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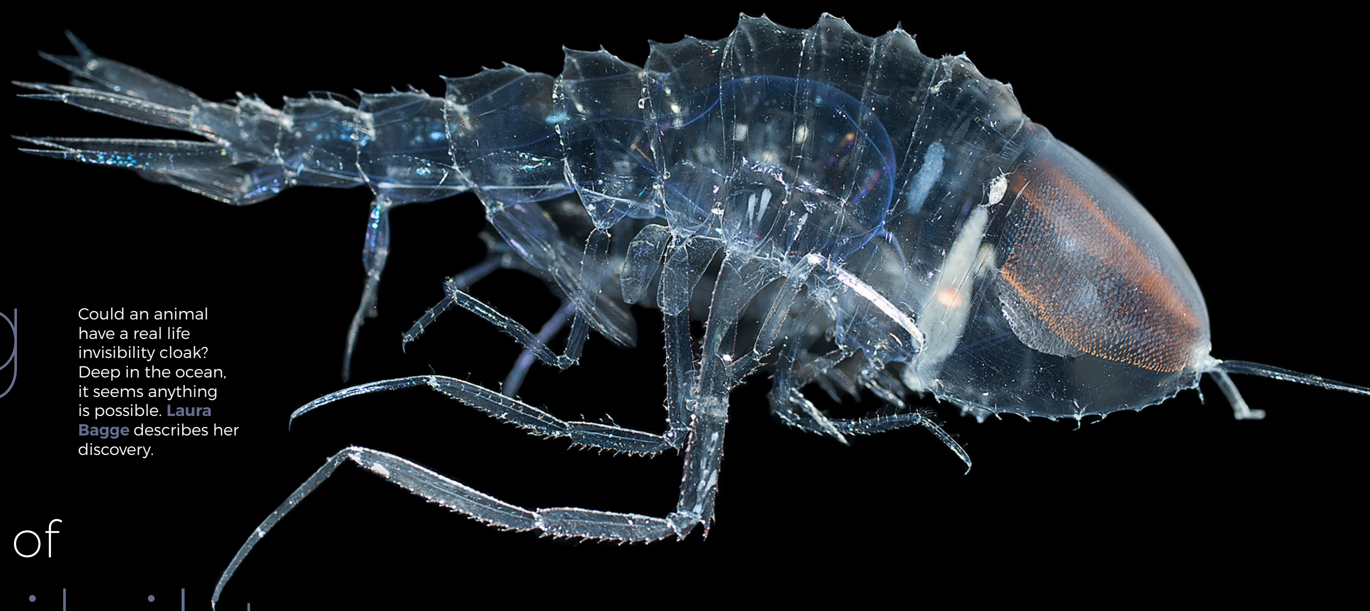

A Living Cloak of invisibility

Could an animal have a real life invisibility cloak? Deep in the ocean, it seems anything is possible. **Laura Bagge** describes her discovery.

MANY OF US HAVE WISHED FOR the superpower of invisibility, allowing us to blend into the background at our most awkward moments, or move about undetected. Well, in a featureless environment like the open ocean, where there is nothing but endless blue water and nowhere to hide, the superpower of invisibility is a reality and a necessity for many animals - they have completely see-through bodies that allow them to avoid being detected by predators.

However, even these transparent animals are not perfectly invisible. Glints of light reflecting off of their bodies can give away their position, just like a flashlight reflecting off of a window pane at night makes even a transparent window clear to see. Our recent research shows that some transparent crustaceans, called hyperiid amphipods, have adapted remarkable ways to reduce these light reflections in order to become even more invisible – they produce an anti-reflective cloak.

