New studies take a second look at coral bleaching culprit

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Close-up of bleached coral sampled in Hawaii's Kaneohe Bay in October 2014. Understanding superoxide's role in coral health may help in the development of bleaching mitigation strategies. Credit: Amy Apprill, Woods Hole Oceanographic Institution

Scientists have called superoxide out as the main culprit behind coral bleaching: The idea is that as this toxin build up inside coral cells, the corals fight back by ejecting the tiny energy- and color-producing algae

living inside them. In doing so, they lose their vibrancy, turn a sickly white, and are left weak, damaged, and vulnerable to disease.

Now, a new study from the Woods Hole Oceanographic Institution (WHOI) is casting a more positive light on superoxide. It suggests that when these molecules are produced at coral surfaces—outside of their cells—they may actually play a beneficial role in coral health and resilience. The research that results from this finding may contribute to future strategies for preventing corals from bleaching.

"Superoxide has largely been vilified as a stress molecule and implicated as the main cause of coral bleaching," said Colleen Hansel, a WHOI biogeochemist and lead author of the study published December 7 in the journal *Nature Communications*. "But when we measured superoxide concentrations at the surface of corals during a natural bleaching event, we saw a completely different dynamic. It appears this 'toxin' may have provided a benefit to the corals—perhaps helping some species to resist bleaching."

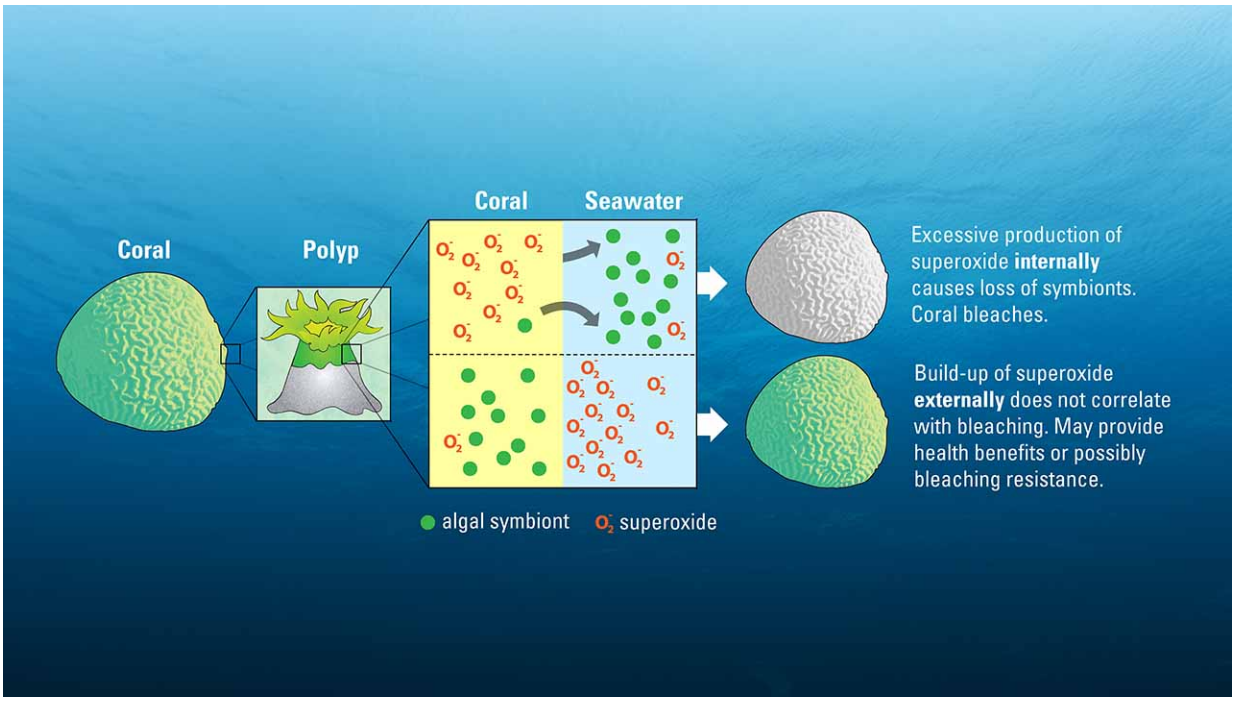
Hansel said the researchers were surprised to see that, during the bleaching event, coral species that were more susceptible to bleaching weren't producing external superoxide, while those that resisted bleaching produced high concentrations.

"While we don't yet understand how corals produce superoxide externally, this discovery points to a fundamental misunderstanding of what this group of compounds does for coral health," she said.

