

First record of imposex in the Horse Conch, *Triplofusus giganteus* (Mollusca: Gastropoda)

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Abstract

We report the first record of imposex in *Triplofusus giganteus*, a top predator in shallow water marine habitats. Imposex is an irreversible disorder in female caenogastropod snails caused by exposure to biocidal organotin compounds identified in vessel antifouling paints. The condition was observed in three of four snails collected from Alligator Harbor, Florida, with affected females developing a non-functional male reproductive organ, the pseudopenis. The presence of imposex is of particular concern for the long-term conservation of the species, given its slow maturation and limited reproductive output.

Research Question

The Horse Conch, *Triplofusus giganteus* (Figures 1, 2), the largest gastropod in the Atlantic Ocean, is a top predator living in shallow-water habitats. The species is found from North Carolina to Florida, the Gulf of Mexico, and the Caribbean coast of Mexico. Four specimens of *T. giganteus*, collected by Gulf Specimen Marine Lab, Panacea, Florida were acquired by the National Shell Museum & Aquarium. Upon arrival, examination revealed that three of the four snails were imposex females each bearing a small pseudopenis behind the right tentacle. The research in question aimed to confirm presence of imposex in these specimens, check the literature to assess whether the condition had already been recorded for the species, and gauge the potential detrimental effects on the species from a conservation standpoint.

Study Design and Methodology

- This investigation was based on four adult *Triplofusus giganteus* collected in December 2023 by Gulf Specimen Marine Lab in Alligator Harbor, Franklin County, Florida. The live snails were transported to the Bailey–Matthews Shell National Museum & Aquarium and maintained in a 398-gallon saltwater aquarium. The design thus combined field collection with anatomical and histological analyses to confirm the presence of imposex. Comparisons were made to published imposex cases in related gastropods (Bryan et al. 1987; Costa et al. 2014; Giulianelli et al. 2020) to ensure accurate interpretation of the structures observed.

Each specimen was carefully examined using the following procedures:

- **Specimen collection and housing:** Four *Triplofusus giganteus* (shell lengths between 307–360 mm) were collected. The specimens were held in controlled aquarium conditions for observation, and were assigned National Shell Museum & aquarium scientific collection numbers (acronym: BMSM).
- **Morphological examination:** Each snail was examined for sex identification and examination of reproductive anatomy.
- **Morphometrics:** Shell dimensions were recorded, and the lengths of reproductive organs were measured.
- **Histological analysis:** Tissue from the pseudopenis and adjacent body wall of the largest imposex female was excised, fixed in formalin, embedded in paraplast, sectioned (transversely and longitudinally), and stained with hematoxylin–eosin.

Findings and Analyses

Upon detailed examination, three of the four snails were confirmed to exhibit imposex. Each of these three females retained gonads and pedal glands but also bore a non-functional pseudopenis on the right side of the body wall. One individual was a normal male. The remaining three had female gonads but each presented a small fleshy outgrowth (pseudopenis) on the right side behind the tentacle, a recognized sign of imposex.