Having a transparent body is an excellent

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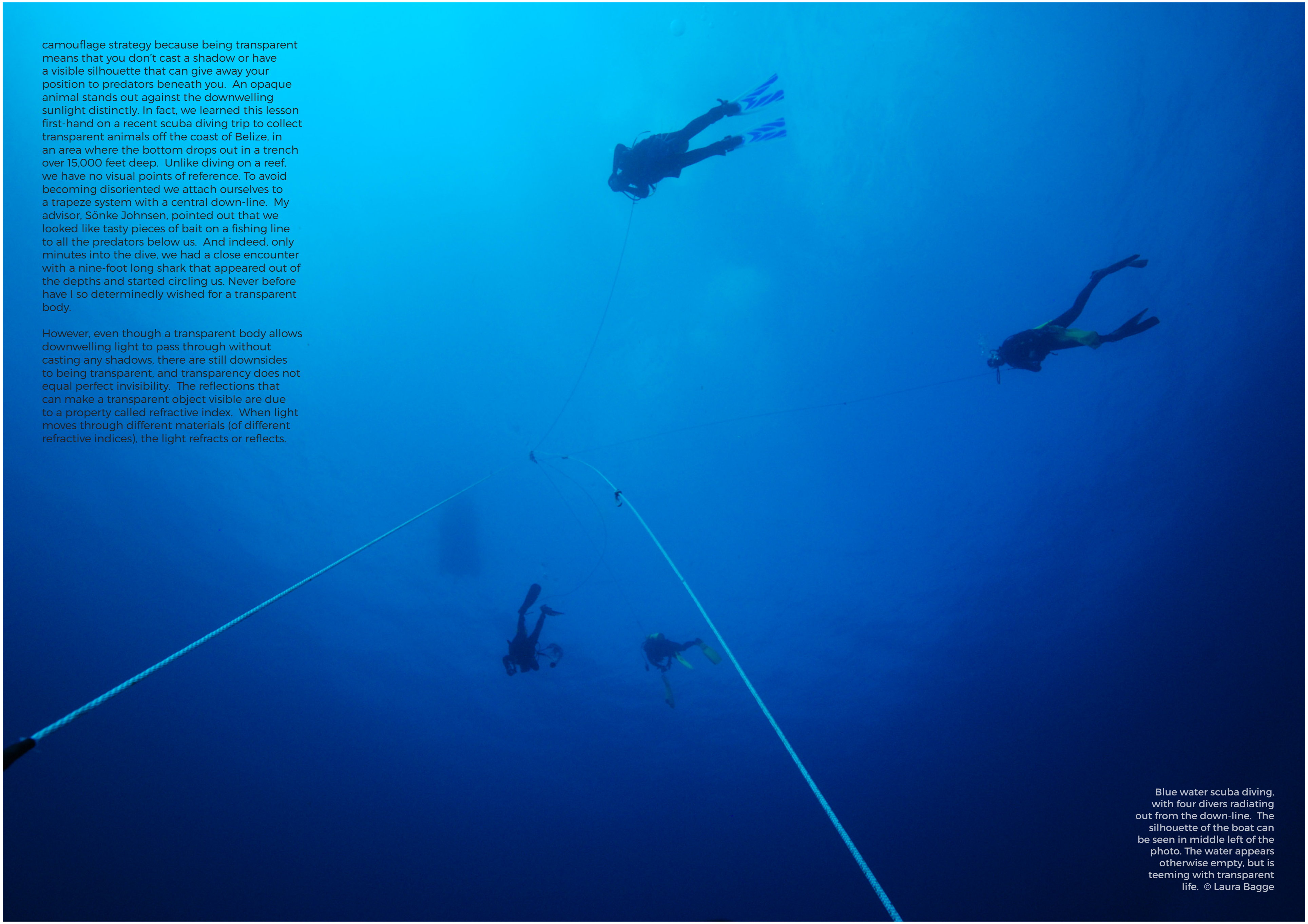
This photograph of the transparent crustacean Cystisoma was maximized for visibility by placing four flashes around the tank holding the animal, demonstrating how both surface and internal reflections can make the animal visible. © Karen Osborn.

camouflage strategy because being transparent means that you don't cast a shadow or have a visible silhouette that can give away your position to predators beneath you. An opaque animal stands out against the downwelling sunlight distinctly. In fact, we learned this lesson first-hand on a recent scuba diving trip to collect transparent animals off the coast of Belize, in an area where the bottom drops out in a trench over 15,000 feet deep. Unlike diving on a reef, we have no visual points of reference. To avoid becoming disoriented we attach ourselves to a trapeze system with a central down-line. My advisor, Sönke Johnsen, pointed out that we looked like tasty pieces of bait on a fishing line to all the predators below us. And indeed, only minutes into the dive, we had a close encounter with a nine-foot long shark that appeared out of the depths and started circling us. Never before have I so determinedly wished for a transparent body.

