacific hagfish), and in both successful and unsuccessful trials. Some animals followed the HG pattern with wagging (C) and then a wide loop (E), which often served to pull the remainder of the body through. The HGC pattern was observed 13 times in both species, and in both successful and unsuccessful animals. The HGCE pattern was observed six times in Pacific hagfish, but only once in Atlantic hagfish, and was only observed in successful animals while passing through less challenging slits. A second pattern, a twist (B) followed by a wide loop (I), was associated with retreat back into the enclosure, and occurred more frequently in Pacific hagfish (16 times, in both successful and unsuccessful animals) than in Atlantic hagfish, for which this pattern was observed only four times, and only in unsuccessful animals. This twisting movement may serve to reorient the relatively stiff branchial chamber in order to aid retreat. Some Pacific hagfish combined these two patterns (HGBI) in a rapid series of attempts to pass through the slit, followed by retreat back into the enclosure. This pattern was observed 12 times in Pacific hagfish, seven times in successful trials and five times in unsuccessful trials.

DISCUSSION

In the current study, hagfishes were able to maneuver through slits with widths that were often smaller than one half of their body width. Atlantic hagfish appeared to be better at getting through narrow openings for a given body size; however, this difference was not statistically significant. In a study of the trap fishery for inshore hagfish (*E. burgeri*), Harada et al. (2007) found that hagfish were retained by holes with perimeters that were about two-thirds of their girth. Our results suggest that hagfish can get through slits that

are considerably narrower and this is probably due to the fact that the slit was essentially unconstrained in the dimension perpendicular to its narrowest aspect, allowing for changes in the body's cross-sectional shape. However, because of the placement of the camera above the slit, we were unable to investigate dimensional changes in the vertical axis of the body.

Our results support the hypothesis that the SCS plays a role in the hagfish's ability to maneuver through narrow spaces by allowing for blood to pool in the trailing region. It is evident, both quantitatively and qualitatively, that fluid is forced posteriorly as a hagfish moves head first through a narrow space. Moreover, the continuity of the sinus along the length of the animal allows for redistribution in either direction, and the mechanism appears to be the same whether the animal moves head first or tail first. Indeed, the trends observed in an Atlantic hagfish moving head first through the slit were similar to those observed in another Atlantic hagfish moving tail first; in both cases, the width of the trailing region increased over time, while the width of the leading region remained relatively constant or decreased. If hagfish were more turgid (i.e. if blood within the SCS were pressurized), it would be more difficult for them to push through a narrow opening, because they would immediately be resisted by the increase in pressure on the trailing side. Flaccidity therefore serves to delay an increase in pressure on the trailing side, and the degree of flaccidity will affect how much blood the trailing side can accommodate. There are, of course, limits to such a system, as more and more fluid is retained in a continuously shrinking trailing side. In an idealized cylindrical model of a hagfish, the diameter of the trailing side approaches infinity as its length approaches zero (Fig. S2).

In many of the trials, swelling was dramatic in the trailing end, but obviously never rose as quickly as predicted by the cylindrical model. There are two possible reasons why. The first is that hagfish skin is not infinitely compliant (as the skin in the model is), and when the trailing end becomes turgid with blood from the leading end, further movement is opposed by the buildup of pressure. Indeed, many of the hagfish we observed proceeded through the slit up to a point and then stopped, appearing to be stuck. In these cases, a buildup of pressure in the trailing end was likely the cause. Another factor to consider is the tapered nature of the hagfish body. While they are approximately circular in cross-section near their middle, hagfish are laterally flattened at the caudal end, and their cross-sectional area is also considerably smaller. This change in shape and area should allow for pressurized blood in the trailing end to move forward, resulting in a release of built-up pressure. Another possibility is that blood is transported from the trailing to leading side via the central circulation through the action of the cardinal and caudal hearts. Indeed, a role for the central circulation is consistent with our observations of head swelling in a hagfish that appeared to be momentarily stuck in the slit (Fig. 3). Swelling may have arisen from the accumulation of arterial blood actively pumped to the head and the simultaneous clamping of the SCS. Alternatively, swelling may have reflected the slow leaking of blood forward within the SCS.

