INSIDE JEB

Flamingos starved in freezing conditions

Camargue flamingos during the freezing conditions of February 2012. Photo credit: ©Jean-François Lagrot.

1985 was one of the worst years in living memory for the flamingo population of the Camargue, France. Over a 15 day period in January, temperatures plummeted, the lagoons, ponds and salt pans where the birds feed froze and by the time the Arctic blast had loosened its grip, almost one third of the population was dead. 'The 1985 mass-mortality shocked a generation of conservationists,' says David Grémillet from the CEFE-CNRS, France. Alan Johnson, a conservation scientist who dedicated 50 years of his life to protecting the iconic animals, was at the forefront of the mission to retrieve the bodies in the hope of discovering what had killed them. Later, when 1500 birds died in another severe cold snap lasting 13 days in February 2012, Grémillet, Arnaud Béchet (Johnson's successor) and Michel Gauthier-Clerc realised that they could look at the effect of both cold snaps on the birds to try to understand how wild populations will be impacted by the increasing number of extreme climatic events that are predicted as a consequence of climate change. Together, with Anne-Sophie Deville and Sophie Labaude, they began investigating what had killed the victims of both unseasonal freezes (p. 3700).

Although the team only had Johnson's weight measurements to go on for the birds that died in 1985, it was clear that the animals were pitifully emaciated. However, they realised that they could get a more detailed impression of the factors that had led to the deaths of the flamingos in 2012 as they had access to six carcasses.

There are three distinct phases of starvation, which occur as the animal systematically consumes its fuel reserves. Each stage can be distinguished by the ratio of lipids to proteins in the body, allowing the team to identify whether starvation was a factor in the birds' deaths. Deville, Labaude and Jean-Patrice Robin painstakingly measured the lipid and protein content of leg muscles, pectoral muscles, skin and carcass. They then compared the flamingo's lipid/protein ratio with that of starving woodchucks and mallards, and could see that the flamingos were in the later stages of starvation. 'We were surprised to see how lean (virtually fat-free) the dead flamingos were,' says Grémillet. The birds had almost certainly died of starvation.

But what role had the perishing temperatures played in the birds' demise if they had not killed the birds directly? The team decided to calculate how much energy the birds required to survive during each month from January 1980 to April 2012. Teaming up with Warren Porter, Megan Fitzpatrick and Paul Mathewson from the University of Wisconsin, Madison, USA, to use their Niche Mapper™ algorithm, the team was able to see that the birds' energy demands naturally peak each year between December and February, decrease through spring and early summer as the temperatures increase, reach a minimum around July and August and then rise again through autumn back to the winter maximum.

However, when they focused on the periods of extreme cold, they found that the cold birds' energy demands increased by 5.7–7.0%, peaking in January 1985 at 2639 kJ d^{-1} for the male birds and 2201 kJ d^{-1} for the females. The team suspects that the flamingos died because the ponds where they feed froze just as their energy demands increased further, tipping the birds over into starvation. However, they suggest that the death toll was lower in 2012 because the temperatures were not as low and returned to normal more quickly than in 1985. Grémillet is also optimistic that this knowledge can be used to help manage the unique saltpan habitats to improve the year-round condition of the flamingos and better protect them from future weather extremes.

doi:10.1242/jeb.114603

Deville, A.-S., Labaude, S., Robin, J.-P., Béchet, A., Gauthier-Clerc, M., Porter, W., Fitzpatrick, M., Mathewson, P. and Grémillet, D. (2014). Impacts of extreme climatic events on the energetics of long-lived vertebrates: the case of the greater flamingo facing cold spells in the Camargue. *J. Exp. Biol.* **217**, 3700-3707.

Kathryn Knight

Electric fish hold clue to voluntary actions

Banded knifefish, *Gymnotus carapo*. Photo credit: Tiago P. Carvalho.

You may not realise it, but in the seconds before you clicked on the link to read this article (or picked the journal off a shelf), your brain was whirling away collecting information about yourself and your surroundings prior to taking action. 'The outcome of a voluntary decision can be predicted by brain activity even before a subject's conscious awareness,' say James Jun and Len Maler from the University of Ottawa, Canada. They add that similar brain activity has been found in other species too. However, the relationship between the timing of the sensory brain activity and the associated voluntary activity was not clear and experiments that could be used to investigate the phenomenon tend to be invasive, leading Jun and Maler to look for an animal that makes more of a display of sensory acquisition. Electric knifefish emit mild electric pulses as they sense their surroundings through distortions in the resulting weak electric field. 'Each pulse corresponds to a discrete active sampling event,' says Jun, and it was this remarkable ability that the duo decided to take advantage of in order to learn more about the relationship

Inside JEB highlights the key developments in The Journal of Experimental Biology. Written by science journalists, the short reports give the inside view of the science in JEB.

between the fish's actions and the sensory sampling that precedes them (p. 3615).

