Food Control 25 (2012) 285–291

Contents lists available at SciVerse ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

First report of Paralytic Shellfish Poisoning (PSP) in mussels (*Mytilus galloprovincialis*) from eastern Adriatic Sea (Croatia)

Ivana Ujević [a], Romana Roje [b,*], Živana Ninčević-Gladan [a], Ivona Marasović [a]

[a] Institute of Oceanography and Fisheries, Šetalište I. Meštrovića 63, 21000 Split, P.O. Box 500, Croatia
[b] Erasmus Mundus Master of Science in Marine Biodiversity and Conservation, Doverska 9, 21000 Split, Croatia

ARTICLE INFO

Article history:
Received 3 March 2011
Received in revised form
14 October 2011
Accepted 22 October 2011

Keywords:
PSP
Shellfish toxicity
Saxitoxin
Mussels
HPLC-FLD
Adriatic Sea

ABSTRACT

The chromatographic HPLC-FLD method was introduced for the first time to identify and quantitatively determine individual Paralytic Shellfish Poisoning toxins accumulated in aquacultured shellfish from Croatian coastal waters. Populations of Mediterranean mussels (*Mytilus galloprovincialis*) were contaminated with PSP toxins throughout January to April 2009 leading to the positive test results by Mouse Bioassay (MBA). Until 2009 there was no evidence of PSP toxins in the examined samples. For the first time an instrumental method revealed the PSP toxin profile of samples taken along the eastern Adriatic coast and identified saxitoxin (STX) as the main representative of this toxin group that may cause paralysis and death in consumers of contaminated shellfish. This phenomenon may have serious health and economic consequences. Following these potential consequences, marine biotoxins (PSP, ASP and DSP) are continuously assessed in bivalves from 25 breeding and harvesting areas along the Croatian Adriatic coast. Positive MBA results were confirmed by instrumental method in two out of three recorded samples. Saxitoxin was the dominant PSP toxin extracted from contaminated mussels within the range of 53.17–1298.17 μg g^{-1}, that contributed more than 70% to the total shellfish toxicity, followed by gonyautoxins 2 and 3 (GTX 2,3) which contributed 27% and decarbamoylsaxitoxin (dcSTX) that accounted for less than 2%, considering all stations.

1. Introduction

1.1. Croatian shellfish production

Shellfish farming is widespread in Croatia along its entire coastline with particularly long tradition on the far south of the Croatian coast (Pelješac Peninsula and Mali Ston Bay; Fig. 1). Mali Ston Bay is the most important shellfish farming area in Croatia with more than one hundred years long tradition. Croatian shellfish industry, with its production of 3000–4000 tons of mussels and 1 million oysters per year is small when compared to the other countries, but it has a high potential for aquaculture expansion within its 33 200 km^2 of territorial waters, 5835 km of coastline and 1246 islands (Anonymous, 2008).

There are 25 sampling sites from shellfish breeding and harvesting areas along the Croatian coast of the Adriatic Sea included in continuous monitoring (in accordance with EU Directives) of shellfish and seawater quality that has begun in July 2000. These sites include: 9 sites near Istria Peninsula, 4 near Zadar, 4 near Šibenik, 1 near Split and 7 sites in Mali Ston Bay, Pelješac Peninsula and Mljet Island (Fig. 1).

1.2. The occurrence of phytotoxic species and shellfish toxicity in the Adriatic Sea

Alexandrium species have been observed in the Adriatic since 1976 (Boni, 1983). Shellfish toxicity outbreaks in the Adriatic Sea were documented in the northwestern part of the Adriatic Sea in 1989 (Boni et al., 1992), however PSP toxicity was first recorded during an *Alexandrium minutum* bloom in the coastal waters of the northern Adriatic (Emilia Romagna) in the spring of 1994 (Honsell et al., 1996). Orhanović, Ninčević, Marasović, and Pavela-Vrančić (1996) reported massive fish kills by intensive red tide bloom of *Lingulodinium polyedrum* accompanied by *A. minutum* in Kaštela Bay (central Adriatic) in the summer of 1994, but the presence of PSP was not specifically stated in the report. Although *L. polyedrum* is considered to produce yessotoxins (Paz, Riobó, Fernández, Fraga, & Franco, 2004) and there is no strong relationship with the PSP toxicity, Bruno, Gucci, Pierdominici, Ioppolo, and Volterra (1990) reported the presence of saxitoxin (STX) in water samples taken

