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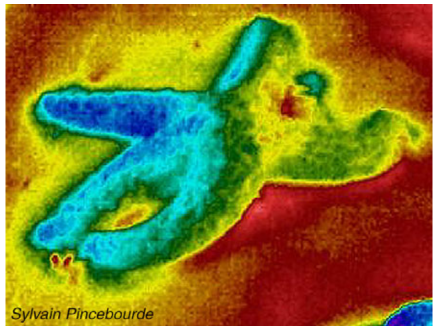
Inside JEB

A SEA STAR'S CHOICE: HOT ARMS OR DEATH


Sylvain Pincebourde

Life isn't easy for a sea star living in the intertidal zone. Daily tides leave them exposed and without access to cooling seawater for up to 6 h. As ectothermic animals, sea stars are unable to control their own body temperatures and if not careful they can find themselves precariously heating up during their daily aerial stints. But how hot is too hot? From the perspective of the sea star's central disc, which houses critical organs such as the stomach, 35°C is the upper limit before death – but what about the arms, do they have a different limit? Sylvain Pincebourde, currently a Centre National de la Recherche Scientifique researcher at the Institute of Research on Insect Biology, France, explains that until now most scientists have assumed that body temperature is even throughout the sea stars' bodies. However, with their characteristic spindly appendages, which should in theory allow a significant amount of heat to be lost *via* convection, their arms could be cooler. So, as part of his post doc at the University of South Carolina, USA, Pincebourde decided to investigate further. He wondered how sea star arms coped during low tide and whether arm temperature affected a sea star's decision to undergo arm abscission, a rare phenomena with an unknown trigger (p. 2183).

With the help of Eric Sanford and Pincebourde's post-doctoral mentor, Brian Helmuth, from the University of California Davis, USA, Pincebourde collected 70 sea stars off the coast of California. After acclimating them to the lab, the team grouped their aquatic testees into 10 groups, placing each group in an empty aquarium. Monitoring them with an infrared camera, the team found that, as expected, body temperature was indeed heterogeneous. However, in contrast to their initial expectations, the arms were warmer, not cooler, than the sea star's vital core. Perhaps convective heat loss played more of a role during their long periods out of water?

To mimic the hot conditions encountered during low tide, the team used overhead heat lamps to set and maintain the sea stars' core temperatures for 6 h. Each group was heated to a different core temperature, ranging from a bearable but warm 26°C to a deathly 42°C. Again, the team found that the sea stars' arms remained consistently higher than core body temperatures, ranging from 29 to 39°C when the core body temperatures were set to 26–35°C. As expected, these sea stars survived their warm spell, whereas sea stars with core temperatures set between 36 and 43°C weren't so fortunate and died within 24 h. Their deaths were expected due to their core temperatures being set so high, but the team were interested to find that these unlucky sea stars were also the only sea stars with arm temperatures lower than their core, ranging from 34 to 40°C.

The team think that the sea stars use their arms as heat sinks, actively drawing away heat from the essential core, to aid survival. While this may ensure a cooler core, it is not without its own disadvantages. When arms reached 33–39°C, after 2 days at least one arm would undergo abscission. Heating the arms to their own thermal limit is a sacrifice sea stars may make to ensure that core remains below 35°C. After all, it's better to lose an arm and save a life than die with five intact arms.

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Pincebourde, S., Sanford, E. and Helmuth, B. (2013). Survival and arm abscission are linked to regional heterothermy in an intertidal sea star. *J. Exp. Biol.* **216**, 2183-2191.

Nicola Stead

SUSTAINED ENERGY INTAKE DEBATE GETS MORE COMPLEX

As the saying goes, you can't get something for nothing, and nowhere is this clearer than in our own bodies. Everything that we do, from breathing to walking to even sleeping, requires energy, which we gain from the food that we eat. In this modern age where food, and thus energy, is available 24/7, surely all the millions of processes our bodies undertake should be limitless? Unfortunately, they are not: at a certain point we are all limited by our physiology – our metabolic rates can simply go no higher. But where, or what, is setting this limit? To answer this, scientists have turned to lactating mice, as this is the most energetically costly activity a mammal is likely to perform. It is thus also the most likely to drive the metabolic rate to its limit, explains John Speakman from the University of Aberdeen, UK, and the Chinese Academy of Sciences, Beijing.

