because of commercial demands in the fishing industry. However, for non-bivalve vectors, no specific monitoring plans have been established to date [26].

The production or capture of echinoderms and tunicates in the EU is small compared to those of fish and other seafood [13]. The commercialization of gastropods and crustaceans has been growing in the last few years [14]. According to FAO reports from recent years [27], crustacean fisheries exceeded the production of bivalve mollusks in the EU, and marine gastropod fisheries have also increased considerably, duplicating in the last two decades.

Therefore, bearing in mind the growing consumers' interest in these species, it is of special importance to evaluate the exposure to marine biotoxins through the ingestion of these new vectors as well as the adjustment of the existing reference methods for this new scenario. In this work, samples of different invertebrate and fish species from the North Atlantic waters were analyzed using the PCOX method in order to detect new vectors for these biotoxins and evaluate their potential as a threat to public health.

2. Results and Discussion

A total of 98 samples were analyzed using the PCOX method [10] with slight modifications [28] to determine and quantify PSTs. The samples were collected from three different sites: Madeira Island, São Miguel Island (Azores archipelago), and the Moroccan Atlantic coast (the sampling sites are described in detail in the Material and Methods section). Several edible (with commercial interest) and non-edible species were selected to search for potential new vectors and the prevalence of the screened biotoxins in the food web: gastropods (*Stramonita haemastoma, Phorcus lineatus*, *Cerithium vulgatum*, *Gibbula umbilicalis*, *Aplysia depilans*, *Charonia lampas*, *Onchidella celtica*, *Patella gomesii*, *Patella aspera, Umbraculum umbraculum, Patella ordinaria*), crustaceans (*Pollicipes pollicipes*), bivalves (*Mytilus* spp.), starfish (*Ophidiaster ophidianus*, *Marthasterias glacialis*, *Echinaster sepositus*), sea-cucumber (*Holothuria* (*Platyperona*) *sanctori*), sea-urchins (*Paracentrotus lividus*, *Arbacia lixula*, *Sphaerechinus granularis*, *Diadema africanum*), and fish (*Sphoeroides marmuratus*).

Since the PCOX method is not validated for these different matrices, optimizations were needed and made for echinoderms and gastropods species, thus adding an additional step prior to the HPLC-FLD analyses and enhancing the reliability of the results [29].

2.1. Madeira Island (Madeira Archipelago)

From a total of 22 samples collected during the summer of 2012, 15 were positive for PSTs, with 7 above the maximum legislated value [30,31] (Table 1).

Table 1. Quantified paralytic shellfish toxins (PSTs) samples in Madeira island, Madeira Archipelago.

Sampling Data	Sampling Site	Sample	Species	Code	µg STX.diHCleq/Kg SM
8 August 2012	Northern coast of Madeira	Limpet	*Patella ordinaria*	336	1123.3
		Limpet	*Patella aspera*	337	122.4
16 September 2012	Reis Magos	Sea urchin	*Paracentrotus lividus*	339#1	<LOQ
		Starfish	*Ophidiaster ophidianus*	341#1	2071
		Starfish	*O. ophidianus*	341#2	2224.1
		Starfish	*O. ophidianus*	341#3	4625.4
		Limpet	*P. aspera*	344	866.4
		Sea urchin	*Arbacia lixula*	345	<LOQ
		Sea snail	*Stramonita haemastoma*	346	964.5
18 September 2012	Caniçal	Limpet	*P. aspera*	350	12.2
		Limpet	*Umbraculum umbraculum*	351	536.8
		Starfish	*Echinaster sepositus*	353	668
		Sea snail	*Charonia lampas*	354	1423.4
		Sea urchin	*Diadema africanum*	355#1	276.3
		Sea urchin	*D. africanum*	355#2	227.9

Values in bold are above the legal limit; STX: saxitoxin; eq: equivalents; SM: Shellfish Meat; LOQ: Limit of quantification.

Regarding toxin uptake, the highest values were detected in echinoderms, more specifically, in the red velvet starfish *O. ophidianus* (4625.4 µg STX.diHCleq/Kg), followed by gastropods *C. lampas*

(1423.4 µg STX.diHCleq/Kg SM), *P. ordinaria* (1123.3 µg STX.diHCleq/Kg SM), and *S. haemostoma* (964.5 µg STX.diHCleq/Kg SM).

Concerning monitoring, bivalves are the selected key sentinel species for which all analytical methods have been validated [22,23,26]. Being the Madeira archipelago located in oligotrophic waters where mussels are quite rare, echinoderms and gastropods showed a good potential as bio-indicators for toxin monitoring, as suggested in previous works [11,32,33].

2.2. São Miguel Island (Azores Archipelago)

From a total of 38 samples from June 2013, 22 were positive for PSTs, with 7 above the maximum legislated value [30,31] (Table 2).

Table 2. Information on Azores samples.

Sampling Data	Sampling Site	Sample	Species	Code	µg STX.diHCleq/Kg SM
7 June 2013	Lagoa	Sea urchin	*Sphaerechinus granularis*	409#3	43.4
		Sea urchin	*S. granularis*	409#4	42.5
		Starfish	*O. ophidianus*	412	1689.6
		Sea snail	*S. haemastoma*	413	939.4
		Limpet	*Patella gomesii*	415	1192.4
8 June 2013	Mosteiros Etar	Limpet	*P. gomesii*	420	902.3
		Sea urchin	*A. lixula*	421	<LOQ
		Sea urchin	*A. lixula*	423	111.7
	Ilhéu S. Roque	Starfish	*O. ophidianus*	424	2588.4
		Sea urchin	*S. granularis*	425#1	<LOQ
		Sea urchin	*S. granularis*	425#2	<LOQ
		Sea urchin	*S. granularis*	425#3	<LOQ
	Cruzeiro	Starfish	*Marthasterias glacialis*	426#2	3.8
9 June 2013		Starfish	*M. glacialis*	428	7744.3
		Sea snail	*S. haemastoma*	431	678.3
	Caloura	Sea urchin	*A. lixula*	432	<LOQ
		Starfish	*M. glacialis*	433#1	47.5
		Starfish	*M. glacialis*	433#2	24.9
		Sea snail	*S. haemastoma*	434	544.7
		Starfish	*O. ophidianus*	435	920.3
10 June 2013	Caloura	Starfish	*O. ophidianus*	440	245
10 June 2013	Caloura	Sea snail	*S. haemastoma*	443	128.6

Values in bold are above the legal limit.

