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Occurrence of palytoxins in marine organisms from different trophic levels of the French Mediterranean coast harvested in 2009

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ABSTRACT

Four sites located in Nice and Villefranche-sur-Mer, on the French Mediterranean coast, were monitored during the summer of 2009 for the presence of epiphytic and planktonic *Ostreopsis* cf. *ovata*, and that of palytoxin (PlTX) and 2 of its analogues (ovatoxin-a (OVTX-a) and ostreocin-D (OST-D)) in different marine organisms.

Several of the 15 species that were sampled between June and September 2009 were found to be contaminated with OVTX-a as the major toxin (90% of the toxin profile) and PlTX; this included fish, echinoderms, gastropods, crustaceans and cephalopods. The contamination levels varied geographically and between species, with the herbivorous species generally having higher toxin levels than carnivorous ones.

The determination of the toxin distribution between the digestive tube (DT) and the remaining tissue (RT) or roe in the case of the sea urchin *Paracentrotus lividus* showed that the toxins were sequestered in the DT. The highest toxin level ever recorded over the course of the study was of 392.2 μg for the sum of OVTX-a and PlTX per kg of DT of the flathead mullet *Mugil cephalus*. No quantifiable levels of toxins were found in the roe of the sea urchins or in the RT of the other marine products. However, in several cases, the toxin level in the whole flesh of the analysed organisms was above 30 μg OVTX-a + PlTX/kg, when knowing that the European food safety authority's opinion is that an adult should not ingest more than 30 μg PlTX + OST-D per kg of shellfish meat to avoid putting the consumer's health at risk. This was observed for the following four species: the sea urchin *P. lividus*, the red-mouthed rock shell *Stramonita haemastoma*, the warty crab *Eriphia verrucosa* and the flathead mullet *M. cephalus*.

The collection of such data is of great importance to refine and complete the risk assessment of PlTX and its analogues and has to be encouraged in order to provide reliable information for setting up a regulatory level that would protect the consumers of edible marine organisms

1. Introduction

Palytoxin (PlTX) is a complex non-protein compound with amphiphilic properties that was first isolated from the zoanthid *Palythoa* sp. after which it was named (Moore and Scheuer, 1971). The production of PlTX and PlTX-like compounds by dinoflagellates of the genus *Ostreopsis* has been reported in the literature (Onuma et al., 1999). *Ostreopsis* blooms were solely reported in tropical areas until the beginning of the 2000s with the first blooms in temperate waters, especially in the Mediteranean (Ciminiello et al., 2006) and the Aegean sea (Aligizaki et al., 2008; Aligizaki and Nikolaidis, 2006). This highlighted the human risks associated with the exposure via aquatic activities and through the consumption of contaminated marine organisms.

During the summer of 2005, about 200 people sought medical treatment in Genoa, Italy, because of cutaneous and respiratory problems after being exposed to marine aerosols; Ciminiello and co-workers (2006) showed the presence of PlTX associated with *Ostreopsis ovata*. In France, the presence of *Ostreopsis* spp. was responsible for similar symptoms between 2006 and 2009 reported by a few people who went swimming and diving on the Mediterranean coast (Tichadou et al., 2010).

PlTX and PlTX-like compounds have been reported in marine organisms collected in various tropical countries including Colombia, Madagascar, Philippines, Japan, Australia, and Micronesia (Aligizaki et al., 2011; Gleibs and Mebs, 1999; Munday, 2008) and some cases of food poisoning were reported in the literature, some being fatal (Aligizaki et al., 2011; Deeds and Schwartz, 2010;

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Munday, 2008; Tubaro et al., 2011b). Several symptoms were reported by the consumers after eating contaminated marine organisms; these included a metallic taste, gastrointestinal malaise, diarrhoea, nausea, vomiting, ataxia, dizziness, numbness of extremities, myalgia, dyspnoea, convulsions and bradicardia (Tubaro et al., 2011b).

No food poisoning has been associated with *Ostreopsis* blooms in the Mediterranean, even though the presence of PlTX-like compounds was reported in shellfish collected in the Aegean Sea (Greece) in 2003 (Aligizaki et al., 2008), in mussels and sea urchin from the Ligurian coastline (Bellocci et al., 2008b), and from the French Mediterranean coast (Amzil et al., 2009) and during this bloom of *Ostreopsis* in the French Mediterranean coast no seafood poisoning was reported by the epidemiological system.

The data reported in the literature regarding the presence of PlTX and PlTX-like compounds in edible marine organisms were obtained with different methods, including biological (mouse bioassay, haemolytic test) and chemical methods (LC–MS). The toxin levels accounted for in these reports vary, some being expressed as mouse or haemolytic units per gram or as microgram of PlTX per kilogram of specimen; this makes it difficult to compare the different sets of data and thereby does not facilitate the risk assessment. Yet, an important step has been made in that direction, as a scientific panel under the aegis of the European food safety authority (EFSA) assessed the risk associated with the presence of PlTX and its analogue ostreocin-D (OST-D) in shellfish and estimated that the toxin level should not exceed 30 μg for the sum of PlTX and OST-D per kg (μg PlTX + OST-D/kg) of shellfish meat to protect the consumers' health (EFSA, 2009). This risk assessment was performed on the basis of the scarce toxicological, occurrence and consumption data available at the time. It pointed out the need for additional information on the toxicity of the different analogues, as well as on the occurrence of these toxic compounds in marine products, other than shellfish, destined to human consumption, in order to refine and complete the EFSA risk assessment that currently only concerns the shellfish. The provision of such data can come from monitoring programmes as well as from research projects.

