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

GLIMPSE OF A TWILIGHT WORLD

Picture by Michael Pfaff

Sönke Johnsen is fascinated by worlds we cannot see, whether it's the ocean depths or the nocturnal world. But just because these worlds are hidden from our eyes doesn't mean they are invisible to the species that populate them; they are well adapted to dim conditions. One sense thought to be of little use to light deprived creatures was colour vision; we only see the twilight world in shades of grey as coloured light receptors fail when the light is low. That was until Almut Kelber discovered that some nocturnal hawkmoths and geckos are capable of colour vision, even in the dimmest starlight. This remarkable discovery set Johnsen wondering. How would the world appear to nocturnal creatures when bathed in the shifting shades of night and twilight? Would nocturnal creatures benefit from a Technicolor world? Johnsen and his colleagues set about collecting spectra at different times of day in his quest to see the twilight world through hawkmoth eyes (p. 789).

Fortunately, physicists Javier Hernández-Andrés and Raymond Lee Jr, had already collected many of the daylight spectra that Johnsen was curious to investigate from a hawkmoth's perspective. But collecting spectra at night was easier said than done; much of the planet is blanketed in the orange haze of light pollution after dusk, making it almost impossible to record natural light spectra between dusk and sunrise. Turning to the International Dark Sky Association's light pollution maps, Johnsen, Alison Sweeney and Edith Widder headed out to particularly remote and dark sites to collect spectra as twilight passed into night on full- and moonless nights. But the team were defeated when they tried measuring starlight's faint spectrum. Even after heading out to sea to escape land's polluted night skies, their equipment wasn't sensitive enough. The team finally resorted to calculating the spectrum.

With thousands of spectra in hand, Johnsen was amazed at the range of colours that illuminate the world as daylight fails. At the height of the day, the spectrum was fairly white, but as the sun descended beneath the horizon during twilight, the shade changed to a deep blue. Johnsen admits that he was puzzled by this colour change until he learned that the spectrum was caused when ozone absorbs certain wavelengths of light, producing the deep blue hue. Johnsen adds he was most surprised when he analysed the starlight spectrum. It was red.

But how would these light changes affect the hawkmoth's vision? Teaming up with Kelber and Eric Warrant, they calculated how the insect saw coloured flowers under different illuminating colours. The team found that yellow and blue flowers were clearly visible in some lights as sunrise and sunset progressed, and when they tested the insect's view of its preferred colour, white, the flower stood out well at all stages of night and day. Kelber also calculated how much the colours shifted in the insect's visual system as the illuminating colours changed, and found that although the flowers became brighter and dimmer as the light varied, they were still more recognisable against a leafy background when viewed in colour than if the moth was restricted to black and white vision. Johnsen explains that although colour vision is less sensitive than monochrome, the added dimension gives hawkmoths a clearer dusk view.

10.1242/jeb.02143

Johnsen, S., Kelber, A., Warrant, E., Sweeney, A. M., Widder, E. A., Lee, R. L., Jr and Hernández-Andrés, J. (2006). Crepuscular and nocturnal illumination and its effects on color perception by the nocturnal hawkmoth *Deilephila elpenor*. *J. Exp. Biol.* **209**, 789-800.

CUTTLEFISH OXYGEN CUT AT EXTREME TEMPERATURES

Over the last decade, the effects of global climate change have become the subject of hot debate. And while the politicians have difficulty agreeing how to deal with the threat, ecologists regularly record species shifting territory as their environment alters. The effect of temperature fluctuations on animal physiology also intrigues Hans-Otto Pörtner. In recent years, Pörtner has investigated the effects of extreme temperature and found that water-breathing vertebrates experience difficulties, resorting to anaerobic metabolism, as their oxygen supply becomes limited at both high and low temperatures. But how widespread is this problem? Pörtner and his colleagues Frank Melzner and physicist Christian Bock decided to investigate the effects of high and low temperatures on oxygen supply in a sophisticated invertebrate: the cuttlefish, *Sepia officinalis* (p. 891).

According to Melzner, cuttlefish are the 'couch potatoes' of the cephalopod world so were perfectly content to sit in Bock's state-of-the-art MRI system for days at a time, having their metabolism monitored with a finely tuned phosphate probe. Melzner explains that animals that derive energy from anaerobic metabolism regenerate spent ATP supplies *via* phospho-L-arginine, liberating inorganic phosphate as ATP is synthesised. Monitoring the cuttelfish's inorganic phosphate levels allowed the team to keep track of the animal's metabolism allowing them to spot the moment when it switched to anaerobic metabolism if oxygen became limited at extreme temperatures.

But cuttlefish mantle muscles also respire anaerobically whenever they set off for a quick jet. How could the team distinguish a bout of activity from an oxygen-limited switch to anaerobic metabolism? By monitoring the pressure surges in the cuttlefish's mantle cavity. Melzner explains that cuttlefish breathe by gently pulsing their mantles, generating pressures around 30 Pa; however, propulsive jets result in massive pressure surges, as great as 10 000 Pa. By measuring the pressure fluctuations in the cuttlefish's mantle cavity, Melzner and Bock were confident they could distinguish between a temperature-induced switch to anaerobic metabolism and a burst of cuttlefish exuberance.

Settling individual cuttlefish in an aquarium surrounded by a powerful MRI magnet, Melzner and Bock began the painstaking task of recording the cephalopod's phosphate levels as the team gently changed the temperature. Melzner remembers that it was a nail-biting experience, keeping the cuttlefish supplied with specially shipped-in clean seawater, while Bock monitored the magnets and water temperature. Measuring phosphate levels as the temperature rose, the team were amazed to see a sudden increase in muscle inorganic phosphate levels; the cuttlefish had switched to anaerobic metabolism at 26.8°C. And as the team dropped the water temperature, they saw the cuttlefish recruit anaerobic metabolism again, at temperatures ranging between 5-8°C. Somehow the cuttlefish's oxygen supply had become limited.