Regarding toxin uptake (Table 2), similarly to Madeira, we detected seven values above the legal limit in starfish (*O. ophidianus* and *M. glacialis*), followed by mollusks *S. haemostoma* and *P. gomesii* (939.4 and 902.3 µg STX.diHCleq/Kg SM, respectively). The maximum uptake value detected was in the yellow spiny starfish *M. glacialis* from Cruzeiro, with 7744.3 µg STX.diHCleq/Kg.

2.3. Moroccan Coast

The northwestern Moroccan coast was surveyed during July 2013, supplying a total of 38 samples, with 28 of them (74%) positive for saxitoxin and its analogs (Table 3).

Table 3. Moroccan samples information.

Sampling Data	Sampling Site	Sample	Species	Code	µg STX.diHCleq/Kg SM
22 July 2013	Casablanca Corniche	Bivalve	*Mytilus* sp.	447	1376.9
		Sea snail	*Phorcus lineatus*	448	929.4
23 July 2013	Sidi Bouzid	Sea snail	*P. lineatus*	449	1404.5
		Limpet	*Patella* sp.	450	1090.5
		Sea slug	*Aplysia depilans*	451	<LOQ
		Bivalve	*Mytilus* sp.	453	2266.4
		Sea snail	*Cerithium vulgatum*	454	158.8
	El Jadida Sâada	Sea snail	*C. vulgatum*	455	2556
		Sea cucumber	*Holothuria (Platyperona) sanctori*	458#1	2.3
		Limpet	*Patella* sp.	459	8.6
		Starfish	*M. glacialis*	463	1852.4

Table 3. *Cont.*

Sampling Data	Sampling Site	Sample	Species	Code	µg STX.diHCleq/Kg SM
24 July 2013	El Jadida Haras	Barnacle	*Pollicipes pollicipes*	464	17.7
	Mrizika	Bivalve	*Mytilus* sp.	465	1140.4
		Barnacle	*P. pollicipes*	466	17.6
		Limpet	*Patella* sp.	467	3622.5
		Bivalve	*Mytilus* sp.	468	1080.9
		Sea snail	*Gibbula umbilicalis*	469	1.6
		Sea snail	*P. lineatus*	470	1043.9
	Oualidia	Starfish	*M. glacialis*	473	1325.4
		Sea slug	*Onchidella celtica*	474	38.8
		Sea snail	*C. lampas*	475	0.02
		Sea slug	*A. depilans*	476	0.6
		Sea snail	*S. haemastoma*	477	384
		Sea snail	*P. lineatus*	482	85.7
		Sea snail	*G. umbilicalis*	483	12.1
		Barnacle	*P. pollicipes*	484	17.4
		Bivalve	*Mytilus* sp.	485	2708.9

Values in bold are above the legal limit.

All positive values were found in mollusks and echinoderms. Thirteen were above the European [30,31] maximum legal limit. It is important to notice that the highest concentration value detected was in a limpet, with a total amount of 3622.5 µg STX.diHCleq/Kg SM, despite the presence of mussels in this region.

2.4. Statistical Analysis

The results of the generalized linear model for PST content as a function of genus and region (*Patella* and *Paracentrotus*) and region (Madeira and Morocco) did not find significant differences for region, despite the southwards increase detected in the whole data set. This could be explained by the low number of samples and organisms included and by the fact that these two sampling regions are closer to each other, relative to Azores. On the other hand, a significant difference was detected in toxin concentrations between genera, with higher concentrations in *Patella* than in the sea urchin ($F_{1,16} = 10.4$, $p < 0.01$), which seems to be a general pattern for sea urchins and limpets in all the three regions (see Tables 1–3).

2.5. General Discussion

One of the primary aims of this work was to screen new vectors for PSTs in order to evaluate public health threats related to seafood consumption. From a total of 66% of positive results, we report 14 new vectors for these hydrophilic phycotoxins, belonging to three different phyla: mollusks (*P. aspera*, *S. haemostoma*, *U. umbraculum*, *P. gomesii*, *P. ordinaria*, *C. vulgatum*, *O. celtica*), echinoderms (*O. ophidianus*, *A. lixula*, *E. sepositus*, *D. africanum*, *S. granularis*, *H. (Platyperona) sanctori*), and crustaceans (*P. pollicipes*). In Figure 1 are displayed some examples of the toxin elution in different matrices, showing that the toxin retention time was dependent on the analyzed matrix used in the PCOX method.

We highlight the latitudinal pattern of PSTs uptake, since the percentage of positive results follows a north-south gradient: Azores (58%) < Madeira (68%) < Morocco (74%). Though many of the causes of dinoflagellate bloom formation are still to unravel, the water temperature and eutrophication play a pivotal role, which is consistent with our results. Water temperature in the Atlantic Ocean rises, in general, following a north-south gradient towards the Equator, and human anthropic pressures are higher in Morocco than in the Portuguese islands screened in this survey [34–39]. We also defined a qualitative pattern of toxin profiles, as shown in Figure 2.