However, even though a transparent body allows downwelling light to pass through without casting any shadows, there are still downsides to being transparent, and transparency does not equal perfect invisibility. The reflections that can make a transparent object visible are due to a property called refractive index. When light moves through different materials (of different refractive indices), the light refracts or reflects.



Blue water scuba diving, with four divers radiating out from the down-line. The silhouette of the boat can be seen in middle left of the photo. The water appears otherwise empty, but is teeming with transparent life. © Laura Bagge

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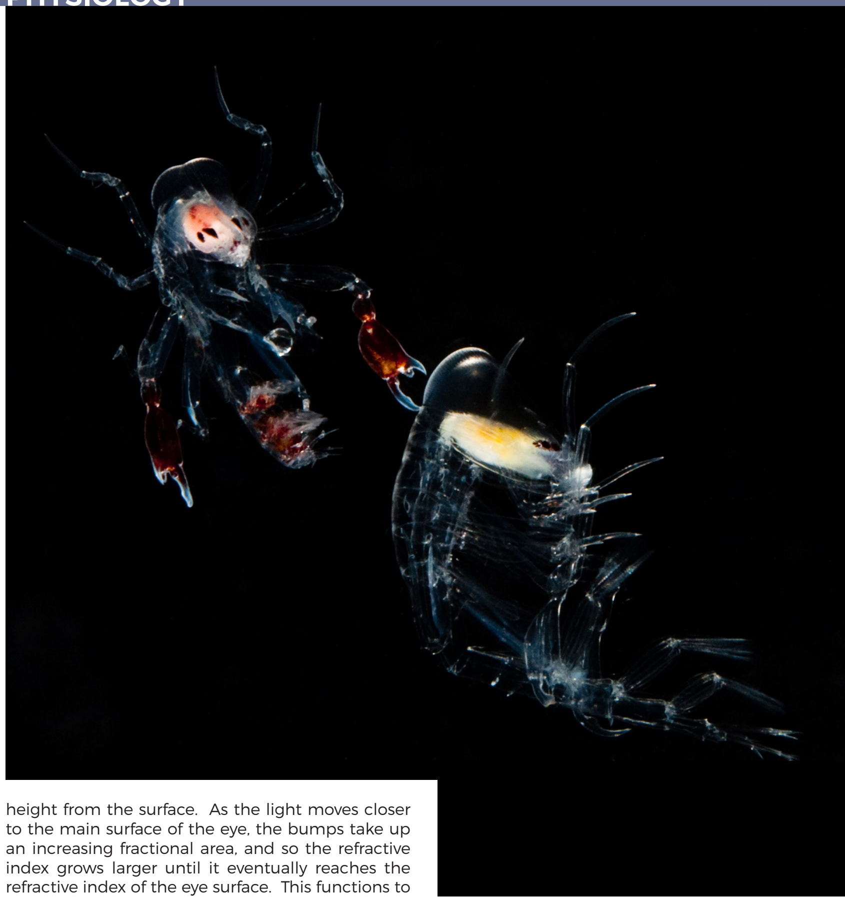
A dragonfish uses photophores underneath its eyes to shine bioluminescent searchlights into the dark to look for reflections from transparent prey. © Sönke Johnsen. © Karen Osborn.