Speakman recalls that 'there was a suggestion back in the 1980s that the

system is probably limited by the capacity of the gut to absorb food.' After 10 days of lactation, MF1 mouse mums reach an average maximum intake of 23 g of food per day. It seemed that they could eat no more and that the gut was indeed the limiting factor. But then Kim Hammond and Jared Diamond from the USA proposed the 'peripheral limit theory' after they found that Swiss Webster mice mums kept at cooler temperatures could increase their intake. Their theory suggested that the limit lay in the process itself, namely that the mammary glands were already working at full capacity and that the extra intake was being used by another process – in this case, thermoregulation. When Speakman joined the debate in the late 1990s and repeated the experiment in his MF1 mice he did indeed find that colder mums ate more. However, he also found that they produced more milk and this allowed the pups to grow bigger. In later studies, Speakman showed that shaving the mums also caused them to eat more and produce more milk. Speakman and his team concluded that it was actually the ability to dissipate heat that was limiting lactation and maximum metabolic rate in general.

Since proposing the heat dissipation hypothesis, Speakman and his team have turned their attention to tracking body temperature changes during pregnancy (p. 2328) and lactation as well as investigating variability amongst animals. Speakman explains that even without changing room temperature the maximum food intake (and thus milk production) can vary widely amongst mums. Although the average maximum intake is 23 g day^{-1}, maximums can range anywhere between 13.2 and 27.6 g day^{-1}. One theory from the 1950s suggested that these differences were pre-programmed during pregnancy by fetal litter size, so that mums with small litters would need to eat less and thus have lower maximum intake. However, this pre-programmed theory was incompatible with the observation that lactation could be adjusted – for example, when mothers were exposed to the cold. To investigate, the team either increased or decreased the size of the litters immediately after birth. They found that maximum food intake did not correlate with fetal litter size – in fact the mum with the smallest fetal litter size of six was the only mum to successfully foster the largest litter size of 16 (p. 2339). To see whether this maximum intake was genetic, the team set up a cross-fostering program where only half the pups were suckling milk from their birth mum. The team measured the pup's maximum food intake when the time came to rear their own offspring, and found that maximum rates were most similar to their birth mums regardless of whether they had been fed by their birth or foster mum, indicating that the variation was genetic (p. 2308).

However, while this work was ongoing, the debate over what was causing the limit carried on. Zhi-Jun Zhao from Liaocheng University, China, found that he couldn't reproduce Speakman's observations in Swiss Webster mice. When he shaved the mums they ate more but the pups didn't grow bigger. Could the peripheral limit theory be right after all? 'We wanted to see if there were other things going on that we'd not thought about or get some resolution of why he got a different result to us. So the best way to do these things is to collaborate', explains Speakman.

So Zhao came over to Aberdeen for a year, and rather than repeat the experiment, the pair along with colleagues from Speakman's lab decided to test the peripheral limit theory in MF1 mice in a new way. Rather than making thermoregulation the 'extra' process that required additional intake, they made exercise the extra activity (p. 2316). The team fed lactating MF1 mice just 80% of the daily maximum, but gave them option to run a set distance to access as much food as they wanted. If running was just an extra process, they could then simply eat enough to replenish the cost of running as well as gain the extra 20% needed to fuel lactation. However, the team found that none of the mums ate more after their run. In fact, mums made to run the furthest before accessing more food did so at the expense of their pups, and weaned the lightest litters. Zhao and Speakman both conclude that it again points towards the inability to dissipate enough of the heat generated through simultaneously running and producing milk.

However, this still didn't explain Zhao's observations in Swiss Webster mice. Luckily, Speakman took up a position the Chinese Academy of Sciences in 2011 and was able to continue the collaboration and help determine why this strain of mice wasn't producing more milk (p. 2349). 'Maybe Zhao's pups just couldn't convert extra milk into more growth', says Speakman. 'So the question is did they not produce the milk because they couldn't or because the pups couldn't use it?' To test this, the team reduced litter sizes down to 1–9 pups – well below their average litter size of 12. This way they knew that the mums already definitely had the capacity to suckle 12 pups. They then placed half the mums and litters at 21°C and the others at 5°C. If pups were limited by the mammary glands' capacity only, mice from the smallest litters would grow the most. If heat dissipation played a role, pups with mums incubated at 5°C would grow more compared with their warmer compatriots. However, the team observed neither: the fewer pups a mum was given the less she ate. Pups reared at 21°C were almost all the same weight regardless of how big their litter was. So, for Swiss Webster mice, it seems that pup demand first and foremost affects how much the mum eats and her milk production.

'I think the bottom line is that it seems that the limit story is much more complex than we imagined and in fact there isn't one solution. In some situations it's very likely to be heat dissipation, in other situations it's very likely to be growth limitation of the offspring, but one thing that we can definitely eliminate is that the limit to sustained energy intake is programmed in pregnancy', concludes Speakman.

