

INSIDE JEB

Kangaroos are not low gas producers



A western grey male and female kangaroo.
Photo credit: Adam Munn.

Ever since the agricultural revolution, humans have lived cheek by jowl with cows, enjoying their meat, milk and cream. However, there is one unfortunate by-product from this relationship: methane. Dairy cows can each produce up to 200 l of methane per day and, with an estimated world population of 1.4 billion animals, that quickly adds up to a serious greenhouse gas problem. However, one family of foregut fermenters seemed to buck the flatulence trend: measurements in the 1970s and '80s of kangaroo and wallaby methane production suggested that they produced much less gas than ruminants. Explaining that the gas is produced by specialised microbes – known as Archaea – in the animals' foreguts, Adam Munn from the University of Wollongong, Australia, says, 'The idea that kangaroos have unique gut microbes has been floating around for some time and a great deal of research has gone into discovering these apparently unique microbes'. However, Munn adds that the kangaroo's seemingly low methane production had never been confirmed. Intrigued by the mystery, Munn and his long-time collaborator Marcus Clauss from the University of Zurich, Switzerland, decided to measure everything that went into and came out of kangaroos to get to the bottom of the problem.

Knowing that methane production is dramatically affected by the length of time that a meal takes to pass through an animal and that food passes through well-fed kangaroos faster than through hungry animals, Munn and Clauss decided to feed alfalfa to the animals at two different

levels (a restricted diet *versus* all they could eat) to find out how that affected the animals' methane production. However, Clauss had little experience of working with marsupials, despite years of measuring methane production in other species, whereas Munn had worked with kangaroos for over 20 years. So, Clauss's student Catharina Vendl travelled to the University of New South Wales' Fowlers Gap Research Station to work with Munn, Matthew Stewart and Keith Leggett to measure the animals' methane production and metabolic rates while also collecting their faeces. Then, back at the ETH Zurich in Switzerland, Michael Kreuzer analysed the nutrient content of the kangaroo's feed and faeces to find out just how much food the kangaroos had digested in relation to the amount of methane they produced.

After months of patiently pulling together the data, the team could see that their kangaroos produced similar quantities of methane to other kangaroos for their body size. However, when the team investigated the kangaroos' methane production relative to their metabolism, it was essentially the same as that of horses; so the kangaroos are no less flatulent than other herbivores, although they still produce less methane than cows. And when the team calculated the amount of methane produced relative to the animals' food intake, they were impressed to see that instead of producing more methane, the kangaroos that were free to eat their fill produced less gas. Just like other species that rely on fermentation as part of the digestion process, the well-fed animals produced less methane because food was passing through the gut faster, leaving less time for the microbes to break down the tough plant material and produce the gas.

'Kangaroos are not mysteriously low methane-producing creatures, but herbivores with an active methane-producing microbe community', says Clauss, adding that he and Jürgen Hummel suspect that the environment in the marsupial's foregut may naturally reduce Archaea methane production. And Munn is keen to find out how the animals' methane production will be affected by changes in diet quality as climate change

takes hold and the frequency of droughts increases.

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Vendl, C., Clauss, M., Stewart, M., Leggett, K., Hummel, J., Kreuzer, M. and Munn, A. (2015). Decreasing methane yield with increasing food intake keeps daily methane emissions constant in two foregut fermenting marsupials, the western grey kangaroo and red kangaroo. *J. Exp. Biol.* **218**, 3425–3434.

Kathryn Knight

Backswimmers use buoyancy aid like a gill



Anisops deanei. Photo credit: Karl Jones.

Ever since he can remember, Karl Jones has been fascinated by the animals that live in streams. 'I grew up next to a river in the Adelaide Hills where I spent many hours catching the creatures that live there', he recalls; and one group of aquatic insects that caught his eye was the backswimmers. However, two members of the Family (*Anisops* and *Buenoa*) have an even more remarkable talent: they can swim at depths that other backswimmers can only reach fleetingly. Explaining that the intrepid insects take down an air bubble that they use to regulate their buoyancy, Jones adds that other aquatic insects depend on air bubbles to extract oxygen from the water. However, it was believed that the surface area of the deep-diving backswimmers' bubble was too small to allow them to use the buoyancy aid as an *ad hoc* gill to supplement their oxygen supply: Roger Seymour and Jones decided to test this.

As the insects consume oxygen from the bubble, a small amount of nitrogen diffuses out during a dive, reducing their buoyancy, and Seymour realised that he could alter the buoyancy changes by replacing the nitrogen in the bubble with other gases, such as helium – which is lost faster than nitrogen – and sulphur hexafluoride – which diffuses away more slowly. By filming the insects while they swam, Jones, Seymour, Edward

Snelling and Amy Watson could see how the different gases affected the insects' buoyancy and dive durations.

Collecting *Anisops deanei* from a local pond, Jones built mini-dive chambers where he could monitor a pair of insects diving simultaneously, and filmed over 300 dives. Analysing the movies with Watson, Jones could see that the insects carrying helium-laced air bubbles had the shortest dives (1.5 min), while the sulphur hexafluoride-doped bubble allowed the insects to remain submerged for an impressive 3 min, compared with the 2 min dives clocked up by the insects diving with regular air. Gas was definitely being lost from the bubbles, suggesting that oxygen could also diffuse in from the surroundings.

