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IMPOSEX IN *Stramonita brasiliensis* (MOLLUSCA: GASTROPODA) IN PORT TERMINAL OF CEARÁ: ENVIRONMENTAL ASSESSMENT AND PERCEPTION

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Abstract

Imposex consists of the appearance of male sexual structures in female gastropods exposed to contamination containing organotin compounds (OTCs). Molluscs of the genus *Stramonita* are highly sensitive to OTC's, making this taxon the world's bioindicator of tin contamination. The aim of the study was to to evaluate the environmental perception of fishermen about the origin and use of OTCs in port areas on the west coast of Ceará. Fifty specimens of the species were collected at each of four fishing ports and evaluated. Interviews were also carried out with fishermen, boat owners and paint dealers to determine the origin and use of OTCs. The incidence of imposex in *S. brasilienses* was observed in three of the locations sampled. The species was not found in Porto Pesqueiro de Acaraú, which may have been the result of years of OTCs use. The highest incidence of imposex was recorded at Praia da Pedra Rachada in Paracuru (69.44%), followed by Porto dos Barcos in Itarema (69.18%) and Porto Pesqueiro de Camocim, with the latter having the lowest incidence of the syndrome (51.22%). The information obtained in the interviews revealed that even after the ban on the use of OTCs in antifouling paints, these contaminants continue to reach ports through alternative sources such as legally marketed insecticides. The occurrence of imposex in these samples strongly suggests that these areas are contaminated by OTC's. Studies based on the development of imposex are important tools for monitoring environmental conditions and conserving biodiversity.

Keywords: benthos, contamination, boats, muricidae, fishing port, tributyltin (TBT).

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Introduction

Biofouling refers to the process resulting from the colonization and growth of a great diversity of organisms on natural submerged surfaces, such as rocks, shells and other organisms, or artificial ones, such as breakwaters, vessels and exposed platforms, in the aquatic environment. This process causes economic problems for structures, in which they increase weight and reduce buoyancy and hydrodynamics in vessels, in addition to making them brittle, reducing their durability and favoring greater pollution in the aquatic environment (Agostini *et al*., 2018).

The solution to this problem came with the development of biocidal antifouling paints based on organotin compounds (OTCs), chemicals widely used to prevent organisms from adhering to hulls (Sierra-Marquez *et al*., 2018). Among the compounds introduced into the marine environment, tributyltin (TBT) and triphenyltin (TPT) have been considered the most toxic, and many studies have demonstrated the relationship between the presence of these compounds in coastal waters and the harmful effects on biota (Rocha-Barreira and Castro, 2021).

In 2003, the International Maritime Organization (IMO) introduced legislation prohibiting the use of OTCs in antifouling paints, but it only came into force internationally in 2008 (Sonak, 2009). In Brazil, the suspension of these products, especially those containing TBT, was regulated by the Navy in 2003, and later, NORMAN-23/DPC came into force in 2007, which determined enforcement and inspection measures in Brazilian ports (CONAMA, 2005).

The syndrome, called imposex, is considered the clearest evidence of endocrine disorders caused by the pollution of OTCs (Matthiessen and Gibbs, 1998). In gastropod molluscs, OTCs act as hormone disruptors, which affect at a biochemical level the synthesis and processing of some hormones, causing the masculinization of females (Shimasaki *et al*., 2003). It even promotes, in some animals, a total and radical change of sex (Castro *et al*., 2005). It can result in reproductive failure and death of the affected organisms and consequently, in the long term, the local extinction of the species due to lack of recruitment (Castro *et al*., 2011).

Approximately 260 species of imposex-exhibiting gastropods are known (Sternberg *et al*., 2010; Titley-O'neal, *et al*., 2011; Ayari *et al*., 2018), among which 76 belong to the family Muricidae (Mollusca: Gastropoda) (Ayari *et al*., 2018), which makes it the taxon most used in the world as a bioindicator of contamination by OTCs. Among the species, *Stramonita brasiliensis* stands out, which has a wide geographic distribution in Brazil and is frequently used to monitor TBT contamination along the Brazilian coast (Otegui *et al*., 2019; Rodrigues *et al*., 2020; Viana *et al*., 2021; França *et al*., 2021). Recently, studies carried out on the coast of Espírito Santo, in southeastern Brazil (Otegui *et al*., 2019; França *et al*., 2021), in Itarema, on the west coast of Ceará (Rodrigues *et al*., 2020) and in the São Marcos Bay, the Brazilian Legal Amazon (Viana *et al*., 2021), deserve highlighting. However, in Brazil, monitoring and studies that address this issue are still

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scarce, especially for the west coast of Ceará, a region of intense fishing activity with small ports in which inspection is often incipient.

Thus, the objectives of this study were: *i*) to evaluate the occurrence of imposex in the gastropod *S. brasiliensis* in fishing port terminals in the western region of the coast of Ceará; *ii*) to carry out a morphoanatomical and size analysis of collected specimens of *S. brasiliensis*; *iii*) on the basis of the study of imposex, to determine the stage of contamination by OTCs in the study areas and; and *iv*) to investigate the origin and use of OTCs in these fishing port terminals.

Material and methods

Study area

The west coast of the state of Ceará is about 194 km long, consisting of 13 municipalities (IPECE, 2020), with 11 being coastal municipalities, of which 4 comprised the study area (Camocim, Acaraú, Itarema and Paracuru). The predominant climate type in the region is the mild tropical hot semi-arid, while rainfall varies between 800 and 1784 mm (IPECE, 2020). The average temperature is 27°C, going through rainy periods, from January to June and dry periods, from July to December (IPECE, 2020).

