

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.

# Inside JEB

## OXIDATIVE PROFILES REFLECT GREENFINCH PERSONALITIES



Katherine Herborn

An individual's personality can have a big effect on their life. Some people are outgoing and gregarious while others find novel situations stressful, and animals are no different. 'Over the last 5 years there has been increasing interest in animal personality,' says Kathryn Arnold from the University of York, UK. Knowing that stress affects wellbeing by raising metabolism and increasing the levels of damaging reactive oxygen metabolites, Arnold and her colleagues wondered whether animal personalities could be reflected in their oxidative stress profiles (p. 1732). Teaming up with graduate student Katherine Herborn at the University of Glasgow, UK, Arnold set about classifying the personalities of 22 greenfinches at Glasgow University's aviary.

First, the duo tested the bird's reactions to a novel situation. Adding a brightly coloured biscuit cutter to each greenfinch's food bowl, Arnold and Herborn timed how long it took for the birds to pluck up the courage to approach their lunches. 'They wanted the food but they had to overcome their fear,' explains Arnold. Repeating the experiments with different coloured biscuit cutters, the team found that the boldest birds took only a few seconds to overcome their fear while more timid birds took up to half an hour to approach their meal.

Having established each bird's courage, Arnold and Herborn measured the greenfinches' motivation to explore by attaching an intriguing object to the birds' perches. Timing how long it took the birds to land next to the object, the duo found that some birds were avid explorers while others were less curious. However, there was no correlation between the birds'

courage and curiosity: some bold birds were unenthusiastic explorers while other shy individuals were very keen to investigate, so would their blood profiles reflect any of their behaviour traits?

Collecting blood samples from the birds several weeks after classifying their personalities, Herborn and Stephen Larcombe measured the animals' damaging reactive oxygen metabolite levels and their defences against these harmful molecules. Herborn also travelled to the WALTHAM® Centre for Pet Nutrition, UK, to measure the amount of oxidative damage sustained by each individual with the help of Lucille Alexander and Jo Coffey.

Comparing the bird's blood oxidative profiles with their personalities, the team found that the most timid birds had the highest levels of damaging oxygen toxins and the weakest defences, so they suffered more oxidative stress than braver individuals. Also, the scientists found that the most curious birds (which approached objects fastest) had better defences than less curious greenfinches, although there was no relationship between the birds' curiosity levels and their toxic oxygen metabolite levels. However, when the team analysed the degree of oxidative damage sustained by cells in the birds' blood, they were surprised to find that the intermediate birds suffered the most damage while the most timid and the boldest birds had much lower damage levels. So the individuals that reacted strongly to novel objects – whether they approached immediately or held back – had lower oxidative damage levels than individuals that reacted calmly.

Having found that the birds' personalities are reflected in their oxidative stress profiles, Arnold is keen to find out how this may impact on birds in the wild. She says, 'Neophobic birds [which are afraid of new things] may suffer high costs of oxidative stress but they might be less likely to be eaten by a predator because they are more wary; however, these individuals may die early because they paid these physiological costs.'

10.1242/jeb.058776

Herborn, K. A., Coffey, J., Larcombe, S. D., Alexander, L. and Arnold, K. E. (2011). Oxidative profile varies with personality in European greenfinches. *J. Exp. Biol.* **214**, 1732-1739.

## HISTAMINE CONTROLS PHOTOTAXIS IN DAPHNIA

*Daphnia* are remarkably versatile little beasts. Living in aquatic environments, they migrate to deeper water by day – to avoid predators and UV damage – and return to the surface when safe at night. Andrew

Christie from the Mount Desert Island Biological Laboratory, USA, and his colleagues Matthew McCooles and Kevin Baer from the University of Louisiana at Monroe, USA, explain that this migration is probably driven by phototaxis – when animals are either attracted to or repelled by light. They add that the tiny crustaceans respond quickly to pollution, so their phototactic behaviour is routinely studied by ecotoxicologists, who use them as a model to understand how environmental change affects behaviour. Yet, shockingly, nothing was known about the neural basis of *Daphnia*'s migrations. Knowing that the amine histamine plays a key role as a neurotransmitter in the visual systems of other arthropods, the team decided to make the first measurements to find out whether histamine contributes to *Daphnia*'s phototactic response (p. 1773).

Collecting *Daphnia magna* and *Daphnia pulex* from lab-based colonies, the scientists were able to trace the tissues containing high levels of histamine using antibodies that recognise the amine. Scrutinising the crustaceans with a microscope, Christie and his colleagues could see that histamine was restricted to the animal's nervous system, and more specifically to the crustacean's eye, optic ganglion and brain.

Having confirmed that the neurotransmitter occurs in *Daphnia*'s visual system, the team began searching through the recently published *D. pulex* genome to look for genes encoding a key enzyme involved in histamine synthesis and ion channels that could be activated by the neurotransmitter. Using *Drosophila* proteins as templates, the team identified three potential histidine decarboxylase proteins, ranging in length from 688 amino acids up to 747 amino acids, and two histamine-gated chloride channels in the *D. pulex* genome. So, the components for histaminergic signalling were in place, but the researchers needed direct evidence before they could be sure that *Daphnia* employ histamine signalling in phototaxis.

