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Hagfish slime ecomechanics: testing the gill-clogging hypothesis

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Summary

Hagfish are able to produce substantial amounts of slime when harassed, but the precise ecological function of the slime is unclear. One possibility is that the slime acts as a defence against gill-breathing predators, whose gills may become entangled with the slime's mixture of mucins and fibrous threads during an attack. We previously demonstrated that hagfish slime does not bind water tightly, but instead behaves like a fine sieve that slows water down *via* viscous entrainment. These properties are consistent with the gill-clogging hypothesis, which we tested here by quantifying the effects of hagfish slime on water flow through an artificial gill model and real fish gills. Our results indicate that the slime is capable of clogging gills and increasing the resistance that they present to the flow of water. We also characterized the behaviour of slime release from live hagfish and the effect of convective mixing on the formation of slime *in vitro*. Our observations show that exudate is locally released from the slime glands as a coherent jet and that hagfish do not appear to use their slime as a protective envelope. We found that convective mixing between the exudate and seawater is necessary for proper slime formation, but excessive mixing leads to the slime's collapse. We suggest that the loose binding of water by the slime may be an optimal solution to the problem of delivering an expanding jet of flow-inhibiting material to the gills of would-be predators.

Key words: biomechanics, slime, hagfish, gill resistance, predator defence, *Eptatretus stoutii*.

Introduction

An agitated hagfish can release an enormous amount of slime from the numerous slime glands lining its body (Ferry, 1941; Strahan, 1959; Downing et al., 1981a; Martini, 1998). In a previous paper (Fudge et al., 2005), we demonstrated that hagfish slime is an extremely dilute assemblage of mucins and seawater held together by a network of fine protein threads. Measurements of water egress from hagfish slime indicated that it is not a coherent material that immobilizes water, but instead a fine sieve that slows water down *via* viscous entrainment. These experiments, along with the many papers on hagfish slime by the late Elizabeth Koch and other researchers (Downing et al., 1981b; Fernholm, 1981; Koch et al., 1991; Fudge et al., 2003), answer several questions about slime morphology and mechanics. However, fundamental questions about the function of the slime persist.

The list of common hagfish predators includes certain species of seabirds, pinnipeds and cetaceans but exhibits a conspicuous lack of fishes (Martini, 1998; Fudge, 2001). This fact has led researchers to speculate that the slime functions as a defence against gill-breathing predators by clogging the gills (Fernholm, 1981; Martini, 1998). The mechanical data we report in Fudge et al. (2005) on slime formed *in vitro* do not contradict this hypothesis. We found that the threads within hagfish slime are extremely effective at catching on projections and making continuous connections across substantial distances. While the slime does not possess the coherence of a solid material, it is capable of trapping large volumes of water *via* viscous entrainment. From these data it is not difficult to imagine that the slime would attach easily to gills and seriously impair respiratory flow across them. Here, we test the gill-clogging hypothesis by measuring the effect of hagfish slime on water flow through an artificial gill analogue and real gills in isolated fish heads and demonstrate that the slime has dramatic effects on flow at physiological water pressures. We also provide information from high-speed video trials on the details of slime release and formation by free-swimming hagfish.

Materials and methods

Experimental animals

Pacific hagfish (*Eptatretus stoutii* Lockington) were collected from Barkley Sound in British Columbia with the assistance of local staff at the Bamfield Marine Sciences Centre. Traps baited with herring were set at a bottom depth

Fig. 1. Apparatus for measuring the effects of hagfish slime on flow rate through and resistance across an artificial gill analogue, which consisted of a piece of test tube brush within polyvinyl tubing. Scale bar, 10 mm.

of approximately 100 m and left overnight. Hagfish were transported to the University of British Columbia, transferred to a 200-litre holding aquarium of cold seawater (9°C, 34‰) and given a monthly diet of squid in accordance with UBC Committee on Animal Care guidelines (protocol A2-0003).

Slime effects on gills

We modelled a gill-breathing predator with two versions of a custom-built 'slime vacuum' that used a siphon to create water flow over an artificial gill analogue and real fish gills. The artificial gills consisted of a 40 mm-long piece of test tube brush inserted inside a 165 mm segment of thick, clear polyvinyl tubing (20 mm inner diameter). The brush was positioned approximately 40 mm from one end of the tube and fitted snugly inside (Fig. 1). The heads from freshly dead China rockfish (*Sebastes nebulosus* Ayres) from a local supermarket provided real gills. Fish had a mean (± s.d.) body mass of 577±118 g and mouth gape area of 580±40 mm^2. The head was severed from the body just anterior to the dorsal fin, and any remaining fins and spines were removed. The isolated fish head was housed within a piece of PVC pipe (150 mm length, 100 mm diameter) fitted with a sheet of extra-heavy dental dam (152×152 mm; Hygenic Corp., Akron, OH, USA) at one end. The head was pushed from the inside of the pipe through a small hole in the dental dam to a point just posterior of the eyes and anterior to the gill operculum (Fig. 2A,B). Heads that were too large to fit inside the pipe had a dorsal portion of muscle removed after severing. A wire oval ring was used to prop the mouth open and hold the tongue down, and small corks were positioned at the front of each opercular cavity to slightly open the opercular flaps. Rubber bands and string wrapped around the edge of the dental dam encircling the head ensured a tight seal. A screw cap closed the other end of the PVC pipe, and a hole in the side provided a passage for water flow out of the pipe.