Our results clearly show that hagfish squeezing through tight openings experience a substantial redistribution of blood within a flaccid SCS, but there may be other advantages to flaccidity. The loose skin of hagfishes may be useful for minimizing strain on the skin during maneuvers that require extreme body flexibility, such as knot-tying (Clark et al., 2016). Flaccidity might also have implications for feeding. One possibility is that it evolved to accommodate gut expansion in deep-sea environments where food availability can change from scarce to abundant with the arrival of a single large carcass. If this is the case, hagfish may regularly become

trapped within carcasses due to the bulk of the ingested food. As oxygen levels are likely to be very low inside carcasses, this scenario may have been one of the factors selecting for the extreme hypoxia tolerance seen in hagfishes (Cox et al., 2011; Gillis et al., 2015).

Behavior

The results of this study reveal that hagfishes use a number of behaviors to move their bodies through narrow openings. Both species are capable of an almost 180 deg bend of the prebranchial region, and this is supported by Wright et al. (1998), who report that the lingual cartilage is similar to the notochord in flexibility. Head flexibility appeared to be far greater in the dorsoventral direction, which is consistent with the organization of the cranial cartilage into longitudinal bars that are thinner dorsoventrally than they are laterally (Wright et al., 1998). For this reason, head bends were often executed after the animal had turned on its side, with dorsal and ventral surfaces in contact with the walls of the slit. Tooth plate eversion was commonly associated with head bends in both species. This may have been an attempt to grasp onto the walls of the enclosure and pull themselves through the slit. Eversion of the tooth plate may also allow the head to become thinner such that it can be squeezed through an especially tight space.

Wagging was seen in both species and resulted in slow and incremental progress through the slit. It is likely that hagfish initiate this motion by contracting the muscles on one side of the body, causing the body to bend laterally toward that side. Bending leads to compression on the concave side of the bend, and lengthening on the convex side. The new length on the convex side must then slide relative to the wall of the slit it abuts. If the sliding can be biased in the direction of desired motion, perhaps by asymmetrical forces exerted by contracting muscles, or by inertial forces exerted by the part of the hagfish that is free to wag, then rocking back and forth in this way can result in slow but steady progress through tight openings.

Lacking limbs or fins, hagfishes employ a number of ways to brace themselves so they can push or pull their body through narrow openings. In the wide loop behavior, the hagfish pressed its body against the walls of the slit, which typically served to oppose the tension needed to pull the anterior portion of the body back into the enclosure. Hagfishes also formed tight loops with the trailing portion of their body to accomplish this same outcome. Tight loops were also formed with the leading portion of the body once a sufficient length of the body had progressed through the slit. These loops were then pressed against the leading side of the enclosure, allowing the portion in the slit to be loaded in tension and pulled forward. In this way, tight loops were used to aid in retreat as well as forward progress. These types of bracing behaviors are similar to the knotting behavior used by hagfishes in order to gain leverage while tearing away pieces of food from carcasses, as well as during escape maneuvers (Jensen, 1966). In the present study, knotting behavior was not observed, although looping could be considered a simplified version of knotting.

The 'gliding' ability of hagfishes appears similar to a type of non-undulatory 'crawling' locomotion exhibited by lampreys when moving through tight spaces, as well as by snakes (Archambault et al., 2001; see Movie 2 for a demonstration of gliding). In contrast to swimming, crawling is achieved via a solitary wave of right and left muscle contractions. This muscle activity appears to be localized to the part of the body near the bend, and propagates caudally, allowing for forward motion (Archambault et al., 2001). Further investigation is needed to determine whether gliding in the hagfish is driven by the same mechanism as crawling in the lamprey. It is interesting that almost all of the behaviors observed involved a

bend of the body around the walls of the slit. Thus, although these behaviors have characteristics that make them unique, they may all be different manifestations of an ability to brace against a solid support to load the body in compression or tension as needed.