Knowing that the drug cimetidine blocks histamine signalling through its receptors, the team decided to see what effect the drug had on the crustacean's ability to respond to light. Together, they tested the reactions of juvenile *Daphnia* to a 10 min UV light exposure and found that most of the animals fled to the bottom of the enclosure. However, when the team added cimetidine to the water, the animals stopped descending to the bottom. The drug had robbed the animals of their ability to respond to light, although they quickly recovered phototaxis when the drug was absent.

'Taken collectively, our results show that an extensive histaminergic system is present in *Daphnia* species, including the visual system, and that this amine is involved in the control of phototaxis in these animals,' says Christie and his colleagues.

10.1242/jeb.058792

**McCooles, M. D., Baer, K. N. and Christie, A. E.** (2011). Histaminergic signaling in the central nervous system of *Daphnia* and a role for it in the control of phototactic behavior. *J. Exp. Biol.* **214**, 1773-1782.

## PINACODERM DRIVES SPONGE CONTRACTION



Travelling back in time along the evolutionary tree you eventually reach sponges; possibly the earliest modern animals. Lacking digestive, nervous and muscular systems, it is sometimes difficult to imagine that they are animals, yet sponges could hold the key to the debate about how muscles evolved. Michael Nickel from the Friedrich-Schiller-Universität, Germany, explains that despite lacking muscles, sponges are able to contract, but no one knew how. Competing hypotheses had suggested that either the connective tissue between sponge cell layers – the mesohyl – might mediate the contraction or the outer cell layer – the pinacoderm – could produce squeezing contractions. However, no one had ever tested these ideas and crucially, neither hypothesis could explain how contracted sponges return to their full size. Intrigued by the mystery, Nickel decided to use an X-ray technique to take a close look at contracted and relaxed *Tethya wilhelma* sponges to find out how they contract without muscles (p. 1692).

First, Nickel made time-lapse movies of a sponge as it contracted and relaxed to see how reproducible the contractions were. Analysing the movies, Nickel could see that each contraction cycle was almost identical. During the first 15 min phase, the relaxed sponge contracted slowly, reducing by 20% of the entire contraction range. But then the contraction accelerated and within 15 mins

the sponge had completed the second phase and contracted completely. During the third and fourth phases, the sponge relaxed, expanding by 60% of the entire contraction range over the first 15 min, but then expanded more slowly until returning to its full size 1.25 h after the cycle began.

Next, Nickel looked for differences between the contracted and relaxed sponges with synchrotron radiation-based X-ray microtomography. Teaming up with Jörg Hammel, Nickel dried a contracted and a relaxed sponge ready for transport to Hamburg to use the Deutsches Elektronen Synchrotron to look at the samples with Felix Beckmann. Scanning the sponges with the high intensity X-ray beam, Beckmann and Julia Herzen reconstructed 1–5 µm thick virtual slices through the sponges to produce a 3D view of the sponges and their internal structures.

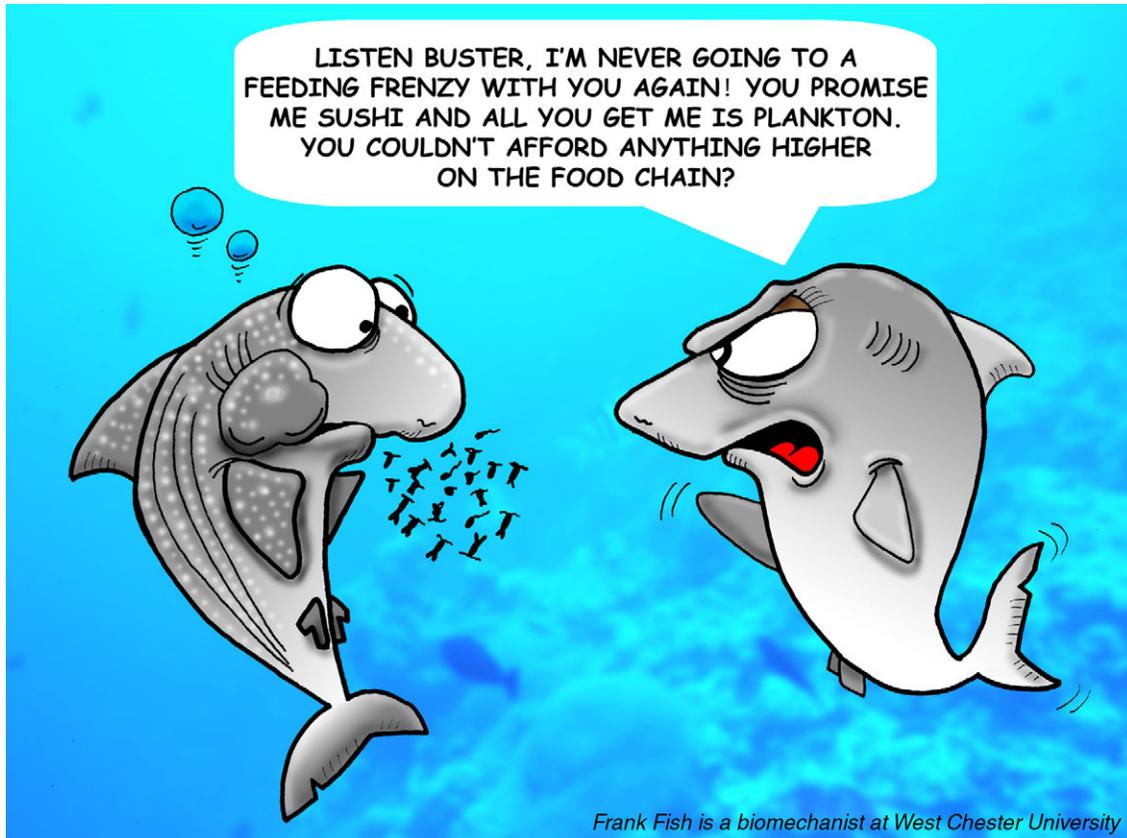
Looking at the sponge reconstructions, Nickel could see that the volume of the open canals in the relaxed sponge, occupying 72% of the body, had closed down in the contracted sponge to 32%. Also, the mesohyl connective tissue comprised 60% of the contracted sponge's body, as opposed to 28% of the relaxed sponge's body. However, when he compared the absolute volume of the mesohyl in the relaxed and contracted sponges, he could see that it was unchanged. The mesohyl was not involved in the contraction phase of the sponge's cycle. Then, Nickel measured the volume of the thin outer pinacoderm layer relative to the mesohyl volume, and he found that the volume of the pinacoderm layer had reduced 2.75-fold. The pinacoderm was driving the sponge's contraction.

