CROATICA CHEMICA ACTA
CCACAA **77** (4) 627–631 (2004)

ISSN-0011-1643
CCA-2969
Original Scientific Paper

Paralytic Shellfish Poisoning (PSP) in the Central Adriatic Sea

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RECEIVED APRIL 7, 2003; REVISED MARCH 31, 2004; ACCEPTED MAY 11, 2004

Key words
Central Adriatic Sea
phytoplankton toxins
Paralytic Shellfish Poisoning (PSP)
Alexandrium minutum

Surveillance of Paralytic Shellfish Poisoning (PSP) in the Kaštela Bay, Central Adriatic Sea, was conducted in the summers of 1995 through 1997. Toxicity was assessed by the mouse bioassay according to the method proposed by the Association of Official Analytical Chemists (AOAC). Incidence of shellfish intoxication by PSP toxins was recorded on several occasions, however at levels not endangering human health. Along with shellfish sampling, seawater samples were taken for analysis of the phytoplankton community and the numerical cell abundance. The phytoplankton composition demonstrates a recurring incidence of *Lyngulodinium polyedra,* and frequent presence of species from the *Alexandrium*, *Gymnodinium* and *Gyrodinium* genera, well known progenitors of PSP and its analogues. Possible recurrence of such phenomena and their distribution to other regions of the Adriatic coast may have serious health and economic consequences, calling for continuous monitoring of phytoplankton composition and toxicological studies of shellfish.

INTRODUCTION

Red tide blooms and mucilaginous aggregations have been known to occur for more than 20 years in the Adriatic area. However, the occurrence of toxic phytoplankton blooms, associated with shellfish intoxication in this region, is a relatively new phenomenon. The first report on the link between the causative organism and shellfish toxicity relates to episodes of Diarrheic Shellfish Poisoning (DSP) in the northern Adriatic.[1] Harmful algal blooms can affect human health through accumulation of toxic substances in the digestive glands of filter-feeding shellfish, thereby leading, through consumption of contaminated seafood, to poisoning in humans. Species of the *Alexandrium, Gymnodinium*, *Gyrodinium* and *Pyrodinium* genera are responsible for the generation of a variety of bioactive compounds (Figure 1) known to produce neurotoxic symptoms called Paralytic Shellfish Poisoning (PSP).[2,3] Today, the PSP syndrome is a worldwide occurrence, in both cold and warm seas. The distribution of PSP in Europe is presented in Figure 2. Ingestion of PSP-contaminated seafood causes a tingling sensation in the finger tips and toes progressing to arms and legs, followed by a general weakness, loss of balance, slight respiratory insufficiency, and finally muscular paralysis, severe respiratory difficulty and death.[4] However, PSP compounds are also important pharmacological tools, used as sodium channel blockers.[5]

Since 1976, incidents of the *Alexandrium* species have been a frequent occurrence in the Adriatic.[6–8] PSP shellfish toxicity was first monitored in the waters of the

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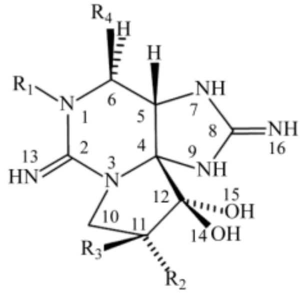

R_1	R_2	R_3	Carbamates $R_4 = H_2N\text{-}COO\text{-}$	*N*-Sulfocarbamoyls $R_4 = {}^{-}OSO_2\text{-}HN\text{-}COO\text{-}$	Decarbamoyls $R_4 = OH$
H	H	H	STX	B1	dc-STX
OH	H	H	NEO	B2	dc-NEO
OH	H	OSO_3^-	GTX I	C3	dc-GTX I
H	H	OSO_3^-	GTX II	C1	dc-GTX II
H	OSO_3^-	H	GTX III	C2	dc-GTX III
OH	OSO_3^-	H	GTX IV	C4	dc-GTX IV

STX: saxitoxin; NEO: neosaxitoxin; GTX: gonyautoxin

Figure 1. Chemical structure of common PSP toxins.

