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Diarrheic shellfish poisoning due to toxic mussel consumption: first recorded outbreak in Greece

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4 1 **Diarrheic shellfish poisoning due to toxic mussel consumption: first**
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6 2 **recorded outbreak in Greece**
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1
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3 **1 Abstract**
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6 2
7 3 During the week 14-20th January 2000, 120 people visited the Emergency Departments of
8
9 4 Thessaloniki (Northern Greece) hospitals complaining of acute gastrointestinal illness
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11 5 after eating mussels (*Mytilus galloprovincialis*). The symptoms indicated diarrhoeic
12
13 6 shellfish poisoning and the toxicity of mussels harvested from Thermaikos Gulf in
14
15 7 Thessaloniki during the outbreak, was investigated using mouse bioassays. The
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17 8 bioassays revealed toxicity to mice by the mussel samples, while high numbers of toxic
18
19 9 algae *Dinophysis acuminata* were identified in water samples from Thermaikos Gulf.
20
21 10 Harvesting of mussels was immediately suspended and a monitoring programme for algal
22
23 11 blooms was established from then onwards. During a follow-up of the mussels' toxicity
24
25 12 from January 2000 through January 2005, two more mussel samples were found positive
26
27 13 for DSP, one harvested in March 2001 from the area of the outbreak (Thermaikos Gulf)
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29 14 and one harvested in January 2001 from North-western Greece (Amvrakikos Gulf).
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31 15 However, no sporadic cases or outbreaks were reported during this period.
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40 17 **Keywords:** Diarrheic Shellfish Poisoning, Greece, mussels, algal toxins, Dinophysis,
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42 18 outbreak
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1 Introduction

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Diarrheic shellfish poisoning (DSP) is a toxic syndrome caused by the ingestion of shellfish contaminated with algal toxins produced by marine dinoflagellates belonging to *Dinophysis* spp. (*D. fortii*, *D. acuta*, *D. acuminata*, *D. caudate*, *D. hastate*, *D. mitra*, *D. rotundata*, *D. tripos*) (Hallegraef 1997; Yasumoto 2000) and *Prorocentrum* spp. (*P. lima*, *P. maculosum*, *P. concavum* and *P. hoffmanum*) (Van Dolah 2000). The DSP toxins belong to a group of fat-soluble polyether compounds, including Okadaic acid (OA) and the closely related Dinophysistoxins (DTX). Gastrointestinal effects have only been proven for OA, DTX-1 and DTX-3 (Murata *et al.* 1982; Rossini 2000; Vale and Sampayo 2002). DTXs are lipophilic and accumulate in the fatty tissue of shellfish. The toxin profiles in shellfish display considerable seasonal and geographical variation (Yasumoto *et al.* 1985; Sechet *et al.* 1990). Ordinary cooking processes do not destroy DSP toxins (Gestal – Otero 2000).

DSP symptoms include diarrhoea, nausea, vomiting, abdominal pain and chills, which manifest within 30 min to a few hours after consumption of contaminated shellfish and persist for 3 to 4 days (Aune and Yndestad 1993). Although short-term effects are relatively mild, long-term effects are more severe and chronic exposure may promote tumour formation in the digestive system (Hallegraef 1997; Rossini 2000). OA and DTX1 are potent inhibitors of protein phosphatases and this mode of action may be linked to the observed diarrhoea, degenerative changes in the absorptive epithelium of the small intestine and tumour development (Hallegraef 1997).

