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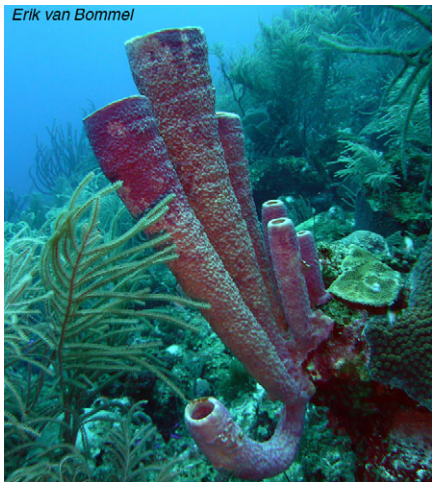
Inside JEB

RECYCLING SPONGES GIVE LIFE TO CORAL REEFS


Erik van Bommel

Coral reefs support some of the most diverse ecosystems on the planet, yet they thrive in a marine desert. So how do coral reefs sustain thriving populations? Marine biologist Fleur Van Duyl from the Royal Netherlands Institute for Sea Research is fascinated by the energy budgets that support coral reefs. According to van Duyl's former student, Jasper De Goeij, *Halisarca caerulea* sponges grow in the deep dark cavities beneath reefs, and 90% of their diet is composed of dissolved organic carbon, which is inedible for most other reef residents. But when De Goeij measured the amount of carbon that the brightly coloured sponges consumed in a day, he found that the creatures were able to consume half of their own weight in organic compounds daily, yet they never grew. What were the sponges doing with the carbon? Were the sponges really consuming that much carbon, or was there a problem with De Goeij's measurements? He had to find out where the carbon was going to back up his discovery (p. 3892).

Travelling to the Dutch Antilles with his student, Anna De Kluijver, De Goeij started SCUBA diving with the sponges. 'It is quite dark and technically difficult to work in the cavities,' explains De Goeij, but the duo collected sponges, placed them in small chambers and exposed the sponges to 5-bromo-2′-deoxyuridine (BrdU). 'The BrdU is only incorporated into the DNA of dividing cells,' explains De Goeij, so cells that carry the BrdU label must be dividing, or have divided, since the molecule was added to the sponge's water, and cells can only divide if they are taking up carbon. But when De Goeij returned to the Netherlands with his samples, he had problems finding the elusive label.

Discussing the BrdU detection problem with his father, biochemist Anton De Goeij, De Goeij Senior offered to introduce his son to Bert Schutte in Maastricht, who had developed a BrdU detection system for use in cancer therapy. Maybe he could help De Goeij Junior find evidence of cell division in his sponges.

Taking his samples to Jack Cleutjens's Maastricht Pathology laboratory, De Goeij was finally able to detect the BrdU label in his sponge cells. Amazingly, half of the sponge's choanocyte (filtration) cells had divided and the choanocyte's cell division cycle was a phenomenally short 5.4 h. 'That is quicker than most bacteria divide,' exclaims De Goeij.

The sponge was able to take up the colossal amounts of organic carbon that De Goeij had measured, but where was the carbon going: the sponges weren't growing. De Goeij tested to see if the cells were dying and being lost, but he couldn't find any evidence of cell death.

Presenting his results to the Maastricht Pathology Department, someone said 'Lets look at this like a human intestine, then you should see shedding where old cells detach from the epithelia'. De Goeij knew that he had seen some loose cells, and thought that they were artefacts from cutting the samples, but when he and his Pathology Department colleagues went back and looked at the samples, De Goeij realised that choanocytes were shedding all over the place. And then De Goeij remembered the tiny piles of brown material he found next to the sponges in the aquarium every morning. The sponges were shedding the newly divided cells, which other reef residents could now consume.

'*Halisarca caerulea* is the great recycler of energy for the reef by turning over energy that nobody else can use [dissolved organic carbon] into energy that everyone can use [discarded choanocytes],' explains De Goeij.