Conclusions

The results of this study confirm that hagfishes are able to pass through narrow openings, and regularly do so through openings that are less than half as wide as the widest part of their body. Our results also show that the flaccid nature of hagfish skin allows for substantial and rapid redistributions of blood within the subcutaneous sinus as hagfish transit narrow openings. Additionally, our results demonstrate that hagfishes employ a number of behaviors while maneuvering through narrow spaces. These behaviors include, but are not limited to, bracing behaviors and a form of non-undulatory crawling locomotion similar to that seen in snakes and lampreys. The ability described here may be an important part of the hagfish lifestyle, which involves entering narrow burrows and crevices for protection as well as entering carcasses for feeding.

Acknowledgements

We would like to thank Ian Moore for helping to design and building the adjustable slit chamber, as well as staff from the Bamfield Marine Sciences Centre and the Shoals Marine Laboratory for help acquiring specimens for this study. We would also like to acknowledge the contributions of four Cornell undergraduates (Charlotte Leape, Andy Wu, Alexandra Nagele, Sarah Wright) and Andy Turko at the Shoals Marine Laboratory, who collected and analyzed the preliminary data that led to this study. Taylor Hicks-Howard assisted with filming trials.

Competing interests

The authors declare no competing or financial interests.

Author contributions

C.R.F. and D.S.F. designed the study. C.R.F. conducted the experiments and analyzed the data. C.R.F. and D.S.F. wrote the paper.

Funding

This work was supported by a Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery grant to D.S.F.

Supplementary information

Supplementary information available online at <http://jeb.biologists.org/lookup/doi/10.1242/jeb.151233.supplemental>