A series of tubing formed the rest of both versions of the slime vacuum. Each gill setup was connected to polyvinyl tubing (1.52 m long, 8 mm inner diameter) followed by a short segment of rubber tubing (225 mm long, 6.6 mm inner diameter), which could be clamped to restrict water flow. Screw adapters joined the consecutive pieces. Experiments were held in a 20-litre aquarium of cold artificial seawater (8–10°C, 32‰). For artificial gill trials, the gill setup was attached to a plastic rod and held in position underwater by clamping the rod to the rim of the aquarium. In fish head trials, the apparatus was kept in place at one end of the aquarium with bricks. A bucket on a top-loading balance placed below the aquarium collected the siphoned water. The free end of the rubber tubing rested in a small overflowing beaker positioned directly above the bucket, reducing the incidence of air bubbles within the tubing. All trials had a starting pressure head of 3.48 kPa, which was determined from the vertical distance between the water level in the aquarium and the top of the overflowing beaker.

A live hagfish was gently placed in the aquarium, and 40–90 s after the start of the siphon the hagfish was pinched on the tail with padded forceps to induce sliming (Fudge et al., 2005). A video camera and VCR recorded the display on the top-loading balance for later review. Outputs from an external timer and a second camera filming a view of the aquarium were recorded simultaneously on to the same tape so that data from the balance could be correlated with events in the tank and time-stamped. Recording was stopped after the balance reached its upper limit (3000 g).

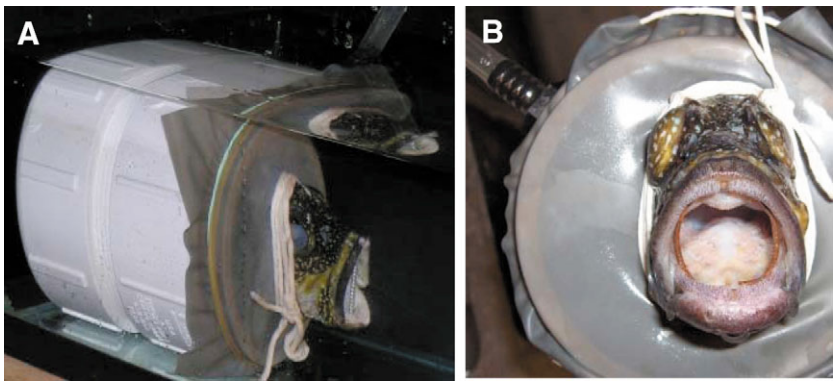

Fig. 2. (A) Apparatus for measuring the effect of hagfish slime on flow through fish gills, consisting of a severed rockfish head with its mouth propped open and housed in PVC piping. (B) Front view.

Water flow rates were determined from the change in mass of water in the bucket and the time interval between mass measurements. To adjust for the decreasing pressure head as water flowed from the aquarium, we calculated standardized water flow rates (ml s^{-1} kPa^{-1}) over time by dividing each flow rate measurement by the pressure head at the time of the measurement. All subsequent calculations involving flow rates used these standardized values. The siphon system consisted of two components in series that contributed to the total resistance (R) that the system presented to the flow of water: the gills (test tube brush or fish head gills) and the narrow tubing connected to the gills. That is,

R_{system}=R_{gills}+R_{tube}. Measurements of flow rates with and without the gills present were used to calculate gill resistance relative to the rest of the siphon, and the pressure drop across the gills. For the artificial gill setup, the test tube brush was removed from its thick polyvinyl tube housing to achieve a gill-less condition. In the fish head setup, the gills were removed by pulling off the dental dam holding the fish head and removing the entire head from the PVC pipe.

High-speed video of slime release

Hagfishes were transferred from their holding tanks to a 20-litre aquarium filled with unfiltered, cold (9°C) seawater. Sliming was initiated by a quick pinch on the body using long forceps. Digital video of the sliming event was captured at 125 frames s^{-1} using a Redlake MotionScope digital high-speed video camera (Redlake-DuncanTech, Auburn, CA, USA). Close-ups of slime release from glands were filmed by constraining hagfish in a specially designed tube that the hagfish voluntarily entered in their holding tank. The 50 mm-diameter tube was 300 mm long and had a window cut in it that allowed us to focus in on a single gland with a 43.5-mm fish-eye macro zoom lens. The window also allowed us to stimulate the skin of the hagfish with forceps or a mild electrical shock (the latter worked best) to induce the sliming response. Two trials using constrained hagfish were clear enough and at the proper orientation to allow us to calculate the velocity of slime exudate expulsion from the slime gland. For velocity measurements, time was measured by the number of frames, and distance was calibrated using the checkerboard pattern on the tubing that held the hagfish.

Convective mixing effects and slime collapse

In the high-speed video trials using constrained hagfish, we observed that exudate released by the hagfish did not hydrate fully, as indicated by it remaining opaque and sinking to the bottom of the aquarium. This observation led us to test the hypothesis that some convective mixing is required for proper slime hydration and formation. To test this hypothesis, we conducted two additional kinds of video trials in which we filmed the introduction of freshly collected slime exudate into still seawater either using a spatula or *via* injection with a syringe fitted with a shortened 18-gauge needle. The capture rate for these trials was 60 frames s^{-1}.

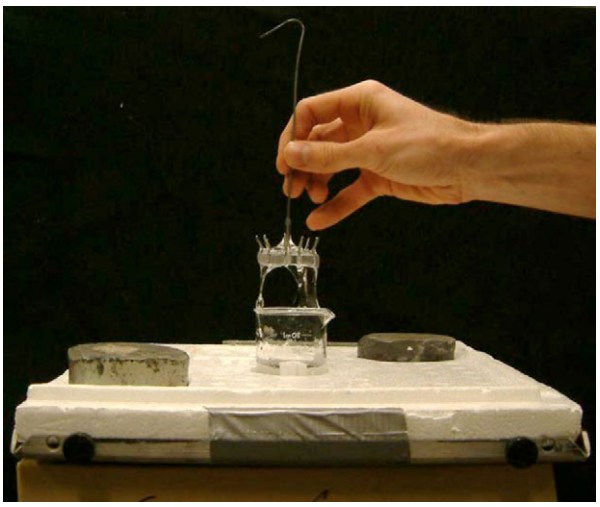

Fig. 3. Apparatus for measuring the removable mass of slime produced from mixing slime exudate in seawater, consisting of a 50 ml beaker mounted on a rotary shaker. A plastic disk fitted with radial spikes hanging on a wire was used to collect removable mass.