First-ever field measurements

Hansel believes the dark shadow cast on superoxide stems from a lack of direct measurements. "To date, our understanding of the role these molecules play in coral health is based on indirect lab measurements of

superoxide inside coral tissue. No one had ever attempted direct measurements of superoxide produced outside corals living within reefs. This is most likely because superoxide is very difficult to measure as it is highly reactive and has a lifespan of only roughly 30 seconds in natural waters. You can't just take the samples back to the lab for analysis."



In this simplified illustration, the top scenario shows what is known about superoxide and coral bleaching: excessive production of superoxide within the coral tissue can cause the loss of symbiotic algae living inside the coral. The bottom scenario illustrates the new finding by WHOI scientists: superoxide produced at coral surfaces -- outside of their cells -- they may actually play a beneficial role in coral health and resilience. Credit: Eric Taylor, Woods Hole Oceanographic Institution

To overcome this, she and her research team devised a novel approach

for taking real-time measurements of coral superoxide production in a natural reef environment. During a week-long field visit to Hawaii's Kaneohe Bay in October, 2014, they lugged a laboratory instrument used for superoxide analysis onto a tiny motor boat and took samples from six different shallow reef sites.

"The measurements were very tricky to collect as we had to sample superoxide produced by the coral using a small tube held just above the coral surface," said Hansel. "From there, it had to be pumped up to the instrument on the boat within 30 seconds, before the superoxide was gone. While all this was happening, we had to hold the boat over the corals being measured and keep the boat steady as waves were coming in."

WHOI microbiologist Amy Apprill was underwater holding the sampling tube in place as her colleagues "shouted out with excitement from seeing the real-time data." Apprill said it was amazing to see wide variations in superoxide production among different coral species, some making "a ton" of superoxide, while others making very little. Their analysis showed the corals producing more superoxide had greater resilience to bleaching than the coral producing very little.

Another noteworthy discovery, according to the scientists, was that corals were producing superoxide even when there were no evident stressors, such as hotter seas, which are known to trigger superoxide production.

In a parallel study published November 24 in the journal *Frontiers in Marine Science*, the team found similar results with corals grown under non-stressful conditions in the lab. The corals, and their microbial partners (symbionts), produced high levels of superoxide at the surface independent of temperature and other variables such as time of day or presence of light.

"This made our eyes open a little bit wider," Apprill added, "and reinforced the idea that, while we have a better understanding of the negative impacts associated with internally-produced superoxide, the role of superoxide produced outside cells has been overlooked and is not been well understood."

Playing a positive role

The possibility that superoxide may help corals resist bleaching was eye opening for the scientists, but it isn't the first time these toxins have proven beneficial to organisms. In fact, research conducted on bacteria and fungi has shown that superoxide is purposely made outside the cell to stimulate cell growth, increase nutrient uptake, and fend off invasive pathogens.

"Superoxide is not always bad," said Hansel. "In fact, it is an essential molecule that all organisms need. Similar to other organisms, we believe that these compounds may be an integral component of the physiology and immune system of corals. It's not as black and white as once believed."

Next steps

Understanding the positive impacts of superoxide on corals may be a stepping stone towards improving coral health and developing bleaching mitigation strategies in the future—particularly if the molecules are in fact protecting corals from stress rather than inducing it. The information could ultimately be used to help determine how to engineer corals to be more resilient. But Hansel feels more work is needed.

"The next step is to conduct a temporal study in the field to get more information on how superoxide is being regulated over time as a

function of stress and during the course of a bleaching event," she said. "This includes when they're completely healthy, as warmer temperatures increase and stress begins, during peak stress and bleaching, and through recovery. We'd also like to couple this with controlled lab studies where we can grow corals that range in bleaching susceptibility and introduce them to stressors including pathogens to see if they trigger superoxide production. We want to mimic a range of natural conditions in the lab to tease out the benefits superoxide is providing."

Hansel said that while the fieldwork made it possible to explore corals in a natural environment that is more realistic to the conditions they experience, lab experiments allow them to change one variable at a time while keeping everything else constant so direct relationships can more easily be seen.

"To truly link variables like heat and stress, we need to minimize variability in other variables, like light, changes in flow patterns, and nutrient inputs," she said.

According to Apprill, the new findings suggest that superoxide may play a variety of roles in coral health, and have led to a new realization of the complexities the toxins play in corals.

"Coral bleaching is one of the biggest problems facing the ocean today," said Apprill. "We understand the factors that contribute to bleaching events, but the mechanisms appear to be more complicated than we had thought. Fortunately, corals are a really good system to focus on in terms of studying the health impacts of superoxide, since drastic changes in coral health are very visible and can be quickly seen—even from space."

More information: Julia M. Diaz et al, Species-specific control of external superoxide levels by the coral holobiont during a natural bleaching event, *Nature Communications* (2016). DOI:

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