



New insights on Tetrodotoxins occurrence in marine invertebrates from Galician coast (NW Spain) and potential implications for public health

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ABSTRACT

Tetrodotoxins (TTXs) are highly polar neurotoxic alkaloids that selectively target voltage-gated sodium channels, causing the well-known pufferfish poisoning. This food-borne disease is mainly prevalent in countries where species of the Tetraodontidae family are part of the culinary tradition. It has also been associated with the consumption of certain edible gastropods in several Asiatic countries for years. However, since TTXs showed up in shellfish from European countries, their presence has raised concerns for public health. In this work, 195 samples of diverse marine invertebrate groups were collected in Northwest (NW) Spain, an important region for seafood harvesting and farming. Hydrophilic interaction chromatography coupled to tandem mass spectrometry (HILIC-MS/MS) was used for identification and/or quantification of TTX and 12 of its analogues. The results show a 9.7% prevalence of TTXs in the analysed samples (19 samples with levels higher than detection limit). TTX was present in mussels and several gastropod species, while 11-deoxyTTX was only detected in limpet samples. TTX was identified in snakelocks anemones, abalones and a velvet swimming crab for the first time in Europe. Considering not only the commercial importance of these organisms but also their edibility in Europe, the present work points to potential risks for seafood safety and highlights the need of further surveillance on TTXs occurrence in this region.

1. Introduction

The pufferfish toxins, named tetrodotoxins (TTXs) after the order Tetraodontiform fishes, are globally known for the potent neurotoxic effects they cause in mammals, including humans (Bane et al., 2014). TTXs are highly polar alkaloids that selectively target voltage-gated sodium channels (VGSC) of cell membranes, blocking the influx of Na⁺ into the cells (Nißl et al., 2025). This results in disruption of action potentials in neuromuscular cells. Early symptoms might include weakness, tingling of the lips, numbness, and dizziness. Gastrointestinal symptoms are often severe and include nausea, vomiting, diarrhea, and abdominal pain. More severe symptoms are paralysis, cardiac arrhythmias, cardiovascular collapse and respiratory failure (Chong & Ruben, 2008; How et al., 2003; Katikou et al., 2022; Martínez et al., 2015). The consumption of a dose as low as 1.2 mg can cause death in a 60-kg adult (EFSA, 2017). The risk of pufferfish poisoning has been mainly restricted to Asian countries where *fugu* (as the pufferfish is also known)

is considered a delicacy and widely consumed (Cheung & Chan, 2023; Noguchi & Ebesu, 2001; Panão et al., 2016). Nevertheless, TTX occurrence has been increasingly reported in new areas and organisms (Guardone et al., 2020). Reports of intoxications related to gastropods and crabs have been common in Japan, China, Taiwan and other nearby countries for decades (Kanchanapongkul et al., 1995; Noguchi et al., 2011; Suleiman et al., 2017). The first case of TTX poisoning in Europe, due to consumption of local seafood, took place in Málaga (Spain) when a 49-years-old person ingested a trumpet shell (*Charonia lampas*) caught in South Portugal (Fernandez-Ortega et al., 2010; Rodriguez et al., 2008). After this first episode, TTX and derivatives have been found in a number of marine organisms in European Union (EU) countries (Varini et al., 2025). The report of TTX contaminated bivalves in England and Greece in 2015 (Turner et al., 2015; Vlamis et al., 2015) led to the release of a scientific opinion on the risk for consumers of TTXs in marine bivalves and gastropods in the EU by the European Food Safety Authority (EFSA) (EFSA, 2017). The EFSA publication establishes

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a recommended limit of 44 µg of TTX eq/kg of shellfish meat, acknowledging though that more data would be needed for a more accurate assessment (EFSA, 2017). With very few exceptions (Gerssen et al., 2018; Sinno-Tellier et al., 2023), most EU countries lack a regulatory framework for TTXs monitoring and control, despite the scientific evidence of TTXs occurrence in edible marine organisms in EU coasts (Rey & Botana, 2026; Varini et al., 2025) and the expectancy of its increase due to ocean temperature rise in the next decades (EFSA, 2020).

Both globally and in EU countries, TTXs have been detected in a range of edible and non-edible marine species, evidencing their prevalence in marine food webs. These marine biotoxins have been detected, for instance, in the pufferfish *Lagocephalus sceleratus*, *Spherooides marmoratus* and *Torquigener flavimaculosus* (Kosker et al., 2018; Pinto et al., 2019; Varini et al., 2025), in the sea stars *Astropecten aranciatus*, *Astrostele scraba* and *Ophidiaster ophidianus* (Oglivie et al., 2012; Pais et al., 2025; Silva et al., 2019), in the trumpet shell *Charonia lampas* (Pais et al., 2025; Varini et al., 2025), the whelk *Buccinum undatum* (Hort et al., 2020), the sea snails *Phorcus lineatus* and *Gibbula umbilicalis* (Silva et al., 2012); and marine bivalves such as mussels (*Mytilus galloprovincialis*, *M. edulis*) oysters (*Magallana gigas*, *Ostrea edulis*, *Saccostrea glomerata*), clams (*Mercenaria mercenaria*, *Ruditapes philippinarum*, *Cerastoderma edule*, *Venus verrucosa*) and cockles (unknown specie) (Dhanji-Rapkova et al., 2023; Katikou et al., 2022; Leão et al., 2018; Turner et al., 2017a; Varini et al., 2025).

This wide incidence in organisms points to the importance of broadening TTXs surveys towards potential new vectors, in addition to bivalves and gastropods. In fact, the Joint FAO/IOC-UNESCO/IAEA guidance on monitoring of algal toxins in bivalve molluscs, has recently indicated that scientific evaluation, legislation and global and regional health standards are urgently required where serious human-health risks have been identified, as in the case with TTXs. Therefore, FAO and WHO recommend assessing TTXs risks in bivalves (FAO IOC-UNESCO and IAEA, 2026).

Despite the effort in data collection and increased number of studies on TTXs occurrence in EU waters, there is still a need for a wider and more regular sampling in certain regions, namely Spain (Rey & Botana, 2026; Varini et al., 2025). This country is the first EU shellfish producer (European Commission, 2025) and specifically, the Galician rias hold the main harvesting areas. These highly productive ecosystems are recurrently impacted by blooms of toxic phytoplankton and the presence of marine biotoxins in molluscs is regularly monitored by laboratories of official control.

Initial claims on their association with dinoflagellates (e.g. *Alexandrium tamarense* and *Prorocentrum cordatum* (Kodama et al., 1996; Vlamis et al., 2015); have not been supported by field observations and only in two strains of *P. cordatum* there appeared C9-based TTX-like compounds (Rodríguez et al., 2017). However, production of TTXs is only confirmed in bacteria (Turner et al., 2017a) and these biotoxins are not included in the monitoring programmes.

To our knowledge, only a few studies have focused on the screening of TTXs in seafood from Spain, and in particular from this region (Blanco et al., 2019; Leão et al., 2018; Rossignoli et al., 2023a). Under this frame, the aim of the present study is to contribute towards filling the gap of information on the occurrence of TTXs in edible marine organisms and potential vectors from Galician production areas.

2. Material and methods

2.1. Chemicals and reagents

Acetonitrile (LC-MS and HPLC grade) was obtained from Scharlab (Barcelona, Spain). Ammonia solution (NH₃, 25% w/w, analytical grade) and LC-MS grade water were also purchased from Scharlab (Barcelona, Spain). Formic acid (≥99%, LC-MS grade) was supplied by VWR (Barcelona, Spain), and glacial acetic acid was obtained from Merck (Madrid, Spain). Type I ultrapure water, in accordance with ISO

3696 recommendations, was produced using a Milli-Q Gradient system coupled to an Elix Advantage-10 system (Millipore Ibérica, Madrid, Spain).

Supelclean ENVI-Carb™ solid-phase extraction (SPE) cartridges (250 mg, 3 mL) were purchased from Supelco (Madrid, Spain).