Melzner admits that he was surprised by how clear the switch was when the cuttlefish shifted to anaerobic metabolism. He adds that he is keen to discover the mechanisms that limit the cephalopod's oxygen supply and suspects that they will be different for high and low temperatures.

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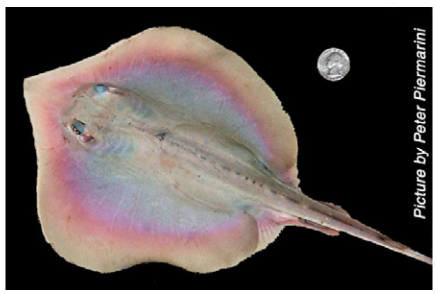
Melzner, F., Bock, C. and Pörtner, H.-O. (2006). Critical temperatures in the cephalopod *Sepia officinalis* investigated using *in vivo* ^{31}P NMR spectroscopy. *J. Exp. Biol.* **209**, 891-906.

ELASMOBRANCHS BALANCE UREA



For most creatures, urea is a nasty toxin best got rid of, but for many elasmobranchs, it keeps them in balance with their salty environment. Yet despite this apparently amicable relationship, high tissue levels of urea still have nasty side effects on proteins. Jason Treberg in William Driedzic's Newfoundland Lab knew from George Somero and Paul Yancey's work that proteins become stable when the ratio of urea to methylamines is 2:1, but there were only a few measurements of this ratio in marine elasmobranchs. Was this ratio wide spread amongst elasmobranchs, and if so, were methylamines alone responsible for balancing their high urea levels? Treberg began measuring tissue levels of methylamines in a range of fish from the marine shark *Chiloscyllium punctatum* to the freshwater Brazilian stingray to find out whether the ratio held up to closer scrutiny (p. 860).

Treberg admits that obtaining samples from a wide range of species was probably the most daunting aspect of this project. James Ballantyne, Ben Speers-Roesch and Alex Ip supplied Treberg with samples from a marine stingray, a shark and two freshwater stingrays (one that retains some urea while the other does not), while Peter Piermarini had access to a euryhaline stingray (able to tolerate marine and freshwater environnments) to add to the marine skate that Treberg could obtain himself.

Sure enough, the methylamine levels behaved as Treberg had expected, with the highest detected in the marine species while the freshwater species had virtually none, and the euryhaline species carrying intermediate levels. And when Treberg calculated the ratio of urea to a methylamine trimethylamine *N*-oxide (TMAO), alone, it was approximately 2:1 in the marine species, while some of freshwater species' had a higher ratio and others lower. Were the freshwater adapted species turning to other osmolytes to stay in equilibrium with their environment?

Turning to HPLC methods to discover which osmolytes these species carried, Treberg found that both freshwater species accumulated more β-amino acids than methylamines. When he calculated the urea to total osmolyte ratio and plotted the relationship on a graph, it was close to 2:1. Treberg explains that the urea levels in freshwater adapted species are so low that the ratio in the Brazilian stingray is only very approximate. Also, he suspects that the ratio isn't always driven by the need to counterbalance urea's detrimental effects, but to maintain the fishes' delicate osmotic equilibria with their aquatic environment.

Curious to know whether the activities of the enzymes responsible for synthesising TMAO and betaine varied in line with the elasmobranch's methylamine levels, Treberg measured TMAoxidase levels from all seven species' and was surprised that only the shark possessed the enzyme; the rest probably derive TMAO from their diet. However, all of the elasmobranchs were capable of synthesising betaine. Treberg explains one possibility: that the shark is the only fish in this study to have retained TMAoxidase, while the rest may have lost the enzyme, deriving TMAO from their diet and supplementing with betaine when TMAO is scarce. But he adds that it is also possible that several species evolved TMAO synthesis independently, and it's impossible to be sure which is most likely.

10.1242/jeb.02145

Treberg, J. R., Speers-Roesch, B., Piermarini, P. M., Ip, Y. K., Ballantyne J. S. and Driedzic, W. R. (2006). The accumulation of methylamine counteracting solutes in elasmobranchs with differing levels of urea: a comparison of marine and freshwater species. *J. Exp. Biol.* **209**, 860-870.

COST OF DEEP DIVING FOR CORMORANTS

Axel Innis is a postdoc at Yale University.

It's tough catching your dinner in icy waters; every time a cormorant fancies a snack it has to plumb the depths. Manfred Enstipp from the Centre d'Ecologie et Physiologie Energétiques in France explains that quite a lot is known about the energetic costs of shallow dives close to the surface, but no one had measured the cost of deep dives. Knowing that birds are less buoyant at depth when the air trapped in their plumage is compressed, the team wondered whether the decreased energy costs of swimming at depth were greater than the cost incurred keeping warm after losing their insulating layer (p. 845). Enstipp, David Grémillet and David Jones measured cormorant oxygen consumption when returning to the surface after shallow horizontal 1 m dives and deep vertical 10 m dives, and found that the bird's metabolic rate was 22% greater after a deep dive than a shallow dive. The team suspect that the metabolic advantage of swimming at depth is far outweighed by the cost of maintaining their body temperature in the chilly depths after losing their thick layer of insulating air.

10.1242/jeb.02142

Enstipp, M. R., Grémillet, D. and Jones, D. R. (2006). The effects of depth, temperature and food ingestion on the foraging energetics of a diving endotherm, the double-crested cormorant (*Phalacrocorax auritus*). *J. Exp. Biol.* **209**, 845-859.

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