

Keeping track of the literature isn't easy, so Outside JEB is a monthly feature that reports the most exciting developments in experimental biology. Short articles that have been selected and written by a team of active research scientists highlight the papers that JEB readers can't afford to miss.

ANHYDROBIOSIS



BIOLOGICAL GLASS: A STRATEGY TO SURVIVE DESICCATION AND HEAT

Anhydrobiosis is an astounding strategy that allows certain insects, crustaceans, nematodes, rotifers and tardigrades to survive severe dry and/or extreme cold or hot conditions they often encounter. During anhydrobiosis the animal enters an almost completely desiccated state which stabilizes its membranes and other cellular structures, preventing otherwise lethal damage caused by environmental extremes. Two, not mutually exclusive, hypotheses propose to explain the mechanisms underlying anhydrobiotic macromolecular stabilization. First, the water-replacement hypothesis proposes that accumulated proteins and non-reducing sugars (e.g. trehalose) interact *via* hydrogen bonds with macromolecules to replace water, thus stabilizing these structures. The alternative vitrification (glass-forming) hypothesis proposes that hydrophilic molecules enter a glassy state during desiccation and this biological glass immobilizes macromolecules, thus preventing denaturation or other structural disruptions.

Steffen Hengherr, Roger Worland and colleagues, from the Universität Stuttgart and the British Antarctic Survey tested the effect of anhydrobiotic glass forming on the heat tolerance of nine Tardigrada (water bears – a sister taxon to arthropods and onychophorans) species. Glass, in this context, doesn't refer to commonly known silica-based window glass but to an amorphous (non-crystalline) biomolecular matrix forming a thermodynamic liquid, i.e. a liquid with an extremely high viscosity, corresponding to a physical solid.

Using tardigrade species from Germany, north-western United States, Kenya and Alaska, Hengherr and Worland's team gradually desiccated individuals until only 5–7% of body mass consisted of water. They then exposed the anhydrobiotic

tardigrades to temperatures from 60 to 110°C for 1 h, rehydrated them and assessed survival. They also used differential scanning calorimetry (DSC) to measure glass transition temperatures – temperatures where the biomolecular matrix changes from a highly viscous glassy state to a less viscous 'rubbery' state, or the 'melting point' of the biological glass.

When fully hydrated these tardigrades are unlikely to survive >40°C. However, during anhydrobiosis the Alaskan species' survival declined only after exposure to 60°C. Five other species could survive 80°C and two others survived 90°C. One extremely tolerant species, *Milnesium tardigradum*, could survive exposure up to 100°C. In six species, excluding *M. tardigradum*, glass transitions were clearly detected. These glass transition temperatures corresponded closely to the thermal survival temperatures, linking these transitions to possible survival-reducing spatial disarrangements of biomolecules.

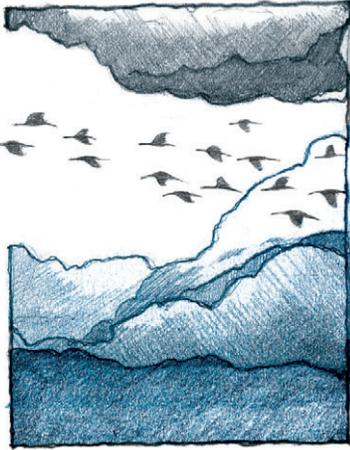
While anhydrobiosis confers markedly higher survivability in tardigrades, the lesser anhydrobiotic heat protection in the Arctic species nevertheless suggests an environmental correlate to the degree of anhydrobiotic protection. Furthermore, previous tardigrade anhydrobiosis research detected no increase in the concentration of trehalose – a key role-player in the water-replacement mechanism's anhydrobiotic biomolecular stabilization in non-tardigrade taxa. In the six species showing glass transition temperatures this supports the vitrification hypothesis. In two species the authors acknowledge that glass transitions could be below the DSC instrument's sensitivity due to small body sizes. And in *M. tardigradum*'s case undetectable glass transitions and undetectable trehalose concentration increases suggest other, as yet unknown, mechanisms conferring unusually effective anhydrobiotic protection against environmental extremes. This study emphasizes our limited understanding of anhydrobiosis and poses further research challenges regarding this remarkable phenomenon.

