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Paralytic shellfish toxins in the Atlantic horse mackerel (*Trachurus trachurus*) over a bloom of *Gymnodinium catenatum*: the prevalence of decarbamoylsaxitoxin in the marine food web

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SUMMARY: This study reports the accumulation of paralytic shellfish toxins (PSTs) in Atlantic horse mackerel (*Trachurus trachurus*) over a bloom of the toxigenic dinoflagellate *Gymnodinium catenatum*. High levels of toxins, up to 4800 µg STXeq kg^{-1}, were registered at the peak of the bloom (5.0 10^3 cells l^{-1}). The suite of individual PSTs was examined. Decarbamoylsaxitoxin (dcSTX) and B1 constituted nearly 90% of toxins (on a molar basis) determined in mackerel. This profile of toxins markedly differs from the known profile of toxins produced by *G. catenatum* strains isolated from the Portuguese coast, which is dominated by N-sulfocarbamoyl toxins, in particular the C1+2 toxins. The prevalence of the potent dcSTX in the pelagic environment and its transfer through the marine food web is highlighted in this study. Atlantic horse mackerel is identified as a high potential vector of PSTs along the Portuguese coast. This fish species has a central position in the marine food web, being an important predator of zooplankton and at the same time an important diet item of top predators. This study reveals bioaccumulation values that are important for evaluating potential impacts of blooms of PST-producing dinoflagellates on marine ecosystems or their components, such as fish.

Keywords: marine toxins, saxitoxin, pelagic fish, phycotoxins, harmful algal blooms.

RESUMEN: DETERMINACIÓN DE TOXINAS PARALIZANTES EN EL JUREL (*TRACHURUS TRACHURUS*) DURANTE UNA PROLIFERACIÓN DE *GYMNODINIUM CATENATUM*: LA PREVALENCIA DE DECARBAMOILSAXITOXINA EN LA RED TRÓFICA MARINA. – Este estudio reporta la acumulación de toxinas paralizantes de molusco (PSP) en las muestras de jurel (*Trachurus trachurus*) durante una proliferación do dinoflagelado *Gymnodinium catenatum*. Los altos niveles de toxinas PSP, alcanzando un valor máximo de 4800 µg STXeq kg^{-1}, se determinaron en el pico de la proliferación (5.0 10^3 células l^{-1}). El perfil de toxinas se examinó y se reveló que los compuestos dcSTX y B1constintuían casi 90% de las toxinas (en base molar) encontrados en las muestras de jurel. Este perfil difiere claramente del perfil de toxinas conocido en cepas de *G. catenatum* aisladas de la costa portuguesa, que está dominado por toxinas N-sulfocarbamoyl, en particular las toxinas C1+2. Este trabajo muestra la prevalencia de dcSTX el ambiente pelágico y la transferencia de este compuesto a través de la cadena alimentaria. El jurel se identifica como un potencial vector de las toxinas PSP a lo largo de la costa portuguesa. Esta especie de pez tiene una posición central en la cadena alimentaria marina, siendo un importante depredador de zooplancton y, al mismo tiempo un elemento importante de la dieta de los depredadores superiores. Este estudio contribuye con valores ecológicos relevantes para evaluar los potenciales impactos de las proliferaciones de dinoflagelados productores de PST en los ecosistemas marinos o sus componentes, como los peces.

Palabras clave: toxinas marinas, saxitoxina, peces pelágicos, ficotoxinas, proliferacions de algas nocivas.

INTRODUCTION

Paralytic shellfish toxins (PSTs) are potent neurotoxins produced by bloom-forming algae species in both marine and freshwater ecosystems. In the marine system the dinoflagellates *Gymnodinium catenatum*, *Alexandrium* spp. and *Pyrodinium bahamense* are the main producers (Hallegraeff 1993, Masó and Garcés 2006). Extensive blooms of these algae species can cause negative impacts on ecosystems and animal health.