1 DSP was first reported in the Netherlands in the 1960s and in Japan in the late 1970s (Kat
2 1985; Yasumoto *et al.* 1980). Since then it has been recorded in several European
3 countries including France (van Egmond *et al.* 1993), Norway (Rossebo *et al.* 1973;
4 Underdal *et al.* 1985, Torgersen *et al.* 2005), Sweden (Krogh *et al.* 1985), Spain (Campos
5 *et al.* 1982; Fernández *et al.* 1996), Italy (Viviani *et al.* 1995; Ciminiello *et al.* 1997),
6 Portugal (Vale and Sampayo 1999), Belgium (De Schrijver *et al.* 2002) former USSR
7 (Konovalova 1993). There are also reports from Japan (Yasumoto *et al.* 1984; Yasumoto
8 *et al.* 1985; Yasumoto 2000; Kawabata 1989), India (Karunasagar *et al.* 1989), Australia,
9 New Zealand, Thailand, North and South America (Hallegraef 1997; Scoging 1998; Gestal
10 – Otero 2000). From the available literature it is concluded that DSP or the presence of
11 DSP toxins in mussels, is increasingly reported particularly in Europe (Table I). The
12 increased reporting may be the result of increasing knowledge and awareness about toxic
13 shellfish poisonings (DSP, ASP and PSP) and the implementation of
14 surveillance/monitoring programmes. Also, it has to be emphasized that toxin producing
15 algae and toxic mollusks are reported from new areas, but well documented cases in
16 Europe are rare and this may be attributed to under-diagnosis and/or underreporting. The
17 shellfish most frequently implicated in DSP are mussels, but it has also been reported
18 from scallops, clams, cockles, oysters and crabs (Gestal – Otero 2000; Vale and
19 Sampayo 2002; Jørgensen *et al.* 2005; Torgersen *et al.* 2005). In the present paper the
20 first DSP outbreak in Greece is reported, together with the laboratory findings from mussel
21 samples collected during the outbreak and the subsequent four years after the outbreak.

1 **Materials and methods**

2 *The DSP outbreak investigation*

3 During the week 14-20th January, 2000, one hundred and twenty people of both sexes,
4 aged from 8 to 70 years old, visited the Emergency Departments of the hospitals in the
5 city of Thessaloniki (Northern Greece), suffering from acute non-febrile gastro-enteritis
6 (Table II). The predominant symptoms reported by the patients were diarrhea, nausea,
7 vomiting, abdominal cramps and chills, which persisted for more than 24 h. All patients
8 reported onset of symptoms within 30 min to 10 h after consuming a mussel meal (deep
9 fried mussels, poached in cheese and tomato hot sauce, mussel pilaf and cooked mussel
10 salads) in various establishments (restaurants, taverns, homes) within the Greater
11 Thessaloniki area. All the hospitalized patients recovered completely within 12 to 36 h
12 after the administration (i.v) of saline solution and were completely asymptomatic upon
13 exit from the hospital. [Insert Table II about here]

14
15 Fecal samples from the hospitalised patients were examined for routine bacterial, viral
16 and parasitic agents causing non febrile gastroenteritis, but they were found negative. The
17 common history of the patients (reporting a mussels' meal), based on relative
18 questionnaires, combined with the clinical and laboratory findings were indicative of
19 diarrheic shellfish poisoning, thus the Veterinary Authorities were immediately notified
20 and consumption of mussels was banned until further notice. Mussel dishes are very
21 popular in the coastal areas of Greece, especially in the vicinity of Thessaloniki and
22 accordingly it was estimated that the actual number of people infected was actually much
23 larger than the 120 cases recorded at the hospitals' admissions desks. Hence, the
24 estimates were for a few hundreds of people infected, who either visited privately a

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3 1 general practitioner or asked no medical assistance because their symptoms soon
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5 2 subsided.
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10 4 The patients who had prepared their meals at home reported purchase of fresh mussels
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12 5 from seafood retails, supermarkets and open markets in the Thessaloniki greater area
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14 6 and similar retail sources were reported by the restaurant and tavern owners. The fresh
15
16 7 mussels (*Mytilus galloprovincialis*) sold in the markets of Thessaloniki area and in most
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18 8 markets in Northern and Northwestern Greece are harvested from aqua cultures located
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20 9 into the Thermaikos Gulf (Figure 1).
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27 11 The Veterinary and Sea Biology authorities reported numbers of dinoflagellates ranging
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29 12 from 3×10^4 to $5,4 \times 10^4$ cells/L of the toxic genus *Dinophysis spp.* in the seawater samples
30
31 13 from Thermaikos Gulf, and specifically of the species *D. acuminata* and *D. sacculus*
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33 14 (Koukaras and Nikolaidis 2002). No other episodes either of sporadic cases or outbreaks
34
35 15 associated with consumption of mussels exposed to toxin-producing Dinoflagellates of the
36
37 16 DSP type have been reported for the following four years, although the presence of toxic
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39 17 *Dinophysis acuminata* was reported again in Thermaikos Gulf in March – May 2001
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41 18 (Koukaras and Nikolaidis 2002). However, suspension of harvesting and withdrawal of
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43 19 mussels from the market are being applied sporadically in Thermaikos Gulf, whenever
44
45 20 algal blooms are detected during the phytoplankton monitoring program, which has been
46
47 21 established after the outbreak. An algal bloom is a rapid increase in the population of
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49 22 phytoplankton algae in an aquatic system. The appearance of *Dinophysis*, even at low
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51 23 densities such as 200 cells per litre, can cause already a toxification of shellfish that is
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53 24 enough to affect humans (Van Egmond *et al.* 2004). According to the Greek Veterinary
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3 1 and Sea Biology authorities, seafood harvesting is suspended when toxic dinoflagellate
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6 2 counts are higher than 200 cells/L.