A tetrodotoxins certified reference material (CRM) was obtained from Cifga S.A. (Lugo, Spain). A stock standard solution was prepared from this material and subsequently used to prepare matrix-matched calibration curves.

2.2. Sampling

Most samples (Supplementary Table 1) were collected on course of two projects: PRIMROSE (Predicting Risk and Impact of Harmful Events on the Aquaculture Sector) and DESCARSEL (Reduction of discards and landing obligation in Spanish fisheries in European Atlantic waters). Further details on both projects are available in Rossignoli et al., 2023b. Samples were obtained from 16 sampling points (Fig. 1). The strategy followed while sampling was to gather as many different marine invertebrate groups/species as possible, within a list previously elaborated by the research team. However, not all the species were available in all locations.

Samples were collected, in most cases manually, from the intertidal zone during low tide. Others were obtained from the subtidal zone by dredging, and those from DESCARSEL were obtained at greater depths by trawl fishing. Additional samples were purchased in local Galician markets originating from the Northeast (NE) Atlantic and Mediterranean Sea on one occasion.

Invertebrates were generally obtained from coastal ponds in rocky shores or other substrates (sand and mud, exposed rocks with or without macrophytes). The samples were collected in intertidal sampling locations (Fig. 1) including a variety of environments, with exposed rocky shores (Cabo Estai, O Carreiro beach, Cabo Silleiro, Pedras Rubias, Portonovo and Punta Balea), tidal flats with estuarine conditions and muddy bottoms (Foz do Miñor, Sada, Campelo) and sandy areas with rocky intertidal ponds (Oza, Samil, Canido, Fontañá). Environmental conditions were obtained from weekly data collected by oceanographic buoys of Intecmar (Xunta de Galicia) located in shellfish production areas of the Galician rias (Intecmar, intecmar.gal/informacion/fito/estacions/Default.aspx).

A total of 195 marine invertebrate samples were analysed, representing five groups: bivalves (mussels, clams, cockles, and oysters), cnidarians (anemones), crustaceans (crabs, prawns, and barnacles), echinoderms (starfish, sea urchins, and sea cucumbers) and gastropods (abalones, whelks, limpets, sea snails and purple dye murex). Supplementary Table 1 indicates the sample species, capture locations and details on whether they are or not commercial in the EU. Invertebrate samples were processed as described by Rossignoli et al., 2023b and Rey et al. (2025).

2.3. Analysis of tetrodotoxins

The extraction procedure for TTXs was performed according to the Standard Operating Procedure (SOP) of the European Union Reference Laboratory for Monitoring Marine Biotoxins (EURLMB, 2017; Turner et al., 2017b), but with slight modifications.

5 mL of 1% acetic acid were added to 5 g of tissue, followed by vortex mixing for 90 s. The sample tube was capped and heated in a boiling water bath for 5 min, then left to cool by placing in an ice bath to room temperature. The mixture was vortexed again for 90 s and centrifuged at ≥4000 g for 10 min. The supernatant was collected in a 15 mL tube. As previously reported (Hort et al., 2020), the final extract volume obtained after sample extraction largely depends on the type of matrix analysed. Therefore, assuming a final volume of 10 mL for TTXs quantification may, in many cases, lead to an overestimation of up to 50% in the final result. In this study, the supernatant volume was adjusted to the nearest

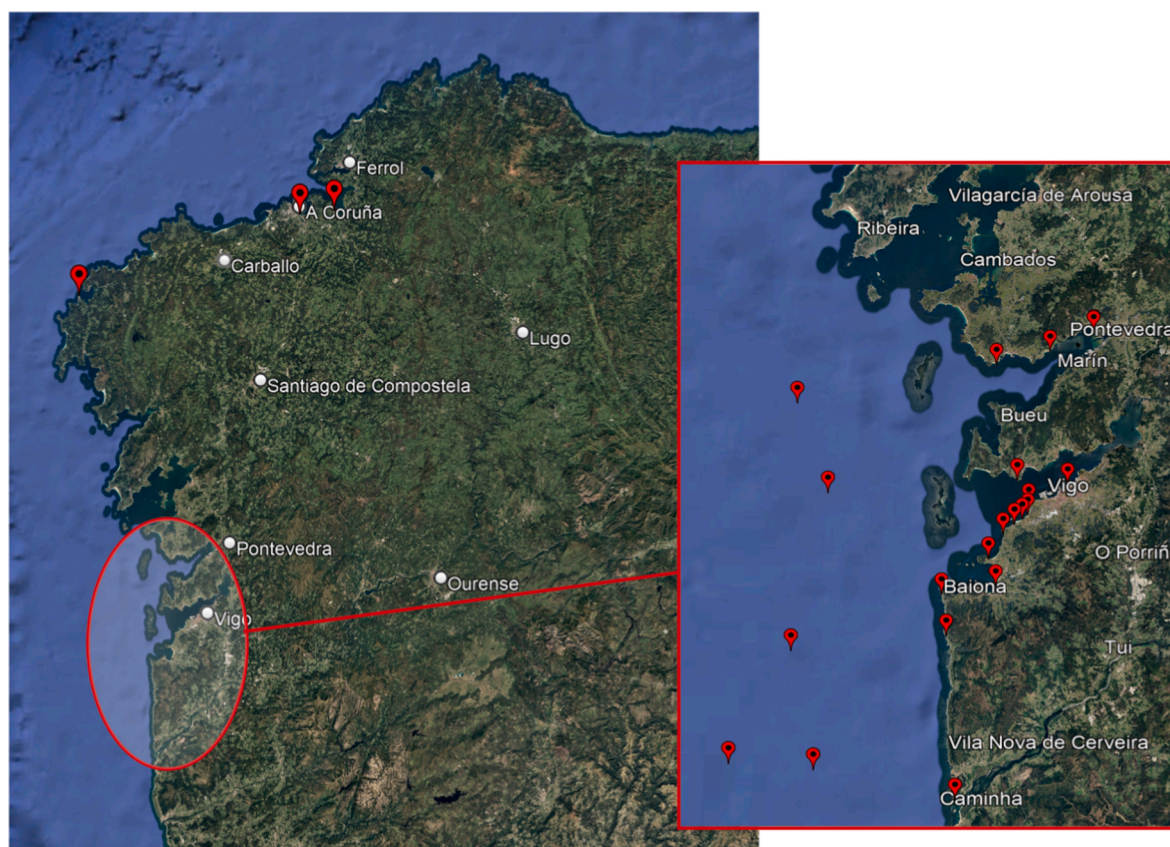


Fig. 1. Location of the sampling sites along the Galician coast (northwestern Spain).

whole-number volume using ultrapure water.

1 mL of the acetic acid extract was transferred to a 1.5 mL Eppendorf tube. Subsequently, 5 μ L of a 25% ammonia solution were added, and the mixture was vortexed. The mixture was then centrifuged at $\geq 10,000$ g for 1 min.

The clean-up was performed using a Supelclean ENVI-CarbTM graphitised carbon cartridge (250 mg, 3 mL), as described in the SOP (EURLMB, 2017). The resulting extract was then diluted by transferring 100 μ L of the extract to 300 μ L of acetonitrile and vortex mixing. The diluted extract was filtered through a 0.22 μ m syringe filter into a polypropylene vial before analysis by LC-MS/MS.

Chromatographic separation was performed using an Agilent 1290 Infinity LC (Agilent, Germany). TTX and its analogues were separated using an InfinityLab Poroshell 120 HILIC-Z column (2.1 $\times$ 100 mm, 1.9 μ m, P/N 685675-924, Agilent, Germany). This was equipped with a Vanguard pre-column system (2.1 $\times$ 5 mm, 1.9 μ m, P/N 821725-947, Agilent, Germany). The operating temperature of the column was set to 30 $^{\circ}$ C. The mobile phase consisted of solvent A (250 mL of water, 37.5 μ L of formic acid and 150 μ L of NH_3) and solvent B (450 mL of AcN, 50 mL of H_2O and 50 μ L formic acid).

