INSIDE JEB

Even light oiling from spills can be catastrophic for birds

An unimpeded western sandpiper flying in the wind tunnel at Western University. Photo credit: Brock Fenton.

It's a depressingly familiar sight when an oil well blows or a tanker runs aground: thousands of stranded, helpless animals wallowing in cloying crude oil. 'Birds are often used as the poster children for the deadly effects of oil', says Ivan Maggini from Western University, Canada, recalling the shocking images of struggling animals that accompanied the catastrophic Deepwater Horizon oil spill in the Gulf of Mexico in 2010. 'However, the effects of oiling go far beyond the unfortunate individuals that become heavily oiled', says Maggini, highlighting the risks of hypothermia and toxin ingestion for lightly oiled birds when they preen. Western University physiological ecologist Chris Guglielmo has been assessing the toxicity of crude oil since 2011. However, no previous study had looked at the effect of oiling on flight, so Maggini, Guglielmo and Karen Dean wondered how even a small amount of contamination might affect the flight of migratory birds that cover great distances.

Selecting western sandpipers as a representative of the migrating species that got caught up in the Deepwater Horizon incident, Guglielmo and Maggini received 80 birds that had been collected in British Columbia just before the animals embarked on their migration flights, 'when they should be highly motivated to fly', explains Maggini. And when Maggini, Guglielmo, Lisa Kennedy, Alex Macmillan and Kyle Elliott tested the birds' stamina in a flight tunnel, all the birds flew comfortably for 2 h.

Having set the baseline for unhindered bird flight, the team gently applied oil – collected from the Deepwater Horizon oil well – to the wing and tail feathers of some of the birds (which they considered lightly oiled) before testing their flight in the wind tunnel. They then added oil to the backs and bellies of the birds 1 week later (moderately oiled), to mimic the natural contamination pattern, before giving them a second run in the wind tunnel: the birds struggled on both occasions. 'They found it hard to keep up with the wind speed', says Maggini, adding that the scientists had to stop half of the flight trials early because the birds kept attempting to land.

Wondering how much of a difference the oil made to the birds as they beat their wings, the team filmed the birds while they flew briefly at wind speeds ranging from 5 to 15 m s^{-1}. The moderately oiled birds beat their wings faster across the entire speed range and when the team calculated the impact of the contamination on the amount of energy that the birds consumed, it rocketed by 45% in the moderately oil migrants. 'Even when oiled only on wingtips and tail, the cost of flight was increased by 20%', observes Maggini, adding, 'It's like trying to fly with clipped wings or carrying a ball and chain'.

Maggini suspects that oil reduces the amount of lift that the birds can generate while also increasing the drag that they experience, which could have significant repercussions for the amount of time that the animals take to recover after a high endurance flight. 'We think that our results set a precedent for future oil spill damage assessments by showing how significant small amounts of oil can be for bird flight. Every movement they make in the air becomes more costly and this can have cumulative effects on migration, survival and reproduction', warns Maggini.

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Maggini, I., Kennedy, L. V., Macmillan, A., Elliott, K. H., Dean, K. and Guglielmo, C. G. (2017). Light oiling of feathers increases flight energy expenditure in a migratory shorebird. *J. Exp. Biol.* **220**, 2372-2379.

Kathryn Knight

Being bullied reduces brain cell birth in harried weakly electric fish

South American knifefish are remarkably adaptable. While the loss of a tail would be a major concern for most fish, some knifefish can replace the lost appendage entirely in only 3 weeks – which is just as well in an environment where hungry catfish continually nibble at them. Despite the knifefish's extraordinary ability to recover from the most extreme injury, Kent Dunlap from Trinity College, USA, noticed that *Brachyhypopomus occidentalis* survivors that had lost their tails naturally to predators in the streams of Panama also had lower rates of cell birth in the part of the brain that controls responses to predators: the telencephalic region. Could this brain deficit be increasing the fish's vulnerability to a catfish attack, raising the number of wounded fish in the predator's vicinity? Or had living in the constant shadow of a predator affected cell birth in the region of the brain that could protect the fish from assault? And, if catfish attacks were the cause of the reduced brain growth, were the knifefish investing more of their resources in tail regrowth at the expense of their own brains?

Intrigued by the possibility that living under the constant threat of predation was affecting the brains of knifefish, Dunlap and his colleagues decided to simulate the impact of the catfish's attentions in the laboratory. Dunlap admits, 'Coming up with an experimental "chase" protocol for the lab that might mimic the experience of living among dense predators was hard'; it was not clear how closely tapping the fish lightly on the tail and amputating the rear portion of the body under anaesthetic reproduces the experience of a harried *B.*

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gauderio. However, when Vielka Salazar from Cape Breton University, Canada, measured the levels of the stress hormone cortisol in the blood of *B. gauderio*, it was apparent that losing the tail was stressful, although the fish didn't seem too bothered when they were harassed with a plastic rod as their cortisol levels did not rise.