The team also noticed that during the initial descent the insects had to swim hard to overcome the bubble's buoyancy. However, they became less buoyant as they consumed oxygen over the course of the dive until the volume of the bubble had shrunk enough for the insects to become slightly negatively buoyant. At this point, the insects began releasing oxygen stored by haemoglobin in the abdomen, stabilising the bubble's volume to maintain almost neutral buoyancy while they remained submerged. Next, the team built a computer simulation of gas loss from the bubble based on the buoyancy changes that they observed, and realised that the insects could extend their period of near-neutral buoyancy by extracting as much as 20% of the oxygen consumed during the dive from the water.

Having confirmed that backswimmers use their dive bubbles as a gill, Jones is keen to find out whether the insects detect the oxygen level in the bubbles that they carry. Explaining that oxygen diffuses faster through helium than through sulphur hexafluoride, Jones was amazed to see that the helium insects only surfaced for 0.18 s to recharge the bubble, compared with the sulphur hexafluoride insects, which took 10 times longer, suggesting that the insects only put themselves at risk at the surface for as long as it takes to recharge the oxygen.

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Jones, K. K., Snelling, E. P., Watson, A. P. and Seymour, R. S. (2015). Gas exchange and dive characteristics of the free-swimming backswimmer *Anisops deanei*. *J. Exp. Biol.* **218**, 3478–3486.

Kathryn Knight

Scent of doom improves larvae's vision



Hemigrapsus sanguineus larvae. Photo credit: Corie Charpentier.

Crab mums will never win the 'Best Zoological Parent' award: as soon as a female releases her young, she's off, leaving the offspring to head for the depths and fend for themselves. Corie Charpentier from the University of Delaware, USA, explains that the minute creatures only ascend to feed under cover of dark, descending again when the sun rises: 'The daytime depth is set by the lowest light intensity that they can detect', Charpentier says. These zooplankton are also sensitive to sudden changes in light intensity – dodging deeper to evade detection when a predator's shadow passes over – and the tiny crustaceans seem to become even more sensitive to light changes after sniffing a predator's odour; 'Crab larvae descend following smaller increases in downwelling light after exposure to predator chemical cues', explains Charpentier. However, it wasn't clear how the threatening odours, known as kairomones, affected the larvae's vision. 'Little work has been done to understand how zooplankton integrate these visual and chemical cues to control swimming behaviour', Charpentier says, so she and her thesis advisor, Jonathan Cohen, set about finding out how the aroma of fear affects crab larval vision.

Fortunately, collecting the larvae was as easy as taking egg-laden crab mums-to-be from nearby estuaries and waiting for them to release their offspring. Charpentier and Cohen also decided to investigate two local species with dramatically different larval lifestyles: dwarf crab (*Rhithropanopeus harrisi*) larvae live in predator-infested estuaries while invasive Asian shore crab (*Hemigrapsus sanguineus*) larvae are transported offshore to safer waters. Then, the duo gently wiped mucus from the bodies of a local fish, the mummichog, and diluted the goo to produce 'eau de

predator' before testing its effects on larval vision.

Filming the larvae's responses as she simulated 3 s flashes of light filtering down to depths of 9–12 m, Charpentier saw that the lowest light levels that triggered the larvae's defensive descent were 1.1×10^{13} photons $\text{m}^{-2} \text{s}^{-1}$ for the dwarf crab larvae and 4.77×10^{12} photons $\text{m}^{-2} \text{s}^{-1}$ for the Asian shore crab larvae. However, when the youngsters had been bathed for up to 3 h in the menacing fish odour, both species became much more sensitive, plunging to safety at intensities that were only a third as bright as the light that had triggered an escape response before they sniffed the odour.

But how had the alarming smell affected the larvae's eyes? Charpentier painstakingly measured electrical activity in the retina of larvae as she exposed the animals to increasingly dimmer flashes of light, and found that the retina's light sensitivity increased after bathing in the kairomone stench. And when she tested the effect of the odour on the speed of the retina's response to light, she was surprised that the sensitised larvae responded as fast, and sometimes even faster, than the larvae that had not experienced the kairomone threat. Charpentier explains that this was unexpected as an increase in light sensitivity is usually accompanied by slower visual responses in most animals.