The gradient started at 87% B and was held steady for 0.5 min before decreasing linearly to 75% over the next 5 min. At 6 min, the proportion of B was 50% and remained constant for 1.5 min before returning to 87% B at 8 min. These conditions were maintained for 4 min. The flow rate was 0.5 mL/min throughout the 12 min run and the injection volume was 1 μ L.

For the analysis of TTXs, a 6495D Triple Quad MS/MS (QQQ) equipped with an iFunnel Jet Stream ESI source (Agilent, Germany) was used. The mass spectrometer operated in dynamic multiple reaction monitoring (dMRM) mode, analysing at least two product ions per compound: one for quantification (Q, the most abundant ion) and another for confirmation (q). The transitions and their corresponding

collision energies are summarised in Table 1. The theoretical limits of detection (LODs) and quantification (LOQs) were calculated based on the signal-to-noise (S/N) ratio, using an S/N ratio of 3:1 for the LOD and 10:1 for the LOQ. The theoretical LODs are 3.12 μ g/kg for TTX, 3.55 μ g/kg for 4,9-anhTTX, and 3.61 μ g/kg for 4-epiTTX, respectively. The theoretical LOQs were subsequently checked by spiking mussel blank sample aliquots ($n = 3$) and assessing the method precision and

Table 1
MS/MS conditions for the analysis of TTXs.

Compound	Precursor ion (m/z)	Collision Energy (V)	Product ion (m/z)
TTX/4-epiTTX	320.1	25/20	302.1 (Q) 162.1 (q)
4,9 anhTTX	302.1	30	256.1 (Q) 162.1 (q)
11-deoxyTTX	304.1	30	286.1 (Q) 176.1 (q)
11-norTTX-6-ol	290.1	30	272.1 (Q) 162.1 (q)
4,4a-anhydro 5,6,11-trideoxyTTX	254.1	30	236.1(q) 162.1(Q)
4,9-anhydro-5,6,11-trideoxyTTX	254.1	30	236.1(q) 162.1(Q)
4-epi-5,11-dideoxyTTX	288.1	30	270.1(Q) 162.1(q)
4-epi-5,6,11-trideoxyTTX	272.1	30	254.1(Q) 162.1(q)
5,6,11-trideoxyTTX	272.1	30	254.1(Q) 162.1(q)
5-deoxyTTX	304.1	30	286.1(Q) 176.1(q)
6-deoxyTTX	304.1	20	286.1(Q) 176.1(q)
trideoxyTTX	272.1	30	254.1(Q) 162.1(q)

accuracy. Practical LOQs are 9.46 µg/kg for TTX, 10.74 µg/kg for 4,9-anhTTX, and 10.93 µg/kg for 4-epiTTX. The samples were analysed using the LC-MS/MS method in dMRM mode for TTX and 12 analogues (Fig. 2). The absence of reference material is one of the major challenges, meaning that only the toxins present in the standard (TTX, 4,9-anhydroTTX and 4-epiTTX) could be quantified. To include additional analogues in the method, a trumpet shell (*Charonia lampas*) reference sample, in which these compounds had been previously identified and confirmed by High Resolution Mass Spectrometry (HRMS) (Pais et al., 2025), was used. Transitions of several TTX analogues have been reported in many studies (Alkassar et al., 2023; Pais et al., 2025; Rossignoli et al., 2023a).

These were used to incorporate additional analogues in the method: 5-deoxyTTX, 11-deoxyTTX, 6-deoxyTTX, 11-nor-TTX-6-ol, 4-epi-5,11-dideoxyTTX, 4-epi-5,6,11-trideoxyTTX, 5,6,11-trideoxyTTX, 4,9-anhydro-5,6,11-trideoxyTTX and 4,4a-anhydro-5,6,11-trideoxyTTX and trideoxyTTX.

Calibration curves were prepared using mussel matrix-matched standards with eight calibration levels. The method was optimised for bivalves, mainly mussels, and no significant matrix effect was observed within this group. To calculate the concentration of 11-deoxyTTX, the TTX calibration curve was used and the TEF provided by the EFSA (2017) was applied.

3. Results and discussion

From a total of 195 marine invertebrate samples collected along the Galician coasts, 19 presented detectable TTXs levels (9.7% prevalence). The toxins detected were TTX and/or 11-deoxyTTX (Table 2).

3.1. Environmental conditions in the sampling locations

The collection areas, located in shallow waters in the intertidal zone, and the sampling seasons (spring to autumn) would favour the presence of TTXs as earlier documented in neighbour NE Atlantic areas like the UK, in particular southern England (Dhanji-Rapkova et al., 2023; Turner et al., 2017a). In that sense, it is worth mentioning that, according to the latter authors, water temperatures of 15–20 °C over a five-year period triggered seasonal TTX accumulation in bivalves in early summer

(starting in June). This seasonality seems to be common in several European countries such as France, Netherlands and Greece (Gerssen et al., 2018; Hort et al., 2020; Vlamis et al., 2015). In agreement, in the present study most samples positive for TTXs were obtained throughout the summer and beyond (mid-June to October), in waters ranging 16.5–19.5 °C. In turn, a few positive ones sampled between April and the beginning of June were collected in cooler waters (12.9 °C).

Samples were collected in diverse benthic environments (see 2.2. Sampling) with different ambient conditions. In particular, samples positive for TTX and 11-deoxyTTX (Table 2) belonged to the most southern locations in the Rías Baixas (Rías de Vigo and Pontevedra, Fig. 1).

Sampling dates with positives for TTX and its analogues spanned from spring to summer (Apr-Sep), excepting one date at the beginning of October in Canido (Table 2). From south to north, locations of interest in the Rías Baixas and their environmental conditions (associated with the detection of TTX and its analogues) could be described as follows.

In the Ría de Vigo, Canido beach displayed recurrent positive TTX results for *H. tuberculata*. This location is next to Fontañá beach which also showed TTX in mussels and limpets. Both sites are under the influence of the nearby river Lagares. Canido is a rocky intertidal flat area, partially sheltered by a small dock while Fontañá (a similar environment), is closer to the river mouth. The general oceanographic conditions in the surrounding area of both locations (vertical profiles of temperature (T), salinity (S) and fluorescence) can be approached by data collected in two sampling stations (Sts. V5 and V7) of the regional monitoring system from Intecmar (Xunta de Galicia: www.intecmar.gal/Ctd/Default.aspx). Though, it must be borne in mind that these are placed in coastal waters away from the intertidal area, which experiences wider ambient oscillations. In that sense, temperature data for the sampling areas in the intertidal environment were approached from the data catalog of IEO-CSIC (datos.ieo.es/geonetwork/srv/search?keyword=Visor).

The T range in Sts. V5 and V7 were similar during the 2021–2022 period, when samples containing TTXs were collected, with minima and maxima of 12.2–12.5 °C and 21.5–21.8 °C, respectively. It must be highlighted that these maxima were reached in September 2022, attaining > 2 °C over the highest records during both years.