However, before the team could begin to unravel the timing relationships, they had to be sure that the fish's movements were made of their own free will. Nothing could inadvertently startle the animals, forcing Jun to become nocturnal and conduct his experiments at night with the fish in a specially constructed light- and vibration-free sensory isolation chamber. Jun then filmed the fish's undisturbed activity while recording their electric fields to understand the relationship between their electric discharge patterns and activity, so that he could later infer the fish's activity levels directly from variation in the strength (amplitude) of the electric discharge.

Next, Jun and Maler teamed up with Andre Longtin to statistically analyse the fish's electrical discharge patterns in relation to their movements and realised that the fish existed in two distinct sensory conditions: one where the fish had a high electric field discharge rate and a second with a low rate. And when the team compared the fish's physical activity levels with their electric discharge patterns, they could see that the fish only moved when the electric discharge rate was high and had been elevated for a period prior to moving. Just like humans, the fish collect sensory information about their surroundings just before making the decision to move voluntarily. Finally, the team compared the fish's electrical discharge rates when moving freely and when startled by a loud soundand found that the discharge rate was fairly constant in the startled fish, but extremely variable in the fish that moved voluntarily.

Having confirmed that weakly electric fish are capable of making voluntary movements and that their sensory activity patterns are essentially the same as the brain patterns of freely moving humans, the team come to the startling conclusion that the ability to make voluntary movements may have been a primitive function in early vertebrate brains that predates the sophisticated cognitive abilities of humans and modern primates. Jun also hopes that it may be possible to discover which part of the fish brain triggers the burst of sensory electrical activity, saying, '[This] may give clues to the initiation sites of human voluntary actions.'

doi:10.1242/jeb.114595

Jun, J. J., Longtin, A. and Maler, L. (2014). Enhanced sensory sampling precedes self-initiated locomotion in an electric fish. *J. Exp. Biol.* **217**, 3615-3628.

Kathryn Knight

Ticks coordinate saliva production with duo dopamine receptors

As the purveyors of unpleasant diseases, ticks have a justly deserved bad reputation and Yoonseong Park, from Kansas State University, USA, explains that salivation is one of the keys to the disagreeable arachnid's success. The tick's complex saliva not only helps it to extract water from the air between feeds and concentrates blood meals by extracting water, but it also contains compounds that defeat the immune system of the host. 'Salivary secretion is crucial for successful feeding and is the mediator of pathogen transmission' says Park. So understanding how this process is regulated by the endocrine (hormone) system is essential if we are to outwit the pernicious pest. 'Dopamine has long been known as the as the most potent inducer of tick salivation,' explains Park, adding that his lab had already discovered two protein receptors (D1 and InvD1L) in the tick salivary gland that are activated by the hormone to regulate saliva production. So he and his colleagues, Donghun Kim and Ladislav Šimo decided to investigate which aspects of the salivation process the two receptors regulate (p. 3656).

'We hypothesised that there are two different dopamine actions mediated through the receptors in different cell types of salivary glands', says Park. However, tracking down the receptors' functions had not been easy as it was not possible to use conventional methods of receptor inactivation to find out which processes they regulated. Instead, the team had to first identify chemicals that independently activate (agonists) and inactivate (antagonists) the receptors in a bid to learn more about their functions.

Inserting the genes for the receptors into animal cells and testing how various chemicals affected the receptors, Kim successfully identified an agonist for the D1 receptor (SKF82958), an antagonist for the InvD1L receptor (fluphenazine) and an agonist that worked on both receptors (SCH23390) that they could use to tease apart their functions in intact salivary glands.

'Dissecting out the entire salivary gland from these tiny animals without damage was challenging,' admits Park, adding that measuring the minute volumes of saliva produced by the minuscule organs was also tricky. However, once Kim had mastered the fiddly techniques, he found that salivary glands that had been stimulated with 100 μmol l^{-1} dopamine produce 1.2 μl of saliva over 30 min. And when he applied the D1 agonist to the salivary glands at the same concentration, they also produced saliva, but at a slower rate.

Next, the team focused on how the receptors functioned in each of the salivary gland's three different types of lobe – known as acini. After finding that the acini expand and contract in response to dopamine (expanding as fluid flows into the acini lumen and contracting as the acinus expels saliva into the acinar duct), Kim filmed the acini as he soaked the gland in dopamine, the two agonists or the antagonist and calculated that dopamine exposure caused the lumen of each acinus to expand by 4 nl.

Finally, the team turned their attention to the type III acinus which produces the concentrated saliva that reduces the volume of blood meals. Analysing how the acinus changed volume as they systematically applied the dopamine receptor agonists and antagonist, the team realised that the D1 receptor regulates uptake of fluid by the acnius while the InvD1L receptor regulates the pumping and gating mechanisms that eject saliva from the acinus into the salivary duct. 'Dopamine acts on the D1 and the InvD1L receptors and leads different physiological actions to orchestrate tick salivary secretion,' concludes Park and his team.

doi:10.1242/jeb.114611

Kim, D., Šimo, L. and Park, Y. (2014). Orchestration of salivary secretion mediated by two different dopamine receptors in the blacklegged tick, *Ixodes scapularis*. *J. Exp. Biol.* **217**, 3656-3663.

Kathryn Knight
kathryn@biologists.org