

Supplementary Information: Unraveling hagfish slime

I. MATERIALS AND MICROSCOPY

Pacific (*Eptatretus stoutii*) and Atlantic (*Myxine glutinosa*) hagfish were maintained at the University of Guelph as previously described [11]. All housing, feeding, and experimental conditions were approved by the University of Guelph Animal Care Committee (Animal Utilization Protocol 2519). Hagfish were anesthetized before the extraction of exudate. Electrical stimulation near the slime glands resulted in the secretion of the exudate locally near the site of stimulation [11]. The exudate was collected directly from fish using a spatula, stored in a microcentrifuge tube at 10°C. No buffer or oil was used to stabilize the exudate from Pacific hagfish and it was used within 2–5 hours from the time of extraction to ensure sample viability. The exudate extracted from Atlantic hagfish was stabilized under mineral oil and stored on ice for transport to the testing location [6].

For microscopy studies, an inverted optical microscope with brightfield imaging was used. To visualize a single skein unraveling, a very small volume of Atlantic hagfish exudate was added to a pool of artificial sea water and an isolated skein was located and observed under a flow created by manually disturbing the sample. For the network images, a precise volume of exudate from Pacific hagfish was introduced into a centrifuge tube containing artificial sea water (1 ml) and the contents were sloshed back and forth in the closed tube six times, similar to prior work [5]. Following this the sample looked like a cohesive mass. Gel samples of various exudate concentrations were transferred to glass slides with small wells. A Nikon 90i Eclipse microscope was used to visualize the network with differential interference contrast (DIC) to image the slime network.

II. MINIMUM PEELING FORCE

Assuming only van der Waals interactions exist at the peeling site, we estimate of the minimum peeling force required for peeling. For an inextensible fiber, with negligible bending energy storage during peeling, the work done by the peeling force F_P in moving a point on the fiber by a distance δ_P is $F_P \delta_P$ (schematic in Fig. 1). The energy involved in creating new surface at the peeling site is equal to the van der Waals interaction energy released at the peeling site, given by $E_A A_P$, where E_A is adhesion energy per unit area for van der Waals surfaces. The area created at the peeling site is $A_P \approx (2r) \delta$, where r is the thread radius and δ is the displacement length of the peeling front created by the fiber displacement δ_P . The energy balance, rearranged, gives $F_P = 2E_A r (\delta/\delta_P)$. For a wide range of peeling angles θ , $\delta_P \sim \delta$ (note that $\delta_P = \delta$ for $\theta = \pi/2$), and we get $F_P \sim 2E_A r$. A typical adhesion energy for van der Waals surfaces is 50–60 mJ/m² [1, 8] and $r \approx 1 \mu\text{m}$, which gives $F_P \approx 0.1 \mu\text{N}$.

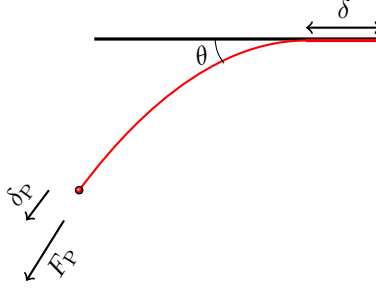


FIG. 1. Schematic of a fiber (red) peeling from a substrate (black).

III. HAGFISH DEFENSE IN SUCTION FLOW

Evidence of hagfish defense with slime secretion is available for both biting and suction feeding predators [2, 12]. Here, we treat the case where the predator creates a suction flow to engulf the prey. A good approximation of suction flow is provided by numerous experimental and computational studies [3, 4, 7, 9, 10]. A one dimensional parametrization of an observed suction flow with bluegill fish is

$$u(x_g^*, t) = u(x_g^* = 0, t)(1 - 2.19x_g^* + 1.86x_g^{*2} - 0.70x_g^{*3} + 0.09x_g^{*4}) \quad (3.1)$$

where $u(x_g^* = 0, t)$ is the flow speed at the mouth of the predator and x_g^* is the non-dimensional distance from the predator mouth normalized by the gape size (the diameter of the open mouth). In (3.1), both the velocity *and* the extensional strain rate decay for $x^* > 0$ (away from the mouth of the predator). The form (3.1) represents a time-varying velocity profile at the mouth of predator. Note that (3.1) applies outside the mouth. We do not have data for the flow profile inside the mouth, but for our purpose here we assume a constant centerline velocity equal to the peak velocity (velocity at the gape), as if the flow was sucked into a constant diameter tube with a negligibly small boundary layer (Fig.2B). The velocity field is continuous, though the spatial derivative is not. This discontinuous strain rate is not a problem since we eventually integrate the velocity to get an average over the unraveled length. To simplify the analysis, we use a time-invariant version of this velocity profile. We note that (3.1) is obtained by polynomial fit to PIV data, and hence should only be used in the range of x_g^* in which it makes physical sense, i.e. monotonically decreasing $\partial u(x_g^*, t)/\partial x_g^*$, which is true only for $x_g^* < 1.36$. We use a gape length of 10 cm, an approximate scale obtained from Fig.2A.