Next, Geoffrey Keane, Elise Lasky and Michael Ragazzi checked the amount of brain cell birth in the fish that had been disturbed with a rod but kept their tails, and found it was reduced to half of that of the undisturbed fish. Living under the constant threat of attack had altered cell birth in the agitated fish's brains. And when the team checked how losing a tail affected brain cell birth after 17 days, it was clear that the fish suffered a similar reduction. In addition, when they tested whether the fish had reduced brain cell birth in favour of regrowing their tails, it was clear that there was no trade-off. 'The reduction of brain cell proliferation following tail amputation was… greatest even before the fish were adding a lot of tail cells and lower during the period of fastest tail generation', says Dunlap, adding that brain cell birth plummeted by 80% within 24 h of the loss of the tail, which is before regrowth has began in earnest.

So, *B. gauderio* fish reduce cell birth in the telencephalic region of the brain when they are bullied, and when Dunlap and his team tried to terrorise brown ghost knifefish (*Apteronotus leptorhynchus*), which are popular in the aquarium trade and more easily available, the fish suffered the same brain impairment. Speculating about possible reasons for the fish to scale back the region of the brain that might provide them with the best protection from lurking predators, Dunlap says, 'We suspect that fish with a lower cell birth rate might be less exploratory and more timid. This could reduce their opportunity to forage and find mates, but might also reduce the probability of falling victim to a predator'.

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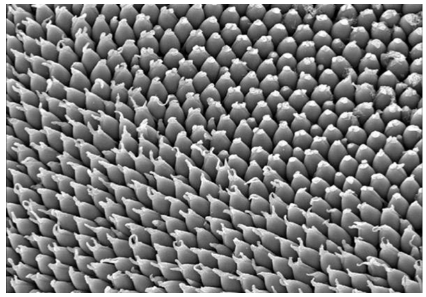
Dunlap, K. D., Keane, G., Ragazzi, M., Lasky, E. and Salazar, V. L. (2017). Simulated predator stimuli reduce brain cell proliferation in two electric fish species, *Brachyhypopomus gauderio* and *Apteronotus leptorhynchus*. *J. Exp. Biol.* **220**, 2328-2334.

Kathryn Knight

Colubrid snake eyes converted rods into cones



A scanning electron micrograph of the photoreceptors in a garter snake retina. Photo credit: Belinda Chang.

Snakes have a remarkably diverse range of eye designs, from moveable lenses for focusing, to slit pupils in species that specialise in a nocturnal lifestyle. The light-sensitive retina at the back of the eye can be equally varied, with some retinas composed entirely of rod photoreceptors packed with highly light-sensitive rhodopsin protein, while others depend exclusively on bright colour-sensing cones that use other less-sensitive photopigments. 'Intriguingly, retinal composition seems to shift between closely related species', says Belinda Chang from the University of Toronto, Canada, who explains that some close relatives have retinas that are composed entirely of rod cells alone, while others depend exclusively on cones. 'In order to explain this, Gordon Wall proposed the theory of transmutation, which hypothesised that instead of losing and re-evolving photoreceptor cell types… photoreceptors could be evolutionarily transformed between rod and cone cell types', explains Chang. Initially, this transformation was confirmed in one species of nocturnal gecko; however, Chang and her colleagues recently discovered rhodopsin in the cone cells of day-active garter snakes. Intrigued by the possibility that other members of the colubrid snake family may also have converted rods into cones, Chang and students Benedict Darren and Nihar Bhattacharyya began investigating the retinas of northern pine snakes.

'These snakes are a good species [to investigate] because they have an all-cone retina and they can burrow and hunt underground, which suggests that dim light vision – supplied by rods – could be useful to the species', says Bhattacharyya. Searching for evidence of the photopigment genes that are essential for vision in the snake's cone-packed retina, Darren found three. And when he analysed the sequences of the genes, he and Ryan Schott realised that two of the genes would produce the coloured opsins that are always found in cones – one that responds to green wavelengths and a second tuned to violet tones. However, the third gene was identical to the rhodopsin genes that are usually found only in rod cells.

Wondering whether the gene was still active and capable of producing the key rod protein in the snake's cone retina, Bhattacharyya and Vince Tropepe painstakingly sliced thin sections from the back of the snake's eye then incubated them with an antibody that only binds rhodopsin proteins, revealing that the rhodopsin protein was present in the pine snake's retina. It was beginning to look as though rod cells in the pine snake retina had transmuted into cones.

However, Chang still needed to be convinced that the rhodopsin pigment was capable of responding to light. Having tackled the challenging task of artificially producing and purifying the protein, Bhattacharyya was then able to test the protein's response to light and was impressed when its light absorption pattern was essentially the same as that of other rhodopsins. However, when they checked the protein's reaction with the chemical hydroxylamine – which is traditionally used to identify cone cells – Bhattacharyya and Chang were amazed to see the chemical react as if the pine snake rhodopsin was a cone pigment.

Considering their observations, Chang says, 'We have found striking evidence in the pine snake of the "relics" of rod photoreceptor origins in their all-cone retina', adding, 'This would suggest that the ability to have dim light vision might have been useful to the snake'. She also suspects that the evolutionary transformation of rods into cones may be widespread in colubrid snakes and that the rod proteins have become more cone-like over time.

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Bhattacharyya, N., Darren, B., Schott, R. K., Tropepe, V. and Chang, B. S. W. (2017). Cone-like rhodopsin expressed in the all-cone retina of the colubrid pine snake as a potential adaptation to diurnality. *J. Exp. Biol.* **220**, 2418-2425.

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