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10 4 ***The mussel toxicity investigation***

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12 5 *Collection of mussel samples*

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15 6 From January 2000 to January 2005, 36 batches (500 g each) of fresh mussels were
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17 7 examined for DSP toxins contamination. The mussel samples (species *Mytilus*
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19 8 *galloprovincialis*) were randomly collected from local seafood markets. Twenty samples
20
21 9 were collected from Thermaikos Gulf (Halastra) in Thessaloniki (Northern Greece) and
22
23 10 sixteen samples from Amvrakikos Gulf (Preveza) (Northwestern Greece) (Figure 1).
24
25 11 [Insert Figure 1 about here] Two out of the 36 samples were sent to the Food
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27 12 Microbiology Unit of the Microbiology Department; University of Ioannina from colleagues
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29 13 at the Hospital for Infectious Diseases in Thessaloniki during the DSP outbreak. These
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31 14 samples were harvested from the Halastra area of Thermaikos gulf at the 20 January
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33 15 2000. Samples after April 2001 until January 2005 were collected every 3 months. All
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35 16 fresh mussel samples forwarded to our lab were deep-frozen (-30°C) immediately after
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37 17 collection and were transported by airplane (flight duration 40 min) in well insulated boxes
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39 18 to the Food Microbiology Unit, within less than 24 hours after collection and upon arrival
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41 19 were stored at -30°C until testing. The samples were tested within 7 days time after their
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43 20 arrival in our laboratory. The samples were defrosted at room temperature and were
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45 21 thoroughly cleaned under a stream of tap water after external materials were brushed off
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47 22 the shells. From each sample, 200 g of flesh was homogenized in a blending mixer and a
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49 23 portion of 100 g was weighed using an Ohaus model 1500D balance (Ohaus Co, P.O.
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51 24 Box 2033 19A Chapin Road Pine Brook, NJ 07058) prior to extraction.
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1 2 3 1 *Extraction of DSP toxins*

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5 2 The extraction of DSP toxins was performed following the method validated by the Marine
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7 3 Laboratory, Aberdeen, UK, which is the EU designated UK National Reference Laboratory
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9 4 (NRL) for Marine Biotoxins. The method is that of Yasumoto et al (Yasumoto *et al.* 1984)
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11 5 with slight modifications. A portion of 100 g of homogenized mussel flesh was suspended
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13 6 three times and thoroughly mixed with 100 ml of acetone (Scharlau, Analar grade) at
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15 7 room temperature. The aliquots were centrifuged at 3000 rpm for 10 min in a Sorvall GLC-
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17 8 4 desktop centrifuge and the supernatants were collected and pooled. Acetone was
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19 9 removed by rotary evaporation for 1h at 60°C or until all trace of acetone had gone. An
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21 10 Eyela type N rotary evaporator (Eyela Co, 3-3-4 Honcho Nihonbashi, Chuo-ku, Tokyo
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23 11 103-0023, Japan) was used. The remaining aqueous-lipid extract was separated with
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25 12 diethyl ether (Scharlau, Analar grade), backwashed with a small volume of water and
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27 13 dried by rotary evaporation at 65°C for 2 to 4 h. The remaining material was suspended in
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29 14 4 ml of 1% Tween 60 (Merck) aqueous solution.
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38 16 *Mouse bioassay*