Having confirmed that the pinacoderm is responsible for sponge contraction, Nickel says, 'I was surprised how clearly our results support one of the two hypotheses,' and adds, 'This leads to the question of whether epithelial contractility is a character in the ground pattern of the Metazoa. I think it is an interesting hypothesis and will fuel the discussion on the evolutionary transition from non-muscular contractile cells to muscle cells.' Also, he does not rule out a role for the mesohyl in the sponge's contraction cycle, suggesting that spindle-shaped cells in the mesohyl could behave as antagonists to the pinacoderm's contraction and return contracted sponges to their full size.

10.1242/jeb.058768

**Nickel, M., Scheer, C., Hammel, J. U., Herzen, J. and Beckmann, F.** (2011). The contractile sponge epithelium *sensu lato* – body contraction of the demosponge *Tethya wilhelma* is mediated by the pinacoderm. *J. Exp. Biol.* **214**, 1692-1698.

BOTTLES SHOW HOW SHARKS FILTER FEED



Some sharks get a bad press from humans while the gentler filter-feeding members of the family rarely get a mention. Whale sharks, megamouth sharks and basking sharks peacefully cruise the oceans and the public barely gives them a thought. However, ecologists and conservationists are keen to find out more about these creatures. Understanding the size of food particles consumed by these elusive animals is key to predicting their migration patterns and population levels; however, measuring how these fish filter food is almost impossible. Which is why Misty Paig-Tran and her colleagues at Friday Harbor Laboratories and the University of California, Irvine, USA, began cutting the bottoms off plastic 1 litre drinks bottles. Figuring that the cut body and neck of the bottle was a reasonable approximation to a small whale shark's gaping mouth, Paig-Tran began using the model mouths to look at how sharks filter-feed (p. 1643).

According to Paig-Tran and her colleagues, water entering a filter-feeding shark's mouth is filtered by gill rakers before passing out through the animal's gills. Adding 20–2000  $\mu\text{m}$  particles to a flow tank, the team varied the number of gill slits and the permeability of the gill rakers (by altering the gauge of the mesh in the gill slits) in their model mouths while changing the water speed to find out how these factors affected the size and distribution of the particles captured by the filter-feeding animals.

Analysing the particle distribution in the mouth models after 3 min of simulated filter feeding, the team realised that some particles were filtered directly by the gill rakers, others were caught in the gill rakers after falling out of the flow and the remainder lodged in the back of the fish's mouths. The team also saw that changing the number of gill slits affected the size of the filtered particles: the model trapped two

classes of particle – small (51–100  $\mu\text{m}$ ) and very large (>1000  $\mu\text{m}$ ) – when it had one pair of gill slits, but as the team increased the number of gill slits, they only found medium-sized particles (101–1000  $\mu\text{m}$ ) lodged in the model fish's head. Finally, changing the model's swimming speed (by increasing the flow tank rate) and the porosity of the gill rakers also affected the size distribution of trapped food particles. The fastest swimming models with the smallest gill pores trapped the most particles, and by simply speeding up the fish could switch to selectively filtering smaller plankton-sized particles.

10.1242/jeb.058784

Paig-Tran, E. W. M., Bizzarro, J. J., Strother, J. A. and Summers, A. P. (2011). Bottles as models: predicting the effects of varying swimming speed and morphology on size selectivity and filtering efficiency in fishes. *J. Exp. Biol.* **214**, 1643-1654.

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