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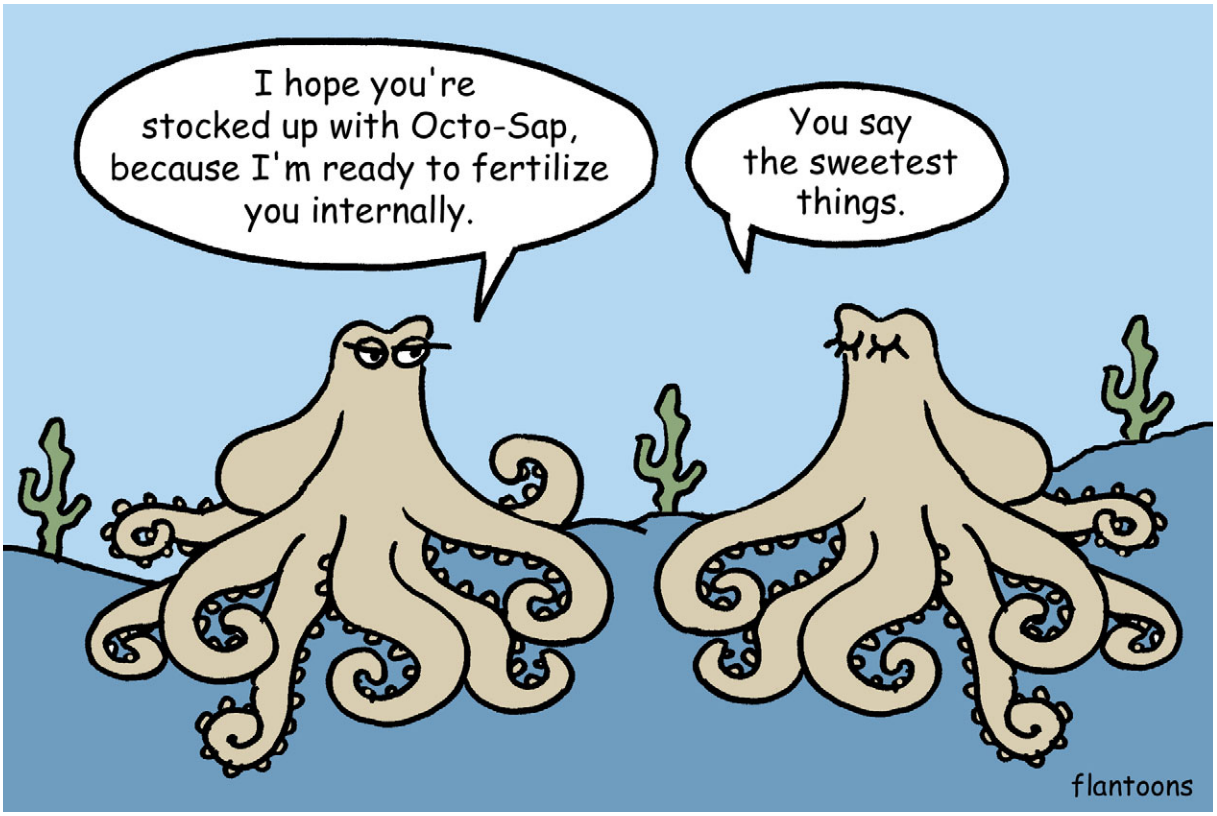
Nicola Stead

ATTRACTING STORED OCTOPUS SPERM



Reproduction for most marine invertebrates is a game of odds: females release their unfertilised eggs into vast oceans and rely on co-released peptides or protein pheromones to tempt sperm towards their eggs. A few species, however, such as the common octopus, have decided to adopt a more mammalian approach and use internal fertilization. It makes sense – surely, in the confined space of the oviduct, at least some sperm should reach the egg by chance without the need for additional attractants to induce chemotaxis (movement towards a signal). However, Anna Di Cosmo from the University of Napoli Federico II, Italy, thought otherwise. She explains that during mating, male octopuses will deposit sperm into the oviduct of the female, but females aren't always ready with an egg and so the sperm will bury themselves into the lining of the oviducal glands. When a mature egg is released, the waiting sperm needs a kick-start to get moving again and Di Cosmo suspected that a chemoattractant similar to those released by free-spawning animals might be involved (p. 2229).

Di Cosmo and her team caught several female octopuses off the coast of Naples and collected their mature eggs. The team then homogenized the eggs and, using a form of chromatography, separated the mixture into fractions of different proteins. Each fraction was then tested for its ability to coax sperm, collected from the oviducal glands, into moving through a fine mesh from one side to the other. One fraction in particular enticed sperm movement and the team identified the attractant as a small 11 kDa protein that they called octopus sperm-attractant peptide (Octo-SAP).

The team further characterized Octo-SAP's properties and showed that chemotaxis occurred in a concentration-dependent manner, with more sperm moving when Octo-SAP was concentrated. Using a microscope to film the tiny movements, the team also showed that the sperm moved up the concentration gradient towards areas of high Octo-SAP concentration. Together, the results suggest that the sperm were using the attractant to home in on what they thought was an egg. So, it seems that chemoattraction isn't just for free-spawning animals after all.

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