Although PSTs are regularly monitored in bivalve mollusks, which are prohibited for human consumption when toxicity exceeds 800 μg STXeq kg^{-1}, less is known regarding accumulation of these toxins in fish. Nevertheless, fish kills associated with PST-producing dinoflagellates have been reported worldwide (White *et al*. 1977, Landsberg 2002, Silva *et al*. 2011, Fire *et al*. in press). PSTs block nerve transmission by reversely binding to voltage-gated sodium channels (Hall *et al*. 1990, Lagos and Andrinolo 2000). They cause impairment of sensor-motor function that affects larval and adult fish survival (White 1981, Robineau *et al*. 1991, Lefebvre *et al*. 2005, Samson *et al*. 2008). Poisoning of fish leading to lethal incidences, as mentioned above, is occasionally reported, but induction of sublethal effects without outward signs of toxicity must occur regularly (Bakke and Horsberg 2010, Costa *et al*. 2012). In this case, fish can play a vectorial role and transfer the toxins to piscivorous predators (Geraci *et al*. 1989, Castonguay *et al*. 1997, Reyero *et al*. 1999).

Changes in the profile of toxins are likely to occur through the trophic web as a result of species-specific metabolism of toxins. The PST family contains around 50 compounds, which differ in chemical structure and toxicity (Llewellyn 2006, Wiese *et al*. 2010). The most common ones can be grouped into three classes in increasing order of toxicity: N-sulfocarbamoyl (B1, B2, C1-C4), decarbamoyl (dcGTX1- dcGTX4, dcNeo, dcSTX) and carbamoyl toxins (GTX1-GTX4, Neo, STX) (Oshima *et al*. 1993). Selective elimination and interconversion of assimilated PSTs increase the complexity of in vivo dynamics of PSTs. While herbivorous organisms mirror the toxin profile of toxigenic algae, predator species may accumulate only those toxins characterized by lower elimination rates or resulting from biotransformation processes.

In fact, investigations on the profile of PSTs in sardines (*Sardina pilchardus*) exposed to blooms of *Gymnodinium catenatum* have shown minor changes between the fish and algae, suggesting that sardines, which have a filter feeding behavior in the presence of algal blooms, ingesting large amounts of toxic algae, are a good tracer for the occurrence of harmful algal blooms and the associated PSTs (Costa *et al*. 2010). Conversely, fishes at higher trophic levels are expected to reveal an altered profile of PSTs (Landsberg 2002).

While most data available in the literature are associated with fish kills, this study addresses the accumulation of PSTs in a fish species over a bloom of *Gymnodinium catenatum*. This study therefore contributes important ecological levels for evaluating potential impacts of blooms of PST-producing dinoflagellates on marine ecosystems components, such as fish and their predators. The Atlantic horse mackerel (*Trachurus trachurus*) was the fish species selected because of its central position in the trophic chain: it has a diet composed of teleosts, cephalopods, larger decapod crustaceans and smaller crustaceans such as copepods, mysids and euphausiids (Cabral and Murta 2002), and is in turn an important prey for piscivorous teleosts and rays (Ebert 1991). Additionally, horse mackerel is one of the most important pelagic fishes in terms of abundance and commercial value off the Iberian coast.

