

Inside JEB 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.

BRIGHT LIFE IN THE BENTHOS



Sinking through the inky ocean, it would seem that there is little light at depth: but you'd be wrong. 'In the mesopelagic realm [200-1000 m] bioluminescence [light produced by animals] is very common', says Sönke Johnsen from Duke University, USA, explaining that many creatures are capable of producing light, yet rarely do so. But how much light do the inhabitants of the ocean floor (benthos) generate? Explaining that some bioluminescence is generated when organisms collide, Johnsen says, 'In the benthos you have a current moving over complicated ground with all the things in the water banging into it, so one idea was that there would be a fair amount of bioluminescence.' However, few people have visited this remote and inhospitable habitat. Intrigued by the animals that dwell there and the possibility that bioluminescent bacteria coating the ocean floor might glow faintly, Johnsen teamed up with long-time collaborators Tamara Frank, Steven Haddock, Edith Widder and Charles Messing to find out just how much light is produced by seabed residents (p. 3335)

Descending to the bottom of the ocean near the Bahamas, switching off all the lights and adapting to the inpenetrable darkness, Johnsen and his colleagues were amazed to find themselves continually surrounded by tiny flashes of light as bioluminescent plankton collided with coral and boulders strewn across the floor. However, there was no evidence of the all-pervasive glow produced by bioluminescent bacteria that the team had hoped to find. 'We weren't in regions where the currents were slow enough to allow for collection of detritus,' says Frank, adding, 'it's not that this phenomenon doesn't exist...we just weren't able to observe it on these dives.'

Next the submariners began searching for bioluminescent inhabitants, gently tapping coral, crabs and anything else they could reach with the submersible's robotic arm to see whether any of the organisms emitted light. The team found that only 20% of the species that they encountered produced bioluminescence. Collecting specimens and

returning to the surface, Johnsen and Haddock then photographed the animals' dim bluish glows - ranging from glowing corals and shrimp that literally vomit light (spewing out the chemicals that generate light where they mix in the surrounding currents) to the first bioluminescent anemone that has been discovered - and carefully measured their spectra. The duo found that most of the species produced blue and blue-green spectra, peaking at wavelengths ranging from 455 to 495 nm. However, a family of soft corals known as the pennatulaceans produced green light, with spectra peaking from 505 to 535 nm. 'We were working at the absolute limits of what the equipment can do', remembers Johnsen, recalling the frustration of working in the cramped, pitch-dark conditions on the boat. 'It gives you respect for our vision, we can see the bioluminescence fine, but getting it recorded on an instrument or a camera is much harder', he adds. And as if that wasn't challenging enough, proving that anything living down there could even see the spectacular light display was even

Devising a strategy for collecting crustaceans ranging from crabs to isopods under dim red light by luring or gently sucking them into light-tight boxes, the submersible's crew then sealed the animals in boxes to protect their vision from harsh daylight when they reached the surface. Back on the RV Seward Johnson, Frank painstakingly measured the weak electrical signals produced by the animals' eyes in response to dim flashes of light ranging from 370 nm to over 600 nm and found that the majority of the creatures were most sensitive to blue/green wavelengths, ranging from 470 nm to 497 nm (p. 3344). Most surprisingly, two of the animals were capable of detecting UV wavelengths. Even though there is no UV left from the sun at this depth, Johnsen explains, 'Colour vision works by having two channels with different spectral sensitivities, and our best ability to discriminate colours is when you have light of wavelengths between the peak sensitivities of the two pigments.' He suspects that combining the inputs from the blue and UV photoreceptors allows the crustaceans to pick out fine gradations in the blue-green spectrum that are beyond our perception, suggesting, 'These animals might be colour-coding their food': they may discard unpleasant-tasting green bioluminescent coral in favour of nutritious blue bioluminescent plankton.