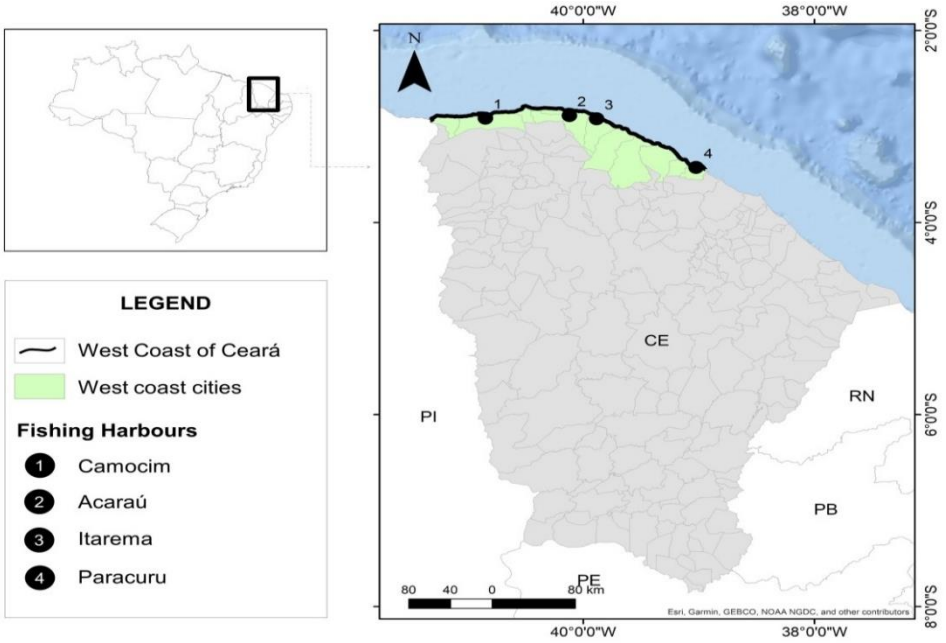

Figure 1. Map representing the sampling points of the fishing ports in the cities along the west coast of Ceará, 1 – Porto Camocim (Camocim); 2 – Porto Acaraú (Acaraú); 3 – Portos dos Barcos (Itarema); 4 – Porto Paracuru (Paracuru). *Soucer: Author (2022)*

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The port terminals on the west coast of Ceará are of great importance for fishing, acting mainly in the mode of catching lobster and tuna. The fishing activities developed in these port terminals directly influence economic, social and food aspects at the local, regional and state level (Rodrigues *et al*., 2020).

The monitoring of the occurrence of imposex in the species *S. brasiliensis* was carried out at four fishing ports on Ceará's west coast (Figure 1): point 1 located in the city of Camocim (2° 54' 47'' S and 40° 50' 47'' W); point 2 in the city of Acaraú (02º 53' 08'' S and 40º 07' 12'' W); point 3 in the city of Itarema (3° 49' 13" S and 38°24'8" W); and point 4 in the city of Paracuru (3° 25' 31" S and 39° 1' 29" W).

Collection of data

The search for molluscs was carried out by direct collection, done by four researchers actively looking for organisms in the intertidal range, where they are usually on some surfaces, such as stakes, trunks, soil, and on the hulls of vessels. In each fishing port, 50 adult specimens of *S. brasiliensis* (approximately 30 mm) were collected (Castro *et al*., 2005).

The collected molluscs were kept and transported alive, under refrigeration, to the laboratory. The freezing of organisms was avoided, and when it was necessary to use the freezing technique, the molluscs were thawed at room temperature before analysis (Guidelines For Monitoring of BiologicaL Effect, 2017).

In the laboratory, the organisms were measured in terms of shell height, referring to the distance from the tip of the spiral to the end of the anterior siphonal canal, with the aid of a caliper (precision = 0.05 mm). The collected animals were then anesthetized in a 3.5% magnesium chloride ($MgCl_2$) solution (Huet *et al*., 1995), for a period of two hours, to obtain maximum muscle relaxation. The shells of each organism were removed with the aid of a table vice, for morphoanatomical analysis of the soft parts. Upon completion of imposex analysis, organisms were preserved in 10% formalin (Guidelines For Monitoring of BiologicaL Effect, 2017).

For analysis of the soft parts, the organisms were examined individually using a magnifying glass. Sexual identification was based on the presence of the seminal receptacle present only in females and prostate gland only in males. Organisms that desplayed a seminal receptacle and penis were considered female at some level of imposex. The penis of males and females with imposex was measured with a millimeter ruler.

The imposex levels were quantified using the following indices: % imposex in females and relative penis length index (RPLI) calculated with the following equation (Gibbs *et al*., 1987).

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$$\text{RPLI} = \frac{(\text{Mean penis length of famales})}{(\text{Mean penis length of males})} \text{x}100 \qquad \textbf{Equation 1}$$

The relative penis size index (RPSI) was calculated using the equation below.

$$\text{RPSI} = \frac{(\text{Mean penis length of famales})^3}{(\text{Mean penis length of males})^3} \text{x}100 \qquad \textbf{Equation 2}$$

To determine the vas deferens development index (VDSI), a six-stage scale proposed by (Gibbs and Bryan, 1994) was used for the species *Nucella lapillus* (Linnaeus, 1758), and adapted by Fernandez *et al*. (2005) for organisms of the genus *Stramonita* (Chart 1).

Chart 1. VDSI scale developed to quantify imposex in organisms of the genus *Stramonita*.