10.1242/jeb.040428

De Goeij, J. M., De Kluijver, A., Van Duyl, F. C., Vacelet, J., Wijffels, R. H., De Goeij, A. F. P. M., Cleutjens, J. P. M. and Schutte, B. (2009). Cell kinetics of the marine sponge *Halisarca caerulea* reveal rapid cell turnover and shedding. *J. Exp. Biol.* **212**, 3892-3900.

BROOD SMELL LIMITS WORKER BEES' LIFE EXPECTANCY

For a few crucial weeks each spring, bees are the most essential labourers on earth, pollinating many of the world's major fruit crops: their survival through winter is fundamental for agriculture. Realising that the survival of the individual was key to a colony's success, Gro Amdam became intrigued by bee longevity. Worker bees' lifespans tend to be only a few weeks;

however, Amdam explains that a new type of bee develops towards the end of the summer, known as *diutinus* bees. These bees have an amazing lifespan of 6 months or more and ensure the colony's survival through winter. Curious to find out what limits worker bees' lifespans and allows the development of *diutinus* bees, Amdam began investigating bee longevity.

Reading the literature, she realised that an egg protein, vitellogenin, could be essential for *diutinus* bee longevity: which is curious, because *diutinus* bees are mostly sterile, do not produce eggs and should not need an egg protein. Another thing that became clear was that the presence of brood (young) in a colony was sufficient to prevent worker bees developing into the long-lived veterans. 'Brood is to *diutinus* bees what kryptonite is to Superman,' laughs Amdam. At first Amdam thought that the hard labour of feeding larvae was preventing worker bees from building up their vitellogenin levels and developing into *diutinus* bees. But then she read a report that the smell of yeast affects the lifespan of fruit flies. Could the smell of brood – brood pheromone – affect the bees' longevity? Could the pheromone prevent workers from building up their vitellogenin levels and developing into long-lived *diutinus* bees? Amdam decided to investigate whether brood pheromone could be the key to the worker bees' short lifespan (p. 3795).

Working with Claus Kreibich, and Margrethe Brynem, Amdam's student – Bente Smedal – set up 12 hives where the team could carefully control the levels of brood and brood pheromone to find out whether the worker bees' low vitellogenin levels, and short life expectancy, were due to burn out or the brood's pheromone smell. Providing the worker bees in each hive with a caged queen (that could not produce brood) so that Smedal could control each colony's brood and pheromone levels, she took control of the hive's brood supply, providing the bees with: brood; synthetic brood pheromone; both brood and the synthetic pheromone; or neither brood nor pheromone.

Monitoring the bees' vitellogenin levels 3–4, 7–8 and 23–24 days after establishing the hives, it was clear that being around brood lowered the worker bees' vitellogenin levels. But exposure to the brood pheromone alone also reduced the bees' vitellogenin levels by the same amount. And when Smedal looked at the vitellogenin levels in the 23–24 day old bees, the bees that had been deprived of both the brood and its pheromone had the highest levels of vitellogenin, just like autumn bees embarking on a winter in the colony. Hard labour caring for the brood did not explain the workers' low vitellogenin levels.

And when Brynem checked the hives 200 days later to find out which colonies had survived and which had died, colonies that had not experienced brood or pheromone had survived best, while the hives that had been provided with both brood and pheromone had the worst survival rates. It was the smell of brood – brood pheromone – that regulated the bees' vitellogenin levels and longevity.

10.1242/jeb.040444

Smedal, B., Brynem, M., Kreibich, C. D. and Amdam, G. V. (2009). Brood pheromone suppresses physiology of extreme longevity in honeybees (*Apis mellifera*). *J. Exp. Biol.* **212**, 3795-3801.