We also assessed the effect of mixing on slime formation using a 'removable mass' assay modified from Koch et al. (1991). A small volume (0.12 ml) of slime exudate stabilized in a high osmotic strength buffer (Downing et al., 1984) was injected into 50 ml of artificial seawater on a shaker table set at 200 revs min^{-1}. After shaking for a precise amount of time (0, 10, 20, 40, 80, 160, 320, or 640 s), a custom hook, which was placed in the beaker before the addition of slime, was removed (Fig. 3). Removable mass was quantified by weighing the hook and adherent slime and subtracting the mass of the hook.

Results

Slime increases gill resistance by one to three orders of magnitude

The relationship between the pressure head, flow rate and resistance in the siphon system can be described by a version of Ohm's Law for fluid flow:

$$\Delta P = \dot{Q}R\,, \tag{1}$$

where ΔP is the pressure head, $\dot{Q}$ is the flow rate, and R is the resistance. Standardized water flow rates, which we will call $\dot{Q}_p$, are given by $\dot{Q}/\Delta P$. Consequently, Eqn 1 can be written in terms of $\dot{Q}_p$, and then rearranged to give:

$$\dot{Q}_p = 1 / R_{system} = 1 / (R_{gills} + R_{tube})\,, \tag{2}$$

where R_{gills} is the resistance of the gills and R_{tube} is the resistance of the tubing. Using measurements of water flow rates with and without the gills present, we determined the relative magnitudes of R_{gills} and R_{tube}. The relative resistance of the gills is given by:

$$R_{gills,rel} = (\dot{Q}_{p,no\ gills} - \dot{Q}_{p,with\ gills}) / \dot{Q}_{p,no\ gills} \tag{3}$$

and the relative resistance of the tubing is simply:

$$R_{tube,rel} = 1 - R_{gills,rel}\,. \tag{4}$$

For the artificial gills, the mean flow rate without gills was 8.3 ml s^{-1} kPa^{-1}, while the mean rate with gills present was 7.9 ml s^{-1} kPa^{-1}; thus, $R_{gills,rel}$ is 0.044±0.0037 (mean ± s.d.; N=3), and $R_{tube,rel}$ is 0.956. That is, the tube resistance is approximately 20 times greater than the artificial gill resistance, which accounts for only 4% of the total resistance in an unslimed system. Because the rockfish heads used in the fish head trials varied in size, the relative resistance of the real fish gills was more variable, ranging from 0.061 to 0.15 (mean ± s.d., 0.11±0.047; N=3). The pressure drop across the gills

Fig. 4. Hagfish slime was difficult to remove from the gills after it was drawn into the rockfish's mouth.

was found by multiplying the relative gill resistance by the mean pressure head in the trial. Mean pressures (± s.d.) across the artificial gills (0.17±0.014 kPa; N=3) and real fish gills (0.35±0.16 kPa; N=3) were comparable to pressures found during normal ventilation in other fishes (e.g. white sucker *Catostomus commersoni*, 0.2 kPa; carp *Cyprinus carpio*, 0.5 kPa) (Saunders, 1961).

Flow rate data from the sliming trials can be used to determine how hagfish slime affects gill resistance, if we assume that the gills intercept all of the slime so that tube resistance remains constant throughout the trial. This assumption is reasonable, considering our observations on the slime vacuum's suction of released slime: most of the slime was stopped at the brush in the gill model or inside the mouth of the fish head. In some instances, slime protruded from the fish's mouth at the end of the trial. Inspection of the slimed gills revealed mucus and threads coating and caught up in the gills (Fig. 4). So, assuming that all changes in system resistance are due to changes in gill resistance, we can derive an expression for the absolute resistance of the gills for a given flow rate, at any time during the trial. First, we must calculate the absolute magnitude of the constant R_{tube}:

$$R_{tube} = (R_{tube,rel})(R_{system}) . \quad (5)$$

We know $R_{tube,rel}$ (Eqn 4), and, because we assume that R_{tube} remains constant, we can calculate its absolute magnitude using data on the pre-slime conditions in the system. Rearranging Eqn 2 (Ohm's Law) and indicating initial conditions before the gills are exposed to slime (denoted by the zero subscript) gives:

$$R_{system,0} = 1 / \dot{Q}_{p,0} . \quad (6)$$

We define R_{tube} as the constant C, and substitute Eqn 6 into Eqn 5 to get the constant value:

$$C = (R_{tube,rel}) / \dot{Q}_{p,0} . \quad (7)$$

Equation 2 can now be written in terms of the flow rate and the gill resistance as functions of time (t):

$$\dot{Q}_p(t) = 1 / [R_{gills}(t) + C] . \quad (8)$$

Rearranging gives:

$$R_{gills}(t) = 1 / \dot{Q}_p(t) - C , \quad (9)$$

which we can use to calculate gill resistance during the experiment from the flow rate data.

