INSIDE JEB

Twists and turns more costly than direct flight for foraging gannets

Northern gannet in flight. Photo credit: David Grémillet

Soaring above the ocean, gannet parents embark on extensive foraging odysseys to find food for their young. For the parents, the voyage is an exercise in balancing the energy that they expend locating food against the rewards of returning with a full belly for their chicks. 'They try to expend as little energy as possible', explains Françoise Amélineau, from the CNRS Centre d'Ecologie Fonctionnelle et Evolutive in France. According to Amélineau and her colleagues, it has long been presumed that foraging birds expend less energy commuting to their foraging grounds than they do when wheeling above a shoal of fish. However, no one had directly measured the birds' energy expenditure in the wild because it was technically too challenging: that was until a new method that estimated energy expenditure based on the birds' acceleration was developed. Amélineau and her colleagues decided to attach accelerometers combined with GPS trackers and depth metres to gannets at their nesting site off the northern coast of France to find out which portions of a gannet foraging expedition cost the birds dear (p. 876).

During several trips to the Rouzic Island nature reserve in consecutive breeding seasons, Clara Péron, Amélie Lescröel, Pascal Provost and David Grémillet successfully tagged almost 50 birds before the animals headed north to their fishing grounds in The English Channel. 'It is quite difficult to catch the gannets, especially the second time, because they have already been captured once so they are more afraid', explains Amélineau. Fortunately, the team successfully downloaded data from 35 of the retrieved loggers, ready to find out which part of the expedition had been most arduous.

However, before they tackled the analysis, the team realised that there was another factor that they must consider. 'We thought maybe the wind was an important factor affecting the cost of movement,' explains Amélineau. Downloading wind speed and direction data from the Laboratory of Oceanography From Space portal for the area covered by the birds, the team was able to include the impact that the prevailing wind had on the gannets' voyage.

Converting each bird's acceleration plot into an estimate of the amount of energy expended at each stage of the journey and comparing it with the path taken at the time, the team could see that the birds used more energy as they twisted and turned over a foraging site than they did during the direct journeys there and back. And when the scientists factored in the impact of the wind direction, they found that the birds used more energy when flying into a head wind than when assisted by a tail wind.

However, the team were surprised to find that instead of taking advantage of a tail wind to assist during their heavily laden return, the Rouzic Island gannets were assisted by the wind on the outbound leg as they headed north, leaving them to battle the elements on their return. Explaining that theory suggests that birds prefer to return home using a tail wind, the team points out that the Rouzic Island gannets are forced to head north from their island home by the location of their prey, pitching them against the prevailing wind on the journey home.

So, Amélineau and colleagues have confirmed that taking tortuous routes over foraging grounds is more costly than flying along direct paths. She adds that it is important to understand the energetic costs of foraging for food to better understand an animal's ecology and the potential impact that climate change might have on them.

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Amélineau, F., Péron, C., Lescroël, A., Authier, M., Provost, P. and Grémillet, D. (2014). Windscape and tortuosity shape the flight costs of northern gannets. *J. Exp. Biol.* **217**, 876-885.

Kathryn Knight

Different wiring for squid's iridescent and pigment displays

Squid iridescent displays are mesmerising – and mysterious. 'Squids are the only invertebrates known to exert neural control over their iridescence,' explains Trevor Wardill, 'but we still don't know for certain why they use iridescence, and which specific neural pathway is responsible for iridescence control.' To shed light on these questions, Wardill teamed up with Paloma Gonzalez-Bellido and other colleagues at the Marine Biological Laboratory (MBL) in Woods Hole, Massachusetts, to take a closer look at how and why squid create their stunning visual displays (p. 850).

Squid have two colour-changing systems in their skin: small pigment-filled sacs called chromatophores, which can be reshaped by muscles to produce rapid colour changes, and cells called iridocytes, which contain stacked thin platelets that interfere with different light wavelengths to generate iridescence. Both types of cell are known to be controlled by nerve fibres. All of the nerve fibres coming from a squid's brain are packed into a single bundle called the pallial nerve, which splits into two branches. One branch, the fin nerve, goes straight to the fin, but the other, the stellate connective, travels into the stellate ganglion – a small bundle of neurons in the periphery, similar to the ganglia found outside each vertebra along a human's spinal column. 'We wondered if we could identify the specific neural pathway controlling iridescence by blocking information from each of these branches', explains Gonzalez-Bellido.

First, the MBL marine resources section collected Atlantic longfin squid outside Woods Hole. Then Gonzalez-Bellido and

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Swimming Atlantic longfin squid (*Doryteuthis pealeii*). Photo credit: Paloma T. Gonzalez-Bellido.

