Inside JEB is a twice monthly feature, which 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

CAROTENOIDS TIP THE BALANCE FOR TIT CHICKS

Picture by Clotilde Biard

Carotenoids are potent guardians of health. With their impressive antioxidant powers they constantly protect us from the ravages of free radical damage. Most species benefit from the physiological effects of carotenoids, but while the beneficial effects of carotenoids for adult birds are relatively well established, their effects on chicks were less clear. Clotilde Biard and Anders Møller from the Université Pierre et Marie Curie, France, were intrigued by the role that carotenoids may play in wild chick development. Would blue and great tit chicks that were kept well supplied with carotenoids have a head start over nest mates on a regular diet? Biard headed out into the Forêt d'Orient to see how chicks on a carotenoid supplemented diet fared (p. 1004).

But getting access to the tiny newborns required a degree of agility; Biard had to clamber 3 m up into trees to retrieve the youngsters from their nesting boxes. Rapidly developing a head for heights, Biard gave half of the chicks in each nest box a 0.05 ml dose of carotenoid-spiked sunflower oil, while the others were given untreated oil, repeating the supplement every two days until the youngsters fledged at 16 days of age. On the day before the chicks left home, Biard measured their weights and tarsus lengths, collected a 100 ml blood sample from a vein in the chick's wing and collected 6 yellow feathers from the breast to see whether the carotenoid boost had benefited them. She also checked how strong the chick's immune system was by injecting a small amount of antigen into the chick's wing, and measured the degree of inflammation 19 hours later.

Back in the Paris lab, Biard began analysing the blood samples to see if the carotenoid boost had improved the chick's health. But when she looked at the proportion of red and white blood cells in each sample there was no difference between the two sets of chicks. Checking the fledgling's feather colour with a spectrometer, Biard saw that the carotenoid-fed great tits' feathers were much brighter than those of unsupplemented great tits. However, the blue tits' plumage hadn't benefited from the carotenoid dietary supplement at all. And when she compared the degree of inflammation produced by the antigen injection, that was also unaffected by the extra carotenoids in the chicks' diet: carotenoids had not improved the youngsters' immune response. Teaming up with Peter Surai at the Avian Research Centre in the Scottish Agricultural College, Biard analysed the levels of antioxidants in the bird's blood and was surprised that the plasma carotenoid concentration was essentially the same in carotenoid supplemented chicks and chicks on a regular diet.

The only detectable benefit to the chick's condition that Biard found was that the smallest chicks on the carotenoid enhanced diet were heavier than equally sized unsupplemented chicks, giving the heavy guys a much greater chance of survival than their lighter nest-mates. 'It seems that dietary availability of carotenoids may have important fitness consequences for tits' says Biard. 'The picture is far more complicated than we initially thought' she adds.

10.1242/jeb.02173

Biard, C., Surai, P. F. and Møller, A. P. (2006). Carotenoid availability in diet and phenotype of blue and great tit nestlings. *J. Exp. Biol.* **209**, 1004-1015.

BLOCKING THE BUZZ OF INTERFERENCE

If you feel overwhelmed in a crowd, spare a thought for electric fish. In their underwater world, the electric organ discharges (EOD) of each passing stranger can interfere with a fish's own EOD-generated view of its surroundings. Intrigued by this problem, Angel Caputi and his team from the Instituto de Investigaciones Biológicas Clemente Estable in Uruguay set out to investigate how electric fish stop the signals of neighbouring fish from jamming their own signals (p. 1122).

'An electric fish "illuminates" its surroundings in an electrical way,' Caputi explains. The electroreceptors in its skin detect changes in the fish's self-generated electric field when it passes nearby objects. Nerve fibres run from the electroreceptors to the electrosensory lobe in the fish's brain, and nestled deep inside this lobe are spherical cells. Spherical cells are highly specialized neurons that transmit information using a latency code: they fire only once, and the latency of this single spike is directly related to the electrical input that caused it. From previous work, the team had good reason to suspect that spherical cells enable electric fish to block jamming signals from other fish.

To take a closer look at the role of spherical cells in the avoidance of jamming, the team caught *Gymnotus* fish in lakes and creeks around Montevideo, Uruguay. Back in the lab, they inserted two tiny wires into a fish's brain to record the spherical cells' activity. Placing this fish in an aquarium, they restrained other live fish in various locations in the tank and recorded the fish's responses to its own EOD and those generated by the other fish. They discovered that after the fish responded to a self-generated EOD, it no longer responded to an EOD generated soon afterwards by a neighbouring fish. In other words, activation of a fish's electrosensory pathway by its own EOD blocks responses to subsequent EODs for a period of time.