We solve for the case of a skein pinned at different locations x^* where the unraveling force is due to the drag on the unraveled fiber under the suction flow given by Eq. (3.1) (Fig. 2C). The governing equations are the same as derived in the paper:

$$(\dot{L})^m = -4\pi\mu\alpha^{-1}L\delta(L)(\dot{L} + \bar{u}(L, X, t)). \quad (3.2)$$

Figure 3 shows the numerical solution for this case. Compared to the case of uniform velocity field of 1 m/s solved in the paper, the unraveling time is longer with a spatially-varying velocity that decays away from the predator’s mouth. The location of the pinning point of the skein has a huge impact on the unraveling timescales as shown in Fig. 3, since being farther from the predator means a lower flow velocity and extensional strain rate. The strain rate for the chosen velocity profile outside the predator’s mouth lies between 2.19 s^{-1} at $x_g^* = 0$ and 0.27 s^{-1} at $x_g^* = 0.9$. From the video evidence of hagfish-predator attack, we note that exudate is released at a distance less than one-third of the gape size. This proximity to the mouth, in this flow field, results in an unraveling timescale close to the physiological one. Note that we used a velocity profile that was experimentally obtained for suction feeding fish with a gape size of only 1.5 cm (bluegill). We therefore expect the magnitude of velocity in the real scenarios to be higher, and hence the unraveling times will be smaller for hagfish predators. It does pose the interesting possibility that smaller fish, with weaker suction flow, may not create a flow field that unravels the hagfish threads within hundreds of milliseconds.

(A)



(B)

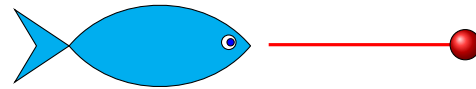
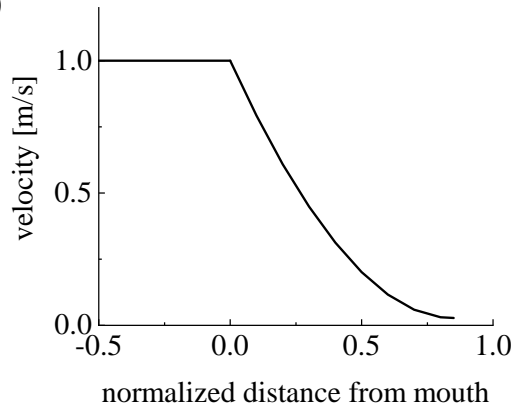


FIG. 2. (A) Evidence of hagfish (*Eptatretus sp. 2*) defense against suction feeding predator (*Polyprion americanus*, Atlantic wreckfish) (adapted from Zintzen et al. [12], by permission from SpringerNature). A suction feeding fish induces flow into its mouth by expanding its buccal cavity and drawing its prey along with the flow. We hypothesize the suction flow to aid in unraveling of thread cells and set up the slime network. (B) The one-dimensional suction flow profile (3.1) with an assumption of constant velocity inside the predator's mouth. (C) A schematic of the fish-thread-skein system (not to scale).

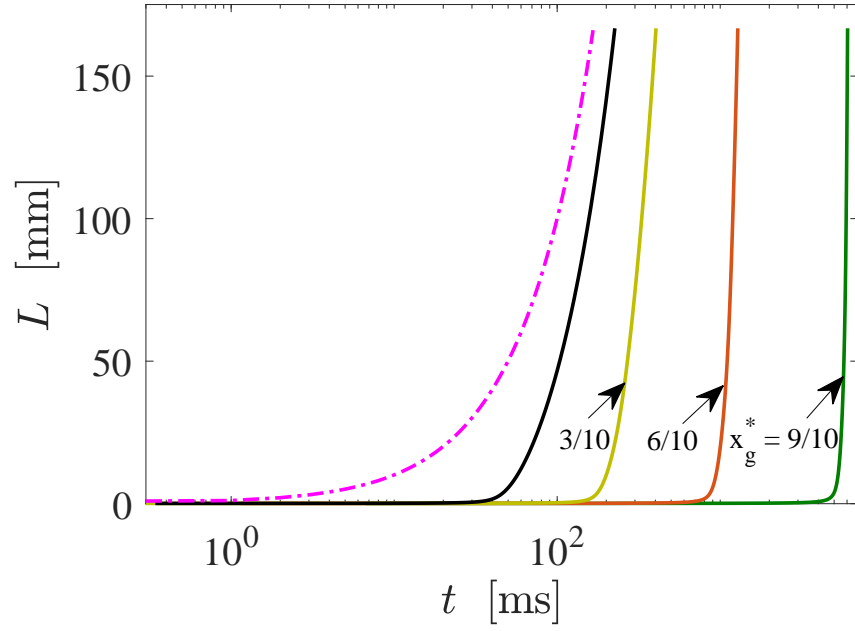


FIG. 3. Numerical solution (dashed black line) of (3.2) for the parameter values $R_0 = 50 \mu\text{m}$, $L_0 = 2R_0$, $\varphi = 0.5$, $m = 1/2$, and velocity profile given by (3.1) with $u(x_g = 0, t) = 1 \text{ m/s}$ with varying the location of the pinned point. Yellow, orange and green are the solution to the cases where the skin is pinned at locations $3/10$, $6/10$ and $9/10$ of the gape size ($= 10 \text{ cm}$). Solid black line is the numerical solution for the case of constant velocity of 1 m/s . The dotted line is the upper bound $L = L_0 + Ut$, with $U = 1 \text{ m/s}$.

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