ZEBRAFISH EMBRYOS EXCRETE CO_2 EARLY

During the early stages of development, most creatures are small enough to meet their metabolic demands by diffusion alone. However, as embryos grow they switch to circulatory systems and gas exchange to satisfy their metabolic requirements. A key molecular component of most circulator systems is the enzyme carbonic anhydrase (CA), which speeds up the conversion of carbon dioxide to soluble bicarbonate for transport. Katie Gilmour, from the University of Ottawa, explains that although the development of the oxygen delivery system is well understood, little is known about the development of the systems involved in carbon dioxide excretion. Intrigued by all aspects of carbon dioxide excretion involving CA, Gilmour and her collaborator Steve Perry were curious to know when a developing animal switches from gas exchange by diffusion to a circulatory system, and whether that is correlated with the expression of CA. Gilmour and Perry turned to the zebrafish to find the answer (p. 3837).

'One of the exciting possibilities of working in zebrafish is the capacity to work in very young fish,' explains Gilmour. Teaming up with Andrew Esbaugh, Gilmour and Perry set out to measure when CA expression kicked in by measuring the relative amount of CA mRNA in zebrafish embryos and larvae, ranging from 3 h to 5 days postfertilisation. Knowing that zebrafish produce various different forms of CA, each specialised for specific physiological functions, Gilmour and Perry focused on the form involved in CO_2 excretion in red blood cells (CAb) and another form, specialised in acid–base regulation found in almost all tissues (CAc). Using real-time PCR, the team saw that the expression levels of the red blood cell form of CA (CAb) were always higher than the more general form of the enzyme (CAc), and there was a substantial increase in CAb at 8 h postfertilisation. But would that correlate with the amount of CO_2 that the embryos and larvae were excreting? Gilmour and Perry teamed up with honours student Kelli Thomas to find out.

'CO_2 chemically reacts with water to give bicarbonate so you have to measure the total CO_2 content, which is very finicky,' says Gilmour. Thomas painstakingly sealed groups of embryos or larvae in a chamber for a period ranging from 20 to 90 min (depending on the age of the embryos/larvae), collected water samples at the beginning and end of the period and measured the total carbon dioxide levels with Gilmour's cantankerous Capni-Con 5. Calculating the CO_2 levels relative to the water's oxygen levels, the team could see that the fish began excreting large amounts of CO_2 at 48 h, around the time that they hatch. In fact the team were surprised to realise that, prior to hatching, CO_2 excretion by the eggs was far less than O_2 uptake. They must be storing CO_2, although Gilmour and Perry are unsure where.