39 17 BALB-C strain white mice weighting 19 to 21 g each were used for the mouse bioassay.
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41 18 The mice were weighed prior to intra-peritoneal injection of 1ml of the mussel extract.
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43 19 Three mice were used for each sample. All injected mice and controls were monitored for
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45 20 a period of at least 24 h after injection. Initially the mice were observed constantly for the
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47 21 first 6 h and from then every 1 h for the remaining 18 h. During the 24 h monitoring any
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49 22 behavioral changes or symptoms were recorded in detail. When no typical symptoms of
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51 23 DSP intoxication were observed after 24 h, the mice were monitored for an additional 24 h
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53 24 period. The symptoms that were characterized as typical of DSP intoxication were initial
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55 25 apathy, general weakness, difficulty in movement, spasms, contractions, bluish skin
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3 1 coloration and respiratory distress and death. According to the Decision 2002/225/EC,
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5 2 death of two out of three mice within 24 hours after inoculation into each of them of an
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8 3 extract equivalent to 5 g of hepatopancreas or 25 g whole body should be considered as a
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10 4 positive result for the presence of one or more of the toxins mentioned, when a mouse
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13 5 bioassay is utilized as a screening method.
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15 6

17 **Results and discussion**

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19 8 During this investigation positive mouse bioassay results were obtained in 4 out of 36
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22 9 samples tested. Specifically, 3 samples out of 9 collected from the area of Halastra within
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25 10 a two-year period from the onset of the DSP outbreak were found positive by the mouse
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27 11 bioassay method. The two samples collected during the outbreak were toxic resulting in
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29 12 death of mice within 85-90 min after the intra-peritoneal injection. The third positive
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32 13 sample harvested from the area of Halastra was collected 15 months after the outbreak
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34 14 when the mussel withdrawal was recalled. However, no sporadic cases or outbreak due to
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36 15 DSP or similar gastrointestinal illness in humans were recorded during the period the DSP
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39 16 positive sample was collected.
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43 18 The fourth positive sample was harvested from the Amvrakikos Gulf in Northwestern
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45 19 Greece, where no positive or suspect DSP cases have ever been recorded. Amvrakikos
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48 20 Gulf is the nearest to Ioannina and like Thermaikos, it is shallow, calm, warm and thought
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50 21 to be the most polluted gulf in the Ionian Sea, which is part of the Adriatic Sea, where
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52
53 22 there are frequent reports for toxic blooms (Boni *et al.* 1990; Fatorruso *et al.* 1992; Draisci
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55 23 1995; Viviani *et al.* 1995; Ciminiello *et al.* 1997; 1998; Caroppo *et al.* 2001; Pavela –
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57 24 Vranic *et al.* 2002). Also, it should be noted that the mice injected with the extract of the
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60 25 Amvrakikos sample, in addition to the typical DSP intoxication symptoms, exhibited a