In this case, hard-shelled crustaceans would have a higher refractive index than the surrounding seawater, and light could reflect off their shells. This can be especially problematic in the twilight zone of the ocean where many of these animals live. The blue-green downwelling light that reaches the crustaceans from the surface is at least 100 times greater than the horizontal light, so even a reflection of only 1% of this downward light can significantly increase the contrast and thus the visibility of an otherwise transparent and hidden animal. Animals in the twilight zone also have to worry about predators that have bioluminescent searchlights, where any light from their bioluminescence that is reflected from the crustaceans' shells will bounce directly back into the predator's eyes.

Given the extraordinary amount of selective pressure these transparent animals are under to stay hidden, or else be eaten, my colleagues and I wondered whether they had any ways to minimise reflections from their surfaces. Although nobody has ever tried looking for anti-reflective features in any ocean animal, a study in the 1960s found that the transparent eyes of moths had a unique ordered array of nanostructures (also called a nipple array) that reduces reflections by reducing or smoothing out the difference in the refractive index between the surface of the eye and the surrounding air. These nanostructures are basically a series of bumps with widths less than a half a wavelength of light. At this size scale, the bumps function to change the refractive index as the light approaches the eye surface. When light first hits the bumps, it touches only the peaks, which take up a small fractional area of the air at that specific



height from the surface. As the light moves closer to the main surface of the eye, the bumps take up an increasing fractional area, and so the refractive index grows larger until it eventually reaches the refractive index of the eye surface. This functions to reduce what was a sharp jump in refractive index to a smooth gradient that reflects less light. This same concept applies to recording studios that often hang shag carpets on the wall to dampen sound.

I decided to look at transparent open ocean crustaceans under the microscope to see if they had any nanostructures similar to what had been discovered on the moth eyes. In particular, I was interested in studying the hyperiid amphipods, a group of mostly transparent crustaceans. I first encountered Cystisoma, a species that can grow as large as 6 inches long, during a research cruise in the Atlantic Ocean when we pulled up a trawl net from 1000 meters deep and placed all the animals we had collected into a large bucket. I reached my hand into the bucket to pick up a black fish, but instead my hand hit something hard. I couldn't even see what I had touched until I pulled it out of the water and saw a large bug-like animal that looked like it was made of glass.

I couldn't even see what I had touched until I pulled it out of the water

You can imagine that such a large, hard-bodied animal has a great need for invisibility. In my study, I examined seven species of hyperiid amphipods, including Cystisoma, that live just beneath the water surface to over 4,000 meters deep. Hyperiids are known for being both a tasty prey animal as well as a vicious hunter of gelatinous animals, so minimising