Regarding S, the range spanned in V5 and V7 from 30 to 35.9, the

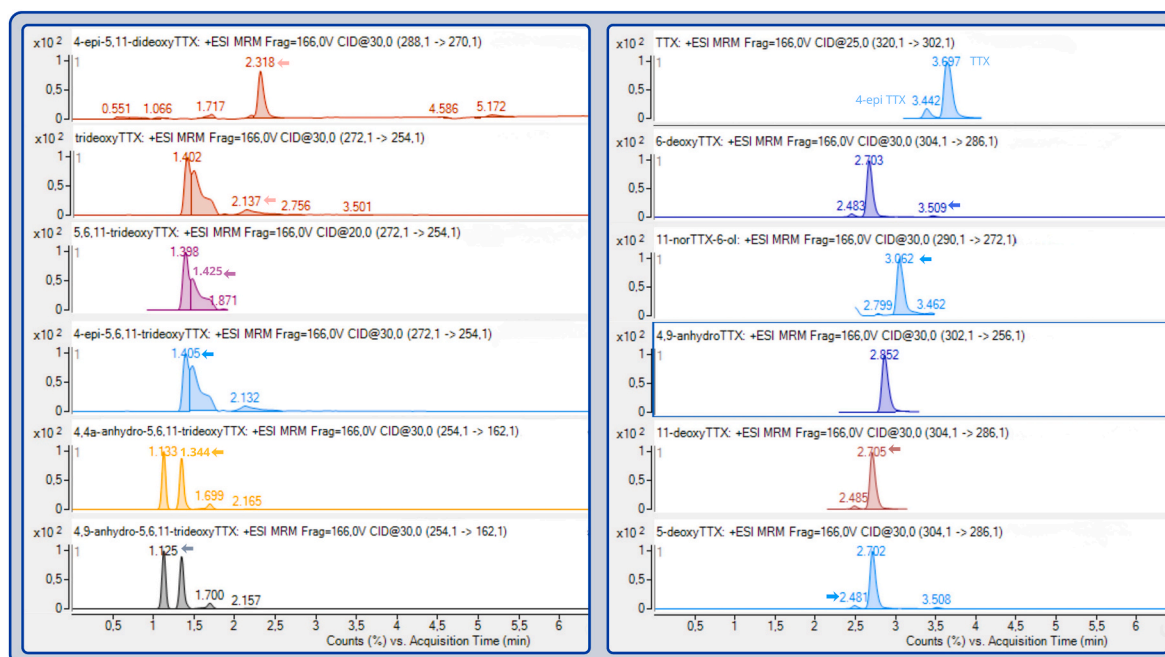


Fig. 2. HILIC-MS/MS chromatograms of TTX analogues included in the analytical method, using a trumpet shell (*Charonia lampas*) as reference sample.

Table 2TTX, 11-deoxyTTX and total toxicity concentration ($\mu\text{g TTX eq/kg}$) in positive samples.

Specie	Sample code	Sampling date	Location	$\mu\text{g TTX/kg}$	$\mu\text{g 11-deoxyTTX/kg}$	Total toxicity $\mu\text{g eq TTX/kg}$
<i>Anemonia viridis</i> ^a	EURLMB/037/22	24/06/2021	Cabo Silleiro (Baiona)	26.0	-	26.0
<i>Haliotis tuberculata</i>	EURLMB/018/22	25/05/2021	Canido beach (Vigo)	19.8	-	19.8
<i>Haliotis tuberculata</i>	EURLMB/245/22	16/06/2022	Canido beach (Vigo)	16.2	-	16.2
<i>Haliotis tuberculata</i>	EURLMB/312/22	09/09/2022	Canido beach (Vigo)	22.4	-	22.4
<i>Mytilus galloprovincialis</i>	EURLMB/014/22	25/05/2021	Fontaíña beach (Vigo)	16.2	-	16.2
<i>Necora puber</i>	EURLMB/065/22	06/10/2021	Canido beach (Vigo)	40.8	-	40.8
<i>Patella</i> sp.	EURLMB/003/22	06/04/2021	Cabo Estai (Vigo)	11.7	<LOQ	11.7
<i>Patella</i> sp.	EURLMB/010/22	25/05/2021	Fontaíña beach (Vigo)	9.4	<LOQ	9.4
<i>Patella</i> sp.	EURLMB/026/22	06/06/2021	O Carreiro beach (Nigrán)	-	1.3	1.3
<i>Patella</i> sp.	EURLMB/033/22	24/06/2021	Cabo Silleiro (Baiona)	9.4	<LOQ	9.4
<i>Patella</i> sp.	EURLMB/249/22	27/06/2022	O Carreiro beach (Nigrán)	<LOQ	-	<LOQ
<i>Patella</i> sp.	EURLMB/267/22	12/07/2022	Cabo Estai (Vigo)	-	1.3	1.3
<i>Patella</i> sp.	EURLMB/085/23	26/05/2023	Campelo (Poio)	10.4	-	10.4
<i>Patella vulgata</i>	EURLMB/019/22	25/05/2021	Canido beach (Vigo)	-	1.3	1.3
<i>Patella vulgata</i>	EURLMB/062/22	06/10/2021	Canido beach (Vigo)	-	1.4	1.4
<i>Patella vulgata</i>	EURLMB/244/22	16/06/2022	Canido beach (Vigo)	<LOQ	<LOQ	<LOQ
<i>Patella vulgata</i>	EURLMB/285/22	11/08/2022	Pedras Rubias (Oia)	<LOQ	<LOQ	<LOQ
<i>Phorcus lineatus</i>	EURLMB/250/22	27/06/2022	O Carreiro beach (Nigrán)	12.1	-	12.1
<i>Phorcus lineatus</i>	EURLMB/266/22	12/07/2022	Cabo Estai (Vigo)	10.9	-	10.9

^a Accepted name following World Register of Marine Species (marinespecies.org). *Anemonia sulcata* is the unaccepted junior subjective synonym, used in the list of commercial names of species permitted in the EU and Spain.

minimum being attained in January 2022. Fluorescence reached maximum values (up to $30 \mu\text{g Chl } a \text{ L}^{-1}$) in both stations in September 2022, though the usual range in spring-summer where TTX positives were registered is approx. $2\text{--}20 \text{ Chl } a \text{ L}^{-1}$.

Cape Silleiro and Pedras Rubias are more exposed and outer locations in the southern margin of the Ría de Vigo that cannot be approached by V5 and V7. In this case, St. B1 would be the closest one, though its conditions could be more representative for another inner location with TTX positives like O Carreiro beach (Nigrán). B1 showed similar T and higher S values than the former stations, ranging $12.4\text{--}21.6^\circ\text{C}$ and $33.6\text{--}35.7$, respectively. The latter is explained by a reduced influence of riverine inputs regarding V5 and V7. In summer 2022, where positives for TTX were registered, there were also recorded the highest fluorescence values during series ($10\text{--}27 \mu\text{g Chl } a \text{ L}^{-1}$).

In the Ría de Pontevedra, the sample that tested positive for TTX in Campelo was collected in May 2023 during a red tide of the PSP

producing dinoflagellate *Alexandrium minutum*. This site is in the inner part of that Ría, dominated by mud sediments and strongly influenced by the discharges of river Lérez. In that month, after heavy rains in the area, T and S attained 20.2°C and 23, respectively, at Campelo during the sampling dates in this work.

Station P0, the closest one in the monitoring system, evidenced the estuarine conditions in that area, with oscillations of T and S (period 2021–2022) between $12.8\text{--}21.2^\circ\text{C}$ and $25.2\text{--}36.3$. Its productivity is remarkable, with fluorescence maxima attaining $39 \mu\text{g Chl } a \text{ L}^{-1}$ in that series, commonly between 5 and $30 \mu\text{g Chl } a \text{ L}^{-1}$ during spring and summer.