10.1242/jeb.036467

Hengherr, S., Worland, M. R., Reuner, A., Brummer, F. and Schill, R. O. (2009). High-temperature tolerance in anhydrobiotic tardigrades is limited by glass transition. *Physiol. Biochem. Zool.* **82**, 749-755.

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COMMUNICATION



BIRDS BEAM ALARM CALLS AT PREDATORS

When a bird sees a predator, it often gives some kind of alarm call. This may serve any, or all, of a number of purposes. Chiefly, the call can alert other nearby animals to the presence of a threat; or, the predator itself might be alerted that it has been spotted, and so be persuaded to leave the area. Distinguishing between these two roles presents a bit of a challenge. In a new approach, Jessica Yorzinski and Gail Patricelli from UC Davis set about measuring the ‘acoustic directionality’ of a range of bird species when presented with a predator – a stuffed owl. Presumably, if a bird is signalling alarm broadly, it would use a largely omnidirectional call, with the sound energy spreading fairly evenly outwards from the caller. In contrast, if the call is intended for a specific recipient (in this case, the stuffed owl), one might expect more of the energy to be targeted towards the receiver.

So, what do birds actually do? By placing small passerines near the centre of an array of eight microphones and four video cameras, and recording their alarm calls when the stuffed owl was revealed, Yorzinski was able to determine the direction of sound energy, and relate this to both the direction of the owl and the orientation of the study bird’s head. Each of the 12 species tested demonstrated a moderate degree of directionality.

Three key species, a junco, a finch and a warbler, allowed more detailed analysis, with 10–17 individuals of each type. Unsurprisingly, if we think in terms of how we humans project our voices, the calls were directed ‘forward’ when the heads pointed at the owl. However, this bias down the barrel of the beak reduced significantly when the birds faced away from the threat. In all three key species, calls were directed towards the right when the owl was on the smaller bird’s right side. What is more, in

two species the bias flipped when the owl was on the left, again the birds successfully directing more of the sound energy towards the owl. It appears that they are capable somehow of directing their calls independently from the orientation of their heads. The juncos, however, continued to project their calls to the right, albeit to a lesser extent, in effect showing a degree of ‘handedness’ in call direction.

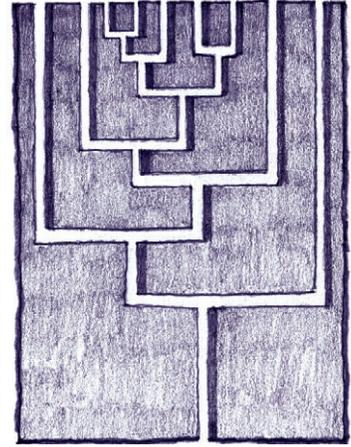
It appears that, at least in some species, alarm calls can be directed towards a threat despite changing head orientation. However, many questions remain. For instance, consider again the benefits of directing a call at an owl. Why exactly would this be beneficial? One option, to conceal the alarm from other nearby birds, does not make much sense – and in any case, the degree of directionality was not really sufficient for this. Might focusing of the sound allow a louder signal for a given energy investment? Perhaps, but models of sound production predict that directional patterns are largely caused by removing the energy heading the ‘wrong’ way, by either interference effects or the sound shadow caused by the head. So, there may be no energetic benefit of directing calls. Yorzinski and Patricelli suggest playback experiments to answer this and similar puzzles: what information might nearby conspecifics be gathering because of call directionality? Are they more willing to help with mobbing if the alarm call is directional? Even, might the direction of the threat be signalled... could the mobbing bird be shouting to its friends ‘it’s behind you’?