All trials showed slowed water flow and an increase in gill resistance following slime release (Table 1). Flow rate and resistance data are presented as normalized values, $\dot{Q}_{p,norm}$ and $R_{gills,norm}$, obtained by dividing $\dot{Q}_p(t)$ and $R_{gills}(t)$ by their mean pre-slime values. The start of slime suction, as observed from

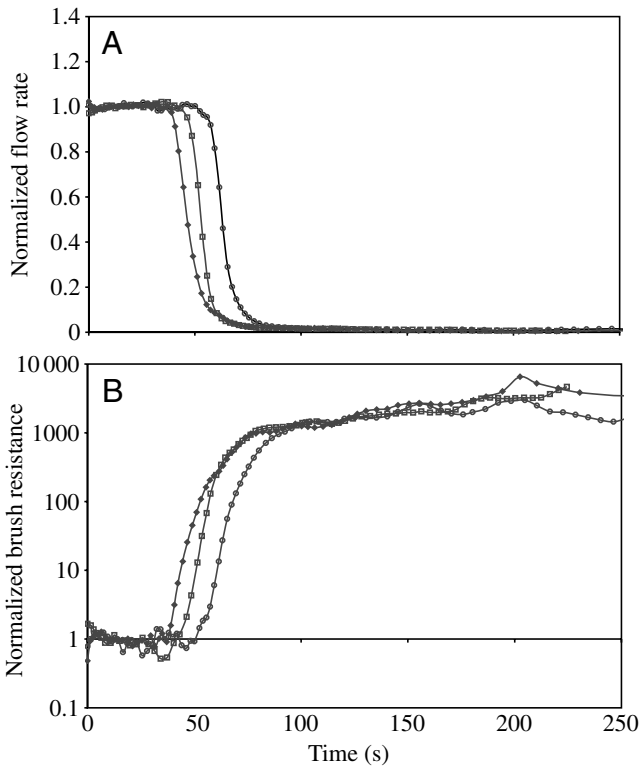

Fig. 5. The effects of hagfish slime on (A) water flow rates and (B) brush resistance in the artificial gill analogue. Slime release occurred at 40–60 s; three trials are shown separately, and the data have been normalized to their pre-slime values. Note the log scale for normalized resistance.

Table 1. *Effects of hagfish slime on an artificial gill analogue and real fish gills*

	Mean $\dot{Q}_{p,norm}$		Mean $R_{gills,norm}$	
	Before slime	After slime	Before slime	After slime
Artificial gills				
Trial 1	1.0	0.015	1.0	1900
Trial 2	1.0	0.013	1.0	2000
Trial 3	1.0	0.014	1.0	2500
Fish head gills				
Trial 1	1.0	0.26	1.0	20
Trial 2	1.0	0.12	1.0	130

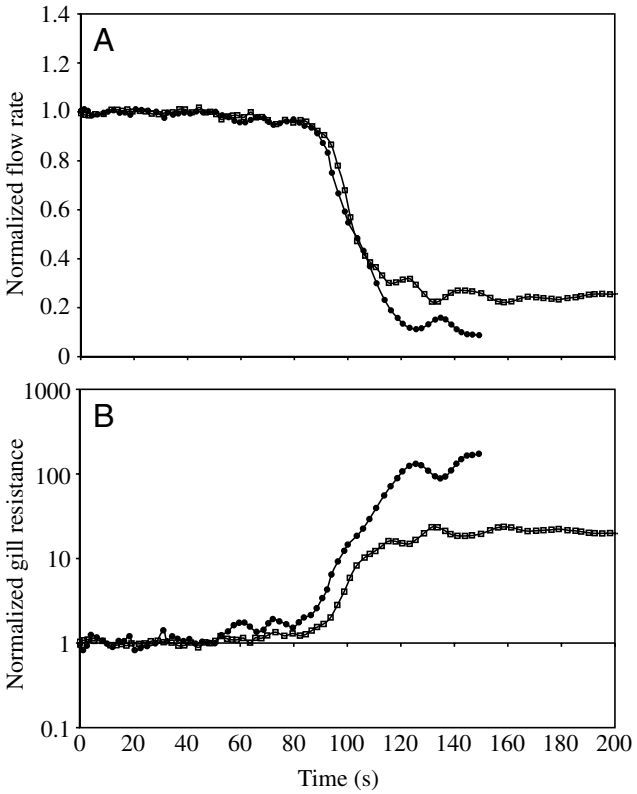

Fig. 6. The effects of hagfish slime on (A) water flow rates and (B) gill resistance in the gills of an isolated rockfish head. Slime release occurred at ~95 s; results from two fish heads are shown separately, and the data have been normalized to their pre-slime values. Note the log scale for normalized resistance.

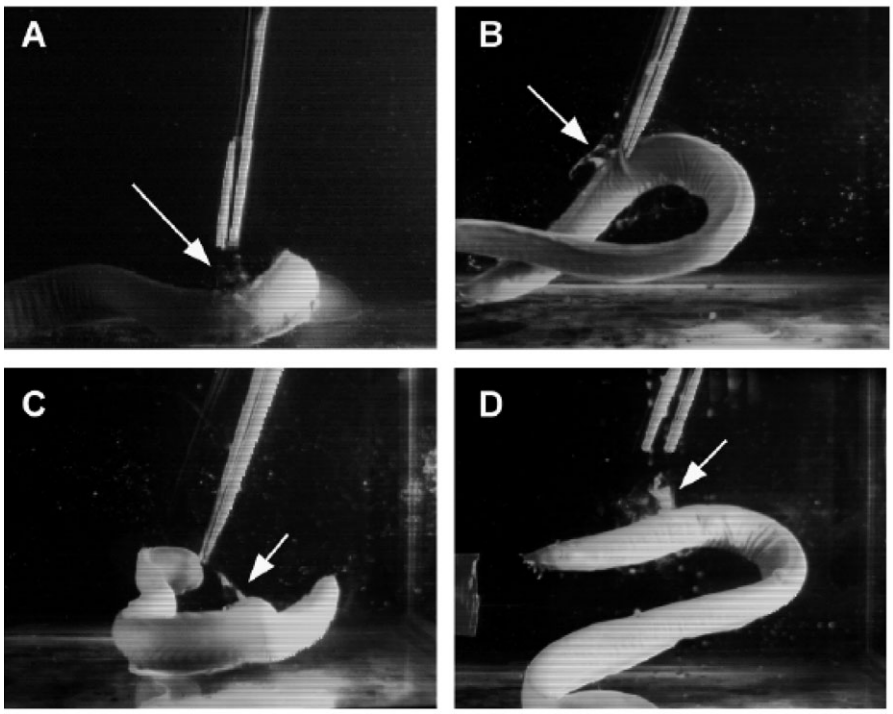

Fig. 7. (A–D) High-speed video images of the local release of slime exudate (arrows) from a hagfish after it has been pinched with forceps. A–D show different hagfish.

video recordings of the aquarium, corresponded well with abrupt changes in flow and resistance. Slime uptake into the artificial gill corresponded with a decrease in flow rate by a factor of 70–80 (Fig. 5A) and an increase in resistance of approximately three orders of magnitude (Fig. 5B). Two trials with the fish head setup were usable for data analysis. In these trials, slime caused the flow rate to decrease by a factor of 4–8 (Fig. 6A) and the gill resistance to increase by one to two orders of magnitude (Fig. 6B).