3.2. Bivalves

A total of 51 samples were analysed within the bivalve group (Supplementary Table 1). The majority of them were mussels ($n = 30$),

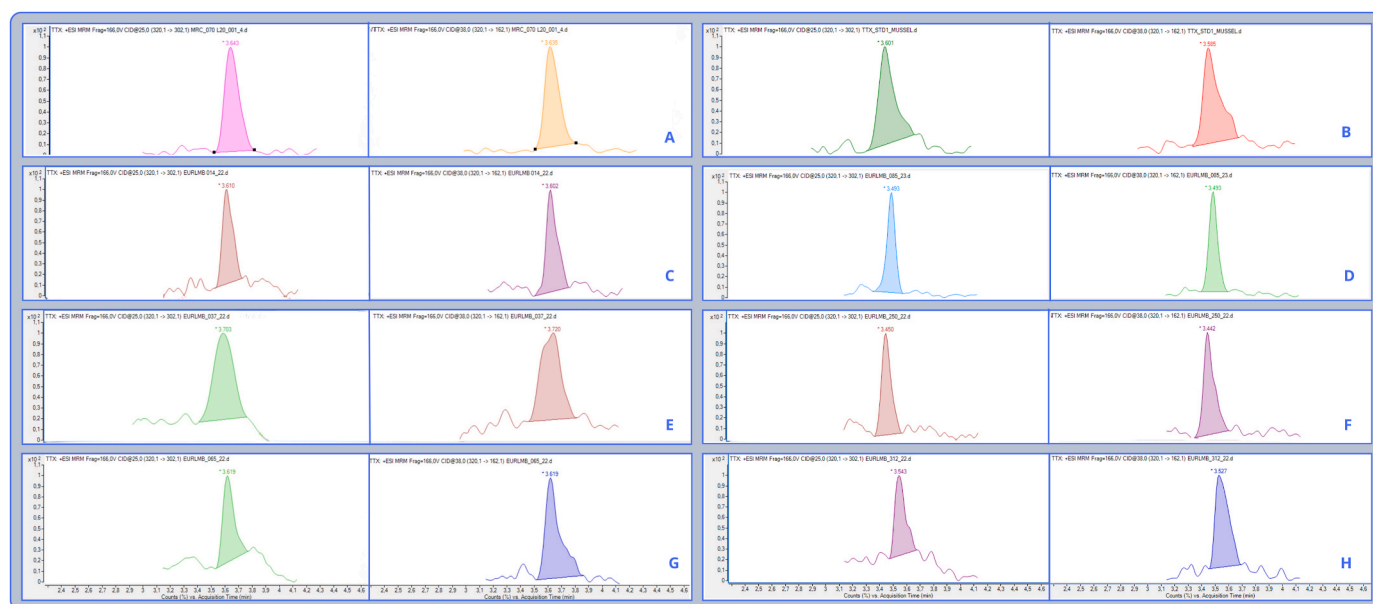


Fig. 3. Representative MRM chromatograms of TTX: (A) TTX standard in solvent; (B) TTX standard in mussel matrix; (C) TTX detected in sample EURLMB/014/22 (*Mytilus galloprovincialis*); (D) sample EURLMB/085/23 (*Patella* sp.); (E) sample EURLMB/037/22 (*Anemonia viridis*); (F) sample EURLMB/250/22 (*Phorcus lineatus*); (G) sample EURLMB/065/22 (*Necora puber*); and (H) sample EURLMB/312/22 (*Haliotis tuberculata*).

followed by clams ($n = 10$), cockles ($n = 7$) and oysters ($n = 4$). These edible species are among the most commonly cultured and consumed in both Galicia and Europe. TTXs were only detected and quantified in one out of 51 samples, a wild mussel (*Mytilus galloprovincialis*) sample (EURLMB/014/22) (Fig. 3C). This resulted in a prevalence of 2.0% within the bivalve group and 0.5% if we refer to the total sample number ($n = 195$). The only congener quantified was TTX at a level of 16.2 $\mu\text{g}/\text{kg}$. No other bivalve species were available for collection at the same point (Fontaña beach). TTXs were not detected in several samples from other groups collected on the same date at the same location, including spiny starfish (*Marthasterias glacialis*) (EURLMB/011/22), beadlet anemones (*Actinia equina*) (EURLMB/012/22) and sea urchins (*Paracentrotus lividus*) (EURLMB/013/22) (Supplementary Table 1). However, limpets (*Patella vulgata*) (EURLMB/010/22) from the same sampling showed quantifiable 11-deoxyTTX levels (Table 2, Fig. 4).

Bivalves are the marine invertebrate group most sampled and analysed in the marine biotoxins monitoring programmes worldwide. Among the bivalves, mussels are frequently used as sentinel species, indicative of the potential marine biotoxins levels of other bivalves and/or certain other marine invertebrate groups (Rossignoli et al., 2023b). However, previous studies from our research group indicate that toxicity levels in mussels may not always be correlated with those found in other invertebrate groups as the correlation can be dependent on the biotoxins groups evaluated (Rey et al., 2025) and this may also be the case for TTXs.

Up to 2025, TTXs have been reported in bivalves (either in mussels, clams, and/or oysters) from 7 European countries (Italy, France, Greece, The Netherlands, Portugal, Spain and United Kingdom (Varini et al., 2025; Vlamis et al., 2015). The highest TTXs levels, 541 μg TTX eq/kg, were registered in Italian mussels (*Mytilus galloprovincialis*) (Bordin et al., 2021), with other high concentrations in oysters (*Magallana gigas*) from the UK, total TTXs 253 $\mu\text{g}/\text{kg}$ (Turner et al., 2017a) and the Netherlands, 253 μg TTX eq/kg (Gerssen et al., 2018), clams (*Venus verrucosa*) from Greece, with 195 μg TTX eq/kg (toxin profile TTX, 4-epiTTX and 4,9-anhydroTTX) (Vlamis et al., 2015), all values

well above the 44 $\mu\text{g}/\text{kg}$ suggested by the EFSA as a safe level. Despite these high levels, no intoxications related to bivalve molluscs containing TTXs have been recorded in Europe so far (Katikou et al., 2022). Other studies from outside Europe also described the TTXs presence in bivalves, in countries such as China, Japan and New Zealand, with very high TTXs levels reported in some samples, such as pipi clams (*Paphies australis*) and greenshell mussels (*Perna canaliculus*) from New Zealand (800–1600 μg TTX eq/kg whole flesh) and in scallops (*Mizuhopecten yessoensis*) from Japan (8000 μg TTX eq/kg digestive glands) (Katikou et al., 2022). In Spain, previous studies were conducted in bivalves from the regions of Catalonia, Valencia and Galicia. Blanco et al. (2019) reported that TTXs were not detected in the mussels, oysters and clams they studied. Leão et al. (2018) found TTX at levels of 2.3 and < 0.9 μg TTX/kg in a Galician cockle and oyster samples, respectively. A recent study by Rossignoli et al., 2023a reported the detection of TTX in a *Tellina donacina* specimen.

To our knowledge, the present study reports for the first time the quantification of TTX in a mussel sample from Spain, at a level lower than the EFSA safe level. The sample was collected in a small but popular urban beach, on the rocks of intertidal zone, with shallow waters, near a small estuarine area, and at the end of May. It is important to highlight that Galicia is one of the main worldwide mussel producers and exporters (Soliño & Figueras, 2025). Although the number of mussel samples analysed in this study is not high, the TTXs prevalence in the samples evaluated is low, and the TTX level in the positive sample was also low, this result points to the need of more studies to collect occurrence data in order to protect consumers.