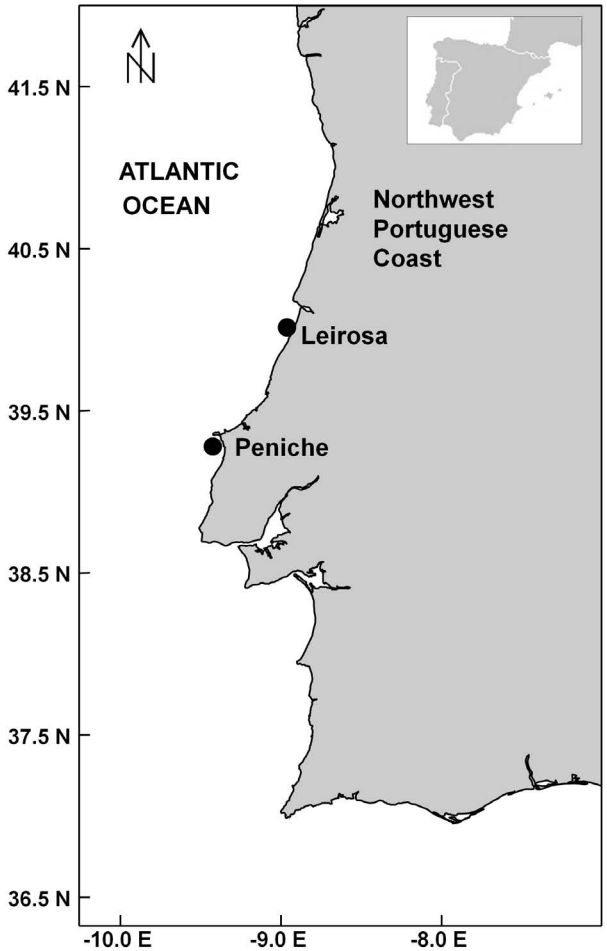

FIG. 1. – Location of seawater and fish sampling.

MATERIALS AND METHODS

Phytoplankton water sample analysis

Surface seawater samples (depth=3 m) were collected fortnightly before the bloom season and once a week during and after the bloom. Samples were collected within the national monitoring programme for toxic marine phytoplankton in 2009. Sampling was carried out at high tide in the harbor of Peniche and Leirosa, NW Portuguese coast (Fig. 1). Seawater samples were preserved with 3% formalin solution. Phytoplankton species were identified and enumerated in subsamples of 50 ml by the Utermöhl technique (Hasle, 1978), using a Zeiss IM35 inverted microscope with phase contrast and bright field illumination. A magnification of 160× and 400× was used for cell counting and species identification, respectively. The detection limit for the lowest magnification was 40 cells l^{-1}.

TABLE 1. – *Trachurus trachurus*: description of samples collected in Peniche (NW Portuguese coast) (*n* = number of individuals).

Sampling Date (2009)	*n*	Whole Body		Viscera
		Weight min-max (g)	Lenght min-max (cm)	Weight min-max (g)
September 9th	30	132 - 224	24 - 33	11 - 25
September 19th	30	147 - 213	24 - 31	19 - 30
October 22sd	30	425 - 566	36 - 43	65 - 84
November 6th	30	72 - 158	20 - 34	10 - 21
November 13th	30	367 - 547	33 - 40	54 - 113

Collection and preparation of Atlantic horse mackerel samples

Atlantic horse mackerel was captured off the NW Portuguese coast on five sampling dates between September and November 2009. Samples were obtained from commercial landings in the port of Peniche. Thirty horse mackerel specimens were obtained from each of the five sampling dates. The specimens were measured, weighed and dissected (Table 1). Fish were grouped in ten pooled samples composed of three specimens on each sampling day. Samples were homogenized and stored at –20°C for toxin analysis.

PST extraction and high-performance liquid chromatography (HPLC) analysis

The frozen samples were thawed at room temperature and 5 g was homogenized with acetic acid 1%. Toxin extraction and cleanup procedures were performed according to Lawrence and Niedzwiadek (2001) and Costa *et al* (2010). Before HPLC analysis, aliquots of the C18 extracts were derivatized with peroxide and periodate oxidants while aliquots of SPE-COOH fractions were only used for periodate oxidation. Because of the lack of analytical standards for C3+4 and B2, their concentrations were estimated after a hydrochloric acid hydrolysis conversion into their carbamate analogues GTX1+4 and Neo, respectively. The limits of detection (LOD) ranged from 0.07 ng STXeq g^{-1} for C1+2 and B1 to 4 ng STXeq g^{-1} for dc-Neo. The toxicity factors stated in EFSA (2009) were used to calculate PSTs in terms of saxitoxin dihydrochloride equivalents.

All solvents and chemical reagents were HPLC or analytical grade. Certified calibration solutions for PSTs were obtained from the Certified Reference Materials Program of the Institute for Marine Biosciences, National Research Council, Canada (CRM-STX-e, CRM-GTX2&3-b, CRM-dcSTX, CRM-dcGTX2&3, CRM-GTX5-b and CRMC1&2).