Slime is locally and forcefully released

Filming hagfish sliming at 125 frames s^{-1} revealed that release of exudate occurs only from glands near the point of contact, as opposed to global release from all of the glands (Fig. 7A–D). These trials also suggested that exudate appears to be forcefully ejected from the slime gland, as opposed to simply oozing out (Fig. 8). To confirm this result, we filmed slime release from constrained hagfish, which allowed us to focus in on single slime glands. These trials clearly indicate that slime is indeed forcefully ejected from the glands (Fig. 9). The jet velocities measured in two different trials were 0.17 and 0.18 m s^{-1}.

Slime hydration requires convective mixing

Slime exudate introduced into still seawater by a spatula or syringe in the absence of mixing failed to form a full mass of hydrated slime. The exudate remained opaque in the water after slipping off the spatula (Fig. 10A) or being ejected from the syringe needle (Fig. 10B) and typically fell to the bottom of the tank. In removable mass trials, mixing duration had a positive effect on removable slime mass up to about 80 s, after which removable mass tapered off (Fig. 11). The minimal amount of hydrated slime produced from short periods of stirring corroborates the results from our spatula and syringe trials. These trials were conducted using slime exudate stabilized in a high osmotic strength buffer, which undoubtedly increased the hydration time of the slime compared with fresh exudate. While the time to peak hydration is therefore not applicable to slime release *in vivo*, the hump-shaped curve is still revealing about the evolution of slime structure and mechanics over time.

Discussion

We tested the hypothesis that hagfish slime functions to deter gill-breathing predators and found that the slime appears to be capable of clogging fish gills and impairing the flow of water through them. The effects of the slime

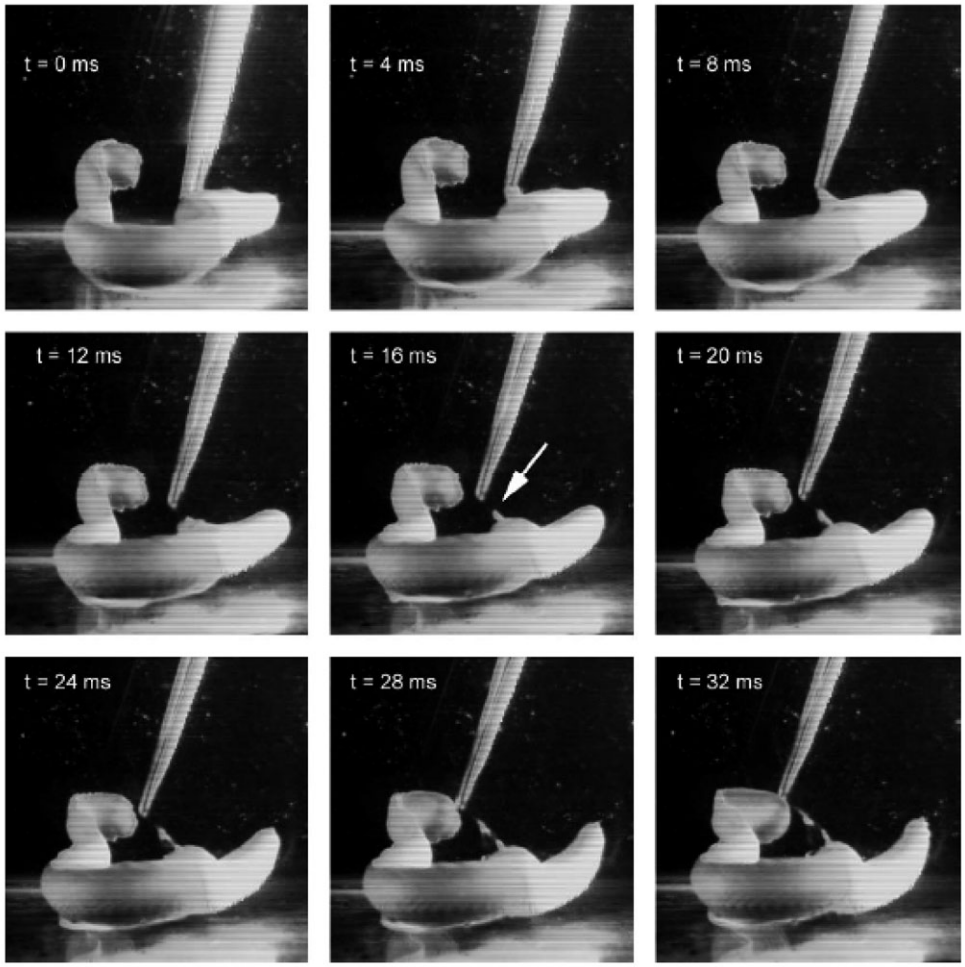

Fig. 8. High-speed video of a single slime gland demonstrates that slime exudate is released as a coherent jet.

on flow rates were apparent in each trial, but the magnitude of the response varied between trials. This is not entirely surprising as the amount of hagfish slime produced was probably variable among different trials. As an example, Fig. 6B shows that resistance at the gills of one fish head increased by a factor of 20, which is not a trivial effect; however, the gills of the second fish experienced a 100-fold increase. The slime effectively increased the resistance of a gill analogue and real gills, consequently slowing the passage of water through them; this result is consistent with the sieve model of hagfish slime structure and function presented in Fudge et al. (2005). The slime's greater effect on the artificial gill model is likely to be due to the multi-layered and densely packed bristles of the test tube brush, which would catch more slime than the single layer of wider-spaced gill rakers in the fish head.