But is this ability to block potential interference from other fish conferred by the fish's spherical cells? To find out, the team stimulated spherical cells in *in vitro* brain slices with stepped current at different delays and examined the cells' responses. Constructing voltage *versus* current plots, the team saw that the cells responded just as the live fish had: after responding to a pulse, spherical cells did not react to further stimuli for a long period. This suggests that the spherical cells enable *Gymnotus* fish to avoid jamming signals from other fish. The team also found that the potassium conductance of the spherical cell membrane increases after a spike, which causes the reduced excitability of the cell.

The team concludes that the intrinsic properties of the spherical cells' cause their low responsiveness to subsequent stimuli shortly after they have fired. 'These cells create a time window that blocks interfering stimuli,' Caputi says. Cleverly, electric fish repeat their self-generated EODs at slightly longer intervals than the cells' low responsiveness window, allowing them to enjoy a continuous stream of self-generated information about their surroundings – even when they're in a crowd.

10.1242/jeb.02172

Nogueira, J., Castelló, M. E. and Caputi, A. A. (2006).The role of single spiking spherical neurons in a fast sensory pathway. *J. Exp. Biol.* **209**, 1122-1134.

Yfke van Bergen

COULD HIF HOLD THE KEY TO COPING WITH THE COLD?

Oxygen is the key to life for every species. But some creatures have evolved to get by with virtually none for short periods, thanks to a suite of specially developed physiological mechanisms known as the hypoxia response. One of the main regulators of the hypoxia response is a transcription factor protein known as hypoxia-inducible factor (HIF), which controls survival mechanisms, such as the upregulation of metabolic enzymes, when oxygen is scarce. When oxygen levels are high, one of the HIF subunits, HIF1a, is degraded, disrupting the transcription factor and inactivating the protective responses. But when oxygen levels fall, HIF1a degradation stops and the active transcription factor bursts into action. When Mikko Nikinmaa identified the first HIF in a cold-blooded fish, it occurred to him that the hypoxia response must be fine tuned for different environmental conditions; the physiological challenges of an hypoxic encounter might be very different at low and high temperatures. Could temperature regulate the hypoxia response? Working with Eeva Rissanen and Hanna Tranberg, Nikinmaa began testing whether temperature regulates HIF in a champion of hypoxia tolerance, the crucian carp (p. 994).

First the team had to catch some fish. Tranberg headed out to a local pond, well known for its crucian carp population, trapping several hundred fish ready to analyse the fish's response. Back in the lab, Rissanen and Tranberg acclimated the fish to 26, 18 and 8°C for several weeks, before she and Tranberg exposed them to hypoxia and measured HIF mRNA levels, protein levels and the protein's DNA binding activity.

First the team analysed the effects of temperature alone on the transcription factor, and the results were intriguing. As the temperature fell from 26°C, the normoxic fish's mRNA levels didn't vary, but their HIF protein levels rose significantly. And when the team tested the protein's activity level it had increased by as much as threefold when the temperature dropped from 26°C to 8°C. The transcription factor's behaviour changed as the fish got colder.