Wardill cut the squids' pallial nerve using micro-scissors. As they had expected, fin movement stopped and the squids' skin blanched, indicating that both chromatophores and iridocytes had stopped working. When they cut the fin nerve instead, fin movement and chromatophore activity stopped, but iridescence remained bright. In contrast, when they severed the stellate connective, the squid were still able to move their fins and control their chromatophores, but their iridescence was extinguished. 'This shows that iridescence signals are routed through the stellate ganglion, while chromatophore signals travel through the fin nerve', says Gonzalez-Bellido. 'The fact that the neural pathway controlling iridescence differs from the pathway controlling the chromatophores suggests that the iridescence pathway evolved at a different time point, perhaps for a different purpose altogether', she adds.

Gonzalez-Bellido and Wardill realised that their discovery would allow them to measure iridescence changes in a live squid for the first time. Measuring iridescence is tricky, explains Wardill, because active chromatophores can mask iridescence changes. Also, fin movements change the angle of light hitting the skin, making it hard to assess iridescence accurately. So, to stop fin movements and chromatophore activity, the team severed the squids' fin nerves. Then they placed the squid in a blackened tent, and after 1 or 2 hours took a flash photo to capture the iridescence at that instant. The dark-acclimated animals showed a decline in iridescence compared with the iridescence seen during an earlier exposure to normal light levels. This is useful, says Wardill: 'being able to reduce iridescence levels in deeper, darker waters could help squid hide from predators.' When they exposed the squid to light again, the animals responded by increasing their iridescence.

The team is now one step closer to elucidating the different functions of squid iridescence. 'Now that we know which specific neural pathway to look for, we can map out on an evolutionary tree which squid species have it and which don't, which may help us discover its function', says Gonzalez-Bellido.

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Fine-tuning the electroglottograph

If you've ever had a problem with your voice, you might have had an electroglottograph taken to find out how your vocal folds are working. Christian Herbst, from the Palacký University Olomouc, Czech Republic, explains that the vocal folds – which are situated in the larynx at the top of the trachea – open and close as we talk, to alter the flow of air exhaled from the lungs to produce a noise that is then modified by our throats and mouths to produce the bewildering array of sounds that we know as human speech. And when the voice is damaged, one of the first things that the doctors investigate is the vocal fold's vibration. However, Herbst explains that assessment of the vocal folds is costly and invasive, 'You have to stick an endoscope down a patient's throat and that is not pleasant', he says. Fortunately, doctors can resort to a less-intrusive approach. They collect electroglottographic data by monitoring variations in high-frequency weak electric current passed through the vocal folds by electrodes that are placed either side of the larynx. 'It is a low-cost, non-invasive alternative', says Herbst. However, the precise details of how well the visible vocal fold vibrations correlated at high speeds with the fluctuations in the electric current were not clear. So, while based at the University of Vienna, Austria, Herbst collaborated with colleagues from several European institutions in a high-speed analysis of the relationship between vocal fold vibrations and the electroglottograph data (p. 955).

Blowing a carefully controlled flow of air through the larynx of a golden retriever that had recently died from natural causes, Herbst filmed high-speed movies (at 27,000 frames s^{-1}) of the vocal fold's vibrations while measuring fluctuations in the weak electric current passing through the vocal folds at a frequency of 44,000 Hz to look for correlations between the vibration and variations in the electric current. Using several graphical methods to analyse the vocal fold's complex rippling motions, Herbst and his colleagues could see that the fluctuations in the electric current were not as tightly correlated with the vocal fold vibrations as had been suggested by earlier video studies at lower speeds.

The team found that the changes in the electroglottograph electric current did not coincide perfectly with the times when the vocal folds opened. Also, the electric current fluctuations – which were thought to indicate the precise moment when the vocal folds opened or closed – were delayed by between 0.02 and 0.61 ms, which doesn't sound like much, but is as much as 10.9% of one of the vocal folds' rippling cycles. Herbst and his colleagues also noticed that there is not a single precise moment when the vocal folds separate or come back into contact. This is because of the complex rippling motions that occur not only from the front to the back of the vocal fold slit, but also down through the depth of the slit – which is about 5 mm deep – so both edges do not separate completely at one defined moment, but open more gradually depending on the way that the rippling motion propagates through the tissue.

So, Herbst and colleagues conclude that variations in the high-frequency electric current measured by an electroglottograph across vibrating vocal folds do not reflect the motion of the tissue precisely during sound production. However, the team are optimistic that high-speed videos of the vocal fold's movements will give us a better understanding of the relationship between peaks in electroglottograph data and the vibrating motions that produce them, to provide a cheap method for assessing vocal fold vibrations in humans and other animals.