* Corresponding author. Tel.: +385 21 467 906; fax: +385 21 358 650.
E-mail address: romana.roje@ugent.be (R. Roje).

0956-7135/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodcont.2011.10.050

Fig. 1. Geographic locations of regular sampling sites (marked with green circles) along the Croatian coast with designated locations (stations S1, 2 and 3, Istria peninsula and Mali Ston Bay are marked with red arrows) where PSP toxins were established during the 2000–2009 period of investigation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

during a bloom of *L. polyedrum* in the Adriatic Sea. During 1995 and 1996, Marasović, Ninčević, Pavela-Vrančić, and Orhanović (1998) speculated that PSP toxins were detected in shellfish, but not at the level considered hazardous to human health based on the MBA test. There are more reports of PSP toxic dinoflagellate occurrence than of PSP shellfish contamination in the Mediterranean waters (Ciminiello, Fattorusso, Fiorino, & Montresor, 2000; Giacobbe et al., 2004; Honsell et al., 1996; Lilly, Kulis, Gentien, & Anderson, 2002; Montresor, John, Beran, & Medlin, 2004; Vila, Garcés, Masó, & Camp, 2001). Marasović et al. (2007) stated that PSP toxicity has never been recorded along the eastern Adriatic coast since the Croatian 'National monitoring programme of shellfish farms' began in 2000, while ASP toxicity was recorded in a very few samples with concentrations below the limits set by the European Commission and there were extremely high DSP shellfish toxicity events recorded in 2005 in breeding areas along the western Istrian coast in the northern Adriatic (Ninčević-Gladan et al., 2008; Ujević et al., 2010).

Therefore, in this study we introduced HPLC-FLD (Lawrence, Niedzwiadek, & Menard, 2005) method for qualitative and quantitative identification of PSP toxin types in Croatian shellfish, as an effort to substitute MBA and to implement it in continuous monitoring. We also established the first PSP toxin types list for Croatian coastal waters and tried to find the relationship between the occurrences of PSP shellfish toxicity and the presence of causative phytoplankton species, based on available data.

2. Materials and methods

From the year 2000 through 2009 sampling frequency per site was once a month from November until May and twice a month from May through October. Istria Peninsula had a weekly sampling frequency from April through November in 2007 and 2008. Starting in 2009, samples were acquired fortnightly from January through March and weekly from April through December, with the exception of sampling sites at Istria Peninsula which had a weekly sampling throughout the entire year. Mediterranean mussel (*Mytilus galloprovincialis*) is the dominant cultured species in this area, while European flat oyster (*Ostrea edulis*), Mediterranean scallop (*Pecten jacobaeus*) and proteus scallop (*Flexopecten proteus*) are present as wild populations at three stations on the West coast of Istria. For this survey all the 3408 shellfish samples have been taken into consideration for PSP toxins testing by MBA. Among all, only 12 mussel samples from S1 location in the northern Adriatic Sea (Fig. 1) showed the PSP toxins presence by MBA, and we subsequently analyzed 11 samples (for one sample there was not enough tissue left after used for DSP analysis). Except these samples, there were 19 shellfish samples that were suspected (death of 1 mouse within 60 min or death time interval in two or three mice between 1 and 24 h, by MBA) for PSP presence during this monitoring period. Among these, 5 were missing, therefore HPLC-FLD analysis was performed on 14 of them.