References

- Adam, H. (1960). Different types of body movement in the hagfish, *Myxine glutinosa* L. *Nature* **188**, 595–596.
- Archambault, P. S., Deliaquina, T. G. and Orlovsky, G. N. (2001). Non-undulatory locomotion in the lamprey. *Neuroreport* **12**, 1803–1807.
- Clark, A. J., Crawford, C. H., King, B. D., Demas, A. M. and Uyeno, T. A. (2016). Material properties of hagfish skin, with insights into knotting behaviors. *Biol. Bull.* **230**, 243–256.
- Clifford, A. M., Guffey, S. C. and Goss, G. G. (2014). Extrabranchial mechanisms of systemic pH recovery in hagfish (*Eptatretus stoutii*). *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **168**, 82–89.
- Clifford, A. M., Zimmer, A. M., Wood, C. M. and Goss, G. G. (2016). It's all in the gills: evaluation of O₂ uptake in Pacific hagfish refutes a major respiratory role for the skin. *J. Exp. Biol.* **219**, 2814–2818.
- Cox, G. K., Sandblom, E., Richards, J. G. and Farrell, A. P. (2011). Anoxic survival of the Pacific hagfish (*Eptatretus stoutii*). *J. Comp. Physiol. B.* **181**, 361–371.
- Davison, W., Baldwin, J., Davie, P. S., Forster, M. E. and Satchell, G. H. (1990). Exhausting exercise in the hagfish, *Eptatretus cirrhatus*: the anaerobic potential and the appearance of lactic acid in the blood. *Comp. Biochem. Physiol. A Physiol.* **95**, 585–589.
- Fernholm, B. (1974). Diurnal variations in the behaviour of the hagfish, *Eptatretus burgeri*. *Mar. Biol.* **27**, 351–356.
- Forster, M. E. (1997). The blood sinus system of hagfish: Its significance in a low-pressure circulation. *Comp. Biochem. Physiol. A Physiol.* **116**, 239–244.
- Forster, M. E., Davison, W., Satchell, G. H. and Taylor, H. H. (1989). The subcutaneous sinus of the hagfish, *Eptatretus cirrhatus* and its relation to the central circulating blood volume. *Comp. Biochem. Physiol. A* **93**, 607–612.
- Forster, M. E., Russell, M. J., Hambleton, D. C. and Olson, K. R. (2001). Blood and extracellular fluid volume in whole body and tissues of the Pacific hagfish, *Eptatretus stoutii*. *Physiol. Biochem. Zool.* **74**, 750–756.
- Gillis, T. E., Regan, M. D., Cox, G. K., Harter, T. S., Brauner, C. J., Richards, J. G. and Farrell, A. P. (2015). Characterizing the metabolic capacity of the anoxic hagfish heart. *J. Exp. Biol.* **218**, 3754–3761.
- Glover, C. N., Bucking, C. and Wood, C. M. (2011). Adaptations to *in situ* feeding: novel nutrient acquisition pathways in an ancient vertebrate. *Proc. R. Soc. B Biol. Sci.* **278**, 3096–3101.
- Glover, C. N., Blewett, T. A. and Wood, C. M. (2015). Novel route of toxicant exposure in an ancient extant vertebrate: nickel uptake by hagfish skin and the modifying effects of slime. *Environ. Sci. Technol.* **49**, 1896–1902.
- Gustafson, G. (1935). On the biology of *Myxine glutinosa* L. *Ark. Zool.* **28**, 1–8.
- Harada, M., Tokai, T., Kimura, M., Hu, F. and Shimizu, T. (2007). Size selectivity of escape holes in conger tube traps for inshore hagfish *Eptatretus burgeri* and white-spotted conger *Conger myriaster* in Tokyo Bay. *Fisheries Sci.* **73**, 477–488.
- Jensen, D. (1966). The hagfish. *Sci. Am.* **214**, 82–90.
- Lesser, M. P., Martini, F. H. and Heiser, J. B. (1997). Ecology of the hagfish, *Myxine glutinosa* L. in the Gulf of Maine I. Metabolic rates and energetics. *Ecology* **208**, 215–225.
- Lim, J. L. and Winegard, T. M. (2015). Diverse anguilliform swimming kinematics in Pacific hagfish (*Eptatretus stoutii*) and Atlantic hagfish (*Myxine glutinosa*). *Can. J. Zool.* **93**, 213–223.
- Martini, F. H. (1998). The ecology of hagfishes. In *The Biology of Hagfishes* (ed. J. M. Jørgensen, J. P. Lomholt, R. E. Weber and H. Malte), pp. 61–64. London: Chapman & Hall.
- Martini, F. H. and Heiser, J. B. (1989). Field observations on the Atlantic hagfish, *Myxine glutinosa*, in the Gulf of Maine. *Am. Zool.* **29**, 38A.
- Martini, F., Lesser, M. and Heiser, J. B. (1997). Ecology of the hagfish, *Myxine glutinosa* L., in the Gulf of Maine: II. Potential impact on benthic communities and commercial fisheries. *J. Exp. Mar. Biol. Ecol.* **214**, 97–106.
- McCarthy, J. E. and Conte, F. P. (1966). Determination of the volume of vascular and extravascular fluid in the Pacific hagfish, *Eptatretus stoutii* (Lockington). *Am. Zool.* **6**, 605.
- Schultz, A. G., Guffey, S. C., Clifford, A. M. and Goss, G. G. (2014). Phosphate absorption across multiple epithelia in the Pacific hagfish (*Eptatretus stoutii*). *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **307**, R643–R652.
- Strahan, R. (1963). The behaviour of myxinoids. *Acta Zool.* **44**, 1–30.
- Wright, G. M., Keeley, F. W. and DeMont, M. E. (1998). Hagfish cartilage. In *The Biology of Hagfishes* (ed. J. M. Jørgensen, J. P. Lomholt, R. E. Weber and H. Malte), pp. 160–169. London: Chapman & Hall.
- Zintzen, V., Roberts, C. D., Anderson, M. J., Stewart, A. L., Struthers, C. D. and Harvey, E. S. (2011). Hagfish predatory behaviour and slime defence mechanism. *Sci. Rep.* **1**, 1–6.