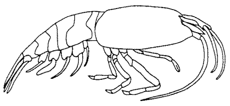
DEEP-SEE SHRIMP

By RICHARD MONASTERSKY



The term "jumbo shrimp" has always tickled oxymoron-lovers. But scientists are chuckling at a new morsel of shrimp humor – the name *Rimicaris exoculata.*

In 1985, when researchers discovered these shrimp swarming around deep-sea geysers of superheated water, they named the species *exoculata,* meaning "without eyes." It seemed a fair and accurate title for a shrimp that lacked the eyestalks and corneas other shrimp use for vision.

But in the Feb. 2 NATURE, marine biologist Cindy Van Dover from the Woods Hole (Mass.) Oceanographic Institution and her colleagues report that the eyeless *R. exoculata* does indeed have eyes. For some reason, the forces of evolution have granted this shrimp a pair of unusual visual organs located on the animal's back instead of in the normal position on stalks in front. Perhaps even more intriguing, the quest to explain the purpose of these peepers has led oceanographers to discover extremely dim light at the bottom of the ocean – coming from hot-water vents on the seafloor.

Now a small but provocative debate is raging concerning whether *R. exoculata's* bizarre ocular organs actually look at this light, or instead serve to detect some other source of light under the waves that has yet to catch the eyes of scientists.

The story of *R. exoculata's* eyes began three years ago when Van Dover, a biology graduate student at Woods Hole, obtained some specimens of the shrimp in order to study their diet. Several months before, scientists had discovered and collected these animals around underwater geysers that sporadically dot the surface of the mid-Atlantic ridge at depths of about 3.5 kilometers. Called black smokers, these formations are sulfide chimneys that continuously shoot out black clouds of 350°C (about 660°F) water laden with dissolved sulfides and other minerals.

While studying the contents of the shrimp's stomachs, Van Dover began to focus on a strange patch located on the backs of the animals. This patch was hardly noticeable on the dead specimens that had been fixed in preservatives or frozen for lab study. But in videotapes of the live animals in their natural habitat, taken from the deep submersible *Alvin,* the patches were reflective. The submarine's lights glinted off them as car headlights might set a cat's eyes aglow.

When Van Dover took a closer look at one of the specimens, she saw that the reflective patches seen on video were actually two lobe-shaped structures sitting underneath a thin, transparent layer of carapace, or shell-type material. A dissection showed that these lobes hooked into the shrimp's brain via a bundle of neurons that looked suspiciously like an optic nerve. She wondered: Could this be an eye?

Like an avalanche triggered by one falling rock, Van Dover's initial musings two years ago have since bounced through the fields of biology, chemistry, physics, geology and several other scientific disciplines.

"Once I started calling it an eye, then it became a matter of proving it was an eye," says Van Dover. She asked biochemist Ete Z. Szuts at Marine Biological Laboratory in Woods Hole to look for characteristic visual molecules in the lobes. Meanwhile, bioengineer Steven C. Chamberlain, a specialist in the structure of invertebrate eyes from Syracuse (N.Y.) University, examined the back organ to determine whether it was actually organized like eyes.

Chamberlain was able to detail the anatomy of the patch, but he could not pin down the function of this novel structure. "I wasn't willing to say it was a sensory organ; it could have been a gland," he says. Ultimately, it would be up to Szuts' lab to provide the key piece of proof that the organ must be a pair of eyes.