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3 1 marked hypersensitivity 2 h prior to death, together with convulsions. The symptoms of
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5 2 the mice following intraperitoneal injection are presented in Table III. [Insert Table III
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7
8 3 about here]
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12 5 There are a number of studies from different countries concerning DSP outbreaks,
13 6 sporadic cases or detection of toxins in a variety of seafood (Table I). The DSP outbreak
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15 7 described in this paper is the first recorded in Greece and it was classified as a diarrheic
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17 8 shellfish poisoning, based on clinical manifestations characteristic for DSP, the common
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19 9 history of all patients reporting consumption of mussels, the absence of any known
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21 10 pathogen in fecal and blood samples from all hospitalized patients, no detection of
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23 11 antibodies to known pathogens, the subsequent analysis of mussels collected during the
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25 12 outbreak and the detection of *Dinophysis acuminata* in the water of Thermaikos Gulf. The
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27 13 actual number of the people affected cannot be precisely estimated, due to lack of
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29 14 sufficient data. However, the number of 120 people needing urgent medical care within a
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31 15 period of less than 7 days indicates a foodborne outbreak, of the group of seafood-borne
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33 16 emerging diseases, the first recorded in Greece. . [Insert Table I about here]
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43 18 The mouse bioassays performed during the outbreak are indicative of the presence of
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45 19 lipophilic toxins. This finding combined with the increased concentration of *D. acuminata*
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47 20 (Koukaras and Nikolaidis 2002), which is the most common DSP producing species in
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49 21 Europe (Kumagai *et al.* 1996; Morono *et al.* 2002), indicates okadaic acid and / or DTX as
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51 22 the potential cause of this outbreak. It should be noted that the third positive sample from
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53 23 Thermaikos Gulf was collected in March 2001 when, according to other researchers
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55 24 (Koukaras and Nikolaidis 2002) an increased number of *Dinophysis* spp. was observed.
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58 25 Furthermore, the predominant DSP toxins detected in European mussels and especially
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3 1 in the mussels of the Adriatic Sea are okadaic acid and DTX – 2 (Draisci 1995; Pavela –
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5 2 Vranic *et al.* 2002). In a recent publication (Ciminiello *et al.* 2005) concerning
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7 3 investigation of the toxin profile of Greek mussels *M. galloprovincialis* by Liquid
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9 4 Chromatography – Mass Spectrometry, the presence of okadaic acid only (at levels 0.10
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11 5 – 0.20 µg/g) is confirmed. The hypersensitivity and the convulsions observed in mice
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13 6 injected with the extract of the positive sample from Amvrakikos gulf, is indicative of the
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15 7 simultaneous presence of an unknown lipophilic toxin along with okadaic acid and / or
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17 8 DTX – 2. Jumping before death, convulsions and short survival time is observed when
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19 9 mice are injected i.p. with yessotoxin (Ciminiello *et al.* 1997). Although the presence of
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21 10 yessotoxin in mussels has been reported in neighboring areas, it is quite improbable that
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23 11 yessotoxins are the cause of these symptoms. Yessotoxins are poorly soluble in diethyl
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25 12 ether and therefore probably could not co-elute with the other toxins (Ciminiello *et al.*
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27 13 1997). Another possible explanation for the observed symptoms in this sample is that it
28
29 14 contained azaspiracid. After intraperitoneal injection of azaspiracid, mice exhibit
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31 15 respiratory difficulties, spasms, paralysis of the limbs and death (Ito *et al.* 2000). Still, the
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33 16 presence of azaspiracid has not been proven by analytical methods in shellfish harvested
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35 17 from the Mediterranean Sea (James *et al.* 2004).
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46 19 Detection of DSP is routinely based on mouse bioassays, HPLC methods and
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48 20 immunoassays. It is generally acknowledged that biological tests suffer from considerable
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50 21 variability and lack of sensitivity and because of increasing opposition to animal testing
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52 22 many efforts have been made to detect DSP toxins by HPLC based methods. However,
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54 23 the lack of availability of analytical standards and Certified Reference materials for all of
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56 24 the DSP toxins currently precludes the sole use of chemical methods in routine monitoring
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58 25 programmes. Additionally, the maximum level for DSP toxins set by regulatory authorities
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3 1 varies considerably from country to country. In general, levels of DSP toxins greater than
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5 200 ng/g in shellfish are considered dangerous for human consumption (Hallegraef,
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7 1997). The potential public health risks and the expanding geographical distribution of the
8
9 causative dinoflagellates have resulted in the issuing by the Commission of the European
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11 Countries of the Decision 2002/225/EC of the 15 May 2002, which is an amendment of
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13 the Council directive 91/492/EEC, which states that the maximum level of okadaic acid,
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15 dinophysistoxins and pectenotoxins together, in bivalves intended for human consumption
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17 (the whole body or any part edible separately) shall be 160 µg of okadaic acid
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19 equivalents/kg.
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27 11 To date, control measures to protect human health against DSP contaminated seafood
28
29 12 involve both biological and chemical procedures. At the time of the outbreak in the Food
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31 Microbiology Unit we were able to apply only the biological method (mouse bioassay).
32
33 The accumulation of algal toxins is one of the most critical problems in bivalve
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35 aquaculture. Amongst the three most important categories of shellfish poisoning
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37 (amnesic, diarrhoeic, paralytic), DSP is the least severe causing only gastrointestinal
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39 16 symptoms. However, the accumulation of toxic algae in many bivalve producing areas has
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41 17 a great economic impact as their appearance leads to the implementation of long term
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43 bans on shellfish harvest, which is happening frequently at Thermaikos Gulf aquacultures
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45 since the reported outbreak. The factors influencing the growth of the microalgal
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47 19 population include: a) changes in water temperature favoring encysting or excysting of
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49 20 dinoflagellates, b) decreased water salinity, c) increased concentrations of organic
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51 21 substances, d) extended duration of sunlight and e) calm waters (Gestal – Otero 2000;
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53 22 Caroppo *et al.* 2001).
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3 1 All these factors are present in Thermaikos Gulf (North Aegean Sea), especially in the
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5 2 Halastra site, where the constantly calm and warm waters favor mussel cultures.
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8 3 However, the outfall of three large rivers in the gulf (Gallikos, Loudias and Axios), the
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10 4 waste effluents of Greater Thessaloniki area (second largest city of Greece) and the
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12 5 drainage of the plain of Central Macedonia (a plain with intensive agricultural activities),
13
14 6 make Thermaikos Gulf, ideal aquatic environment for the growth of microalgal population
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16 7 and possible toxin production by dinoflagellates. Similar conditions exist at Amvrakikos
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18 8 Gulf (North Ionian Sea), which is part of the South-central Adriatic Sea. Red tide blooms
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20 9 are known to occur in the central Adriatic Sea since the 80's (Boni *et al.* 1990). However,
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22 10 toxic blooms associated with shellfish intoxication are becoming more frequent during the
23
24 11 last decade in this geographic area (Fattorusso *et al.* 1992; Viviani *et al.* 1995; Draisci *et*
25
26 12 *al.* 1995; Ciminiello *et al.* 1997; Ciminiello *et al.* 1998; Draisci *et al.* 1999; Caroppo *et al.*
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28 13 2001; Pavela – Vranic *et al.* 2002).
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36 15 DSP intoxication is still an under reported illness, which is attracting interest after
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38 16 notification of outbreaks (Gestal – Otero 2000; Yasumoto 1985; Vale and Sampayo 2002).
39
40 17 Therefore knowledge of its existence along with the recently increasing reports of red tide
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42 18 blooms spreading from Europe to America to Australia should trigger a constant
43
44 19 awareness for this emerging seafood zoonotic disease.
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52
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54
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Table I

