

Freshwater stingray venom varies according to sex and age

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There is no antidote or specific treatment for freshwater stingray venom, although accidents involving these animals are frequent on rivers in the Amazon and other regions.

A groundbreaking study conducted at the Butantan Institute in São Paulo, Brazil, analyzed [freshwater stingray](#) toxins to elucidate their

mechanism of action and highlighted methods of treatment. One of the key findings was that the composition and effects of the venom produced by members of the family *Potamotrygonidae* vary even among individuals of the same species.

A sting by a young ray is extremely painful and is used help to frighten off predators, whereas the toxins injected by adult rays cause necrosis and are therefore effective weapons in the hunt for small fish, shrimp and other crustaceans.

"Environmental pressure leads to a modification in the venom's composition and its toxicological effect. When the environment changes, the type of food also changes, and all this causes evolutionary pressure that can lead to a change in toxin composition," said Carla Lima, Vice Director of Butantan Institute's Special Applied Toxicology Laboratory (LETA).

The study was conducted by Lima and Mônica Lopes-Ferreira, the director of LETA, under the aegis of the Center on Toxins, Immune Response and Cell Signaling (CeTICS). The results were published in the journal *Toxicon*.



Toxins produced by young female stingrays cause more pain, whereas toxins produced by adult stingrays cause tissue necrosis. Credit: Carla Lima

"Several studies have been performed with freshwater stingrays to compare the toxicity of venom from their stinger and mucus. However, studies demonstrating the influence of sex and maturation stage on the composition of stingray venom and its toxic effects are still scarce," Lopes-Ferreira said.

"We set out to discover whether the stage of development and gender of *Potamotrygon rex*, a freshwater stingray found in rivers throughout South America, influences the composition of its venom and its capacity to trigger an acute inflammatory response using mice as a model. Our results suggest that nociception is induced mainly by the toxins produced by young females," Lima said.

Nociception is the reception, conduction, modulation, central processing and perception of sensory information elicited by [tissue injury](#) that is transmitted to the central nervous system by nociceptors, which are peripheral sensory neurons that respond to damaging stimuli in skin and tissue. The brain interprets the signals received by nociceptors as pain.

"In contrast, adult ray venom is more effective in producing protein exudation," Lima added.

Exudation is the discharge of organic liquids via cell walls and membranes in response to injury or inflammation.

"Our findings showed that the composition of the venom of *P. rex* is influenced by the animal's development to maturity. The production of peptides and proteins capable of influencing the leukocyte-endothelium interaction and favoring neutrophil infiltration into damaged tissue is modulated according to the stage of development," Lopes-Ferreira said.

Potamotrygon rex

The researchers collaborated with colleagues at the Federal University of Tocantins—UFT (also in Brazil), who coauthored the article, to collect venom samples from stingrays captured in the Tocantins River.

All the stingrays belonged to the species *P. rex*, which is endemic to South America and common in the middle and upper Tocantins, although it was only first scientifically described in 2016.

"To verify toxicity, venom from young and adult rays was applied directly to the skin of anesthetized mice and the resulting alterations to tissue were analyzed under the microscope," Lopes-Ferreira said.

According to the researchers, venom from rays up to two years old, especially females, was found to be more potent and capable of causing acute pain due to the presence of neuroactive peptides.

"Schools of young rays spend a great deal of time hiding in the mud in the riverbed, feeding on microcrustaceans and leaving only rarely. Their sting causes a painful wound and probably serves to ward off predators," Lima said.

Two-year-old rays are sexually mature. They leave their siblings and hiding places in the riverbed to live as lone predators in the water column, which is more or less turbid.

"Toxin composition changes at this point," Lima said. "Pain-causing peptides give way to proteins that cause inflammatory wounds and tissue necrosis."

According to the study, sexual maturity is required for this alteration in the venom composition to occur. Rapid changes in the river water and

the natural environment may delay an animal's development and the transformation of its toxins.

This can be observed, for example, in the fish that inhabit the rivers affected by the January 2019 dam burst and the mine tailings spill in Brumadinho, in the Brazilian state of Minas Gerais.

"A sudden change in pH or the transformation of crystal-clear water into turbid water can impair the maturation of stingrays and their arsenal of toxins. Massive contamination will also destroy much of their food and force a change in diet," Lopes-Ferreira said.

Venom composition is known to change in snakes depending on the life stage and sex. According to the authors, this is the first time the same phenomenon has been observed in river or marine stingrays.

Accidents becoming more frequent

"The number of accidents involving stingrays in the Amazon Basin is substantial and continuously rising. To date, there are no antidotes for freshwater stingray venom; therefore, it must be treated with medication to control pain and tissue necrosis. For this reason, it is important to study toxin compositions and to discover how toxins vary between young and adult stingrays or between males and females," Lopes-Ferreira said.

One of the reasons for the increasing number of accidents may be the growing demand for rays in the global ornamental fish trade. According to a report produced by Brazil's Environment Ministry as part of its commitments under the Convention on International Trade in Endangered Species ([CITES](#)), Brazil legally exported 68,600 specimens belonging to six freshwater stingray species between 2003 and 2016. The highest price was fetched by *P. leopoldi*, the polka-dot river stingray, which accounted for approximately 40 percent of the total (27,700).

Freshwater stingrays are captured at a young age, when their disks are approximately 6 cm in diameter. In an aquarium, they can grow to approximately 20 to 30 cm. They may then be considered too large, in which case they may be released into a river, lake or dam reservoir. This is why there are now many of these stingrays in rivers in southern and southeastern Brazil, which may be another reason for the increasing frequency of accidents.

"Now that we know the [venom](#) changes depending on age and sex, we'll be able to provide more suitable treatments for victims when they come to the emergency room. If they report an encounter with a small ray, for example, the attending or nurse can opt for antivenom serum therapy with peptides. However, if the individual has been stung by an adult ray, medical staff should consider a protein antivenom," Lopes-Ferreira said.

Freshwater stingrays are found only in South America. They evolved from a marine ancestor that took up residence inland after part of the continent was flooded by rising sea levels during the Eocene Epoch, approximately 50 million years ago, and possibly in the Miocene, approximately 20 million years ago.

When the sea retreated from what is now Amazonia, saltwater species had to adapt to freshwater environments or they would disappear. Some species succeeded, including the Amazon river dolphin (*Inia geoffrensis*) and manatee (*Trichechus spp.*), as well as the stingrays.

There are four genera with a total of 34 species. *Potamotrygon* alone comprises 27 species; 21 are found in Brazil, and 11 are endemic to Brazilian rivers.

More information: Juliane Monteiro dos Santos et al, Stingray (*Potamotrygon rex*) maturity is associated with inflammatory capacity of the venom, *Toxicon* (2019). [DOI: 10.1016/j.toxicon.2019.03.013](https://doi.org/10.1016/j.toxicon.2019.03.013)

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