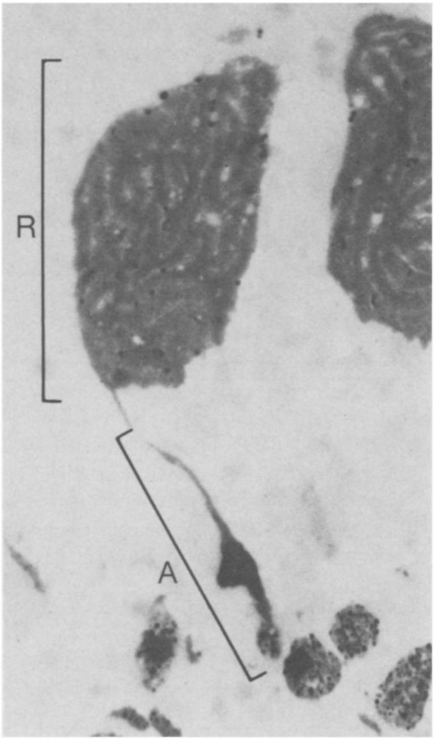
Szuts was looking for a visual pigment known as rhodopsin, which is the light-sensing molecule in all known types of eyes. Rhodopsin molecules are the switchboard in the eye, absorbing photons of visible light and initiating a neurologic message to the brain.

At the start, Szuts did not expect to find any rhodopsin when he ground up several of the organs for analysis. An animal's back just seemed to be the wrong place to put a pair of eyes. After all, he thought, other species of deep-sea shrimp have eyes in the normal place; some even live near the vents, although they are not nearly as numerous as *R. exoculata.*

Besides, even if the back patch turned out to be two visual organs, other problems would seem to hinder the detection of any pigment. Normally, biochemists need concentrated extracts from some 50 to 100 shrimp in order to detect any visual pigment molecules, says Szuts. But the number of available *R. exoculata* was limited, and he was dealing with extracts from five to 10 shrimp.

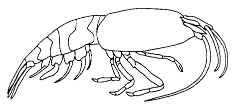
Szuts' skepticism turned to surprise when he found the "eyeless" shrimp did have rhodopsin. "It turns out *R. exoculata* has a visual pigment, and it has it in very large quantities – at the very least five times more than the usual amount of pigment in other shrimp," he says. Because the bright lights of the submersible

This photoreceptor cell for R. exoculata *devotes most of its body space to catching light. The tiny A-segment contains the nucleus and the normal cellular machinery. The R-segment contains light-absorbing pigments. Photoreceptor cells in most animals have a reverse arrangement with large A-segments and small R-segments.*



Chamberlain

Can 'eyeless' shrimp see jets of hot water spouting from the ocean floor?



most likely damaged much of the pigment in the collected shrimp, Szuts believes the pigment he found must be only a small fraction of the amount the animal truly possesses.

With the identification of rhodopsin, Chamberlain took a new look at the patch. He showed that the surface of the lobes is made of specialized sensory cells. Organized in clusters of six, these cells are jammed together so that a single lobe might contain from 1,300 to 1,500 clusters. Rhodopsin molecules sit embedded within the folds of the sensory cell's outer membrane.

Chamberlain says these cells are unlike any he has seen in other marine invertebrates. Animals such as the horseshoe crab, which mates at night, and *Bathynomus giganteus,* a giant deep-sea cousin of the pillbug, have evolved eyes suited for a world of dim light. But the *R. exoculata* eye seems to be even more highly specialized, he says.

It doesn't take a microscope to see some of *R. exoculata's* adaptations for sensing extremely weak light. Perhaps the most striking feature about the eyes is their size. Chamberlain believes they developed on the animal's back because that is the only spot where such large organs would fit. "You want a big space; these things are huge," he says. If *R. exoculata* had its eyes on stalks in front, "the animal would look like a lollipop. It would have this great big blob in front."

The elusive reflective quality – which can't yet be preserved in laboratory specimens – may also help the shrimp make the most of dim light. Chamberlain surmises that some kind of reflective structures sit under the layer of receptor cells. In that case, if rhodopsin molecules in the receptor cells failed to absorb a photon as it passed through the eye, the reflective layer underneath would send the photon right back up, giving the rhodopsin molecules a second chance. Cats and other animals that see at night have developed such a scheme for catching the most light possible.