Year/month	Country	Number of cases	Seafood	Toxins determined
1984 October	Sweden ^a	Several hundred	Mussels	>170 MU/kg
1984 –1985	Norway ^b	~ 400	Blue mussels	-
1990 August	Canada ^b	16	Mussels	DTX-1: 100µg/100g
1990	France ^c	415	Mussels imported from Denmark	OA: 170 µg/100 g
1990	Mexico Gulf ^d	NR	Oysters (<i>Crassostrea virginica</i>)	OA: 0.162 µg/100g
1991 January	Chile ^b	120	Mussels	OA DTX – 1
1991-1994	Ireland ^e	Several hundred	Mussels (<i>Mytilus edulis</i>)	OA: <0.7 µg/g DTX-2: 6.3 µg/g
1993 Summer- 1994 Summer	Italy ^f	NR	Mussels (<i>M. galloprovincialis</i>)	OA: 32–151 µg/100g DTX-1: 0–0.32 µg/100g
1994 February	Portugal ^g	18	Donax clams (<i>Donax trunculus</i>)	OA: 130 µg /100 g
1994 June	UK ^h	2	Mussels	OA
1997 March	UK ⁱ	49	Mussels	OA: 25.3–36.7 µg/100 g
1997 June	UK ^j	55	Mussels	-

1997 February-June	Croatia ^k	NR	Mussels	OA: 0.133 µg/g DTX-2: 0.1 µg/g
1997 March-November	Norway ^l	NR	Blue mussels	OA/DST-1: 349 µg
1998	Slovenia ^m	Several hundred	Blue mussels	ND
1998 September-November	Spain ⁿ	NR	Mussels	OA: 18–1010 µg/100g
2000 January	Greece ^o	>120	Mussels (<i>M. galloprovincialis</i>)	ND
2001 June-July	Portugal ^p	7	Clams, Razor clams, Green crabs	OA: 32 µg/100 g
2002 February	Belgium ^q	403	Blue mussels imported from Denmark	OA: 22.9 µg/100g
2002 Summer	Norway ^r	~200	Brown crabs (<i>Cancer pagurus</i>)	OA: 29 µg/100g DTX-2: 2 µg/100g
2002 September	Portugal ^s	32	Blue mussels	OA: 1328–1862 µg/100g
2002 August-2004 March	Denmark ^t	NR	Surf clams – Blue mussels	OA: 4.3–163.1 µg/100g
2003 February	Greece ^u	NR	Mussels (<i>M. galloprovincialis</i>)	OA: 0.10-0.20 µg/g
2003 May-October	China ^v	NR	Clams, Scallops Gastropods	OA: 3.2-17.5 µg/100g
2003 December	Australia ^w	NR	Oysters	DSTs total: 0.253