Stage	Characteristics of development of imposex
0	Normal female
I	Small papilla appearing next to the right tentacle
II	Small penis forming to the side of the right tentacle (<2 mm)
III	Penis measuring 2 mm or more and visible vas deferens
IV	Fully formed penis and vas deferens
V	Vulva blocked by the vas deferens forming epithelium
VI	Dark mass composed of aborted eggs inside the capsule gland

Source: Fernandez et al. (2005)

Statistical analysis

The normality of the data was tested through descriptive analysis, the results of these tests indicated that the data met the prerequisites for performing parametric tests. Thus, a two-way analysis of variance (two-way ANOVA) was used to verify the relationship between the size of the shells and the penis of the animals. When differences between the means were detected, at a significance level of 5% ($p < 0.05$), Tukey's multiple comparison test was used.

Determination of the origin of TBT-based paints

To determine the origin of the OTCs, at each collection point, interviews were conducted with fishermen and boat owners, as well as with those responsible for the businesses that sell paints at each sampling site. Individuals over 18 years of age participated in the sample survey.

Interviews were conducted with a total of 50 fishermen and boat owners. These individuals included 14 fishermen in the fishing port of Acaraú (Acaraú), 14 fishermen in Porto dos Barcos (Itarema), 12 fishermen in the fishing port of Camocim (Camocim) and 10 fishermen in the fishing port of Paracuru (Paracuru). With the business owners, a total of 12 interviews were carried out.

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In which 3 were in the city of Acaraú, 2 in the city of Itarema, 4 in the city of Camocim and 3 in the city of Paracuru.

The interviews were carried out in the semi-structured mode (Boni and Quaresma, 2005). The "snowball" method (Goodman, 1961), was adopted, which refers to indications of people interviewed to find others who have information on the subject being studied. The interviews took place through a script in which subjects were asked about the use of tributyltin-based paints (TBT), ways of using and handling the compound, and how to acquire the compound.

All interview participants were invited to an informed consent form, and this plan was submitted to the Research Ethics Committee of the Federal Institute of Education, Science and Technology of Ceará-IFCE (Process No. 55435922.7.0000.5589).

Results

Imposex in fishing ports

Four fishing ports on the west coast of Ceará were monitored, using the species *S. brasilienses*, to determine the occurrence of imposex in port areas. In three of the port areas, where the study was carried out, imposex was observed at some level of development, from the presumed sources of OTCs. In one of the monitored areas, the presence of *S. brasilienses* was not observed.

Praia da Pedra Rachada, in Paracuru, showed the highest incidence of imposex, followed by Porto dos Barcos, in Itarema, which had a high RPLI value. Meanwhile, VDSI varied between stages 0 and IV in all sampled locations (Table 1) (Figure 2). In Acaraú, no specimens of *S. brasiliensis* were found.

Significant differences were observed between shell size (F_2, 98 = 9.2205, $p < 0.00021$) and penis length between males and females affected by imposex in the study areas (F_2, 98 = 4.7236, $p < 0.01100$). Thus, the size of the shell and the length of the penis of male organisms are greater than those of females affected by the syndrome.

At all sampling points, the size of the male organisms' shells was larger compared to the females' shells (Figure 3). In Porto dos Barcos, in Itarema, males had larger shells compared to organisms of the same sex collected in Fishing Port of Camocim and Pedra Rachada Beach in Paracuru. The female organisms, collected at Fishing Port of Camocim, displayed the largest shells, followed by Porto dos Barcos and Praia da Pedra Rachada Beach.

At all sampling points, the penis length of male organisms was longer and similar when compared to the penis of females (Figure 4).

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Table 1. Imposex indices in *Stramonita brasiliensis* in fishing ports on the west coast of Ceará

LOCATION	N	♂ /♀	% IMPOSEX	RPLI	RPSI	VDSI
Fishing Port of Acaraú	0	0	0	0	0	0
Porto dos Barcos/Itarema	50	6/44	69.18%	39.93	6.37	0, I, II, III and IV
Fishing Port of Camocim	50	9/41	51.22%	25.87	1.73	0, I, II, III and IV
Pedra Rachada Beach/Paracuru	50	14/36	69.44%	27.43	2.06	0, I, II, III and IV

Source: Author (2022)

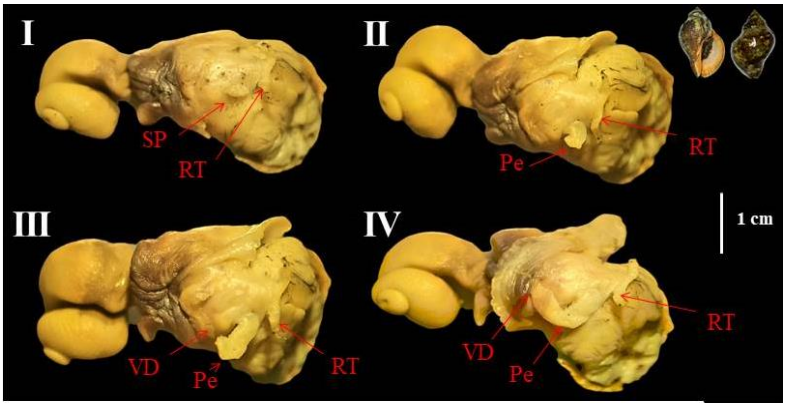

Figure 2. (I) *Stramonita brasilienses* female, stage VDSI I. (II) *Stramonita brasilienses* female, stage VDSI II. (III) *Stramonita brasilienses* female, stage VDSI III. (IV) *Stramonita brasilienses* female, stage VDSI IV. Legend: SP (small papilla); Pe (penis); RT (right tentacle); VD (vas deferens). In the upper right corner, ventral and dorsal view of the shell of the species *Stramonita brasilienses*. *Source: Author (2022)*