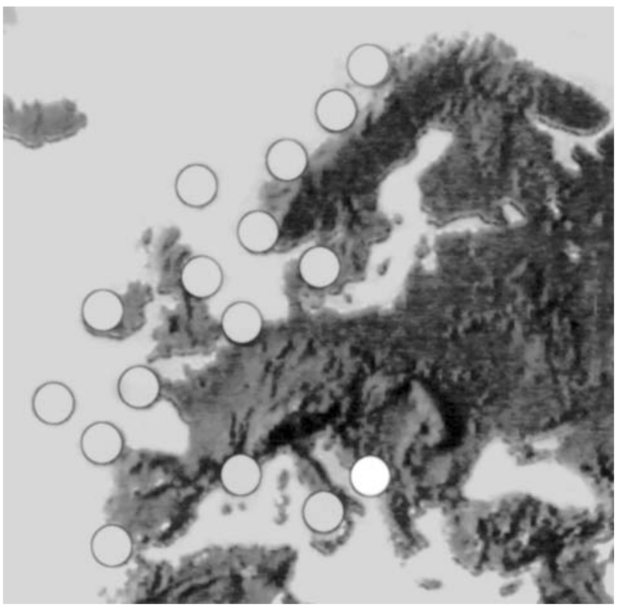

Figure 2. The geographical distribution of PSP in Europe. The investigated location in the Adriatic is marked white.

northern Adriatic in the spring of 1994, during a bloom of *A. minutum.*[9] However, little attention has been given to the extent to which this and related organisms cause intoxication of shellfish in the coastal waters of the central Adriatic Sea. The most aggravated region in the central Adriatic is the eastern part of the Kaštela Bay (Vranjic Basin), heavily polluted by household and industrial wastes. Since 1980, a *Lyngulodinium polyedra* bloom has been regularly occurring in the Bay every summer, often with lethal effects on marine organisms.[10] In addition to *L. polyedra*, the predominant species, several other toxic and potentially toxic phytoplankton species have been identified, including *A. minutum* and members of the *Dinophysis* genus (*D. fortii, D. caudata, D. sacculus, D. acuta,* and *D. acuminata*). Analysis of shellfish samples from the Kaštela Bay in 1994 established the presence of toxins from the DSP and PSP class of compounds.[11] The occurrence of DSP toxicity could be associated with the incidence of species from the *Dinophysis* genus,[12] whereas PSP toxicity was ascribed to a more frequent occurrence of *A. minutum* in the Bay,[13] emphasizing the necessity of exploring the PSP phenomenon essential in evaluating the risk of human poisoning by consumption of PSP-contaminated seafood.

EXPERIMENTAL

Sampling

Wild populations of the mussel *Mytilus galloprovincialis* were harvested at regular intervals from a localized site in the eastern part of the Kaštela Bay, Vranjic Basin, Central Adriatic Sea (FI: 43°29'–43°33', LA: 16°15'–16°29'). The mussels were collected at a depth of 50 cm, and stored at –20 °C.

Phytoplankton Analysis

The composition of the phytoplankton community in waters surrounding the sampling site was determined according to Utermöhl (1958).[14] Seawater samples were preserved in formaldehyde (Φ = 2 %) solution. Counts and identification of the organisms were conducted using an Opton inverted microscope. The live material was analyzed using an Olympus IMT-2 microscope with a Nomarski difference contrast attachment.

Mouse Bioassay

PSP analysis by the mouse bioassay was conducted according to the method proposed by the Association of Official Analytical Chemists (AOAC).[15] Homogenized shellfish tissue (100 g) was treated by boiling for 5 min in 100 mL acidified water (0.1 M HCl, pH < 4). After cooling to room temperature, the pH was adjusted to pH 2–4 by addition of either 5 M HCl or 0.1 M NaOH in order to prevent local alkalization or decomposition of the toxins. Water was added up to a total volume of 200 mL, and the mixture was centrifuged at 3000 r.p.m. for 5 minutes. The supernatant was decanted and acidified if necessary. The acidic extract (1 mL) was inoculated into the peritoneum of the mouse (strain BALB/C, weight limits 18–20 g). Three parallel tests were done, and the reaction of the mice was observed for 24 hours after treatment, or until death.