R. exoculata's eyes have no lenses, so they cannot see actual images of an object. Just about all they can do is sense the strength and direction of a light source. Yet while they seem limited by the standards of animals living in bright light, the strange eyes are particularly well adapted for their dark environment. Chamberlain says the shrimp's eyes can best sense large, faint objects.

But what are the shrimp seeing down there? This question has perplexed Van Dover's group throughout their study of the shrimp's eyes.

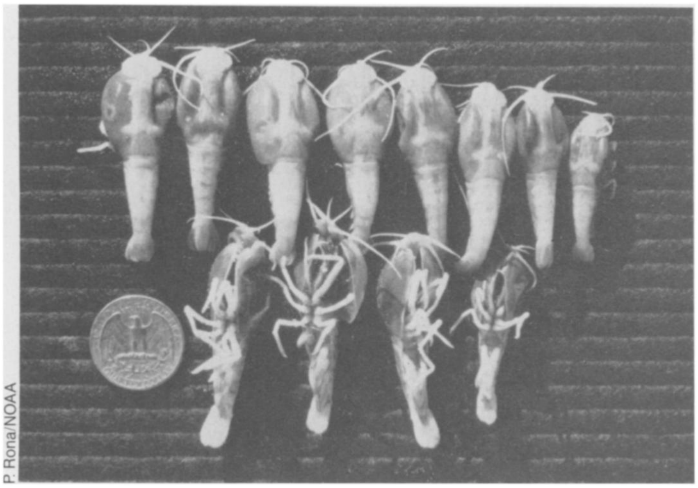
Though the sun's pale rays do not penetrate more than a few hundred meters below the water's surface, the deep sea is not completely dark. Occasional flashes of light signal the presence of bioluminescent fish, which emit visible photons to find prey and attract mates.

Yet Van Dover and her cohorts considered it unlikely that *R. exoculata* uses its eyes to see bioluminescent creatures. "Normal crustacean eyes can see bioluminescence, and actually form images of whatever is luminescing. So it seemed reasonable to suspect that this eye might be looking at something different just because the eye is so highly modified," she says.

Barring bioluminescence, the researchers turned to the most obvious objects within the shrimp's habitat: the vents themselves. Van Dover could think of several good reasons why the shrimp might want to sense light coming from the plumes of superhot water.

R. exoculata is thought to feed on bacteria that live directly around and on the chimneys. If these vents glowed, the shrimp might use such light as a beacon to find their feeding ground.

The same light could also protect the shrimp from the dangers of the vent environment. There is little room for error in this neighborhood, where water spews out of smokers at 350°C before quickly mixing with the 2°C ambient seawater. Darting about as they feed, *R. exoculata* often swim within centimeters of the plume water, which is more than hot enough to cook a shrimp. The researchers imagined the animals might use their eyes to sense the proximity of the hot water and thereby avoid a searing end. A heat-sensing organ could do a similar job for the shrimp, but visual organs respond to stimuli much more quickly, says Chamberlain.


P. Rona/NOAA

Shrimp platter: Specimens of R. exoculata *brought up from more than 2 miles below the Atlantic surface. Ranging between 1 and 2 inches in length, this species has two novel eyes located on its back (not clear in photo). Sulfide minerals trapped under shell create dark blobs on the animal's sides.*

Van Dover suggested this idea to John R. Delaney from the University of Washington in Seattle, who was taking the *Alvin* down to study oases of black smokers along the Juan de Fuca ridge off the Washington coast. *R. exoculata* is not known to exist in Pacific waters, but Van Dover figured if the Atlantic vents were producing light, then so should those in the Pacific. *Alvin* was set to carry a sensitive charge-coupled device (CCD) camera on this cruise, and Van Dover suggested the camera might be able to detect the light.

Although simple in concept, this seafloor photography session was not as easy as point-and-shoot. Delaney and his two companions in *Alvin* covered the port-holes so no light would leak out of the sub, then tried to keep the sub motionless as they took 10- to 20-second exposures of the vents with the CCD. With the stiff current at the bottom, the pilot had to push the sub full-throttle into a rock to keep it steady.