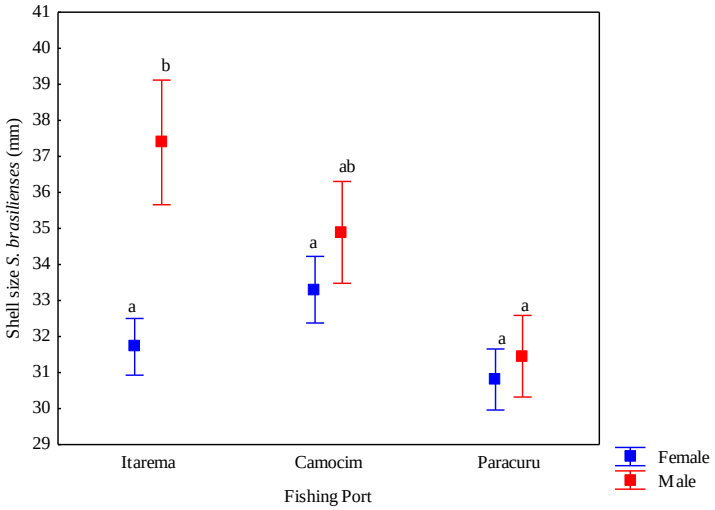

Figure 3. Mean ± standard deviation of the shell size of *Stramonita brasilienses* males and females, affected by imposex, between fishing ports. Different letters indicate significant differences according to Tukey's multiple comparison test. *Source: Author (2022)*

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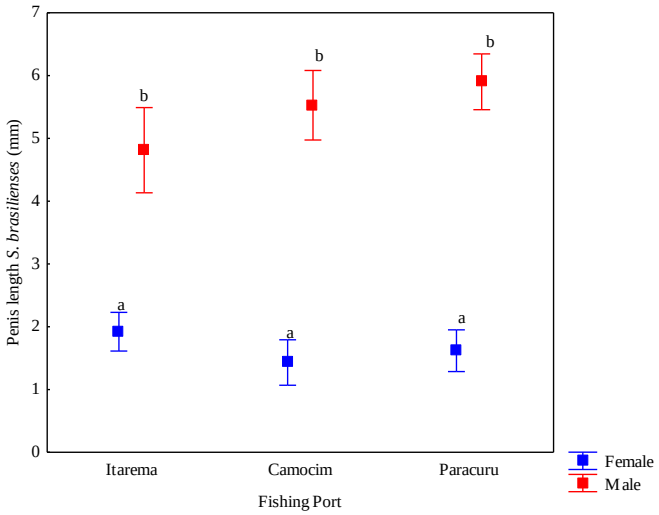

Figure 4. Mean ± standard deviation of penis length of *Stramonita brasilienses* males and females affected by imposex, between fishing ports. Different letters indicate significant differences according to Tukey's multiple comparison test. *Source: Author (2022)*

Origin and use of TBT in fishing ports

In each fishing port, interviews were conducted with fishermen and boat owners to identify the origin and use of TBT-based paints in each location. The results of the approach are summarized in Chart 2.

According to the respondents, the compounds most used as potentiating biocidal agents are Barrage®; Baygon® or Jimo Cupim®, which are mixed with the paints for maintenance of vessels. These products are legally sold, facilitating the acquisition of the compound, and their main chemical compound is cypermethrin, a substance from the pyrethroid group, and has a toxicological classification level II (very dangerous product). It is photostable and does not degrade in sunlight. Like many pyrethroids, it is a very broad-acting insecticide and acaricide. This compound falls within the third generation of antifouling paint biocides. The third generation of antifouling biocides belong to different chemical groups encompassing organic and organometallic compounds.

According to boat owners and fishermen, the use of antifouling paints known as “poisoned paint” is used to paint boats once a year, as it is an expensive product. Also according to them, one of the alternatives to reduce the maintenance costs of vessels is to use synthetic enamel together with biocidal compounds to reduce maintenance costs.

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Chart 2. Results of the interviews with the questionnaire administered to fishermen and boat owners in the fishing ports used in the study.

Category	Responses
Use of paints to paint boats	Q/1: 90% use antifouling paints to maintain the painting of vessels; 10% do not use them.
	Q/2: 90% use synthetic enamel paints as an alternative source to paint boats; 10% revealed not using these paints.
Acquisition of paints	Q/3: 92% buy their paints from fishing supply stores; 8% buy from building material stores.
Handling	Q/4: 38% stated that they paint the boats three times a year; 22% two or more than five times a year; 14% four times a year; 4% once a year.
	Q/5: 100% of respondents revealed that they paint the whole hull of the vessel for maintenance.
Potentiating compound	Q/6: 60% said that they use biocidal compounds to enhance synthetic enamel paint; 40% revealed that they do not use these compounds.
	Q/7: 46% throw the paint containers in the trash after use; 42% take them home to reuse; 12% throw them in the river.
Environmental perception	Q/8: 70% said that the use of paints with TBT or its derivatives does not cause any harmful effects or damage to the environment; 30% stated that they pollute the water, affect fish and kill sea plants.

Legend: Q (questions) in the questionnaire used in the study. *Source: Author (2022)*

The results of the interviews carried out with the business owners of selling paints in each municipality where the fishing ports are located are summarized in Chart 3.

At all sampling points, the official sale of antifouling paints was evidenced. However, the fishermen revealed that they use synthetic enamel paint together with biocidal compounds and insecticides to maintain the painting of the boats. According to the fishermen, this mixture replaces antifouling paints, being a cheaper alternative to reduce maintenance costs.