RESULTS AND DISCUSSION

The present work contributes to the current topic on the production of bioactive secondary metabolites by the dinoflagellates, their involvement in fish and shellfish poisoning, and its impact on public health and the shellfish

TABLE I. Incidence of PSP toxicity in shellfish collected from the Kaštela Bay (Central Adriatic Sea) from 1995 to 1997, and the abundance of the potentially toxic species in the accompanying phytoplankton community

Time of sampling	Abundance *L. polyedra* cells dm^{-3}	Abundance *A. minutum* cells dm^{-3}	Abundance *Gyrodinium sp.* cells dm^{-3}	PSP toxicity (mouse bioassay)
1995				
July	1.5×10^7	1.0×10^6	–	+
1996				
May	–	1.8×10^4	4.5×10^4	–
June	7.8×10^4	8.1×10^3	4.8×10^5	+
July	mucilage	mucilage	mucilage	+
August	–	–	4.2×10^5	+
September	–	–	–	+
October	–	–	–	–
1997				
May	6.6×10^4	–	–	–
June	–	–	–	–
July	–	–	–	–

industry. Shellfish toxicity was assessed by the mouse bioassay. Along with shellfish sampling, seawater samples were taken for analysis of the phytoplankton community and the numerical cell abundance (Table I).

In the Adriatic, PSP toxicity was first recorded in the spring of 1994 in the coastal waters of the northern Adriatic (Emilia Romagna) during a bloom of *A. minutum*.[9] In the summer of 1994, an intensive red tide bloom of L. *polyedra* resulted in massive fish kills in the Kaštela Bay, Central Adriatic Sea.[11] Despite a rather low abundance of *A. minutum*, the mussels displayed contamination with PSP toxins, however at concentrations below the alert safety level. In the summer of 1995, during the usual *L. polyedra* bloom, both *A. minutum* and species from the *Gymnodinium* genus were present in the phytoplankton community.[12] *L. polyedra* was markedly dominant, its numerical abundance ranging from 7.2×10^4 to 1.5×10^7 cells dm^{-3}. *A. minutum* was observed only in July, and although its abundance was high, 1×10^6 cells dm^{-3}, only traces of PSP toxins were recorded in shellfish. In the summer of 1996, the presence of PSP was established again, however at levels not considered dangerous for mammals or humans based on the mouse survival time of more than one hour (the level of PSP shellfish toxicity endangering human health). The phenomenon occurred in June and continued throughout the summer season, but in October 1996 shellfish intoxication with PSP toxins could no longer be detected. The highest level of toxicity was recorded in September 1996, substantiated by a shorter mouse survival time. The uptake and depuration of PSP toxins by shellfish is species and location specific.[16] Shellfish from the *Mytilus* group rapidly accumulate PSP compounds, and depurate them faster than any other shellfish culture. At the beginning of the summer bloom, *A. minutum* appeared at a numerical abundance of 1.8×10^4 cells dm^{-3}, whereas *L. polyedra* was present at a concentration of 7.8×10^4 cells dm^{-3}. Besides *A. minutum* and *L. polyedra*, a bloom of an unusual species from the *Gyrodinium* genus (Figure 3), known as a potential causative organism of PSP intoxication, has been recorded. The toxicity assay conducted in the summer of 1997 presented a negative response, demonstrating the absence of PSP toxins in shellfish. Although *L. polyedra* occurred at an abundance of 6.6×10^4 cells dm^{-3}, *A. minutum* and the *Gyrodinium* species were not detected in the phytoplankton community.

The incidence of PSP toxicity in the summers of 1995 and 1996 was associated with the occurrence of *A. minutum*, an organism worldwide known as the causative agent of Paralytic Shellfish Poisoning. *A. minutum* is a widely distributed species (Egypt, Greece, Spain, Portugal, Ireland, Italy, Turkey) with varying toxicity. Cases of a toxicogenic strain of *A. minutum* were documented in 1986 along the Australian coast,[17] and in 1994 in the Harbour of Alexandria,[18] but significantly lower levels of toxicity were recorded in species from Spain in 1989,[19] and no toxicity was found in shellfish during an *A. minutum* red tide in Turkey in 1983.[20] Moreover, PSP toxin profiles and total toxicity per cell may vary among the different *A. minutum* strains. If PSP toxicity in the Kaštela Bay in the summers of 1995 and 1996 can be associated with the occurrence of *A. minutum*, an explanation for the discrepancy between the numerical cell abundance and the level of toxicity may lie in the interrelationship of factors governing phytoplankton toxicity, such as nutrient concentration, environmental factors and the growth phase. It has also been suggested that the interac-