While the trio in *Alvin* was trying to image the vents, Van Dover waited on the support ship, pacing in and out of the radio room in search of any news that might appear in the half-hourly reports from the submersible. The day passed without mention of the imaging project. Then, as *Alvin* was returning to the surface, a two-worded message came in over the radio, saying simply: "Vents glow."

The plumes of hot water did, in fact, appear clearly on the CCD images. The hydrothermal water glowed brightest where it left the opening in the seafloor, and then dimmed sharply as it mixed with the cold seawater.

These images delighted the crew, yet they didn't necessarily prove that the vents produce visible light. Human beings can only see photons with wavelengths between about 400 nanometers and 700 nanometers, while the CCD camera can pick up invisible infrared light with wavelengths as long as 1,000 nm. Delaney's crew wondered if all the vent light was in the invisible end of the spectrum.

To test what wavelengths the camera was sensing, the *Alvin* crew made a second dive and put filters on the CCD that cut out all infrared light. In these images, the vent's brightness dropped dramatically, but a faint glow remained. Delaney is still analyzing the data and is reluctant to discuss the results from the cruise until they are published in a peer-reviewed journal. But the initial findings suggest the vents *do* emit visible light, albeit an extremely slight amount, Van Dover reported in December at a meeting of the American Geophysical Union in San Francisco.

The vents were so dim that crew members (whose eyes were not adapted to the dark) could not see them through the porthole. Nor did the vent light turn up in photographs from a 35-millimeter camera with 1000 ASA film.

Van Dover and her colleagues have suggested a handful of mechanisms that might be producing the vent light, but they have focused on one possibility called thermal radiation. It is well known that hot objects emit electromagnetic radiation – and if an object is hot enough, it can even produce a substantial amount of visible light. For example, take the heating element in an oven. While it emits invisible waves of infrared light that heat the air in the oven, it also sends out photons in the red range of the visible spectrum, giving the element a reddish glow.

Another explanation of the vent light might involve something called Cherenkov radiation, which comes from radioactive elements such as potassium-40. When unstable atoms decay, they give off photons, some of which are visible. In most parts of the ocean, such radioactive elements are so dilute that Cherenkov radiation can be detected only with the most sensitive photomultipliers. But vent water acquires many minerals and elements while flowing through cracks in the ocean crust, and it may contain concentrated amounts of radioactive elements.

In addition to thermal radiation and Cherenkov radiation, Van Dover and her colleagues have thought of several other mechanisms that might be producing the light. But for now, they say the most likely contender is thermal radiation.

It will certainly require a good deal more work to come up with a final identification of the light source. A return visit to the vent neighborhood will help, and Van Dover is trying to arrange such a project. Jeffrey Steinfeld, a physical chemist from MIT, says it should be possible to determine whether thermal radiation is the sole cause of the vent light by analyzing its spectrum.

If thermal radiation does in fact produce the vent light, then *R. exoculata* should be able to sense it. At least this is the conclusion of Chamberlain and Syracuse colleague Denis G. Pelli, who have published a separate report in the Feb. 2 NATURE.

In their theoretical analysis, Pelli and Chamberlain calculate the amount of visible thermal radiation that should be coming from the vents, and compare this number to the estimated sensitivity of the *R. exoculata* eye. Their work suggests the shrimp's eyes are responsive enough to see the dim vent light, whereas even a well-adapted human would probably be left in the dark down there.