Also, the smaller area of the model gill's opening compared with the open area of the fish mouth makes the model gills easier to block with a given amount of slime; the slime is more concentrated in this small area, and water flow is impaired to a greater extent.

For a live fish predator, sustained low water flow over the gills might lead to insufficient oxygen delivery and reduced gas exchange. Furthermore, the increase in diffusion distance

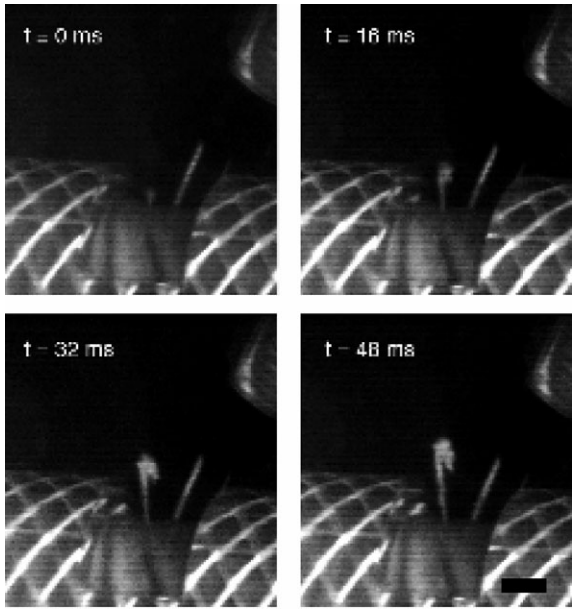

Fig. 9. Close-up of slime release from a single slime gland of a hagfish constrained in a tube with a window cut in it. These events were filmed at 125 frames s^{-1}, and the mean jet velocity was 0.17 m s^{-1}. Scale bar, 5 mm.

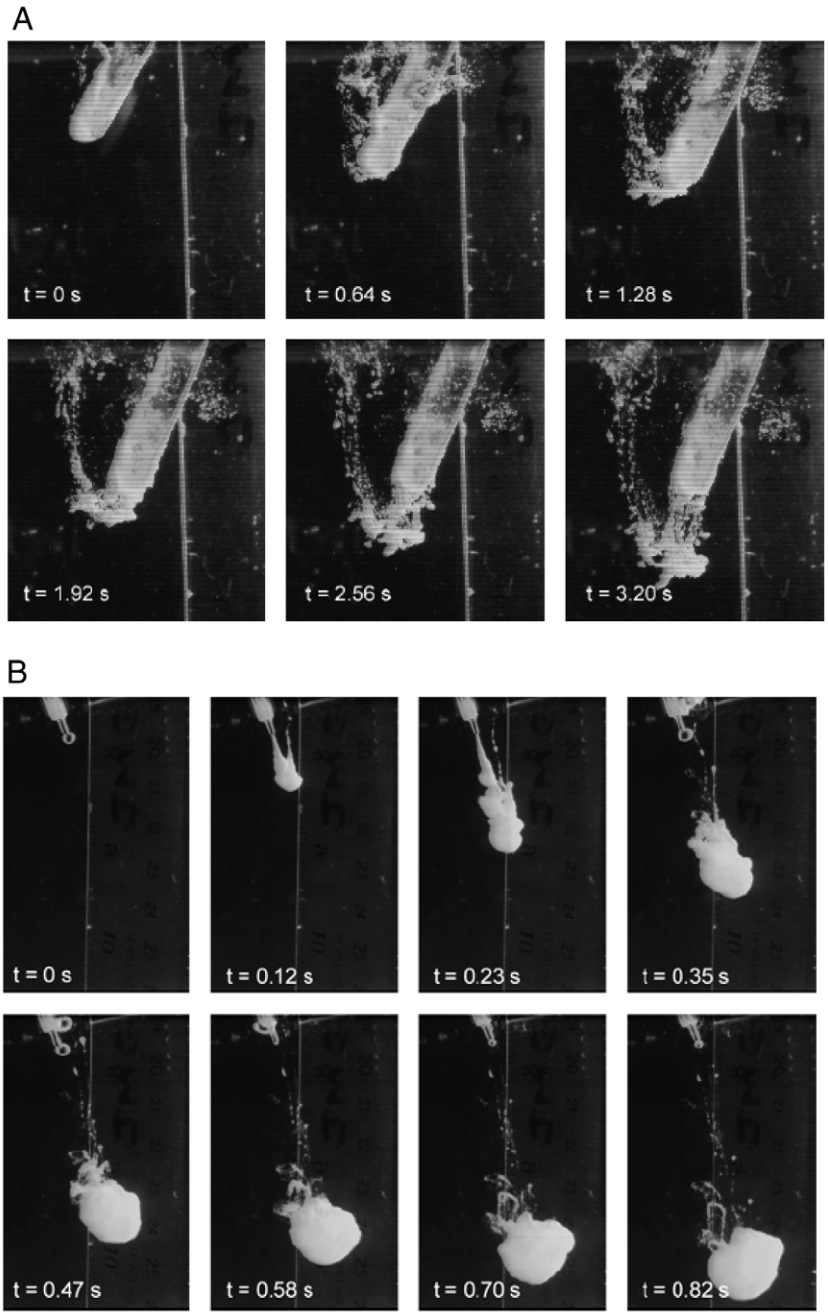

Fig. 10. Slime exudate introduced into still seawater from (A) a spatula or (B) injection from a syringe fails to hydrate as it does *in vivo*.

across the gills caused by a slime coating should also decrease gas exchange, as diffusion rates are inversely proportional to distance (Fick's Law) (Vogel, 2003). This hypothesis will be tested in future experiments using respirometry of live fish exposed to hagfish slime. The potential for suffocation through one or both of these mechanisms might discourage gill-breathing predators from preying on hagfish.