3.3. Gastropods

The gastropod group was the largest studied one, comprising 64 samples, of which limpets (*Patella vulgata* and *Patella* sp.) and sea snails (*Phorcus lineatus*) represented the most numerous sample sets, with $n = 27$ each of them. In addition, 6 abalone (*Haliotis tuberculata*), 1 whelk (*Nassarius reticulatus*) and 3 purple dye murex (*Bolinus brandaris*)

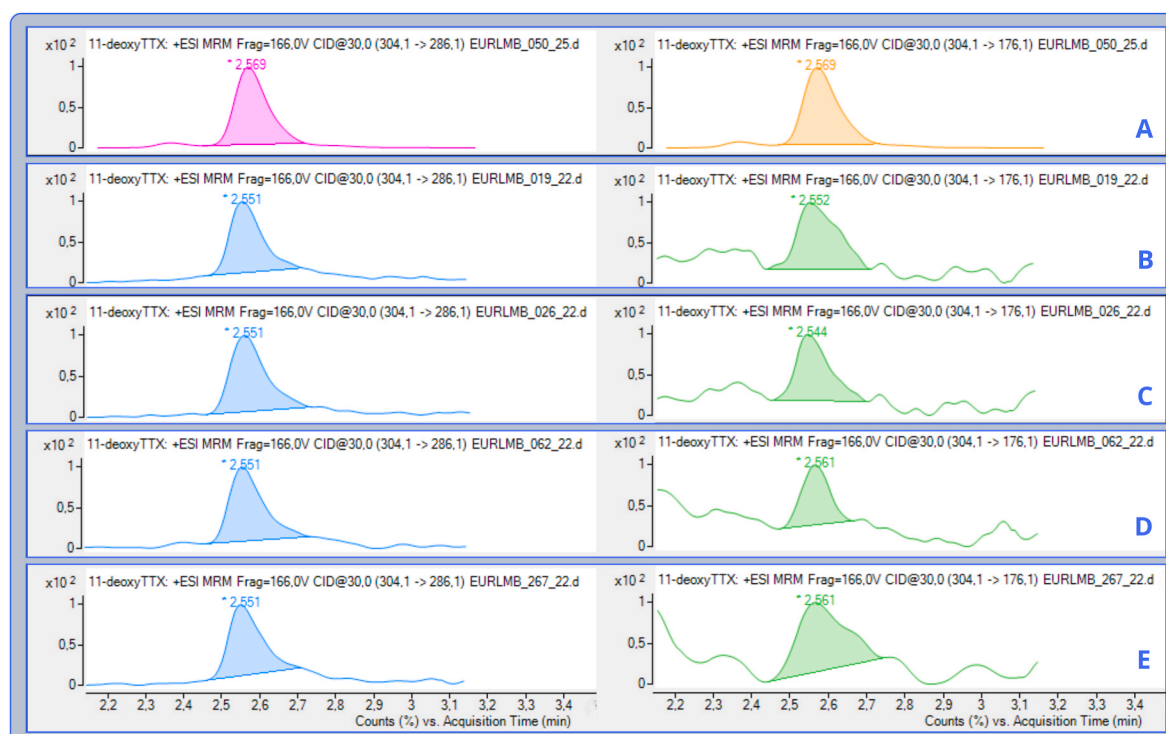


Fig. 4. Representative MRM chromatograms of 11-deoxyTTX: (A) reference sample EURLMB/50/25 (*Charonia lampas*); (B) sample EURLMB/019/22 (*Patella vulgata*); (C) sample EURLMB/026/22 (*Patella* sp.); (D) sample EURLMB/062/22 (*Patella vulgata*); and (E) sample EURLMB/267/22 (*Patella* sp.).

samples were evaluated (Supplementary Table 1). TTX was the only analogue detected and quantified in three abalone samples (EURLMB/018/22, EURLMB/245/22 and EURLMB/312/22). These samples were collected at Canido beach in the 2021 and 2022 late spring and summer seasons. Sample EURLMB/061/22, was the only abalone, collected at the same sampling point, in which TTXs were not detected. TTX concentrations in the 3 positive samples ranged from 16.2 to 22.4 µg TTX eq/kg (Table 2, Fig. 3H), these levels are around half of the 44 µg eq TTX/kg EFSA safe limit. This is the first report of TTXs presence in *Haliotis tuberculata* from Europe. We also would like to highlight that samples EURLMB/018/22 and EURLMB/312/22 showed paralytic shellfish toxins (PSTs) levels around 100 µg STX.dHCl eq/kg in a previous study from our group (Rey et al., 2025). Although PSTs levels are one eighth of the EU legal limit, the combined neurotoxic effect of both PSTs and TTXs in abalones could pose a risk for consumers. Abalones, which are considered a delicacy, are consumed in some European countries. Over the last years, they were commercialised from Galicia (0.4, 0.1 and 0.3 mt in 2023, 2024 and 2025, respectively) (Xunta de Galicia, 2026).

Out of the 27 sea snail samples (*Phorcus lineatus*) analysed, only 2 showed quantifiable TTX levels. EURLMB/250/22, with 12.1 µg TTX/kg (Fig. 3F), and EURLMB/266/22 with 10.9 µg TTX/kg (Table 2). No other TTX analogues were detected. Sample EURLMB/250/22 was collected at O Carreiro beach (Nigrán) while EURLMB/266/22 at Cabo Estai (Vigo), both sampled in the middle of summer 2022. Other sea snail samples, gathered at the same sampling points but on different years (2021 and 2023) did not present detectable TTXs levels (Supplementary Table 1). This could be due to individuals' variability or to more favourable climatic conditions in 2022. To our knowledge, this is the first time that TTX is reported in sea snails from Spain. Nevertheless, as we describe below TTXs were previously reported in the same species in Portugal (Silva et al., 2012) TTX was not detected in the only whelk sample analysed.

Interestingly, limpets (*Patella vulgata* and *Patella* sp.) samples showed the highest rate of positive results. Out of the 27 samples analysed, TTX and/or 11-deoxyTTX were detected in 11 (Table 2). To further confirm the presence of 11-deoxyTTX, limpet samples containing this analogue were additionally analysed by LC-HRMS/MS at CCMAR (Faro, Portugal), using the same instrumentation and analytical conditions described by Pais et al. (2025). However, the concentration of the analyte was too low to obtain a product ion spectrum suitable for structural confirmation. Therefore, the representative MRM chromatograms of the samples in which 11-deoxyTTX was quantified by LC-MS/MS are shown in Fig. 4. Total TTX levels ranged within 9.4 to 11.7 µg/kg. Moreover, there were 3 samples in which TTX was under the method LOQ. This is the first study reporting the presence of TTXs in limpets from Spain. In Europe, 11-oxo-TTX had previously been detected in *Patella depressa* from Portugal (Silva et al., 2019). Consequently, our research describes the presence of TTX and 11-deoxyTTX in *P. vulgata* from Europe for the first time. If we take into account all the gastropod samples in which TTXs were detected ($n = 16$), the TTXs prevalence was 25% within the gastropods group and 8.2% if we refer to the total sample number ($n = 195$). It is important to remark that limpets and sea snails are edible in Europe. In Galicia, low limpet and sea snail amounts are usually commercialised, this may be related to scarce exploitation plans. In particular, data from shellfish auctions were 0.5 and 0.04 mt of common limpets (*Patella vulgata*) in 2024 and 2025, respectively, and less than 0.1 mt for sea snails (*Littorina littorea*) in both years (Xunta de Galicia, 2026). However, it is important to mention that both gastropod subgroups, very common on the Galician rocky shores, are easily accessible to beachgoers, especially in summer when beaches are crowded.

Gastropod samples with TTXs (Table 2) were all collected on the rocks of the intertidal zone where small ponds are formed at low tide. The sampling points were in shallow waters. Some of them were located in small urban beaches, in semi-enclosed bays into the Ría de Vigo. However, others were placed in open coastal areas, on rocky shores with

difficult access. All the samples were gathered between spring and summer 2021-2023.

Guardone et al. (2020) reported that gastropods are the second source of TTXs intoxications worldwide with 20.9% of the cases. Most cases occurred in Taiwan and China because of the traditional consumption of small detritivore gastropods, considered an economic, nutritive and delicious food. Studies from other Asiatic countries, such as Japan and Vietnam, have also reported TTX human intoxications related with this marine invertebrate group (Noguchi et al., 2011). Examples of species in which TTXs were reported are: *Babilonia japonica* (Japanese ivory shell), *Natica lineata* now named *Tanea lineata* (lined moon shell), *Niotha Clathrata*, now named *Nassarius conoidalis* (cone-shaped nassa or jewelled dog whelk), *Nassarius glans* (acorn dog welk), *Tutufa lissostoma* now named *T. bufo* (frog shell), *Charonia sauliae* now named *C. lampas*, *Oliva* spp., *Rapana* spp. (Biessy et al., 2020; Hwang et al., 1990, 1992; Noguchi et al., 2011; Taniyama et al., 2009; Yasumoto et al., 1981). TTXs reported concentrations in gastropods from Asia are generally much higher than the ones found in Europe (except for *C. lampas*) and over the EFSA recommended safe limit. The fact that TTXs are usually found at highest levels in the digestive glands, suggest that accumulation may be via dietary sources (Biessy et al., 2019). On top of these data, dog intoxications possibly associated to TTXs present in *Pleurobranchaea maculate* (grey side-gilled sea slug) were reported in New Zealand (McNabb et al., 2010).