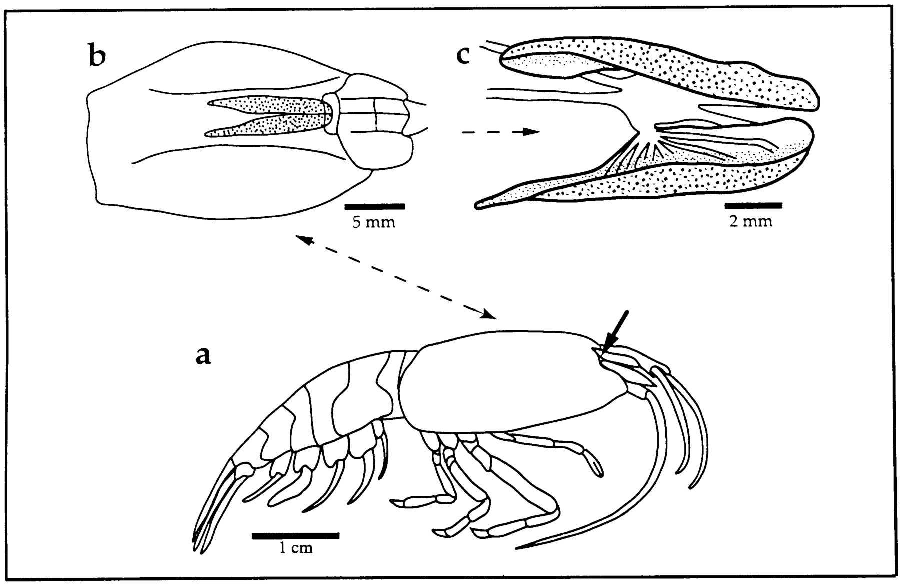
Such calculations might seem to wrap up *R. exoculata's* story, but they don't. For even if future investigations prove Pelli and Chamberlain correct – that the shrimp can indeed sense the vents – the animals might actually be spending most of their time looking at something else. Szuts claims the shrimp's eyes are designed to sense a completely different kind of light.

An expert in visual pigments, Szuts believes the rhodopsin from the shrimp offers an important clue that cannot be overlooked. His work indicates the particular rhodopsin in *R. exoculata* absorbs 500-nm photons most strongly. Light with this wavelength sits on the blue end of the visible spectrum, far from the visible red and even farther from the invisible infrared.

The vent, however, sends out many more red photons than blue ones, and this puts a kink in the theory that the shrimp are looking at the vents, says Szuts. Simply stated, the shrimp rhodopsin is poorly suited for absorbing vent light.

Szuts points out that red-absorbing pigments and blue-absorbing pigments differ by just a few amino acids. He reasons that *R. exoculata* went through major changes as it evolved its unusual eye. If this development was truly an adaptation in order to see light from the vents, he asks, then why didn't the shrimp make a slight alteration in pigment? "Usually an animal will match their sensitivity to the light source in the environment," he says.

Even with the blue-absorbing rhodopsin, the shrimp might still be able to see the vents, Szuts says. But that doesn't mean they are looking primarily at such light, he says. "You and I, if we were



Van Dover et al.

(A) indicates where other shrimp have eyes (solid arrow) and where R. exoculata *has its unusual eyes (broken arrow). (B) and (C) show details of visual lobes.*

adapted to the dark, could probably detect [thermal] radiation from a 375°C oven, but that doesn't mean we principally use our eyes to look at ovens," he says.

Szuts thinks the shrimp use their unusual eyes to sense a different source of light – one in the blue end of the spectrum, which is better matched to the animal's rhodopsin. "I think there's lots of blue-green light down there that we must have not yet found," he says.