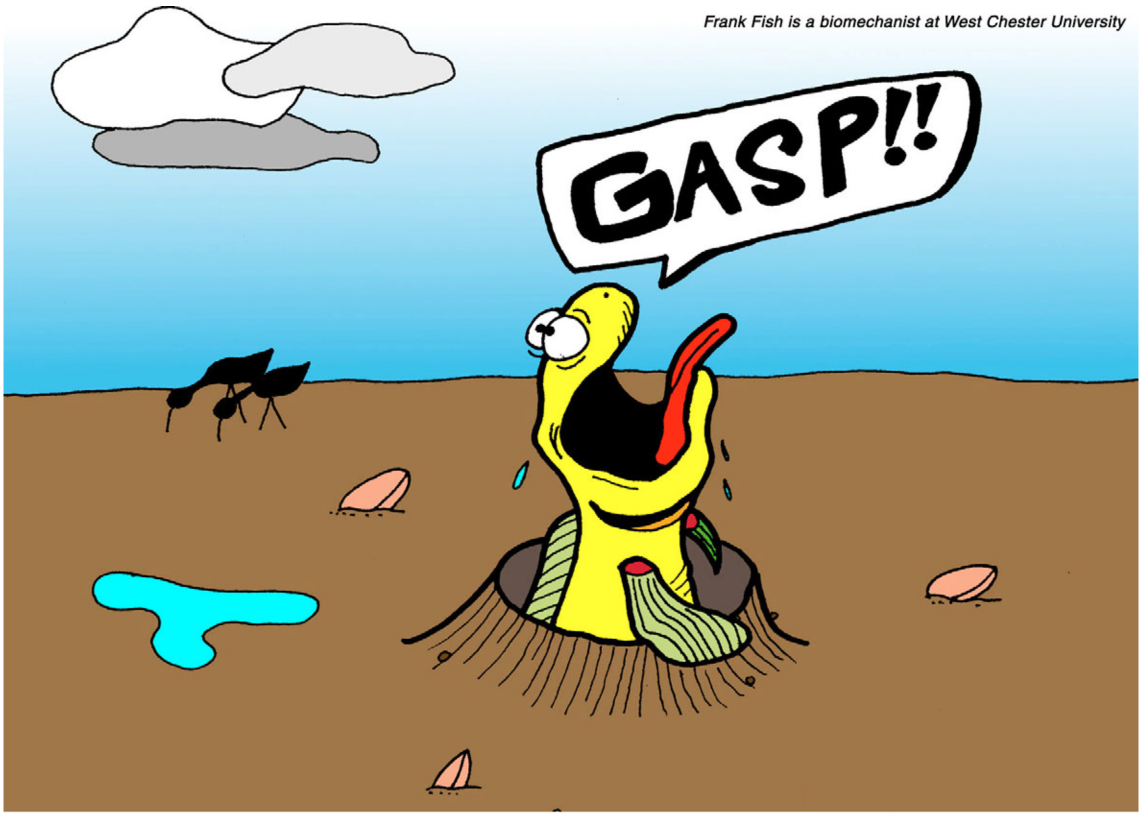
Turning to the effect of temperature on the fish's hypoxia response, the team were surprised to see that the mRNA levels did rise a little, even at 26°C, even though it is known that HIF mRNA levels remain unchanged in hypoxic mammalian cells. And when the team measured the fish's HIF protein levels at all temperatures, they saw them rise too. However, the protein's DNA binding activity increased only in the cold fish. The transcription factor's response to hypoxia had been modified by the cold.

Rissanen explains that when the temperature falls, chilly enzymes become more sluggish. But if the fish can synthesize higher levels of metabolic enzymes by activating HIF to compensate for the activity loss, they can maintain higher levels of metabolism than expected in the cool conditions. The team suspect that HIF could play a major role in temperature acclimation in cold-blooded creatures.

10.1242/jeb.02174

Rissanen, E., Tranberg, H. K., Sollid, J., Nilsson, G. E. and Nikinmaa, M. (2006). Temperature regulates hypoxia-inducible factor-1 (HIF-1) in a poikilothermic vertebrate, crucian carp (*Carassius carassius*). *J. Exp. Biol.* **209**, 994-1003.

EEL GOBIES SURFACE FOR AIR



As the tide recedes on the mudflat, the eel goby decides it's time for a breather.

How the first land dwellers adapted to breathe air after emerging from the depths has fascinated us for decades. While many estuarine air-breathing fish have adopted an amphibious life style, absorbing oxygen through blood vessels in the mouth when roaming at low tide, little was known about air-breathing fish that stay put in flooded burrows at low tides. Tomas Gonzales, Masaya Katoh and Atsushi Ishimatsu decided to go down a mudflat near Nagasaki, Japan, to put the aquatic air-breathing eel goby under surveillance (p. 1085).

First the team recorded burrow oxygen levels while waiting for eel gobies to emerge and start breathing; the fish surfaced when the oxygen fell to 13% of normoxic levels. Watching the eel gobies breathe, the team saw that the fish took great gulps of air, holding it in their mouths for up to 25 min before exhaling. Wondering how the fish absorbed oxygen from the mouthful of air, the team looked to see if the eel gobies had converted their mouths into blood-rich impromptu lungs like other air-breathers, but they hadn't. The fish must be using their collapsed gills to absorb oxygen.

Gonzales suspects that breathing air has given eel gobies the upper hand in their rich mudflat homes, allowing them to stay put and reap the ecosystem's benefits at times when other fish would be left high and dry.

10.1242/jeb.02175

Gonzales, T. T., Katoh, M. and Ishimatsu, A. (2006). Air breathing of aquatic burrow-dwelling eel goby, *Odontamblyopus lacepedii* (Gobiidae: Amblyopinae). *J. Exp. Biol.* **209**, 1085-1092.

Kathryn Phillips
kathryn@biologists.com