During the sample survey, it was possible to observe that the painting maintenance procedure of the vessels is carried out on the river, and all paint remnants such as the old paint scraped off are discarded in the river, contributing to the process of sedimentation of the compound. It was also observed that the packages of the compounds are thrown in the river (Figure 5).

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Chart 3. Results of the interviews with the questionnaire administered to the business owners who sell paints in the municipalities where the fishing ports are located.

Category	Responses
Sale of paints	Q/1: 53.8% sell antifouling paints for painting boats; 46.2% do not sell the paints. Q/2: 84.6% said that boat owners buy synthetic enamel paints to paint their boats; 15.4% revealed that they do not sell these paints.
Potentiating compound	Q/3: 69.2% sell biocidal compounds to be mixed with paints to enhance the antifouling action; 30.8% said they do not sell these compounds.
Sales frequency	Q/4: 76.9% stated that paints and additional compounds are sold often; 23.1% revealed that they do not sell these items regularly. Q/5: 100% of respondents revealed that sales are made throughout the year.
Quantity	Q/6: 100% said that generally the owners of vessels buy 2 gallons (3.5 L) to carry out maintenance on the painting of the vessels.
Environmental perception	Q/7: 100% stated that the use of paints containing TBT or its derivatives does not cause any harmful effect on the environment.

Legend: Q (questions) in the questionnaire used in the study. Source: Author (2022)

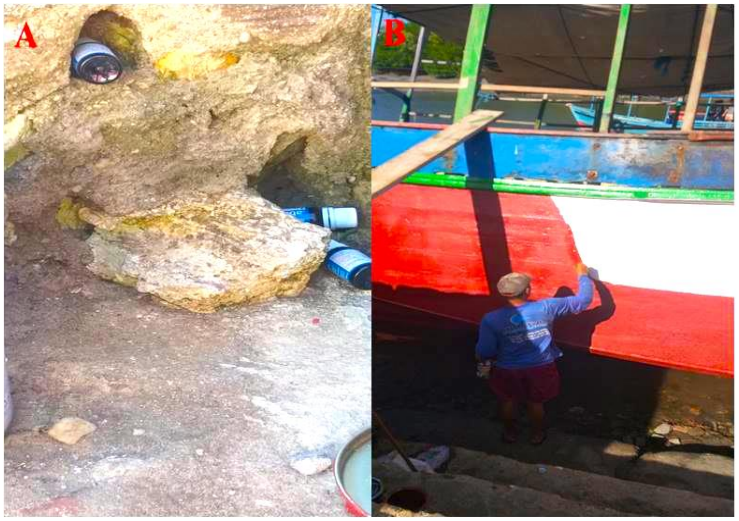

Figure 5. (A) Dam compound used as a biocidal agent to enhance the action of paints; (B) fisherman performing maintenance on the painting of the vessel on the river bed, with biocidal compound mixed with paint. *Source: Author (2022)*

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In view of the above, the data obtained during the interviews help to understand the origin of the contamination of organic tin compounds in the fishing ports of the west coast of Ceará, and can serve to justify the high incidence of the syndrome in the species *Stramonita brasiliensis* even after the ban on use of paints based on organic tin compounds.

Discussion

Studies carried out around the world reveal large concentrations of TBT, TPT and their derivatives in port areas, generally associated with the circulation of vessels (Strand and Jacobsen, 2005). The results obtained in the present study indicate that despite the ban on the use of TBT-based paints having occurred internationally in 2008, imposex was found in all areas sampled during monitoring.

In the fishing port of Acaraú, during the sampling, the absence of the species *S. brasilienses* was demonstrated. According to Rodrigues *et al*. (2020), the species was recorded in the municipality of Acaraú, in an area close to Porto (about 8 km), on Arpoeiras Beach. In view of the above, it is inferred that the environmental impact caused by human actions, among them TBT contamination, is influencing the distribution of the species in the location.

Similar results were found by Azevedo (2011), in Porto do Mucuripe/Fortaleza, where the species *S. haemastoma* was found to be absent during monitoring of imposex in the port area. However, during studies carried out in the region, the species was reported in the same port area (Castro *et al*., 2005). This fact may be indicative of the extinction of that population at this place, since it had high rates of imposex in 2004/2005 (Castro *et al*., 2005). The absence of organisms during imposex monitoring was also recorded by Azevedo (2011), in 5 areas of Baia da Ribeira, coast of Rio de Janeiro, where imposex was recorded in 2002 in the species *S. haemastoma*, and during reassessment in 2008, in the same place, the species was not found, being possibly extinct due to the action of OTCs.

One factor that may be contributing to the absence of the species *S. brasilienses* in the port of Acaraú is the operating time of the fishing port, the oldest among those evaluated, and the frequent use of TBT-based paints for years, a practice that may have led to the local extinction of the species. According to Castro *et al*. (2011), the constant use of TBT-based paints compromises the quality of the aquatic environment, directly affecting the local fauna, and in the long term, the harmful effects of the compound can lead to the death of the animals, and consequently to local extinction. of the species. However, there are no past data for the Porto de Acaraú region.

The results found during the monitoring show that there was a reduction in the incidence of imposex in Porto dos Barcos, in Itarema, compared to the study carried out by Rodrigues *et al*.

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(2020), in the same location. In the previous study, the incidence of imposex was 82.2%, while in the current monitoring this value was 69.18% of females affected by the syndrome. However, in the present study, the indices used to quantify imposex revealed that the levels of development increased significantly at the site, compared to the previous study, where RPLI was 18.8 and VDSI varied between stages 0 and III (Rodrigues *et al*., 2020). This indicates that on-site development of imposex is increasing, while the incidence of contamination is decreasing.

