© 2018. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2018) 221, jeb188037. doi:10.1242/jeb.188037

INSIDE JEB

De-slimed hagfish take a month to recover

Sarah Schorno holding fresh hagfish slime showing the reinforcing threads. Photo credit: Douglas Fudge.

Hagfish have some repulsive habits, but to their fans, they are awe inspiring. From disabling the gills of assailants by spraying them with reinforced mucous, to surviving hours without oxygen inside carcasses while dining, the revolting creatures have even inspired a national day celebrating their unique qualities. 'I've always been interested in what most consider to be "odd" animals', says Sarah Schorno from the University of Guelph, Canada, who first encountered the nauseating creatures during an invertebrate zoology class taught by hagfish doyen Douglas Fudge. After 15 years of painstakingly revealing the secrets of the animals' defensive slime, Fudge knew that the animals expel minute quantities of mucous, which expand rapidly, and tightly wound skeins of super-strong protein filaments, which ensnare attackers in a fraction of a second. In contrast, recharging the spent slime glands takes as much as 4 weeks. Wondering how the animals replenish their stocks of mucous and thread cells, Fudge, Schorno and Todd Gillis monitored the process.

After flying the animals from their ocean home off Vancouver Island to land-locked Guelph in bags of saltwater, Schorno gently touched each slime gland along the animals' sides with an electrostimulator to release their slime. 'Sometimes full slime glands forcefully expel their slime cells, similar to a pimple being popped', chuckles Schorno. Then, over the period of a month, she re-emptied some of the glands every consecutive week, so that after 5 weeks the slime glands of each hagfish were at different stages of recharging when she collected them. After measuring the glands, which ranged in size from 1.8 mm when empty to 2.6 mm when full, Schorno cautiously embedded each in a block of paraffin ready to slice into 5-µm-thick slivers for viewing in a microscope. 'If the slime glands were not embedded in the paraffin properly, they would sometimes pop out halfway through slicing, never to be seen again', she recalls.

Viewing the gland cells and comparing the area filled with thread cells with the area filled by mucous cells over the 4-week period, Schorno could see that the thread cells developed at a much slower rate. They only comprised 15% of the gland area during the first 3 weeks of the refilling process; however, by the time the glands had recharged fully, the thread cells comprised almost 44% of the gland area. In fact, the trio suspects that the slow pace of thread cell development, which is probably a result of the complex process that produces the tightly packed skeins, determines how long the gland cells take to refill.

Next, Schorno used a specially modified antibody, which targets cells that are dividing and glows when exposed to ultraviolet light under a microscope to reveal where new cells are forming, and found that the newly formed mucous and thread cells only occur in the lining of the gland. She says, 'We had hypothesized that the slime cells would undergo a linear growth pattern, continuously growing as they were pushed towards the centre of the gland'. However, it was clear that most of the growth occurs near the edge of the gland before the cells are pushed to the centre by new cells that are produced below.

Having discovered why it takes so long for de-slimed hagfish to recharge their glands, the team suggests that the lengthy period accounts for the fish's policy of only releasing a few glands when attacked. 'We suspect that this is a way for them to conserve slime, so that they will not be left defenceless', says Schorno.

10.1242/jeb.188037

Schorno, S., Gillis, T. E. and Fudge, D. S. (2018). Cellular mechanisms of slime gland refilling in Pacific hagfish (*Eptatretus stoutii*). *J. Exp. Biol.* **221**, jeb183806.

Kathryn Knight
kathryn.knight@biologists.com