Emergent marine toxins in the temperate north Atlantic coast

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Summary
The occurrence of toxins typical from tropical environments in waters from the north Atlantic coast has been sporadically reported in the past years. However, these infrequent reports have been preceded by human intoxication episodes. For this reason it became evident that the analysis of coastal water needed a more detailed survey of marine toxins. The detection of new vectors, particularly those potentially used as food by human beings, suggests that monitoring of marine toxins should be extended to species that currently are not being monitored, and that regulated limits of toxin should be closely monitored. In this work, first reports on the occurrence of tetrodotoxin (TTX) and its analogues, of Spirolides (SPX), and of ß-methylamino-L-alanine (BMAA) in temperate north Atlantic coast (Portugal and Morocco), are presented. This highlights the changing environment, in terms of toxin patterns, and suggests potential pathways of human exposure.

Introduction
Phycotoxins may travel along the food chain, reaching edible organisms, resulting in intoxication incidents. Organisms such as bivalves, crustaceans, gastropods, and fish have been reported as phycotoxin vectors. Tetrodotoxin (TTX) is a potent neurotoxin that has been reported in several taxa not genetically close, such as bacteria, marine invertebrates, and terrestrial and marine vertebrates. Neither its biochemical path nor its origin is clear. Several poisoning incidents due to TTX have been reported (Silva et al. 2012). Spirolides (SPXs) are marine toxins, which have a cyclic imine group that is responsible for their neurotoxicity, and are produced by dinoflagellate species, such as *Alexandrium ostenfeldii* and *A. peruvianum*. SPXs have shown acute toxicity in mouse bioassays; however, poisoning incidents have not yet been reported, despite the fact that SPXs have already been reported in shellfish in a number of countries (Silva et al. 2013). ß-methylamino-L-alanine (BMAA) is a non-protein amino acid shown to be neurotoxic on cellular and *in vivo* level (Karamyan and Speth 2008). A possible pathway for human exposure to BMAA is through cyanobacterial blooms in water or through consumption of aquatic organisms exposed to such blooms. Recently, it was reported that planktonic diatoms and dinoflagellates might also contain BMAA (Faassen 2014). The possible occurrence of emergent toxins and new vectors in the Atlantic Ocean poses new challenges for monitoring programs. In this study we searched for vectors of marine toxins along the coast of Portugal and Morocco, surveying benthic species including gastropods, bivalves, sea-urchins and starfishes.

Material and Methods
Organisms were collected from the intertidal area during low tides in temperate north Atlantic coast (Portugal and Morocco) and *Charonia lampas* were purchased at local Portuguese fish markets. TTX and SPXs were extracted and analysed as described in Silva et al. (2012) and Silva et al. (2013), either by LC-MS/MS or UPLC-MS/MS. BMAA was extracted after lyophilization of the animals and acidic digestion (2 mL 6 M HCl, at 90ºC, for 20 min), using a high-pressure microwave system (Milestone - Ethos 1). The samples were evaporated under a low flux of nitrogen, reconstituted in 0.5 mL 20 mM HCl, and filtered (0.22 μm Millipore) prior to analysis by LC-MS/MS. The analyses were performed in a Thermo LCQ Fleet Ion Trap LC/MS^n system (Thermo Scientific), using a 2.1 x 100 mm, 5 μm diameter ZIC-HILIC column (SeQuant), and a 14 x 1 mm, 5 μm guard column (SeQuant). The mobile phase consisted in eluent A, acetonitrile (0.1% formic acid) and eluent B, deionised water (0.1% formic acid). In the first 20 min a 90–60% linear gradient of acetonitrile was achieved, and afterwards 60% acetonitrile was maintained for 15 min. The system was then equilibrated to the initial conditions during 5 min. The flow rate was 0.4 mL min^{-1}, the injection volume was 10 μl, and the column temperature was 40 ºC. The electrospray ionization (ESI) was operated

in the positive mode. Nitrogen was used as sheath gas, at a rate of 45 (unitless), and auxiliary gas at a rate of 20 (unitless). The capillary temperature was held at 250 ºC. Mass-to-charge ratio (*m/z*) scan was performed from 50 to 150, and the ion *m/z* 119 was monitored. At collision energy of 14 V the presence of more abundant product ions *m/z* 102, 88 and 76 was verified. The most abundant ion *m/z* 102 was used for BMAA quantification.

Results and Discussion

Table 1 reports the positive results obtained for the analysed samples A sample was considered positive when the toxin level was above the limit of detection (LOD) of the analytical method. The LOD for TTX analysed by LC-MS/MS was 16 ng mL^{-1}, and by UPLC-MS/MS was 1.7 ng mL^{-1}. The LOD for SPXs analysed by LC-MS/MS was 0.1 ng mL^{-1} and by UPLC-MS/MS was 1.6 ng mL^{-1}. The LOD for BMAA analysed by LC-MS/MS was 10 ng mL^{-1}.

Table 1 - Species sampled and their results (positive -☑ - or negative - ×) for the presence of toxins

	Morocco	Portugal					
Species	BMAA	BMAA	TTX	4-*epi*TTX	MonodeoxyTTX	5,6,11-trideoxyTTX	SPX
Mytilus galloprovincialis	×	×	×	×	×	×	☑
Marthasterias glacialis	*n/a*	*n/a*	×	×	×	×	☑
Paracentrotus lividus	*n/a*	*n/a*	×	×	×	×	×
Charonia lampas	*n/a*	*n/a*	×	×	×	☑	☑
Gibbula umbilicalis	*n/a*	×	×	×	☑	×	☑
Monodonta lineata	☑	×	☑	☑	×	×	☑
Nucella lapillus	*n/a*	*n/a*	×	×	×	×	☑
Patella intermedia	☑	×	×	×	×	×	☑

n/a - not analysed

These results highlight the necessity to improve monitoring programs in order to minimize risks to human health. Close monitoring, in conjunction with reliable detection methods, might help to reduce the number of poisonings. In addition, these results contribute knowledge on the role of toxins in benthic food chains and might help reveal seasonal, geographical or interspecific patterns of toxin accumulation.

References

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