10.1242/jeb.036459

Yorzinski, J. L. and Patricelli, G. L. (2010). Birds adjust acoustic directionality to beam their antipredator calls to predators and conspecifics. *Proc. R Soc. Lond. B* **277**, 923-932.

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OXYGEN SENSING



THE VASCULAR TISSUE OXYGEN SENSOR STINKS!

Blood vessels of the vertebrate circulatory system are known to react to local changes in oxygen tension and thus the vasculature is finely adjusted to match perfusion to the physiological needs of each region of the body. In arteries of the systemic circulation, which provide oxygen-rich blood to the body, reduced levels of oxygen in tissues (hypoxia) trigger a dilatation of the vessels to improve delivery of blood and therefore oxygen. For many years it has been known (and become a paradigm) that in mammals the vessels that deliver oxygen-depleted blood to the lungs, the pulmonary arteries, react to pulmonary hypoxia *via* the exact opposite reaction, they constrict and thus reduce blood flow to hypoxic areas. This hypoxic vasoconstriction is a major mechanism for ensuring that blood is shunted to areas of the lungs that are highly ventilated (i.e. high in oxygen); the result is more efficient or complete gas exchange, which is of course the primary function of the lungs. The mechanisms by which the vascular cells sense and react to oxygen are not fully understood, but it is known that several cell types are involved. Ken Olson and colleagues at the Indiana University School of Medicine were investigating the role of hydrogen sulfide (H₂S – which smells of rotten eggs) signaling in the control of vasculature dilatation state in sea lion lungs when they found something new – hypoxic vasodilatation in pulmonary arteries. Further, they provide strong evidence that H₂S is the primary signal that the vessels respond to, and not reduced oxygen *per se*.

The team used a variety of methods to investigate the role of hypoxia and H₂S in controlling the dilatation state of pulmonary arteries from both cows and sea lions. First, they exposed chunks of isolated pulmonary resistance arteries (those that are critical for controlling blood flow changes in the lungs) to hypoxia and H₂S

(100–300 $\mu\text{mol l}^{-1}$) and measured their response to the signals using vessel myography. They also checked for expression of the major enzymes known to be important in the production of H_2S using immunohistochemistry and western blotting. Finally, using probes specific to oxygen and H_2S , they measured the ability of the tissues to produce H_2S and investigated the relationship between levels of oxygen and levels of H_2S in homogenates prepared from lung tissues.

Their results were surprising. Cow lungs responded to hypoxia and H_2S in a similar fashion and as expected by constricting. However, sea lion lungs responded to both signals by dilating. The team found that the enzymes required to produce H_2S were present and H_2S was produced in the lung tissue of both species. Further, they found that levels of H_2S and oxygen were inversely related, and H_2S levels began to rise at physiologically relevant levels of hypoxia that elicit a reaction from the lung arteries. Thus, it appears that cows and sea lions may both use H_2S to signal low oxygen conditions in the pulmonary arteries, but they respond to the same signal with opposite responses.

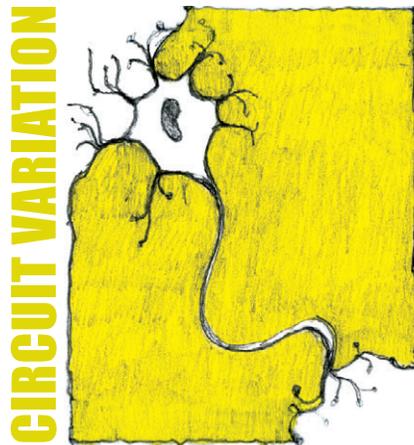
The discovery of hypoxic vasodilatation in the pulmonary arteries of sea lions is surprising and significant. The reason for this difference is likely adaptive and related to the diving physiology of marine mammals. However, perhaps the more interesting conclusion is that H_2S may act as an oxygen sensor in these tissues. The model that Olson and colleagues propose for H_2S as an oxygen sensor requires continuous cytoplasmic production of H_2S . Under normal oxygen levels the H_2S is simultaneously removed by mitochondrial oxidation; however, when tissues become hypoxic and oxygen scarce, H_2S oxidation is reduced, leading to a rise in H_2S levels.