RESULTS

Presence of *Gymnodinium catenatum*

Gymnodinium catenatum cells were observed in seawater samples collected from both locations (Peniche and Leirosa) between late June and early September (Fig. 2). The bloom peaked in early September with densities of 1.9 10^3 and 5.0 10^3 cells l^{-1} at Peniche and Leirosa, respectively.

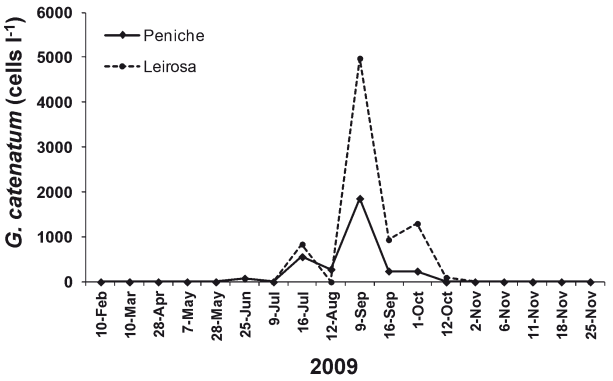

FIG. 2. – Concentration of *Gymnodinium catenatu*m (cells l^{-1}) in surface seawater samples collected in Peniche and Leirosa, NW Portuguese coast, during 2009.

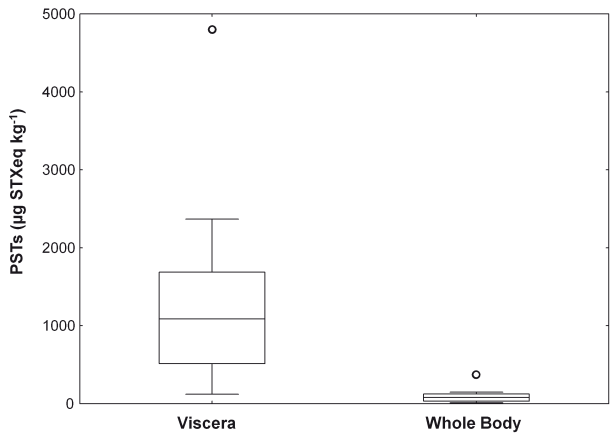

FIG. 3. – *Trachurus trachurus*. Total concentration of paralytic shellfish toxins (µg STXeq kg^{-1}) determined in fish viscera and estimated for the whole body (median, 25% and 75% quartiles, minimum, maximum and outliers). Sample collected on 9 September 2009.

PSTs in fish

PSTs were detected in fish collected on 9 September but were not detected in fish samples collected afterwards. The toxins were restricted to the viscera of fish. High levels were determined, ranging from 120 to 2370 µg STXeq kg^{-1}, with an extreme high level reaching 4800 µg STXeq kg^{-1}. In terms of whole body, estimated from the proportion of viscera to the whole body, the

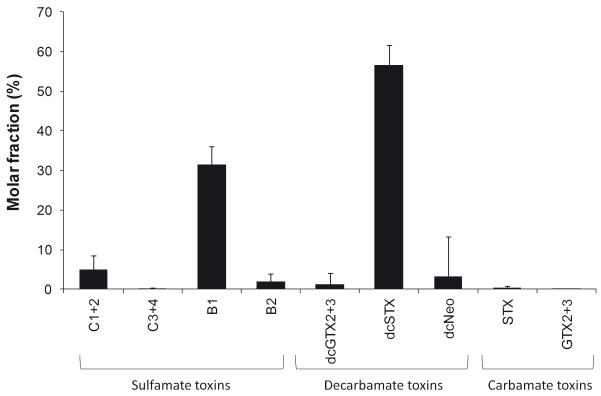

FIG. 4. – *Trachurus trachurus*. Profile of paralytic shellfish toxins (% molar fraction, means ± SD) in fish collected on 9 September 2009.

toxicity ranged from 10 to 370 μg STXeq kg^{-1} (Fig. 3). The profile of toxins in the horse mackerel was dominated by dcSTX, with a molar ratio of 56.5%, followed by B1 with 31.6% (Fig. 4). The minor toxins were C1+2 (4.9%), dcNeo (3.2%), B2 (1.9%), dcGTX2+3(1.3%), STX (0.4%), GTX2+3 (0.1%) and C3+4 (0.1%).