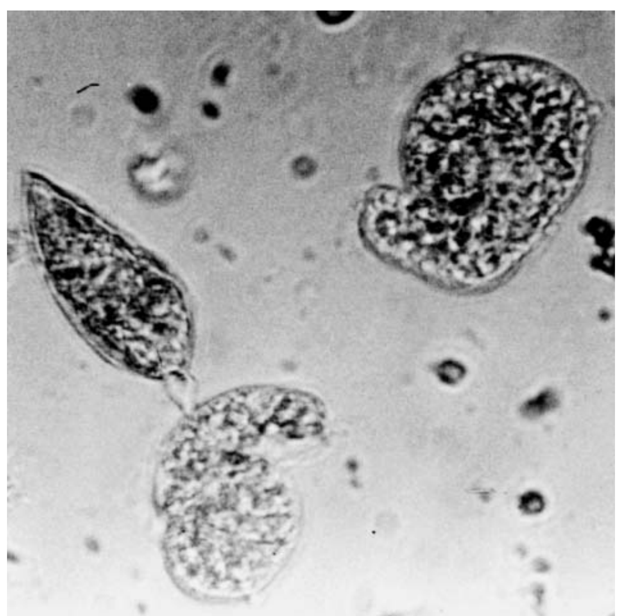

Figure 3. Unidentified species from the *Gyrodinium* genus found in the Kaštela Bay.

tion of bacteria with dinoflagellates could be responsible for the production of PSP toxins by the dinoflagellates.[21]

Another possible explanation for the occurrence of PSP toxicity in the Kaštela Bay in the summer of 1996 is the presence of an unusual species from the *Gyrodinium* genus (Figure 3). It has been established that several unidentified species from the *Gymnodinium* and *Gyrodinium* genera may cause PSP toxicity. Although the species was detected in May, first signs of shellfish intoxication were recorded in June, when the shellfish had accumulated enough toxic phytoplankton cells. Toxicity was maintained throughout the summer, with a concomitant occurrence of the *Gyrodinium* species in the Bay. The highest abundance of the *Gyrodinium* species was recorded in August, followed by increased shellfish toxicity in September.

Although recorded only in trace amounts, the incidence of PSP in shellfish from the central Adriatic implicates potential problems with serious health and economic consequences. If the accumulated toxins are not depurated following exposure, the relative amount of the decarbamoyltoxins is likely to increase over time from further biotransformation, leading to a dramatic increase in toxicity, even if the PSP source is removed.[4,22] Hence, the possible recurrence of this phenomenon, the variability of toxin composition, the metabolic activity in shellfish digestive glands known to modify the algal toxin structure and consequently its toxicity level, and the wide diffusion of these species to other regions of the Adriatic necessitates continuous monitoring of the toxin content in the shellfish population and the accompanying phytoplankton community.

Acknowledgement. – This work was supported by grants from the Ministry of Science and Technology, Republic of Croatia, No. 177050 and No. 000100101.

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SAŽETAK

Paralitičko trovanje školjkašima (PSP) iz srednjega Jadrana

Maja Pavela-Vrančič i Ivona Marasović

Pojava paralitičkih toksina (PSP, Paralytic Shellfish Poisoning) u Kaštelanskome zaljevu (Srednji Jadran) praćena je analizom školjkaša u ljetnim mjesecima od 1995. do 1997. godine. Prisutnost toksina ustanovljena je pokusom na miševima u skladu s preporukom AOAC (Association of Official Analytical Chemists). U nekoliko navrata zabilježena je intoksikacija školjkaša PSP toksinima, ali u količinama koje ne ugrožavaju ljudsko zdravlje. Pored uzoraka školjki, sakupljeni su i uzorci morske vode za analizu fitoplanktonske zajednice i brojnosti stanica. Sastav fitoplanktonske zajednice ukazuje na učestalo pojavljivanje vrste *Lyngulodinium polyedra* te prisutnost vrsta iz roda *Alexandrium, Gymnodinium* i *Gyrodinium*, poznatih uzročnika PSP trovanja. Mogućnost opetovanoga pojavljivanja ovoga fenomena i njegovo rasprostranjivanje na druga područja uz jadransku obalu može biti uzrok ozbiljnih zdravstvenih i gospodarskih problema. Stoga se nameće potreba stalnoga praćenja sastava fitoplanktonske zajednice te toksikološke analize školjkaša.