According to Azevedo (2011), in the re-evaluation of imposex at the port terminal of Mucuripe, in Fortaleza, there was a reduction in the incidence of imposex in all stations examined compared to the study carried out in the years 2004/2005. The reduction of imposex was also observed at dos Coqueiros Beach, in Fortaleza, during the campaign carried out in 2005, when 100% of the animals were affected by the syndrome, and in the 2011 campaign, only 34.6% of the organisms were affected with the syndrome imposex.

According to Azevedo (2011), the reduction in the incidence of imposex in some regions is related to the efficiency of the applicability of Normam 23 and IMO legislation. Monitoring based on the determination of imposex revealed, in general, a gradual decrease in TBT levels, as well as a reduction in indices such as the percentage of affected females, RPLI, RPSI and VDSI, worldwide (Rocha-Barreira and Castro, 2021). A significant drop in rates was observed in populations of the species *Haustrum scobina* (Quoy and Gaimard, 1833) in New Zealand, after a few years of the implementation of restrictions. In the North Sea and Iceland, a reduction of the indices for populations of *Nucella lapillus* was also observed (Linnaeus, 1758). Comparing the indices obtained in 2003 and 2008 in Europe, it became clear that the syndrome was reduced in the species *Nassarius reticulatus* (Linnaeus, 1758) (Rocha-Barreira and Castro, 2021). In Brazil, according to Castro *et al*. (2012), the drop in imposex levels was demonstrated in several port terminals in Brazil's Northeast, Southeast and South regions in *S. brasiliensis* populations.

In the fishing port of Camocim and Pedra Rachada Beach in Paracuru, imposex incidence was first recorded for these two regions of the west coast of Ceará through the present study. In the fishing port of Camocim, the incidence of imposex was found to be 51.22, and the rates of imposex development observed were RPLI= 25.87 and VDSI= 0 to IV. At Pedra Rachada beach, in Paracuru, 69.44% of the organisms were found to be exhibitors of imposex, with the values RPLI=27 and VDSI= IV. The results reveal that despite the ban on the use of OTCs, these two small port areas have high levels of OTC contamination.

Contamination cases in small port areas seem to be related to small vessels, especially leisure and fishing vessels, identified as the main current sources of TBT in Latin America. According to the restrictions implemented by the IMO, these vessels are authorized to use paints based on OTCs (Rocha-Barreira and Castro, 2021). In this sense, these data are in line with the present study,

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because during the sampling process it was observed that the fleet of vessels in the fishing port of Camocim and in Pedra Rachada Beach consists of small artisanal vessels (3 m in length) used for fishing and local tourist excursions.

All the fishing ports studied comprise small port areas, where inspection is still incipient in relation to the restrictions established by the IMO in 2008, which may explain the occurrence of the syndrome in these places. Another reason is that these vessels spend more time moored close to the coast, favoring the bioaccumulation of the compound. These results corroborate the data obtained during interviews with fishermen and business owners who sell paints in each sampled location.

According to Borges (2012), contamination by OTCs continues to occur in small port areas because of lack of inspection, since the applicability of the restrictions established by the IMO are put into practice only in large port terminals with circulation of international vessels.

The results of the present monitoring demonstrate that there are significant differences between shell size and penis size in both males and females, revealing that male shells and penises are larger compared to females affected by the syndrome. Similar results were observed by Azevedo (2011) in studies carried out at Porto do Mucuripe in Fortaleza, Ceará, Brazil. According to (Galante-Oliveira, 2009), the size of the shells of the animals is considered one of the factors that can influence comparisons about the levels of imposex as they influence the length of the male's penis, which can interfere with the results of the RPLI value.

Merchants confirmed that they often sell synthetic enamel paints and biocidal compounds or insecticides to fishermen, which are mixed and used to paint boats. Respondents stated that the painting procedure is often carried out on the river, in the estuarine area. This practice was reported by Rodrigues *et al*. (2020), in a study carried out on the west coast of Ceará, where during the sampling, the researchers observed that the fishermen added an insecticide to the paint that contains OTCs to potentiate the effects of antifouling paints.

According to Borges (2012), the use of paints containing other toxic biocides started to be used frequently. As these paints contain different types of biocides, and are in direct contact with water, they can have a greater impact locally.

According to Rocha-Barreira and Castro (2021), after the ban on paints containing TBT and/or TPT, the boat industry started to produce and make available on the market, for more than ten years, paints that contain alternative biocides, without the presence of OTCs. About 23 different chemicals are currently used in new marine antifouling systems, in which, among these molecules are endocrine disruptors, herbicides and toxic organometallics. However, studies of Castro *et al*.

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(2011) and Abreu *et al*. (2020) have demonstrated environmental damage caused by contamination with the new generation of biocides, this is very worrisome because the impacts of OTCs have not yet been overcome.

The way in which the painting of vessels is maintained and the use of alternative sources such as synthetic enamels mixed with compounds of biocidal action, belonging to however group of pyrethroids, can cause damage to aquatic communities, especially to invertebrates however the harmful effects to biota are still were not measured.

When aquatic organisms are exposed to pyrethroid compounds, which mostly have xenobiotic action, they cause endogenous changes in these animals such as liver dysfunction, changes in biochemical enzyme levels and behavioral changes, since pyrethroids act on the inhibitory neurotransmitter of the central nervous system. (CNS). In addition, the potent stressful effect that pesticides cause on these organisms lead to hematological changes, and ultimately, can also cause damage in the reproductive phase (Montanha and Pimpão, 2012).