DISCUSSION

The PSTs determined in Atlantic horse mackerel were associated with a bloom of the toxin producer *Gymnodinium catenatum*. While a good correlation was previously reported between cell densities of *G. catenatum* and sardines (Costa *et al.* 2010), the Atlantic horse mackerel does not feed directly on algae, which means that toxin levels may not correlate well with the toxigenic algae. The relatively weak intensity of the bloom, expressed by the maximum cell density of 5.0 10^3 cells l^{-1}, may suggest that mackerel must have ingested a high biomass of contaminated prey to accumulate such high PST levels. Atlantic horse mackerel is known to prey on copepods and decapod larvae in coastal areas (Cabral and Murta 2002), which in turn may have taken up large quantities of toxins from algae. The transfer of contaminants from one trophic level to the next with increasing concentrations would suggest biomagnification of PSTs. However, toxins were not stored or retained in fish. Throughout the survey of the present study, toxins were only found in fish caught on 9 September. Although one study points to a progressive accumulation of PSTs in the liver of mackerel *Scombrus scombrus* throughout their life (Castoguay *et al*. 1997), our results are in accordance with studies carried out under controlled laboratory conditions showing that PSTs are rapidly depurated from fish tissues (Kwong *et al*. 2006, Bakke and Horsberg 2010, Costa *et al*. 2011). The fact that PSTs are hydrophilic compounds with low accumulation efficiencies (Costa *et al*. 2011) further supports the hypothesis that detection of PSTs is restricted to samples caught during the bloom.

The high levels observed in the present study, up to 4800 μg STXeq kg^{-1} in the fish viscera, are not commonly reported unless associated with fish kills (Mortensen 1985, Jester *et al*. 2009, Fire *et al*. in press). Species-specific differences in sensitivity of fishes to PSTs have been previously investigated (White 1981) and mortality of caged salmon has even been attributed to levels as low as 40 μg STX eq. kg^{-1} (Sephton *et al*. 2007). Our results point to the high potential vectorial role of Atlantic horse mackerel in the marine food web. This fish species is one of the most important pelagic fish species in terms of abundance, it is widely distributed and is a prey item of top predators, such as marine mammals (Silva 1999). In terms of whole body fish toxicity, samples collected revealed a maximum estimated value of 370 μg STXeq kg^{-1}.

Characterization of the profile of PSTs in the fish samples revealed important data. While dcSTX is a minor component of the profile of *G. catenatum*, usually corresponding to approximately 4% of the toxins (Negri *et al*. 2007, Costa *et al*. 2010, 2012), it accounted for more than 50% of the amount of toxins in Atlantic horse mackerel. B1 was the second most important toxin analogue detected in fish. The present study is in line with results obtained from laboratory experiments showing a prevalence of dcSTX and B1in white seabream (*Diplodus sargus*) orally challenged with cockles contaminated during a *G. catenatum* bloom (Costa *et al*. 2011). A higher elimination rate was calculated for B1 than for dcSTX in white seabream (Costa *et al*. 2011), which may justify the higher concentration of dcSTX than B1 in mackerel. Moreover, these toxins may be the result of inter-conversion of PSTs (Costa *et al*. 2011). Interestingly, the octopus, which also plays a central position in the marine food web, has been found to accumulate high levels of these two PSTs in the digestive gland (Monteiro and Costa 2011). Selective elimination of these toxins with higher elimination of B1 and retention of dcSTX were suggested for octopus (Monteiro and Costa 2011). The present study, together with those cited above, shows the importance and prevalence of dcSTX and B1 in the marine food web after blooms of *G. catenatum*.

The pervasiveness of dcSTX in the marine ecosystem may well require a risk evaluation of routes of exposure, species-specific and population-specific sensitivities, depuration rates, and compartmentalization. Furthermore, the potency of PSTs was recently re-evaluated. The same (the highest) level of potency was attributed to STX and dcSTX (Vale *et al*. 2008, EFSA 2009).

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