				mg/kg
2004 April	Chile ^x	NR	<i>Mytilus chilensis</i>	DTX-1:19 µg/100 g

NR=Not Reported, ND=Not Determined, OA= Okadaic acid, DTX = Dinophysitoxin,

DSTs=Diarrhetic Shellfish Toxins

^a Krogh et al. 1985.

^b Aune and Yndestad 1993.

^c Van Egmond et al. 2004.

^d Dickey et al. 1992.

^e Carmody et al. 1996

^f Draisci et al. 1995.

^g Vale and Sampayo 1999.

^h Anon. 1994.

ⁱ Scoging and Bahl 1998.

^j Anon. 1997.

^k Pavela – Vrančič et al. 2002.

^l Ramstad et al. 2001.

^m Mozetic and Bozic 2001.

ⁿ Moroño et al. 2003.

^o Papadopoulou et al. (present study)

^p Vale and Sampayo 2002.

^q De Schrijver et al. 2002.

^r Torgersen et al. 2005

^s Vale et al. 2003

^t Jorgensen et al. 2005.

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2 ^u Ciminiello et al. 2005
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4 ^v Wu et al. 2005
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6 ^w Madigan et al. 2005
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8 ^x Garcia et al. 2004.
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Table II.

Characteristic	Values
Demographics	
Male No. (%)	73 (60.8)
Female No. (%)	47 (39.2)
Mean age in years (range)	35 (8-70)
Clinical characteristics	
Mean duration of symptoms in h (range)	5 (0.5-10)
No. (%) of patients with DSP symptoms seeking Hospital assistance	120 (100)
Estimated No. of patients with DSP not seeking medical assistance	>500
Laboratory tests	
No. of patients/no of faecal samples studied	120/120
No. of mussels' samples bought in the same venders as the cases during the onset of the outbreak	2
No. of unconsumed mussels (leftovers) tested	None
No. of mussels' samples bought in the same market as the cases after the end of the outbreak	18

TABLE III.

Sampling Date	Place (one sample/place)	Symptoms observed	Median Mouse survival Time (number of mice dead/number of mice tested)		DSP toxins Result
Outbreak 15/1/2000	Thermaikos Gulf	Apathy, weakness, difficulty in movement, respiratory distress, spastic contractions just before death	1 h 25 min	(3/3)	Positive
Outbreak 19/1/2000	Thermaikos Gulf	Apathy, weakness, difficulty in movement, respiratory distress, spastic contractions just before death	1 h 30 min	(3/3)	Positive
15/4/2000	Thermaikos Gulf	No symptoms	> 48 h	(0/3)	Negative
6/12/2000	Thermaikos Gulf	No symptoms	> 48 h	(0/3)	Negative
19/1/2001	Amvrakikos gulf	Apathy, weakness, difficulty in movement, hypersensitivity, respiratory distress	9 h	(3/3)	Positive
22/2/2001	Thermaikos Gulf Amvrakikos gulf	No symptoms	> 48 h	(0/3)	Negative
23/3/2001	Thermaikos Gulf	Apathy, weakness, difficulty in movement, respiratory distress	5 h	(3/3)	Positive
10/9/2001	Thermaikos Gulf Amvrakikos gulf	No symptoms	> 48 h	(0/3)	Negative
20/12/2001	Thermaikos Gulf	No symptoms	> 48 h	(0/3)	Negative

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	Amvrakikos gulf				
15/4/2002	Thermaikos Gulf	No symptoms	> 48 h	(0/3)	Negative
20/7/2002-	Thermaikos Gulf	No symptoms	> 48 h	(0/3)	(0/3)
20/7/2005	Amvrakikos gulf				

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FIGURE 1.