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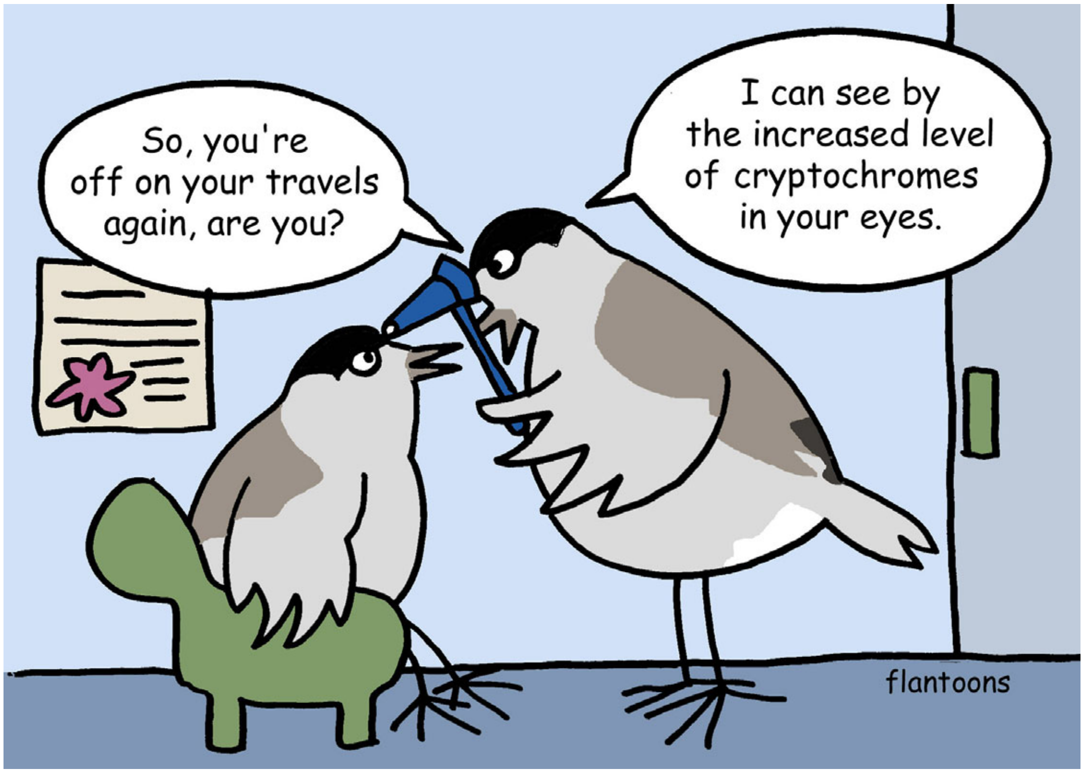
Herbst, C. T., Lohscheller, J., Švec, J. G., Henrich, N., Weissengruber, G. and Fitch, W. T. (2014). Glottal opening and closing events investigated by electroglottography and super-high-speed video recordings. *J. Exp. Biol.* **217**, 955-963.

Kathryn Knight

Cryptochrome could help blackcaps find way



Setting off on their 9500 km migration south from their summer home in Latvia, blackcaps set a bearing toward their wintering grounds in Kenya that guides them on their way. Augusto Foà and collaborators from the University of Ferrara, Italy, explain that the tiny aviators are probably able to detect the earth's magnetic field, and use it to set their bearing as they head south. Some migratory species sense magnetic fields using a chemical interaction between cryptochrome proteins in the eye and blue light, which they use as a compass during migration. Could migrating birds increase cryptochrome levels in their eyes in preparation for migration in order to guide them on their way (p. 918)?

Leonida Fusani and Cristiano Bertolucci travelled to the birds' nesting site near the Pape Ornithological Station, Latvia – where the birds prepare for migration – in August 2011 and intercepted the voyagers just before they embarked on their ambitious journey. Having transported the animals back to the University of Ferrara, the team then waited for the birds to begin showing the tell-tale signs that they were ready to set off – they become more active by night, are more sedentary during the day and have gained weight. The team then allowed some of the birds to continue hopping around at night to simulate their migration. However, in another group they switched off the urge to migrate by recreating the return to normal behaviour that birds experience when they stop to refuel after two days of migration. The team did this by not feeding the birds for two days – to simulate the flight – and then resumed feeding the birds on the third day to induce the return to normal behaviour during the refuelling stop. The team then collected samples of the birds' eyes to look for evidence of cryptochrome production and compared them with samples from birds that were hopping around ready to embark on migration, birds that had completed their virtual migration for the year and were back to their normal daily routine, and birds that had just gone through a night of virtual migration

They found that the birds that were experiencing the simulated migration at night had high levels of the mRNA that produces the crytochrome proteins. However, the birds that had returned to their normal behaviour patterns had much lower levels of the cryptochrome RNAs. So, blackcaps that are on the verge of migration and those that are experiencing migration have access to one of the key components for detecting magnetic fields that might guide them on their way. And although this is not conclusive evidence that the birds set their bearing using a magnetic compass, it is another piece of compelling evidence that they probably do.

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Fusani, L., Bertolucci, C., Frigato, E. and Foà, A. (2014). Cryptochrome expression in the eye of migratory birds depends on their migratory status. *J. Exp. Biol.* **217**, 918-923.

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