New research sheds light on how harmful algal species use nitric oxide to grow

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University of Delaware Associate Professor Kathyrn Coyne and Emily Healey, who received her masters at UD in marine biosciences and is currently a doctoral student at the University of Maryland School of Public Health, looked at how Heterosigma akashiwo, a globally distributed toxic species of alga, is accessing nitric oxide in the environment and how that may give it an advantage over other harmful algae species. Credit: Edward Whereat, Tammy Beeson/University of Delaware

Ever since it was discovered to be a driving force behind economically

and environmentally destructive harmful algal blooms (HABs) throughout the world, researchers have been trying to discover more information about the effects of different types of nitrogen, such as nitrate or ammonium, on the proliferation of HABs.

At the University of Delaware, Kathyrn Coyne, associate professor in the School of Marine Science and Policy (SMSP), has spent several years studying HABs—including Heterosigma akashiwo, a globally distributed toxic species of alga. About 10 years ago, with funds from Delaware Sea Grant (DESG), Coyne's lab discovered that Heterosigma akashiwo is able to use nitric oxide as a nitrogen source.

It does this by using a unique modification of an enzyme called nitrate reductase. This enzyme typically catalyzes the first step in the process of converting nitrate to ammonium, a more useable form of nitrogen. The modification of nitrate reductase in Heterosigma akashiwo allows it to use nitric oxide instead of nitrate as a source of nitrogen.

"This kind of raised a lot of questions," Coyne said. "Where would they be accessing nitric oxide in the environment? What kind of advantage does it give Heterosigma akashiwo to have this modification in their enzyme? And how do other types of nitrogen affect its ability to use nitric oxide?"

The last question was answered in a recent article about the results of the research in *Scientific Reports*.

The lead author on this paper, Emily Healey, received her master's degree at UD in marine biosciences and is a doctoral student at the University of Maryland School of Public Health. Along with Coyne, other co-authors include Joanna York, professor in the School of Marine Science and Policy and Director of DESG, and Robinson Fulweiler, professor in the Department of Earth and Environment at Boston

University, as well as past members of Coyne's lab, Stacie Flood and Patience Bock.

REU participant

Healey said that while she joined Coyne's lab as a master's student in the summer of 2019, she came to UD and worked with Coyne earlier as a participant in the SMSP Research Experiences for Undergraduates (REU) summer program.

"I have always been interested in microbiology and to have the opportunity to spend my summer at the beach through the REU program was pretty amazing," Healey said. "Dr. Coyne runs a great lab. She had really helpful Ph.D. students working for her, and I just loved the work. I had never worked with algae before—I had been doing more with bacteria so it was interesting to get immersed in a different microbe."

After her REU experience, Healey was able to come back the next year to start her master's program. During that time, she began the research which would eventually lead to this recent publication.

Nitrate reductase

Healey said one of the goals of her research was to see if other nitrogen sources affect the activity of nitrate reductase in Heterosigma akashiwo and its ability to take up nitric oxide. For example, if there is a lot of ammonium present in an environment from agricultural sources, most species will down-regulate the nitrate reductase enzyme, which might affect Heterosigma's ability to use nitric oxide.

"We thought if there's a lot of ammonium, maybe Heterosigma akashiwo isn't able to use nitric oxide as a source of nitrogen," said Healey.

"Maybe if there's ammonium present, they simply turn off that enzyme."

By shutting off the enzyme in the presence of ammonium, the possibility existed that Heterosigma akashiwo would be inhibited or even prevented from accessing nitric oxide and turning it into biomass.

What they found, however, was that even when there was ammonium present, providing nitric oxide to cultures of Heterosigma akashiwo actually increased activity of the enzyme so that it was able to successfully take up nitric oxide and convert it to biomass in the presence of ammonium.

To determine the effects of different types of nitrogen on nitrate reductase, the researchers conducted three experiments. They grew Heterosigma akashiwo in the lab first with only nitrate, then with only ammonium, and finally with a 50/50 mix of nitrate and ammonium.

In each of the experiments, the activity of the enzyme nitrate reductase not only increased when nitric oxide was added, but results showed that Heterosigma was incorporating nitric oxide into biomass even in the presence of ammonium.

Coyne said this ability potentially gives Heterosigma akashiwo an advantage over other species, by allowing them to access a novel source of nitrogen.

"If that were the case, then Heterosigma may be able to use nitric oxide as an alternative that other species simply don't have access to," said Coyne.

Nitrogen in the sediments

This ability to use nitric oxide as a nitrogen source may have something

to do with how Heterosigma akashiwo moves throughout the day—as the species will move up to the surface of the water during the day and return to the bottom of the water column during the night.

"At night, Heterosigma migrates to the sediments and then during the day time, they're photosynthetic so they move up to the surface where they are exposed to sunlight," said Coyne. "There is a lot of data showing that nitric oxide seeps up through the sediments in some coastal areas, so we think Heterosigma may be accessing that nitric oxide as a nitrogen source at night."

Healey said this ability to use nitric oxide may have a few implications. One would be that it gives Heterosigma akashiwo an advantage over other algae when other types of nitrogen have been depleted.

In addition, Healey said that nitric oxide should be considered a factor in bloom formation for Heterosigma akashiwo.

"People often study the effects of nitrogen on blooms and how to prevent blooms in the first place by reducing nitrate or ammonium," said Healey. "Now, we're saying, 'Ok, add nitric oxide to that list because it could be important.'"

More information: Emily M. Healey et al, Effects of nitrate and ammonium on assimilation of nitric oxide by Heterosigma akashiwo, *Scientific Reports* (2023). DOI: 10.1038/s41598-023-27692-3

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