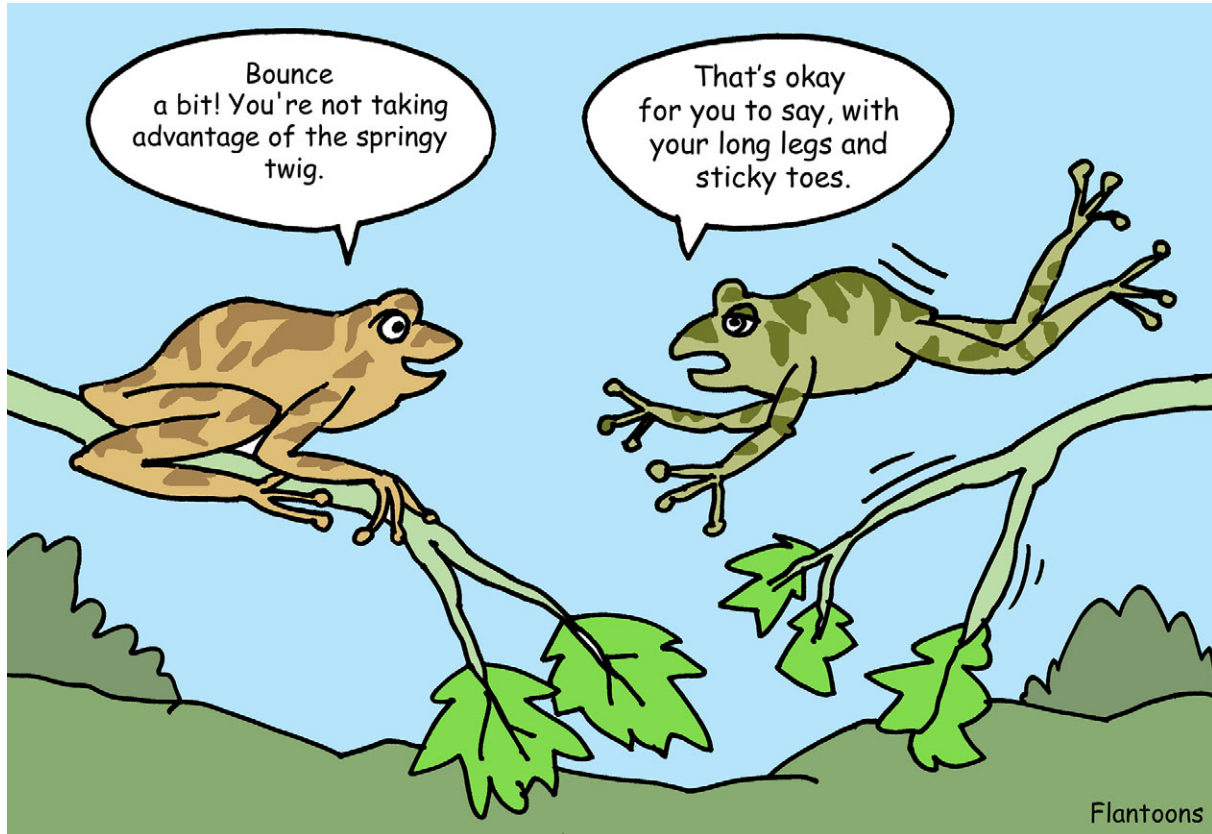
Having confirmed that kairomones affect the visual sensitivity of the larvae's retina, Charpentier took a closer look at the structures that comprised the crustacean's compound eye to see whether they had altered, and she was impressed to discover that each eyelet (rhabdom) became wider, increasing the structure's light sensitivity, while the dwarf crab's eye structures also became shorter. Exposure to kairomone odours released by predatory fish was altering the sensitivity of the larvae's eyes to light by directly affecting the eye structure and retina activity to give them a head start when danger looms.

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Charpentier, C. L. and Cohen, J. H. (2015). Chemical cues from fish heighten visual sensitivity in larval crabs through changes in photoreceptor structure and function. *J. Exp. Biol.* **218**, 3381–3390.

Kathryn Knight

Leaping Cuban tree frogs benefit from rebound boost



Springboard divers get a major boost when bouncing repeatedly on a flexible board. However, for most animals that bound from a bendy surface, the results are disappointing. Tree frogs and other arboreal species that don't bounce up and down – and are unable to capitalise on the rebound – lose mechanical energy when they launch from a springy surface. Henry Astley, Alison Haruta and Tom Roberts from Brown University, USA, say, 'In all species previously examined, animals lost contact with the substrate prior to recoil, preventing any recovery of the energy imparted to the substrate'. The trio wondered whether Cuban tree frogs could be even more compromised, thanks to their extraordinarily long legs. Building a springy frog perch from a length of latex

tubing stretched to varying degrees to alter the stiffness, Astley, Haruta and Roberts tickled the frogs with a brush and filmed their take-offs with high-speed cameras to capture every detail of the launch.

Describing how the most flexible perch deflected by 34% of the frog's leg length, while the least flexible perch only extended by 10%, the team then calculated how much energy was stored in the perch during a leap and was impressed to see that almost half of this energy was returned to the frog by the stiffer perches as they recoiled just before the animal's feet lost contact to thrust the animal into the air. 'The recoil of an elastic perch presents an

opportunity to recover some of this energy to reduce the potential detriment to jump performance', says the team, and Astley adds, 'These tree frogs are the first known examples of an animal recovering perch energy from the perch during a jump from a static posture'. They also suspect that the frogs' long legs and sticky toes could help them to stay in contact with the perch for longer to take advantage of the rebound boost.

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Astley, H. C., Haruta, A. and Roberts, T. J. (2015). Robust jumping performance and elastic energy recovery from compliant perches in tree frogs. *J. Exp. Biol.* **218**, 3360–3363.

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