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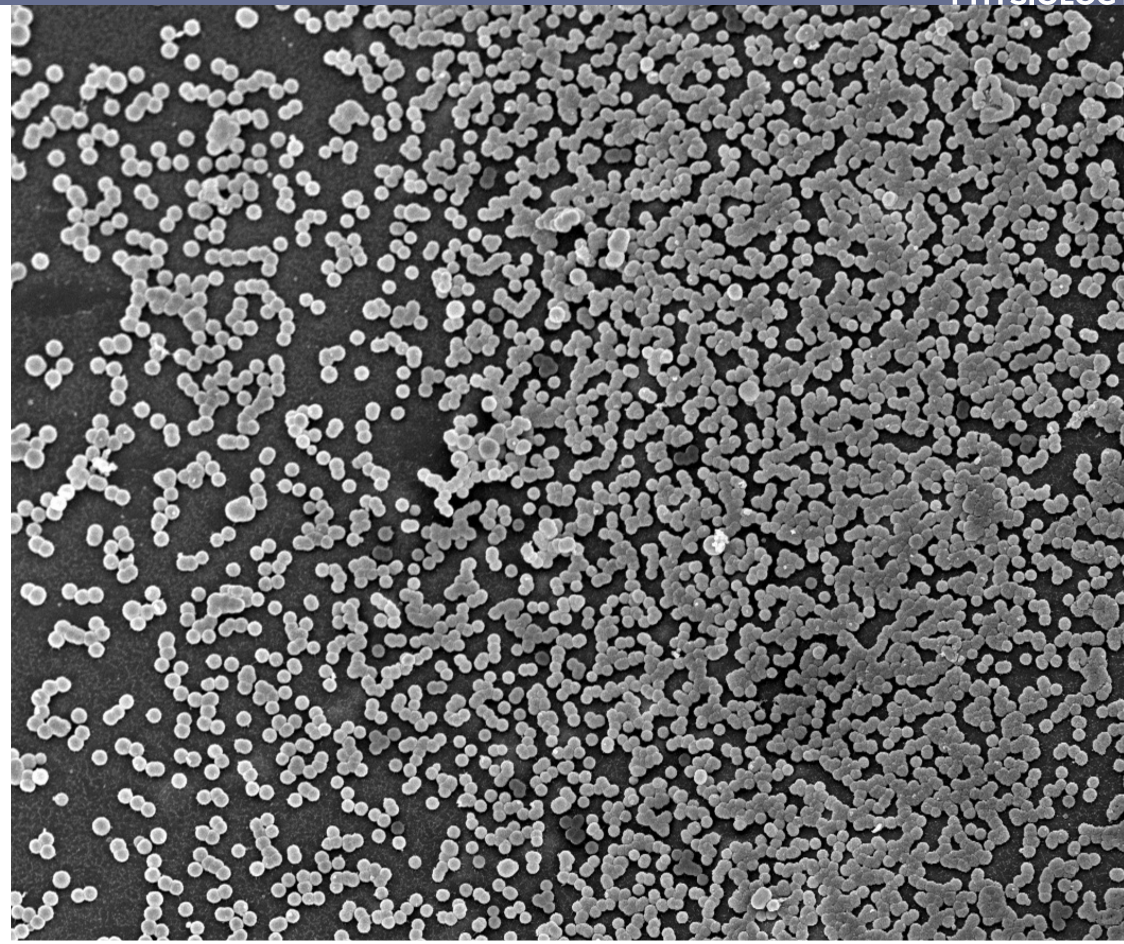


Left: Five species of hyperiid amphipods, coated in gold-palladium, ready to be examined by the scanning electron microscope. ©Laura Bagge. Right: Monolayer of spheres on Phronima. ©Laura Bagge

reflections from their hard chitinous shell could be important.

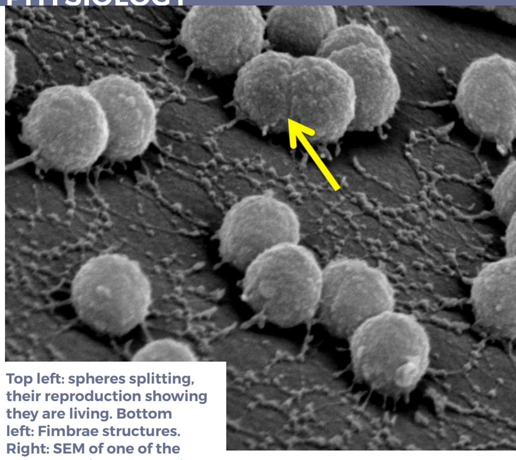
To study the surfaces of the hyperiid amphipods, I had to use a powerful microscope called a scanning electron microscope (SEM). This microscope can show us things beyond the resolution of a typical light microscope by using beams of electrons. I prepared specimens of hyperiid amphipods that I had recently caught during diving or deep-sea trawling trips. Karen Osborn, from the Smithsonian Institution National Museum of Natural History, also provided access to many previously caught hyperiid amphipods that were nicely preserved in jars. To look at the animals using SEM, we have to first coat them in metal, such as gold, which prevents buildup of static electric fields and also increases the signal that the electron beam can detect. The prepared specimens ended up looking a lot like pieces of jewellery, carefully arranged in my laboratory.