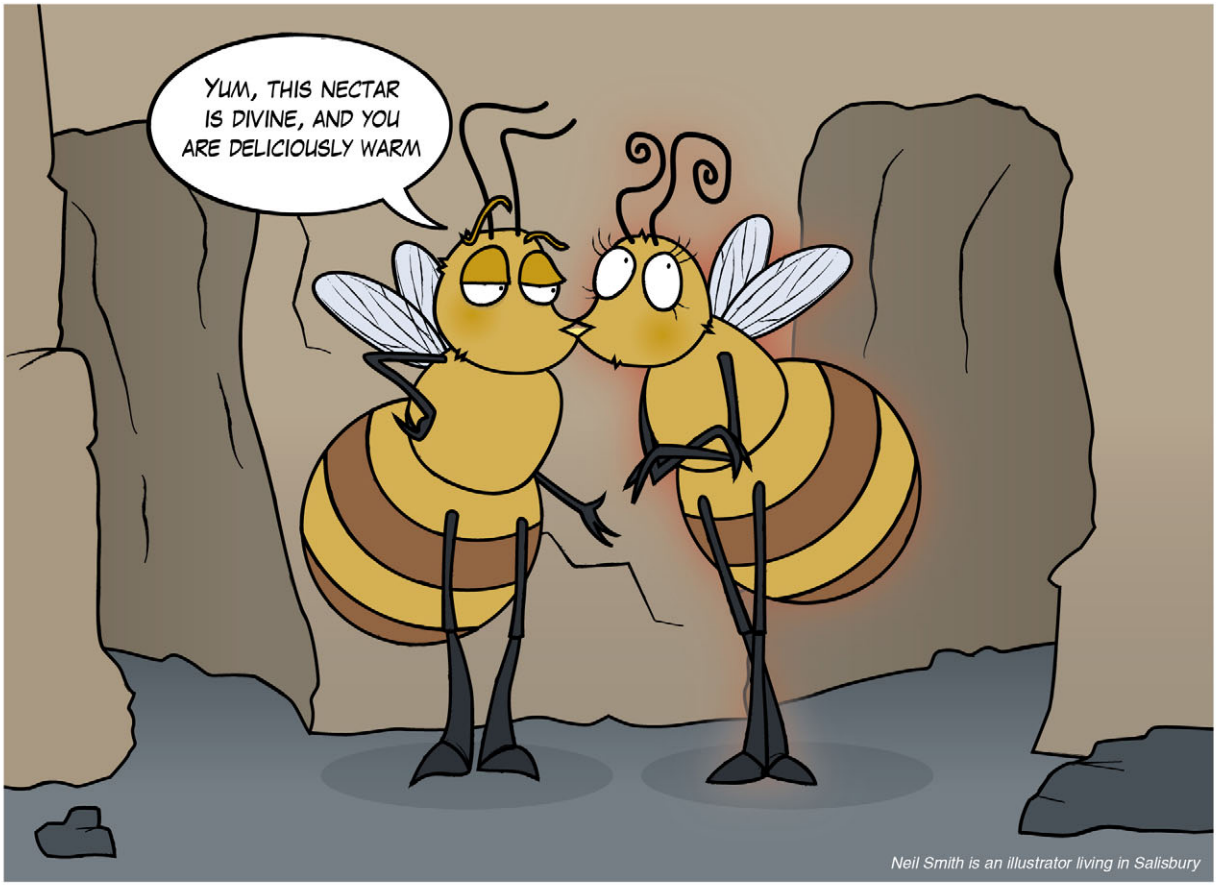
Having found the point at which the larvae begin excreting CO_2, Gilmour and Perry were keen to find out which type of CA was responsible for the massive increase in CO_2 excretion. Using a molecular technique only available in zebrafish, the team were pleased to see that CAb was partially responsible for the increase in CO_2 excretion. But they were amazed to see that CAc was involved in CO_2 excretion too. 'That isn't the case in adult animals,' says Gilmour.

Having found that CAb is a key player in CO_2 excretion, Perry and Gilmour are keen to discover whether red blood cells are actively involved in CO_2 excretion at 48 h, which is long before they are essential for oxygen transport, at 14 days.

10.1242/jeb.040451

Gilmour, K. M., Thomas, K., Esbaugh, A. J. and Perry, S. F. (2009). Carbonic anhydrase expression and CO_2 excretion during early development in zebrafish *Danio rerio*. *J. Exp. Biol.* **212**, 3837-3845.

BEES DISCRIMINATE BETWEEN HOT AND COLD FOOD



Getting a temperature is a bad thing for most mammals, but returning forager bees always warm up on the home run and a high temperature can indicate the quality of nectar or pollen that they return with. Flowers also warm up: the temperature inside a *Narcissus longispathus* can be 8°C higher than the surroundings. Knowing that bees sense temperatures with their antennae, James Nieh, and his colleagues Tobin Hammer and Curtis Hata from The University of California San Diego, wondered whether bees can use this thermal information about food. Can bees learn to discriminate between food sources at different temperatures (p. 3928)?

Training bees to stick out their tongues in return for a sugary reward when the team touched a warm surface to a bee's antenna, the team discovered that bees can learn to identify warmth with food. Next the trio tested whether the insects could learn to associate temperature differences with a food reward, and discovered that the bees do associate temperature differences with food. The bees' ability to recognise the temperature difference increased dramatically as the difference increased, but the insects were better at recognising warm temperature differences than cold temperature differences.

So, bees can learn to recognise different temperatures and could be guided by this information when foraging for, or receiving, food.

10.1242/jeb.040436

Hammer, T. J., Hata, C. and Nieh, J. C. (2009). Thermal learning in the honeybee, *Apis mellifera*. *J. Exp. Biol.* **212**, 3928-3934.

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