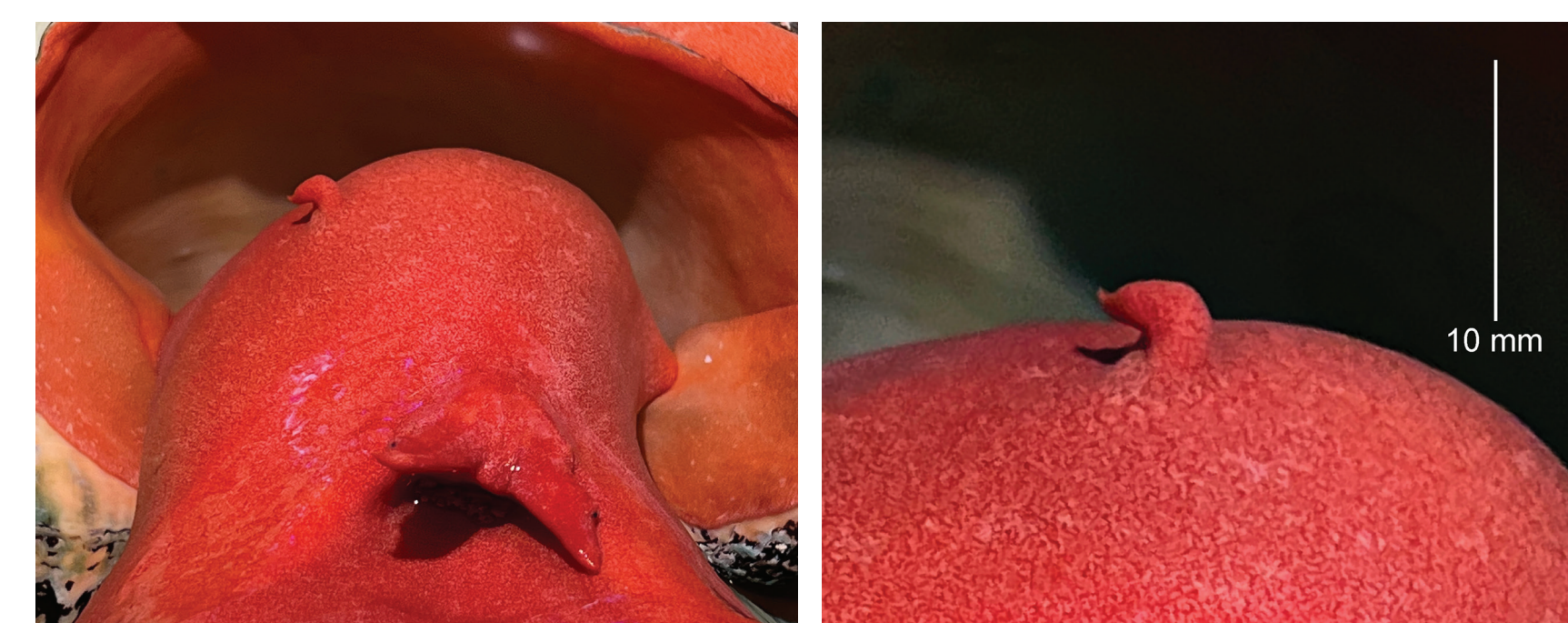
The disparity in organ size between the imposex females and the only male individual was noticeable: the largest imposex female (BMSM 135084, shell length 360 mm) (Figures 3, 4) had an approximately 4 mm-long pseudopenis, and the second imposex female (BMSM 135086, shell length 327 mm) had a pseudopenis of similar size (Figure 5). In contrast, the male of comparable size (BMSM 135085, shell length 330 mm) had a normal penis measuring 65 mm in length (Figure 6). The third imposex female (BMSM 135083, shell length 307 mm) (not illustrated) had a pseudopenis of comparable size.

Histological sections (Figures 7, 8) revealed that the pseudopenis contained a tubular vas deferens lined by columnar epithelium and surrounded by longitudinal and circular muscle layers (luminal diameter 50–90 µm). These features match the vas deferens anatomy verified in other neogastropod species.

These conclusions allow us to definitely add *Triplofusus giganteus* to the list of gastropods affected by the imposex condition. From a phylogenetic standpoint, the closest species to *T. giganteus* for which imposex has been verified are the fasciariids *Leucozonia nassa* and *Leucozonia ocellata* (Costa et al. 2014). The most likely cause for imposex is exposure to



Figure 2. Largest imposex female BMSM 135084 in aquarium (shell length 360 mm).



Figures 3, 4. Details of the body wall showing the pseudopenis of imposex female BMSM 135084.



Figure 1. *Triplofusus giganteus* attacking *Sinistrofulgur sinistrum* at low tide. On Kice Island, Collier County, Florida. Photo: Amy Tripp (modified).