High-speed video of free-swimming hagfish revealed that they do not generally release slime and then hide within it (Fig. 12). The local release of exudate supports this idea; simultaneous slime release from all of the glands would likely be more effective at producing a mass of slime for an instant refuge. Hagfish have an ingenious behaviour, however, that implies that they do occasionally have to free themselves from their own slime. Covered in slime and facing eventual suffocation, a hagfish will tie its body in a knot and pass the knot toward its head to slough off the slime (Strahan, 1963; Martini, 1998). While not a protective shroud, the behaviour of slime release suggests that it may have a more active role in defending hagfish against predators. When pinched, slime glands near the region of contact respond by forcefully ejecting exudate as a coherent jet. It is possible that the combination of local and forceful release of slime is functionally important in 'targeting' the gills of an attacking fish predator.

To test this argument, we address here the mechanics of slime release in more detail. We develop a simple model of slime ejection, determining whether the muscular contraction of the gland capsule is sufficient to eject the exudate at the velocity observed or whether the surrounding myotomal muscle must also be recruited. The first thing we need to know is the pressure that the gland can generate. This can be calculated from the Law of Laplace for a sphere:

$$\sigma_{\text{sphere}} = pr / 2d \,, \tag{10}$$

where σ_{sphere} is the wall stress, p is the pressure, r is the radius and d is the wall thickness. Using a typical muscle stress of 200 kPa, a gland radius of 0.65 mm and a wall thickness of 45 mm (Lametschwandtner et al., 1986), we get a pressure inside the gland of 28 kPa, or about double the blood pressure of a mammal.

To calculate the velocity of the exudate as it exits the gland, we use the Hagen-Poiseuille equation for flow through a pipe:

$$\dot{Q} = \pi \Delta p a^4 / 8 \mu l \,, \tag{11}$$

where $\dot{Q}$ is flow, a is the radius of the pipe (45 μm), Δp is the pressure head, μ is the dynamic viscosity and l is the duct length. Since we already know the jet velocity (0.175 m s^{-1}) from high-speed video, we can use this equation to calculate the viscosity of the exudate. If it gives us a reasonable value, then we know that the muscular gland capsule is capable of ejecting the slime without help from the surrounding myotomal muscle. Rearranging the equation above, we get:

$$\mu = \pi \Delta p a^4 / 8 \dot{Q} l \,, \tag{12}$$

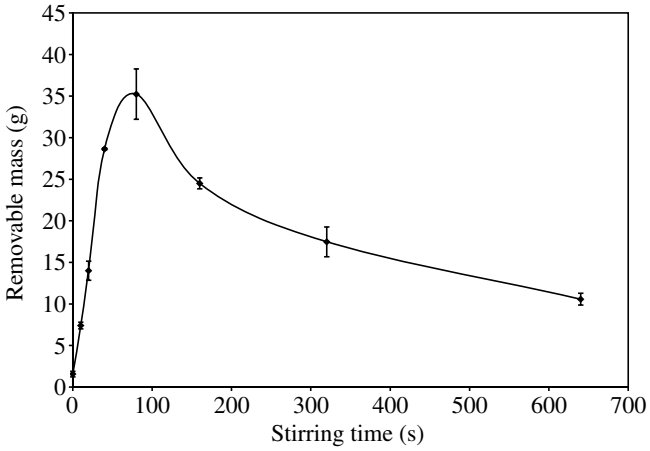

Fig. 11. The removable mass of slime plotted against stirring time demonstrates that stirring is required for proper slime hydration and cohesion and that excessive stirring eventually leads to slime collapse. Values are means ± s.e.m.

and with a pressure head of 28 kPa (from LaPlace), a pipe radius of 45 μm and a duct length of 500 μm (Spitzer and Koch, 1998), we get a viscosity of 0.08 Pa s, which is about 60 times the viscosity of seawater at 10°C and not an unreasonable value. If the calculation predicted a viscosity considerably less than water, then clearly we would need to invoke another source of pressure. Our estimates indicate, however, that the gland capsule can eject a fluid with a viscosity 60 times that of seawater at the velocities we have measured; thus, another mechanism, such as compression of the gland *via* contraction of nearby myotomal muscle, is not required to explain our data.

After exudate is discharged into seawater, convective mixing is essential for rapid hydration and full expansion of the slime. The Reynolds number (*Re*) of the exudate jet is informative on this point. Using the values for exudate viscosity and jet velocity that we calculated above, and the gland duct diameter, *Re* within the duct is ~0.1. Because flow immediately outside the duct is unlikely to differ much from the flow inside the duct, the *Re* indicates that the exudate jet is laminar. As a result, the exudate experiences very little mixing from inherent turbulence in the jet despite its seemingly forceful ejection. Also, given the relatively large size-scale of the slime, diffusion alone is insufficient to cause formation once the exudate is in seawater. In nature, convective mixing is likely fulfilled by the hagfish itself, as escape behaviours often include vigorous thrashing after slime release. While this requisite mixing appears at first to be a limitation, it may serve an important function: if expansion were faster, the slime would form closer to the slime gland pore. This could decrease the distance that the slime is shot and potentially even clog the gland pore. The laminar character of the exudate jet and the full formation of slime some time after release from the gland also support the idea that the jet is more important in the targeting of predator gills than other functions, such as mixing.