The incidence of imposex recorded in the present study in the small fishing ports of the west coast of Ceará indicates that organic tin compounds continue to be sold and used illegally in the process of maintaining the painting of vessels, since during the interviews, the participants revealed that sell and use “poisoned paint” to paint boats. It is therefore suggested that the paint mentioned by them contains organic tin compounds. Another factor that may help to clarify the reasons why imposex is still found in these areas may be related to the fact that the ports studied are located within estuaries, where water circulation is restricted, favoring the local concentration and accumulation of the compound. In this sense, carrying out studies aimed at monitoring environmental conditions, as well as monitoring imposex are of great relevance in these areas.

The imposex results recorded for the species *S. brasilienses* in fishing ports on the west coast of Ceará state geographically expand the known limits for the problem in Brazil. The monitoring of these areas along with the establishment of environmental education programs would be one of the forms of remediation and awareness of the damage to the environment by the use of paints containing TBT and its derivatives.

Conclusion

Small port areas can be the providers of OTCs in coastal areas. The populations of *S. brasilienses* from the fishing ports sampled show high levels of imposex, even with the ban on the use of paints containing organic tin.

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The increase in insecticides used as biocidal agents in paints, combined with the lack of knowledge on the part of fishermen, boat owners and business owners about the damage to the environment may be contributing to the increase of the incidence of the syndrome in fishing ports on the west coast of Ceará.

The biocidal compounds identified in the present study can lead to ecosystem compromise that has not yet been measured.

The results obtained contribute to the understanding of the origin of imposex and TBT in the west coast of Ceará, and more investigations are needed to evaluate and mitigate the presence of OTCs in small port areas.

The constant use of COEs without supervision and non-compliance with international legislation, causes a series of implications for the fauna, such as the mortality of organisms most sensitive to contamination, in addition to contaminating other non-target organisms. The effects of COEs are more visible in port estuarine regions, where the geographic location, as well as the dynamics of the environment, contribute to the accumulation of the compound, causing irreversible consequences such as the local extinction of species.

It is hoped, with data collected in this study, to alert the fishing community and the scientific community for problematic inherent to contamination by the use of organotins.

It is suggested that long-term monitoring, along with interventions through environmental education programs, can contribute to raising awareness about the use of tin compounds and their derivatives, mitigating the effect of contamination on the environment.

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Bibliographic references

Abreu, F., Silva, J. L., Castro, I. B., Fillmann, G. (2020) Are antifouling residues a matter of concern in the largest South American port? *Journal of Hazardous Materials,* **398,** 122937. https://doi.org/10.1016/j.jhazmat.2020.122937

Revista AIDIS de Ingeniería y Ciencias Ambientales:
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Diciembre 2023

Agostini, V. O., Macedo, A. J., Muxagata, E. (2018) The role of bacterial biofilm in benthopelagic coupling during the biofouling process. *Liberato Magazine, Novo Hamburgo*, **19**(31), 01-134.

Ayari, T. E., Bierne, N., Menif, N. T. E. (2018) Imposex incidence inStramonita haemastoma (Gastropoda: Muricidae) from the Mediterranean and Atlantic coast after Tributyltin global ban. *Journal of Sea Research,* **134**,10–1511. https://doi.org/10.1016/j.seares.2017.12.004

Azevedo, D. B. (2011) *Imposex in Stramonita haemastoma (Mollusca: Gastropoda): reassessment in the metropolitan area of Fortaleza and induction under laboratory conditions.* Dissertation (Master's in Tropical Marine Sciences) - Institute of Marine Sciences, Federal University of Ceará, Fortaleza, 88 pp.

Boni, V., Quaresma, S. (2005) Learning to interview: How to do interviews in Social Science. *Electronic Journal of Graduate Students in Political Sociology at UFSC*. **2**(1), 68-80.

Borges, C. L. L. (2012) *Temporal evaluation of Pollution by organotin antifoulings on the coast of the State of Rio de Janeiro: Before and after the national and international ban.* Dissertation (master's degree in oceanography). State University of Rio de Janeiro,120 pp.

Castro, Í. B., Braga, A. R. C., Rocha-Barreira, C. A. (2005) High rates of imposex in stramonite rustica (mollusca: gastropoda) in port areas of the states of Alagoas and Sergipe, Brazil. *Tropical Oceanography,* Recife, **33**(2),121–128.

Castro, Í. B., Westphal, E., Fillmann, G. (2011) Third generation antifouling paints: new biocides in the aquatic environment. *Kim. New,* **34**(6),1021-1031.

Castro, Í.B., Rossato, M., Fillmann, G. (2012) Imposex reduction and residual butyltin contamination in southern Brazilian harbors. *Environ. Toxicol. Chem*. **31**, 947–954. http://dx.doi.org/10.1002/etc.1793

Conama. (2005) Provides for the classification of bodies of water and environmental guidelines for their classification, as well as establishing the conditions and standards for the release of effluents, and other measures. 357, Official Gazette, Brazil, 2005. Accessed on March 5, 2022, disponível em: http://pnqa.ana.gov.br/Publicacao/RESOLUCAO_CONAMA_n_357.pdf

Fernandez, M. A., Wagener, A. L. R., Lima-verde, A., Scofield, A. L., Pinheiro, F. M., Rodrigues, E. (2005) Imposex and surface sediment speciation: A combined approach to evaluate organotin contamination in Guanabara Bay, Rio de Janeiro, Brazil. *Marine Environmental Research,* **59,** 435–452.