Finally, after recording the crustacean's spectral sensitivity, Frank measured how much light the animals' eyes had to collect before sending a signal to the brain (the

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flicker rate). She explains that there is a trade-off between the length of time that the eye collects light and the ability to track moving prev. Eyes that are sensitive to dim conditions lower the flicker rate to gather light for longer before sending the signal to the brain. However, objects moving faster than the flicker rate become blurred and their direction of motion may not be clear. The crustaceans' flicker rates ranged from 10 to 24 Hz (human vision, which is sensitive to bright light, has a flicker rate of 60 Hz) and the team were amazed to find that one crustacean, the isopod Booralana tricarinata, had the slowest flicker rate ever recorded: 4 Hz. According to Frank, the isopod would have problems tracking even the slowest-moving prey. She suggests that as it is a scavenger, it is possible that it may be searching for pockets of glowing bacteria on rotting food and it might achieve the sensitivity required to see this dim bioluminescence with extremely slow

Having shown that bioluminescent benthic species are scarce but the phenomenon itself is not, Johnsen is keen to return to the ocean floor to discover more about the exotic creatures that reside there. 'We would love to go back, get more basic data. We've only scratched the surface', he says, adding, 'When you are down there you are cramped and cold and stiff, but at the end of a dive I never want to come back up.'

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Kathryn Knight

SYMBIOTIC ALGAE DELIVER GLUCOSE TO ANEMONE HOST

Organisms that have struck up symbiotic relationships have found a wonderful *quid pro quo* lifestyle solution. For example, animal hosts provide essential nutrients and a safe refuge to their algal lodgers, while the algae provide a plentiful supply of energy harnessed by photosynthesis. 'Most



earlier studies had pointed to glycerol as being the primary photosynthetic metabolite [energy source] transferred from dinoflagellate algae to their cnidarian animal hosts', explains John Pringle from Stanford University. However, other studies had suggested that symbionts may supply their hosts with alternative materials, such as photosynthetic glucose. Pringle, a yeast geneticist by trade, says that he was attracted to the problem because of his fascination with coral reefs. However, he recalls that when he began working on symbiosis he had no preconceptions about which materials the symbionts might deliver to their hosts. 'When you change fields you look at everything with a sceptical eye', he remembers. Teaming up with graduate student Matthew Burriesci, Pringle wanted to tackle the question of material transfer from a fresh - and softer perspective. He decided to find out which materials the symbiotic anemone Aiptasia receives from its algal occupants (p. 3467).

According to Pringle, symbiotic algae are remarkably robust, surviving separation from their host, and in the past, many researchers had measured the materials released by the isolated algae. Instead, Burriesci and Pringle supplied the intact anemone with heavy carbon dioxide where the ¹²C atoms were replaced with ¹³C atoms – and allowed the algae, secure inside their symbiotic hosts, to photosynthesise for a day before separating the two. Then Burriesci and Theodore Raab analysed which materials in the host tissue had acquired the heavy carbon marker by isolating individual compounds with gas chromatography and identifying them by mass spectrometry.

'Glycerol does eventually appear in the host's body along with succinate, fumarate, various amino acids and other compounds', recalls Pringle, adding that most host materials become labelled eventually if left for long enough. However, in order to find out which metabolic compound was being delivered directly to the host by its residents, Burriesci and Pringle realised that they would have to separate the symbiotic partners more quickly.

Burriesci went off and designed and built a bespoke filter holder; 'He did all the machining himself. I didn't know about it until after it was done,' recalls Pringle, adding, 'Then Matt could take an anemone sitting in a tank that had been provided with ¹³CO₂, disrupt it, separate the mixture into an algal fraction and an animal cytoplasm fraction and then freeze them in liquid nitrogen, all within about two minutes'. Under the right conditions, this would allow the team to capture the first carbon compounds that the algae delivered to its anemone host after exposure to the heavy carbon dioxide.

Switching the lights on for a few hours – to make sure that the algae were happily photosynthesising – before supplying the anemone with the heavy carbon dioxide, Burriesci swiftly separated the algae from the anemone 2–10 min later. Then he began analysing the animal tissue for any evidence of transfer of the heavy carbon from the photosynthesising algae to the anemone.

'The results were, to my eye, remarkably clear cut', recalls Pringle. Mass spectral analysis showed that the only compound in the anemone tissue carrying the ¹³C signature was glucose. Instead of delivering glycerol to its host, the algae were supplying it with glucose. However, Pringle is quick to point out that this does not necessarily mean that all symbiotic algae provide glucose to their hosts, adding that they could deliver glycerol or other compounds under different circumstances.

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