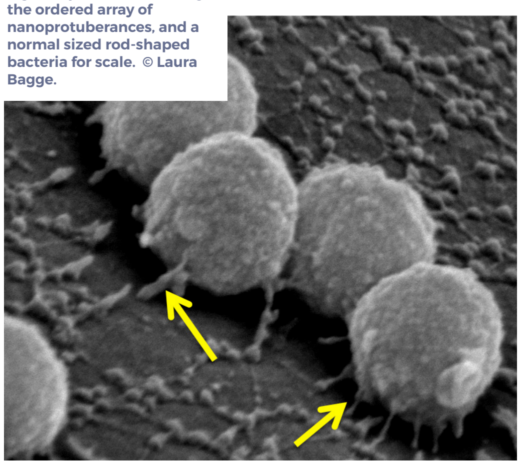
When I first looked at a Cystisoma under the SEM, I was excited to see that the legs were covered with a beautiful ordered array of nanoprotuberances that looked strikingly similar to what had previously been found on moth eyes. I measured the heights and widths of the bumps, and with the help of my mathematically gifted advisor, we used optical modelling to figure out how much light would reflect from the bumpy surface of the legs. This showed that the bumps are just the right size to reduce reflections by as much as 100-fold over a broad range of angles and light wavelengths, including the most commonly encountered blue-green wavelengths (480 nm). Interestingly, we only found these bumps, which were a feature of the shell surface, emerging from the legs of Cystisoma and not any of the other six smaller species that I examined.

This was fascinating, but we found something even weirder on other surfaces of Cystisoma, such as their backs, and on all six other species of hyperiid amphipods. We found nano-sized spheres that appeared to be perfectly uniform, arranged in a monolayer. When I first viewed theses spheres under the SEM, I had no idea what I had discovered. I had been using the microscopy lab at the University of North Carolina at Wilmington, and I immediately called in several professors to look at these weird spheres and guess what they might be. Nobody had seen anything like it before.

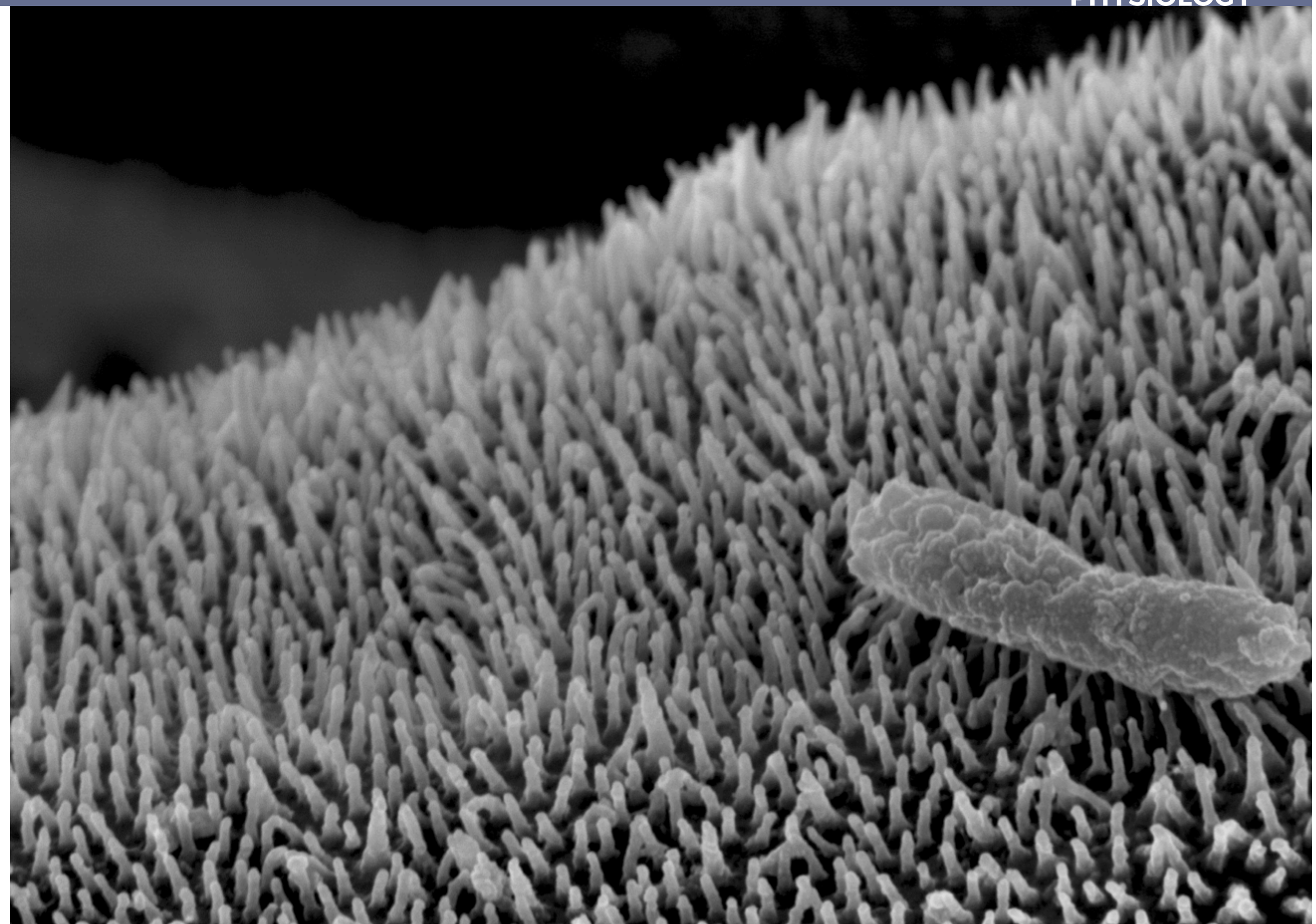
These spheres had many morphological features that spherical shaped bacteria have, such as fimbrae - little thread-like structures coming out and attaching the sphere to the surface. Also, there was evidence that the spheres were replicating, which indicated that this spherical layer was alive!