Given that the enzymes required to produce H_2S are widespread in animal tissues, it is likely that this simple, elegant, and powerful mechanism may underlie oxygen sensing and signaling in many physiological contexts where oxygen sensors have been postulated to exist, but are yet to be identified.

10.1242/jeb.036475

Olson, K. R., Whitfield, N. L., Bearden, S. E., St Leger, J., Nilson, E., Gao, Y. and Madden, J. A. (2010). Hypoxic pulmonary vasodilation: a paradigm shift with a hydrogen sulfide mechanism. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **298**, R51–R60.

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CIRCUIT VARIATION

NEURAL CIRCUITS GOOD ENOUGH DESPITE INTERINDIVIDUAL VARIATION

Simulated neural circuits can generate similar output despite variability in the intrinsic properties of individual neurons and the connections among them (synaptic properties). But few studies have attempted to actually measure just how much a particular neural circuit varies between individuals in an animal population. Jean-Marc Goaillard, Adam Taylor, David Schulz and Eve Marder took on this challenge in a recent paper published in *Nature Neuroscience*.

The researchers work with a small neural network in the crab nervous system: it consists primarily of a pair of coupled, rhythmically active cells (pacemakers), which make inhibitory synapses onto two types of follower neurons. The follower neurons are inhibited during periods of pacemaker activity, but then rebound and become active (with characteristic delays) as the pacemaker falls silent. This ends up producing a rhythm with three distinct activity bursts within each cycle.

First, the team measured animal-to-animal variability in the output of their network and found that within an animal, the speed of the rhythm was constant, but between animals, rhythm speed varied 2- to 4-fold. They also found that, in every animal, each burst of activity occupied the same percentage of a cycle, regardless of the speed of the rhythm in that particular animal. It was as if, from animal to animal, the same burst pattern was simply stretched or squished horizontally so that its duration varied but occupied the same fraction of the cycle in every individual. This suggests that some features of the network must remain constant even at different cycle speeds.

To examine the underlying causes of population variability, Goaillard and colleagues measured parameters thought to

play important roles in determining the phases of network activity. First, they measured the relative strengths of the synapses from the two pacemaker cells onto one follower cell. They found that the conductances (strengths) of both synapses varied widely between crab individuals; however, the conductances of the two synapses were always negatively correlated with each other such that the total synaptic conductance of the two synapses was held approximately constant. Since follower neuron activity can also be modulated by neuropeptides, the team measured a modulator evoked intrinsic current and found that the modulator current was highly variable, but positively correlated with the strength of one of the pacemaker–follower synapses and the firing properties in the follower. In a final *tour de force*, the team plucked out each pacemaker and follower cell and measured expression levels of a whole battery of ion channels known to affect neuronal firing properties. Again, there were large variations from animal to animal, but they also found strong correlations between expression levels and various circuit parameters.

The work of Goaillard and colleagues is important because it provides experimental evidence for the idea that neural circuits can find more than one way to produce functional output that is ‘good enough’ for the role it has to satisfy. This work also establishes a new standard for detailed circuit analysis. The authors’ multi-dimensional analysis techniques reveal a complex network of correlations between intrinsic properties, synaptic properties and the network output. Lists of correlations may seem like dull accounting at first but, taken as a whole, they provide important insights into how multiple parameters within a neural network are co-regulated and which parameters are functionally critical. These kinds of insights will be essential if we want to understand how circuits with variable underlying components organize themselves.

10.1242/jeb.036483

Goaillard, J.-M., Taylor, A. L., Schulz, D. J. and Marder, E. (2009). Functional consequences of animal-to-animal variation in circuit parameters. *Nat. Neurosci.* **12**, 1424–1430.

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