In Europe TTX was identified for the first time in 2008 as the causal agent of a food poisoning case linked to the consumption of *C. lampas*, with very high TTXs levels, harvested in southern Portugal and purchased in Malaga, Spain (Rodriguez et al., 2008). Since then, several studies have evaluated TTXs in certain gastropods. Silva et al. (2012) evaluated TTXs levels in several gastropod species from Portugal (*P. lineatus*, *Monodonta turbinata*, *Gibbula umbilicalis*, *Gibbula magus*, *Littorina littorea*, *Littorina saxatilis*, *Nucella lapillus*, *Ocenebra erinacea*, *Calliostoma zizyphinum*, *Patella intermedia* and *C. lampas*). The authors first reported the co-occurrence of TTX (90.5 µg/kg) and 4-epiTTX (21.5 µg/kg) in *P. lineatus* and the presence of monodeoxyTTX at a concentration of 63.8 µg/kg in *G. umbilicalis*. Silva et al. (2019) reported the detection of TTXs under their UHPLC-HRMS method LOQ in *P. depressa*, *P. lineatus*, *C. lampas*, *N. lapillus*, *G. umbilicalis* from Portugal. They also evaluated some gastropods from the Moroccan coast and found levels under their LOQ in *Aplysia depilans* and 24 µg TTX eq/kg in *Onchidella celtica*. Hort et al. (2020) evaluated TTXs levels in 66 whelk (*Buccinum undulatum*) samples. They only found TTX in one of them, reporting 7.4 µg TTX/kg in a pool of the gonads, digestive gland and stomach, and levels below the LOD (1.7 µg TTX/kg) in the remainder tissues. In Spain, Blanco et al. (2019) reported that the TTXs levels found in gastropods from Galicia (*Patella* spp. and *Calliostoma* spp.) and Catalonia (*Bolinus brandaris*) were under 10 µg TTX eq/kg in the 15 tested samples. Moreover, Rossignoli et al., 2023a, analysed 175 gastropod samples from Galicia including limpets (*Patella* spp.), whelks (*Nassarius* spp.), sea snails (*Monodonta* sp.) and abalones (*Haliotis* spp.), among others. They only detected TTXs in a *N. reticulatus* sample, reporting 5,6, 11-trideoxyTTX and its isomer at levels under 0.6 µg TTX/kg. The prevalence of TTXs in gastropods in the Rossignoli et al., 2023a study is very low (0.6%) as compared to 8.2% in our study. Most of the gastropod samples they analysed were collected in different Galician sampling areas. They gathered a sea snail and a limpet sample in Patos beach at the beginning of April 2022. Patos is very close to one of our sampling points (O Carreiro beach), where we detected and/or quantified low TTXs levels in one limpet and one sea snail sample at the end of June 2022 (Table 2). Although the number of positive samples to compare is reduced and the TTXs levels quantified were low, there exists the possibility that the summer temperatures could have a positive effect on TTXs production. In Italy, Bacchiocchi et al. (2025) analysed 100 samples belonging to eight different genera and species of gastropods (including *N. mutabilis*, and *P. caerulea*). However, they did not detect TTXs in any of the samples tested. In turn, Pais et al. (2025) reported

additional TTXs data on *C. lampas* from Portugal.

3.4. Crustaceans

A total of 40 crustacean samples (27 crab, 9 shrimp and 4 barnacle samples) were analysed. Only one of them showed levels above the method LOQ. This was a velvet swimming crab (*Necora puber*) (EURLMB/065/22), collected at the beginning of October 2021 at Canido beach intertidal zone, with TTX levels of 40.8 µg/kg, a value close to the EFSA recommended limit (Fig. 3G). The TTXs prevalence was 2.5% within the crustacean group and 0.5% if we refer to the total sample number (n = 195). There were 2 limpet and 3 abalone samples gathered at the same location that contained quantifiable levels of either TTX or 11-deoxyTTX (Table 2). Moreover, there was another limpet sample in which both toxins were detected (Table 2). All these samples were collected in or close to the summer seasons of 2021 and 2022, when the sea temperatures rise and the potentially optimal temperature range (15–20 °C) (Turner et al., 2017a) for TTXs production can be achieved, especially in shallow ponds that are exposed to sun at low tides. By contrast, TTXs were not detected in two other velvet swimming crab samples (EURLMB/242/22 and EURLMB/313/22), collected at the same location in the 2022 sampling season (Supplementary Table 1). Similarly, other crustacean species analysed: *Eriphia verrucosa*, yellow crab (2 samples), *Palaemon serratus*, shrimps (2 samples) and *Pollicipes pollicipes*, goose barnacle (1 sample), from the same sampling campaigns (Supplementary Table 1) did not show detectable TTX levels.

Studies on the evaluation of TTXs in European crustaceans are scarce. In fact, up to now, most investigations focussed on either bivalves or gastropods. The only available data are from Portugal, Spain and Italy. A study conducted in Portugal coasts and north western coast of Morocco (Silva et al., 2019) reports TTXs analysis in barnacles (*P. pollicipes*) but they did not detect any analogues. In Portugal, Lage et al. (2023), reported the presence of TTXs in *Afruca tangeri* (European fiddler crab) and *Carcinus maenas* (green crab) from Ria Formosa lagoon, in the south. Total TTXs concentration (µg TTX eq/kg) of the European fiddler crab soft tissues were 1.5 and 2-fold higher than the EFSA recommended safe limit of 44 µg TTX eq/kg, for the samples collected at Ramalhete Marine Station and Faro beach, respectively. However, in the green crab levels were 16-fold lower than in European fiddler crab, and below the EFSA recommended safety limit. Although TTX was not found in the crab samples, three TTX analogues were reported: an unknown TTX epimer, deoxyTTX, and trideoxyTTX. In Spain, the only data available are those from Rossignoli et al., 2023a, who evaluated 44 crustacean samples from the Galicia region and only detected 5,6,11-trideoxyTTX in a *Liocarcinus corrugatus* sample (levels under 0.6 µg TTX/kg). In Italy, Bacchiocchi et al. (2025) conducted a TTXs survey on non-bivalve marine invertebrates, including crustaceans, from the North-Central Adriatic Sea. A total of 33 samples were collected, comprising 30 blue crab samples (*Callinectes sapidus*), one of true crab (*Brachyura* spp.), one of European spider crab (*Maja squinado*), and one of mantis shrimp (*Squilla mantis*). The samples were pooled taking a certain number of specimens and analysed by HILIC-MS/MS. However, TTXs were not detected in any samples.

Although not much information is available on TTXs levels in European crustaceans, we must not ignore that there exist many studies in Asiatic countries reporting TTXs in crustaceans since the 90s. Several publications link TTXs to human intoxications related to the ingestion of *Carcinoscorpius rotundicauda* (horseshoe crab) and even deaths (Ha et al., 2025; Kanchanapongkul, 2008; Kanchanapongkul & Krittayapoositpot, 1995; Suleiman et al., 2017).

Among the crustacean TTXs vectors reported are: *Atergatis floridus* (floral egg crab) in Japan, Taiwan and Philippines (Arakawa et al., 1994; Asakawa, 2014; Tsai et al., 1997), *C. rotundicauda* in Thailand, Bangladesh, Vietnam, Malaysia and China (Dao et al., 2009; Kanchanapongkul & Krittayapoositpot, 1995; Suleiman et al., 2017; Tanu & Noguchi, 1999; Zhu et al., 2022), *Tachyleus tridentatus*

(horseshoe crab) in China (Zhu et al., 2022), *Lophozozymus pictor* (mosaic reef crab) in Taiwan (Tsai et al., 1995), *Demania reynaudi*, (common name not found) in Taiwan (Tsai et al., 1997) and *Zosimus aeneus* (devil crab) in Japan (Zhang et al., 2021).