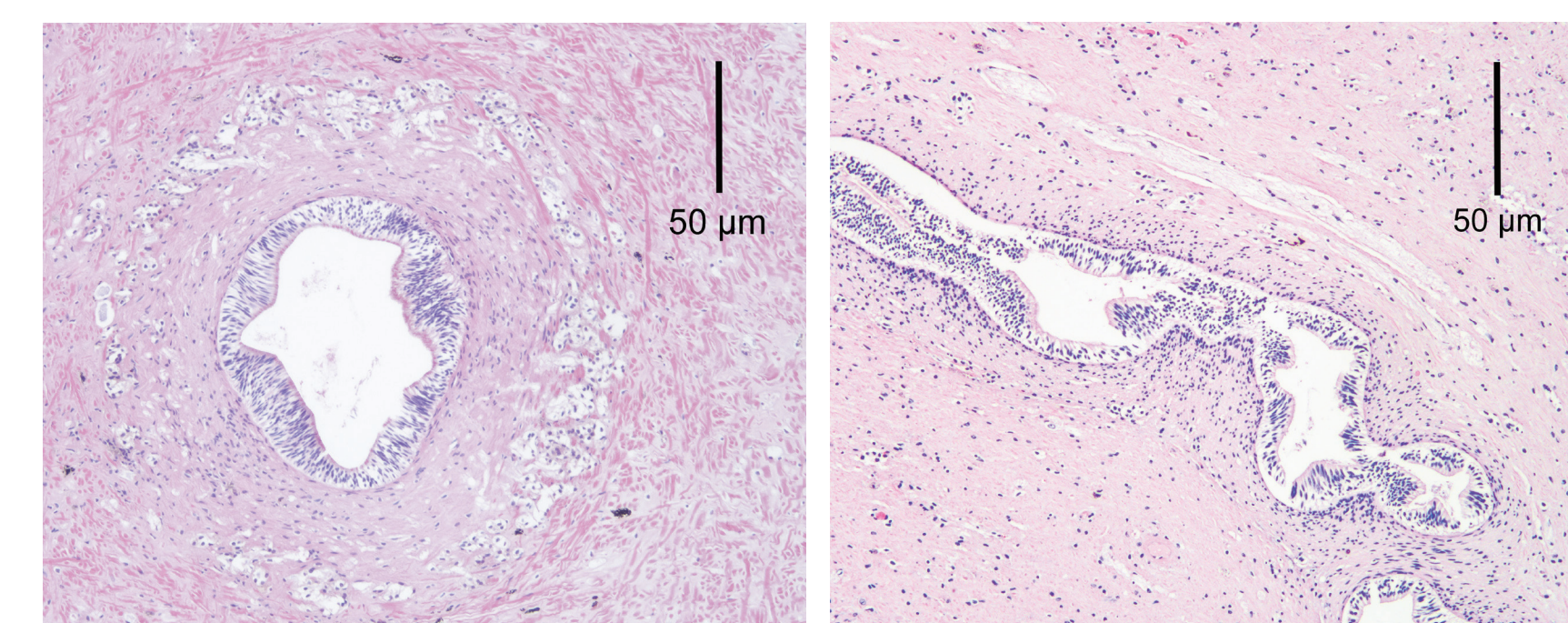
organotin compounds present in the environment. Organotin compounds such as tributyltin (TBT) and triphenyltin (TPT) are present in vessels antifouling paints (Bryan et al. 1987; Giulianelli et al. 2014). We are aware that imposex can be induced by very low concentrations of TBT/TPT in the water, although this study did not assess chemical levels in the snails' environment. Notwithstanding, the presence of imposex in three out of four individuals from the same location suggests that the sea water at Alligator Harbor has levels of environmental organotin contamination sufficient to trigger the imposex condition.



Figure 5. Detail of the body wall showing pseudopenis of imposex female BMSM 135086 (shell length 327 mm).



Figure 6. Dissection detail showing penis of male BMSM 135085. (Shell length similar to female BMSM 135084).



Figures 7, 8. Transverse section of pseudopenis (Figure 7) and longitudinal section of adjacent body wall (Figure 8) of imposex female BMSM 135084 revealing the lumen of vas deferens lined by columnar epithelium. Luminal diameter ranging from 50–90 µm.

Conservation Implications

The presence of imposex in *Triplofusus giganteus* is of particular concern for the long-term conservation of this apex predator. Herbert et al. (2022) determined that the lifespan of the species is relatively short, with large specimens not living longer than 16 years. Additionally, females show delayed maturation: first maturity apparently occurs after six to seven years and large females only spawn three to five times in a lifetime (Herbert et al. 2022), which restricts the reproductive output of the species. Further research is needed to assess the incidence of imposex within the sampled population of *T. giganteus*, confirm the presence and source of organotins in the Alligator Harbor marine environment, and understand the impact on species fecundity or presumptive loss of reproductive-age females.

Potential Follow-up Studies

The present study suggests that there is a need for more comprehensive surveys of *T. giganteus* populations along the Florida coast to determine the eventual presence of imposex in other locations. Concurrently, chemical analysis of Alligator Harbor's sediments and waters may confirm the presence of organotins and assist in the location of their sources. More broadly, this case underscores the lingering threat of organotin pollutants: although TBT and TPT have been banned for decades in many regions, residues may remain in the marine environment and may continue to bioaccumulate. The results highlight the need for continued monitoring of persistent pollutants and stronger conservation measures for vulnerable marine mollusks like *Triplofusus giganteus*.

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Publication

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