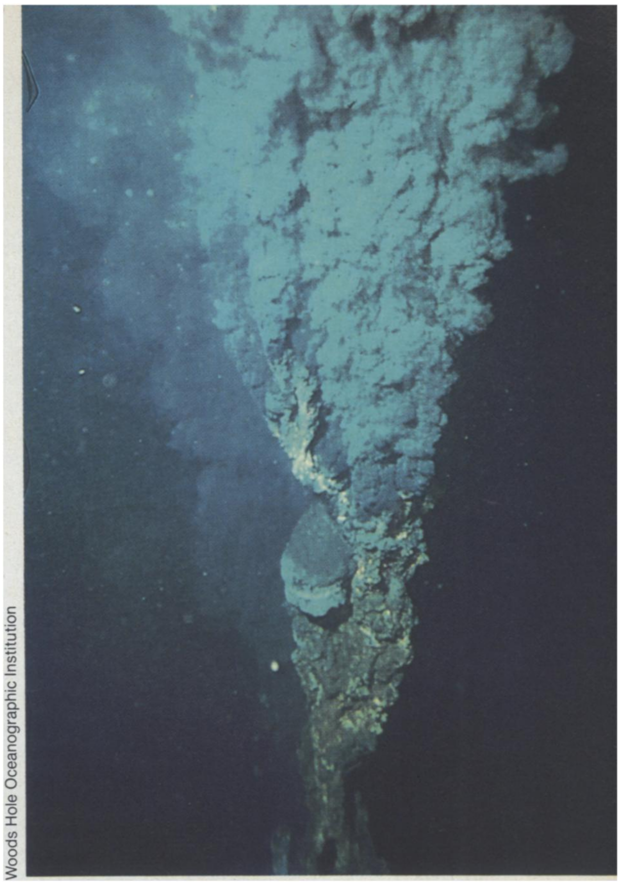
It is quite possible, says Szuts, that blue-green light from an unknown source has escaped detection because scientists have spent relatively little time looking for light near the vents.

Alternatively, the answer might be bioluminescence after all. In an editorial accompanying the two NATURE papers, biologist Michael F. Land from the University of Sussex in England notes that several other deep-sea creatures have "naked-retina" eyes, similar in structure to those of *R. exoculata*. He finds it hard to believe that all these animals live near hot vents. Instead, he proposes this type of lens-less eye may provide enough information to let shrimp sense the direction of bioluminescent organisms.

At this point, the *R. exoculata* saga awaits an ending, and it may be a while before a resolution comes into sight. While physicists and chemists may soon determine what process actually creates the vent light, biologists will have a more difficult time telling whether the shrimp actually use their eyes to sense this energy. And if *R. exoculata* is indeed relying on such light to steer clear of the hot plumes, scientists will have to answer why the shrimp never evolved a more appropriate form of rhodopsin.

Certainly, behavioral studies using live specimens will offer important lessons concerning the habits of the shrimp, but biologists have never had the opportunity to study living examples, and it is not clear whether these animals can survive in a laboratory environment. It may also be quite difficult to study the shrimp's activity in their natural home because bright light, such as that from the *Alvin*, seems to damage their eyes. It will take some ingenuity to study the shrimp without blinding them, or to collect samples without harming the very structures researchers are trying to study.

Moreover, Chamberlain raises the possibility that the shrimp act differently when the submarine's lights are not trained on them. Perhaps "it's the shrimp version of the Heisenberg Uncertainty Principle," he says. "As soon as humans look at them, they don't do what they normally do." □



Woods Hole Oceanographic Institution

Clouds of hot water and dissolved minerals spew out from black smoker chimneys on the East Pacific Rise that are similar to Atlantic chimneys around which the shrimp have been found.

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In "Germ Wars," Melissa Hendricks accurately portrays the work of Army microbiologists striving to provide a medical defense against the threat of biological attack. The fact that she also catalogs all available criticisms of that work is not taken as a slight against the Army program. She told us from the outset that she was writing about the controversy over the work, not just about the work.

However, she concludes her piece with a call for "openness," as if there were more of the program to be revealed at some future point of rapprochement, presumably to be brought about by the critics of the BDRP program.

Ms. Hendricks, and the many other writers who have covered this story, have had unimpeded access to the Army medical defense program, through interviews with key investigators and laboratory managers, copies of documents and tours of facilities. It's an unclassified program, conducted by investigators whose careers will benefit most from published results, and not at all from nefarious and secret perversions of biotechnology. Openness is already here, in a much greater degree than any of the myopic critics can bear to admit.

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