The idea of an animal having a living biofilm is not a new one, but this particular thin layer didn't have





Top left: spheres splitting, their reproduction showing they are living. Bottom left: Fimbrae structures. Right: SEM of one of the legs of Cystisoma, showing the ordered array of nanoprotuberances, and a normal sized rod-shaped bacteria for scale. © Laura Bagge.



the same properties as biofilms we know about. I examined multiple specimens of the Phronima species from the Pacific and Atlantic oceans, and they all had similarly sized (~300 nm in diameter) spheres. Other species had their own unique monolayers, with spheres that ranged from as small as ~50 nm to as large as 320 nm. When Sönke and I modelled how a nano-sized layer of spheres would affect reflection, we found that in all cases, reflections were reduced. The optimal scenario occurs when an animal has a layer of spheres on its surface that is uniform – where the spheres are all close enough to be touching one another – at a size of 110 nm. When an animal possesses this, it can, reduce reflections by as much as 250-fold. However, even if we assume that the spheres aren't uniform and even when they range in diameter from 50 nm to 350 nm, we still see that reflection is reduced by at least four-fold. An additional calculation demonstrated that the spheres reduce reflection enough to make a functional difference in the contrast, or visibility of the hyperiid's surface.

Hyperiid amphipods' nanoprotuberances and their monolayer of spheres that reduce their surface reflections, are two things that haven't been documented before, so it's an exciting discovery. But the identity of the living spheres remains unclear.

I'm currently working with colleagues to positively identify the spheres as bacteria, but we can't yet confirm this. It's possible that the presence of the monolayer of spheres may be a happy accident, due to hyperiids swimming in an ocean surrounded by abundant nanobacteria, but we would like to discover whether there is any kind of symbiosis occurring between the spheres and their crustacean hosts. If these crustaceans, and perhaps other transparent, alien-looking ocean species, are indeed making their own living anti-reflective cloaks, then move aside Harry Potter, because these ocean creatures have found the ultimate super power of invisibility, no magic required.

Bagge, L. E., Osborn, K. J., & Johnsen, S. (2016). Nanostructures and monolayers of spheres reduce surface reflections in hyperiid amphipods. Current Biology, 26(22), 3071-3076.