The present study aimed at collecting information on the occurrence and the distribution of PlTX and 2 of its analogues (OVTX-a and OST-D) in a wide variety of edible marine organisms harvested in four recreational sites located in Nice and Villefranche-sur-Mer on the French Mediterranean coast. The sampling campaign took place between June and September 2009, in locations where *Ostreopsis* cf. *ovata* had previously been observed during the same periods.

2. Materials and methods

2.1. Sampling locations

The choice of the first two sites (Fig. 1), site N (43°41′27.19″ N and 7°17′35.09″ E) located east from Nice harbour and site V1 (43°42′6.87″ N and 7°19′14.45″ E), located in Villefranche, south from "Plage de la Réserve", was based on high *Ostreopsis* cf. *ovata* abundances recorded in 2008.

Two other locations were also sampled when blooms of *Ostreopsis* occurred in the area; these are site V2 in Villefrance, which corresponds to "Plage des jeunes" (43°42′10.81″ N and 7°19′13.04″ E) and site V3, also known as "Rochambeau" (43°41′34.83″ N and 7°18′31.66″ E).

2.2. Sampling periods

The edible marine organisms targeted in the study as well as the planktonic and epiphytic *Ostreopsis* samples were collected between the 2nd of June (week 23) and the 31st of September 2009 (week 39), with variations depending on the site. During the course of the sampling period, samples were taken on a bimonthly basis in sites N and V1. The sampling frequency was increased to weekly when the toxic bloom was detected.

Additional sampling on a wider range of marine organisms in sites V2 and V3 was carried because *Ostreopsis* cf. *ovata* blooms occurred in these areas.

2.3. Sampling of marine organisms

A wide variety of marine organisms available on site were sampled in the four locations and included molluscs (gastropods and cephalopods), echinoderms, fish, and crustaceans (Table 1).

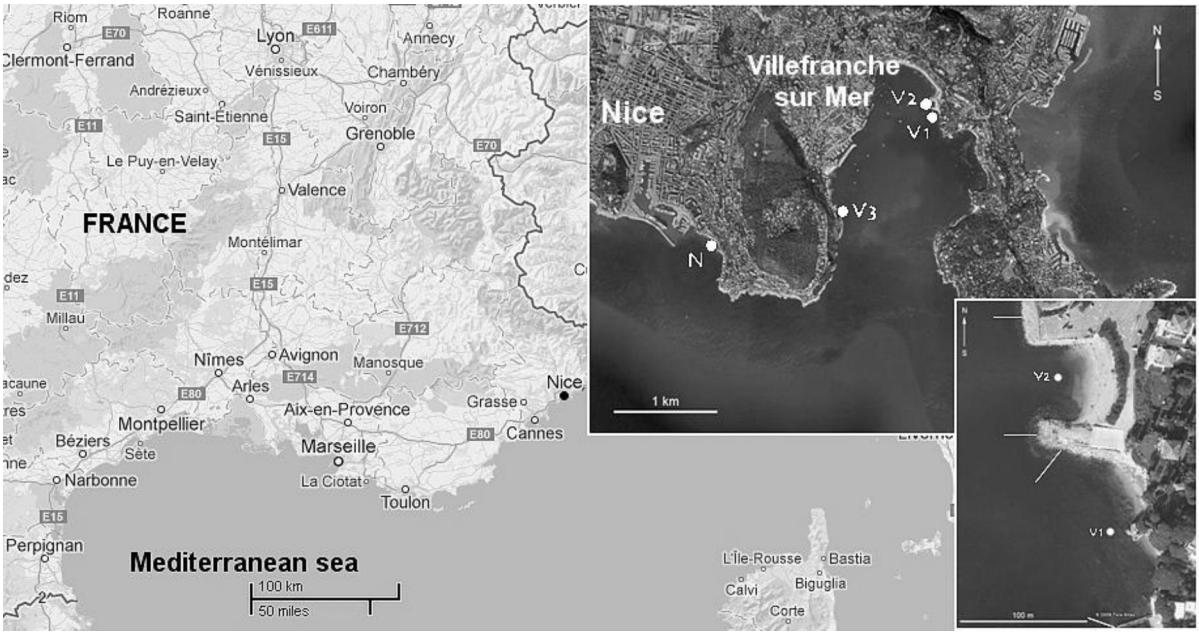

Fig. 1. Location of the sampling sites selected for the study. N = Nice, Plage de la Réserve; V1 = Villefranche, beach located below Plage des Jeunes; V2 = Villefranche, Plage des jeunes; V3 = Rochambeau.