França, M. A., Otegui, M. B. P., Zamprogno, G. C., Menário, J. M. F. S., Costa, M. B. (2021) Imposex and ecological quality status in Stramonita brasiliensis (Claremount & Reid, 2011): A temporal (2007 to 2018) and spatial evaluation on the southeastern coast of Brazil. *Journal of Sea Research,* **174**. https://doi.org/10.1016/j.seares.2021.102080

Galante-Oliveira, S., Oliveira, I., Jonkers, N., Langston, W.J., Pacheco, M., Barroso, C. M. (2009) Imposex levels and tributyltin pollution in Ria de Aveiro (NW Portugal) between 1997 and 2007: evaluation of legislation effectiveness. *Journal of Environment Monitoring*, **11**,1405–1411.

Gibbs, P. E., Bryan, G. M. (1994) *Biomonitoring of tributyltin (TBT) pollution using the Imposex response of neogastropod mollusks.* In: Biomonitoring of Coastal Waters and Estuaries. KEES, J. M. KRAMER (ed.) CRC Press Inc, Boca Ratón, EUA, 2005-226.

Gibbs, P.E., Bryan, G.W., Pascoe, P.L.C., Burt, GR. (1987) The use of the dog-whelk, Nucella lapillus, as an indicator of Tributyltin (TBT) contamination. *Journal of the Marine Biological Association of the United Kingdom*, **67**, 505-507.

Goodman, L. A. (1961) Snowball Sampling. *The Annals of Mathematical Statistics,* **32**, (1),148–170. Acesso em: 19 mar. 2020. Disponível em: https://bit.ly/3bfzFHn

Guidelines For Monitoring of Biological Effect. (2017) *Imposex and intersex.* Acesso em: 05 Mar. 2021. Disponível em: https://www.semanticscholar.org/paper/Guidelines-for-monitoring-of-biological-effect-%E2%80%931/cd9034c32d3640bc3f3228e043c39ea72a5de999

Huet, M. Fiorini, P. Oehlmann, J. and Stroben, E. (1995) Comparison of imposex response in three Prosobanch species. *Hydrobiologia,* **309**, 29 – 35.

Revista AIDIS de Ingeniería y Ciencias Ambientales:
Investigación, desarrollo y práctica. ISSN 0718-378X
http://dx.doi.org/10.22201/iingen.0718378xe.2023.16.3.83883
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Diciembre 2023

Ipece, Institute of Research and Economic Strategy of Ceará. (2020) Ceará on maps. Acesso em: 09 de nov. 2021. Disponivel em: http://www2.ipece.ce.gov.br/atlas/capitulo1/11/147x.htm

Matthiessen, P., Gibbs, P. E. (1998) Critical appraisal of the evidence for Tributyltin – mediated endocrine disruption in Mollusks. *Environmental Toxicology and Chemistry,* **17** (1), 37 – 43.

Montanha, F.P., Pimpão, C.T. (2012) Toxicological Effects of Pyrethroids (Cypermethrin and Deltamethrin) In Fish – Review. *Electronic Scientific Journal of Veterinary Medicine*, **1** (18), 1-58.

Otegui, M. B.P., Zamprogno, G. C., França, M. A., Daros, B. N., Albino, J., Costa. M. B. (2019) Imposex response in shell sizes of intertidal snails in multiple environments. Journal of Sea Research, **147**, 10–1812, https://doi.org/10.1016/j.seares.2019.02.003

Rocha-Barreira, C., Castro, I. (2021) *Triorganotin compounds.* In: Organic contaminants in aquatic environments Editora: Imprensa Universitária, 246pp. Acesso em: 22 de Dez. 2021. Disponível em: https://www.researchgate.net/publication/349118209_COMPOSTOS_TRIORGANOESTANICOS

Rodrigues, J. A, M., Santos, M. R., Maia, R. C. (2020) Study of imposex in the gastropod stramonita brasiliensis (claremont; dg reid, 2011) in the municipalities of Acaraú and Itarema, west coast of Ceará, Brazil. *arch. science sea,* **53**(1), 126 – 133.

Shimasaki, Y., Kitano, T., Oshima, Y. (2003) Tributyltin causes masculinization in fish. *Enviromental Toxicology and Chemistry,* **22** (1), 141-144.

Sierra-Marquez L., Sierra-Marquez, J. J., Rosa, De La., Olivero-Verbel, J. (2018) Imposex in *Stramonita haemastoma* from coastal sites of Cartagena, Colombia**.** *Braz. J. Biol*, **78** (3), 548-555. Acesso em 04 Fev. 2021. Disponível em: Imposex in Stramonita haemastoma from coastal sites of Cartagena, Colombia (scielo.br)

Sonak, S. (2009) Implication of the ban on organotions for protection of global coastal and marine ecology. *Jornal of environmental management,* **90**, 96-108.

Sternberg, R., Gooding, M., Hotchkiss, A., Leblanc, G. (2010) Environmental-endocrine control of reproductive maturation in gastropods: implications for the mechanism of tributyltininduced imposex in prosobranchs. *Ecotoxicology,* **19**, 4 – 23.

Strand, J., Jacobsen, J. (2005) Accumulation and trophic transfer of organotins in a marine food web from the Danish coastal waters. *The Science of the total environment.* **350** (1-4), 72-85.

Titley-O'neal, C. P., Munkittrick, K. R., Macdonald, B. A. (2011) The effects of organotin on female gastropods. *Journal of Environmental Monitoring,* **13**, 2360-2388.

Viana, J. L. M., Mendes, V. J. C., Costa, M. B., Otegui, M. B. P., Diniz, M. S., Santos, S. R. V., Franco T. C. R. S. (2021) First evaluation of imposex in Stramonita brasiliensis (Claremont and Reid, 2011) (Caenogastropoda: Muricidae) from Brazil's Legal Amazon. *Journal of Sea Research,* **174**. Acesso em 21, out. 2021, Disponível em: https://doi.org/10.1016/j.seares.2021.102064