Our study reports the first description worldwide of TTX in *Necora puber*. This species is edible and much appreciated in Spain, and particularly in the Galician region for its savoury flesh. In Galicia the volume of velvet swimming crabs commercialised over the last 3 years was 86.2, 72.7 and 81.7 mt in 2023, 2024 and 2025, respectively Xunta de Galicia, 2026. The consumption of crabs in Europe is important especially in the Mediterranean countries. Therefore, wider studies covering more areas, different species and ampler sampling periods are urgently needed.

3.5. Echinoderms

Seventeen sea urchin samples, all belonging to the species *Paracentrotus lividus*, except for an unidentified one, were analysed. Seven starfish samples, including 5 spiny starfish samples (*Marthasterias glacialis*), 1 starfish (*Luidia sarsi*), and 1 unidentified sample were also evaluated. The echinoderm group was completed with two sea cucumber samples (*Stichopus regalis*) (Supplementary Table 1). TTXs were not detected in any of the 26 echinoderm samples analysed. Some *P. lividus* samples belonged to the same samplings done at Fontaíña beach and Canido beach on the dates (May and October 2021, June 2022) in which TTXs were detected in other matrixes (mussels, abalones, limpets and a velvet swimming crab). This could be an indication of different TTXs accumulation rates in diverse marine invertebrate groups or species.

Data on the evaluation of TTX levels in echinoderms are uncommon worldwide. Silva et al. (2012) analysed 8 starfish (*M. glacialis*) and 10 sea urchins (*P. lividus*) samples from the Portuguese coast, but they did not detect TTXs on these samples. In Silva et al. (2019) published an article covering the analyses of TTXs in multiple invertebrate vectors, including echinoderms: sea urchins, starfish and sea cucumbers. They reported the detection of 11-oxoTTX in the sea urchin *Echinus esculentus* from continental Portuguese coast and the quantification of high levels of 2 unknown TTX analogues in several samples of red velvet starfish *Ophidiaster ophidianus* collected in São Miguel, Azores archipelago. In Spain, Rossignoli et al., 2023a, analysed 26 echinoderm samples from our region (Galicia) and only detected 5,6,11-trideoxyTTX and its isomer in an *Asterina gibbosa* sample (levels under 0.6 µg TTX/kg). They also analysed 11 *P. lividus* and one *M. glacialis* but, as in our study, they did not detect TTXs. However, Bacchiocchi et al. (2025), analysed 23 *P. lividus* samples and reported for the first time the quantification of trace TTX levels (~5 µg/kg) in only one of the samples. Finally, Pais et al. (2025) evaluated TTX levels in 25 *Astropectem arancianus* (sea stars) samples from Portugal. They reported estimated toxicities ranging from 7 to 64 µg TTX eq/kg in the digestive glands and from 0.3 to 27 µg TTX eq/kg in the stomach. Although several TTX analogues were identified, 4,9-anhydro-5,6,11-trideoxyTTX and an unknown trideoxyTTX were the most frequently detected. The authors suggest the potential transfer of TTXs from sea stars to trumpet shells, therefore acting as vectors for this gastropod which is related with potential food safety issues and the only EU reported intoxication (Rodríguez et al., 2008).

3.6. Cnidarians

Three cnidarian species (*Actinauge richardi*, *Actinia equina* and *Anemonia viridis*) were included in this study, with a total of 14 anemone samples (Supplementary Table 1). The only sample that showed detectable TTXs levels was EURLMB/037/22 (Fig. 3E), snakelocks anemones (*A. viridis*), with a concentration of 26.0 µg TTX/kg. This species is edible and very appreciated in Spain and in several European areas. Data from Galician shellfish auctions for snakelocks anemones (*Anemonia viridis*) were 16.5, 17.2 and 15.6 mt in 2023, 2024 and 2025, respectively (Xunta de Galicia, 2026). The sample was collected at Cabo

Silleiro in summer 2021 (Table 2). On the same sampling date and area, we collected the beadlet anemone (*A. equina*) EURLMB/036/22 sample, where TTXs were not detected. However, the limpet sample EURLMB/033/22, gathered on the same sampling campaign, presented TTX levels of 9.4 µg/kg and 11-deoxyTTX under the method LOQ (Table 2). This could be an indication of different TTX accumulation patterns on diverse Galician marine invertebrate species from the same marine environment. However, a deeper investigation should be considered with the collection of additional samples to support the hypothesis. Regarding TTXs in cnidarians, the only data we found in literature was that of Rossignoli et al., 2023a. Their study comprised 34 cnidarian samples (most of them unidentified), from Galicia (Spain), describing the quantification of TTX and 5,6,11-trideoxyTTX in the non-edible cnidaria *Calliactis parasitica* (49.7 and 233 µg TTX eq/kg, respectively). Moreover, they reported the TTX detection in an unidentified cnidarian and the detection of 5,6,11-trideoxyTTX in another one. We would like to highlight 3 out of the 34 samples tested were *Actinia* sp. samples where TTXs were not detected. Our study, and the general lack of data, indicate the need conduct further studies in snakelocks anemones aimed to ensure the food safety of these products.

4. Conclusions

The present study provides new evidence of TTXs accumulation in invertebrates from Galician coasts, including edible and highly appreciated species. The most relevant findings are the first report of quantifiable TTXs levels in mussels, sea snails and limpets from Spain, as well as the first findings of TTX in abalone and snakelocks anemones in Europe. On top of that, this is the first worldwide description of TTX in a velvet swimming crab.

Limpets (*P. vulgata* and *P. sp.*) showed the highest rate of positive results, 40% of the 27 samples analysed, loading TTX and/or 11-deoxyTTX. Although their trade is not significant, limpets may be directly collected from rocks for self-consumption by locals, thus representing a direct risk for humans. Interestingly, these gastropods could also act as TTXs vectors transferring them to their predators such as crabs.

Abalone samples were also positive for PSTs, indicating that the risk for consumers may be underestimated, since TTXs are not considered in national monitoring programs in Spain, so far. The potential synergistic effects of both neurotoxins groups should not be disregarded.

For other species where TTXs were detected, the levels found in this study were comparable to previous works performed in the same region, although showing a higher prevalence in gastropods. TTX was also quantified in the edible cnidarian snakelocks anemone. Although TTXs were found in other cnidarian species before, they were not previously detected in this species. On the other hand, TTXs were not detected in any of the 26 echinoderm samples analysed, which is consistent with other reports from the same area.

The areas and the season where these organisms were collected may favour TTXs accumulation (shallow waters, high temperatures), although the levels quantified varied among organisms from a determined spot. This may point to species specific accumulation.

These findings are relevant for their potential implications for food safety and indicate that the coasts of Galicia may deserve further survey for a better understanding of TTXs incidence, evolution of temporal trends in the current context of global warming, and risk.

CRediT authorship contribution statement

Verónica Rey: Conceptualization, Data curation, Formal analysis, Investigation, Validation, Writing – original draft, Writing – review & editing. **Lucía Soliño:** Data curation, Resources, Supervision, Writing – original draft, Writing – review & editing. **Francisco Rodríguez:** Conceptualization, Resources, Writing – original draft, Writing – review & editing. **Eva Eiriz-Pazo:** Investigation, Writing – original draft. **Julio Valeiras:** Resources, Writing – review & editing. **Begoña Ben-Gigirey:**

Conceptualization, Resources, Writing – original draft, Writing – review & editing.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodcont.2026.112547>.

Data availability

The authors do not have permission to share data.

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