'Hide or get eaten,' urine chemicals tell mud crabs

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A blue crab in a tank in a research lab at Georgia Tech. In the tank behind it, small mud crabs are hiding in plastic tubes. Credit: Georgia Tech / Alex Draper

Psssst, mud crabs, time to hide because blue crabs are coming to eat you! That's the warning the prey get from the predators' urine when it spikes with high concentrations of two chemicals, which researchers have identified in a new study.

Beyond decoding crab-eat-crab alarm triggers, pinpointing these compounds for the first time opens new doors to understanding how chemicals invisibly regulate marine wildlife. Insights from the study by researchers at the Georgia Institute of Technology could someday contribute to better management of crab and oyster fisheries, and help specify which pollutants upset them.

In coastal marshes, these urinary alarm chemicals, trigonelline and homarine, help to regulate the ecological balance of who eats how many of whom—and not just crabs.

Blue crabs, which are about hand-sized and are tough and strong, eat the mud crabs, which are about the size of a silver dollar, and thin-shelled. Blue crabs also eat a few oysters, but mud crabs eat a lot more oysters than they do. When blue crabs are going after mud crabs, the mud crabs hide and stay still, so far fewer oysters get eaten than usual.

Humans are part of the food chain, too, eating oysters as well as blue crabs that boil up a bright orange. The blue refers to the color of markings on their appendages before they're cooked. Thus, the blue crab urinary chemicals influence seafood availability for people, as well.

Predator pee-pee secrets

The fact that blue crab urine scares mud crabs was already known. Mud crabs duck and cover when exposed to samples taken in the field and in the lab, even if the mud crabs can't see the blue crabs yet. Digestive products, or metabolites, in blue crab urine trigger the mud crabs' reaction, which also makes them stop foraging for food themselves.

"Mud crabs react most strongly when blue crabs have already eaten other mud crabs," said Julia Kubanek, who co-led the study with fellow Georgia Tech professor Marc Weissburg. "A change in the chemical balance in blue crab urine tells mud crabs that blue crabs just ate their cousins," Kubanek said.

Figuring out the two specific chemicals, trigonelline and homarine, that set off the alarm system, out of myriad candidate molecules, is new and has been a challenging research achievement.


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Principal investigator Julia Kubanek loads a sample into a nuclear magnetic resonance spectroscopy device. Credit: Georgia Tech / Christopher Moore

"My guess is that there are many hundreds of chemicals in the animal's urine," said Kubanek, who is a professor in Georgia Tech's School of Biological Sciences, in its School of Chemistry and Biochemistry, and who is also Associate Dean for Research in Georgia Tech's College of Sciences.

The researchers applied technology and methodology from metabolomics, a relatively new field used principally in medical research to identify small biomolecules produced in metabolism that might serve as early warning signs of disease. Kubanek, Weissburg, and first author Remington Poulin published their results the week of January 8, 2017, in the journal *Proceedings of the National Academies of Science*.

The research was funded by the National Science Foundation.

Peedle in a haystack

Trigonelline has been studied, albeit loosely, in some diseases, and is known as one of the ingredients in coffee beans that, upon roasting, breaks down into other compounds that give coffee its aroma. Homarine is very similar to trigonelline, and, though apparently less studied, it's also common.

"These chemicals are found in many places," Kubanek said. But picking them out of all those chemicals in blue crab urine for the first time was like finding two needles in a haystack.

Often, in the past, researchers trying to narrow down such chemicals have started out by separating them out in arduous laboratory procedures then testing them one at a time to see if any of them worked. There was a good chance of turning up nothing.

The Georgia Tech researchers went after all the chemicals at one time, the whole haystack, using mass spectrometry and nuclear magnetic resonance spectroscopy.

"We screened the entire chemical composition of each sample at once," Kubanek said. "We analyzed lots and lots of samples to fish out chemical candidates."

Mud crabs hiding in reaction to chemicals taken from blue crab urine and introduced into their tanks in a lab at Georgia Tech. Credit: Georgia Tech / Alex Draper

Crabs are 'walking noses'

The researchers discovered spikes in about a dozen metabolites after blue crabs ate mud crabs. They tested out those pee chemicals that spiked on the mud crabs, and trigonelline and homarine distinctly made them crouch.

"Trigonelline scares the mud crabs a little bit more," Kubanek said.

More specifically, high concentrations of either of the two did the trick. "It's clear that there was a dose-dependent response," said Weissburg, who is a professor in Georgia Tech's School of Biological Sciences. "Mud crabs have evolved to hone in on that elevated dose."

"Most crustaceans are walking noses," Weissburg said. "They detect chemicals with sensors on their claws, antennae and even the walking legs. The compounds we isolated are pretty simple, which suggests they might be easily detectable in a variety of places on a crab. This redundancy is good because it increases the likelihood that the mud crabs get the message and not get eaten."

Ecological and fishery effects

Evolution preserved the mud crabs with the duck-and-cover reaction to the two chemicals, which also influenced the ecological balance, in part by pushing blue crabs to look for more of their food elsewhere. But it influenced other animal populations as well.

"These chemicals are staggeringly important," Weissburg said. "The scent from a blue crab potentially affects a large number of mud crabs, all of which stop eating oysters, and that helps preserve the oyster populations."

All of that also impacts food sources for marine birds and mammals: Just by the effects of two chemicals, and there are so many more chemical signals around. "It's hard for us to appreciate the richness of this chemical landscape," Weissburg said.

As scientists learn more, influencing these systems could become useful to ecologists and the fishing industry.

"We might even be able to use these chemicals to control oyster consumption by predators to help preserve these habitats, which are critical, or to help oyster farmers. That's becoming important in Georgia fisheries," Weissburg said.

Pollutants in pesticides and herbicides are known to interfere with estuaries' ecologies. "It will be a lot easier to test how strong this is by knowing specific ecological chemicals," Weissburg said.

Fear-o-mone small molecules

By the way, trigonelline and homarine are not pheromones.

"Pheromones are signaling molecules that have a function within the same species, like to attract mates," Kubanek said. "And blue crabs and mud crabs are not the same species."

"In this case, the mud crabs have evolved to chemically eavesdrop on the blue crabs' pee. You might call trigonelline and homarine fear-inducing cues."

Identifying such metabolites, also called small molecules, and their effects is the latest chapter in constructing the catalog of life molecules. "Everyone knows about the human genome project, identifying genomes; then came transcriptomes (molecules that transcribe genes)," Kubanek said. "Now we're pretty far along with proteomics (identifying proteins), but we're just now figuring out metabolomes."

More information: Remington X. Poulin el al., "Chemical encoding of risk perception and predator detection among estuarine invertebrates," *PNAS* (2017). www.pnas.org/cgi/doi/10.1073/pnas.1713901115

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