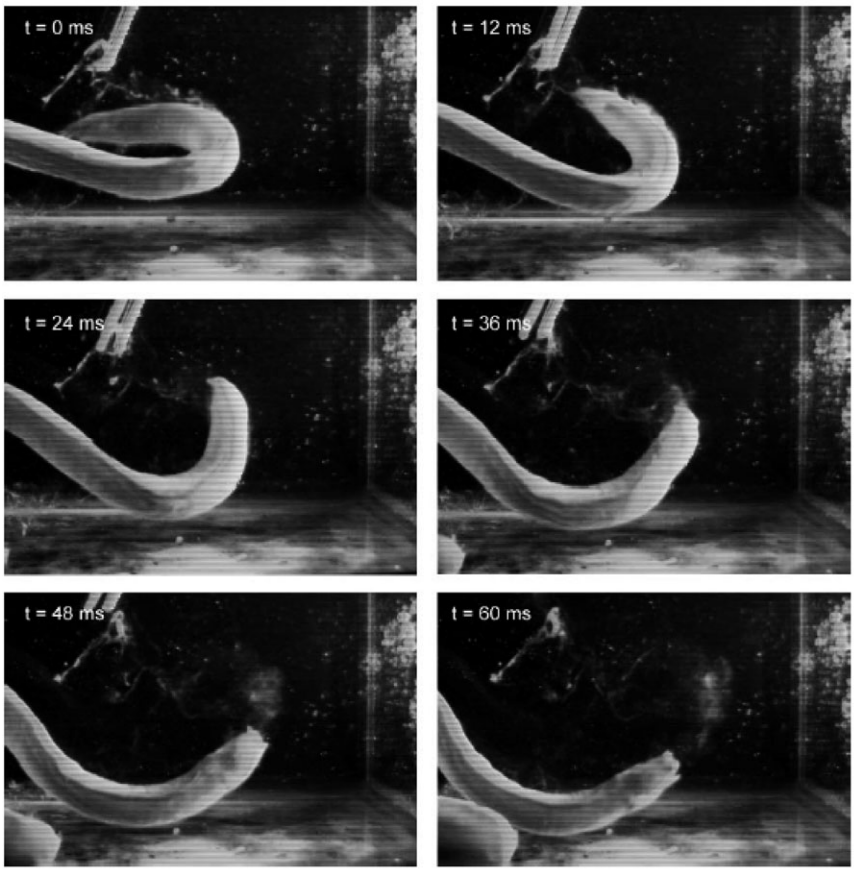

Fig. 12. High-speed video (shot at 125 frames s^{-1}) of a sliming event demonstrating that released slime rarely envelops the hagfish and often is dispersed by an evasive manoeuvre that mixes the exudate with seawater.

Removable mass trials showing the non-linear relationship between the amount of final slime product and stirring time underscore the convective mixing result. They also indicate, however, that mixing past a certain point decreases the mass of slime produced. This agrees with previous studies that have demonstrated that the slime collapses when it is disturbed (Ferry, 1941; Fudge et al., 2005). In a future study, we will explore in more detail the mechanism by which the mucins and fibrous threads interact with seawater and each other to form fully hydrated slime.

The sieve model of hagfish slime in which water is loosely bound is consistent with the anti-predator role of the slime when one considers the functional trade-offs between a slime that binds water loosely *versus* a gel that binds it tightly. Hydration is slower in a loosely binding slime,

meaning the exudate jet can travel farther than it would if it had a greater affinity for water. In addition, the resulting slime has a greater volume and is less mechanically coherent. Such slime may have more opportunity to initially stick to the gills of a predator and tangle between the gill rakers compared with a more coherent and smaller slime mass. Prolonged agitation of the slime from any subsequent thrashing will also cause the slime to collapse more completely on the gills, as the results of our removable mass experiments imply. At one extreme, slime with little coherence might be more likely to catch on the gills but may not interfere much with respiratory flow. At the other extreme, coherent slime might effectively block water flow but may be ineffective at lodging in the gills in the first place. In addition, a tight plug of slime would be easier for a fish to dislodge *via* 'coughing'. Thus, the strength of the interaction between the slime and seawater may be a compromise among several requirements for effective anti-predator activity. While the focus of the present study has been the anti-predator function of hagfish slime, the slime should be equally effective at endangering gill-breathing competitors. Hagfish also release slime during feeding (Martini, 1998) and this could serve to deter competitors from imposing themselves on a hagfish's meal.

Conclusions

We demonstrate here that hagfish slime can clog fish gills, which increases gill resistance and slows water flow through them. The potential for entrapped slime to interfere with gill respiration suggests that the slime may have evolved to deter gill-breathing animals from preying on hagfish. We have shown that the release of slime exudate is local and that its forceful ejection from the slime gland can be accomplished by contraction of the gland capsule muscle alone. Once slime is released into the water, the extent of its hydration and expansion depends on the amount of convective mixing in the water. The mechanical consequences arising from different models of how tightly water is bound to the slime imply that hagfish slime's loose water binding is functionally important in defending hagfish against gill-breathing predators.

Symbols and abbreviations used

a	duct radius
C	constant value of R_{tube}
d	wall thickness
l	duct length
p	pressure inside gland
$\dot{Q}$	flow rate
$\dot{Q}_{p}$	standardized flow rate
$\dot{Q}_{p,no\ gills}$	standardized flow rate without gills present
$\dot{Q}_{p,norm}$	normalized flow rate
$\dot{Q}_{p,0}$	pre-slime standardized flow rate
$\dot{Q}_{p,with\ gills}$	standardized flow rate with gills present
r	gland radius
R	resistance
Re	Reynolds number
R_{gills}	gill resistance
$R_{gills,norm}$	normalized gill resistance
$R_{gills,0}$	pre-slime gill resistance
$R_{gills,rel}$	relative gill resistance
R_{system}	siphon system resistance
$R_{system,0}$	pre-slime system resistance
R_{tube}	tubing resistance
$R_{tube,rel}$	relative tubing resistance
t	time
Δp	pressure head in the gland duct
ΔP	pressure head in the slime vacuum
μ	dynamic viscosity
σ_{sphere}	gland wall stress

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