The first observation of okadaic acid in *Mytilus edulis* from the Gulf of Finland

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Levels of okadaic acid, a DSP toxin, were determined in the soft tissues of the blue mussel *Mytilus edulis*. The mussels were collected by divers at Långskär, an island a few kilometres off Tvärminne in the western Gulf of Finland. The dived mussels were placed in plastic baskets at two stations, at Storgadden (59°47´N, 23°19´E) in the western Gulf of Finland, and at Huovari (60°24´N, 27°40´E) in the eastern Gulf of Finland. Due to economical difficulties the mussels couldn't be systematically sampled by divers. At both stations, between April and September, the mussels were kept for 3–4-week periods in baskets at depths of 5 m and 30 m. After 3–4 weeks, the sampled mussels in the baskets were replaced with the new ones. During each sampling, in Storgadden and Huovari, the mussels from both depths were combined for toxin analysis. Plankton samples were taken from the surface water layer (0–10 m) during mussel sampling. At Storgadden, the mussels sampled in May and at Huovari in July contained small amounts of okadaic acid: 21.7 ± 3.6 (mean $\pm$ method error) ng g^{-1} d.w. and 82.5 ± 16.5 (mean $\pm$ method error) ng g^{-1} d.w. in May and July, respectively. No toxins were detected in samples from August–September. This preliminary study shows that okadaic acid can be detected in *Mytilus edulis* in the Gulf of Finland, although toxic levels were not found, nor was there found any correlation between the density of dinoflagellates and the okadaic acid levels.

Introduction

Okadaic acid (OA) is a polyether toxin, one of the toxins causing diarrhetic shellfish poisoning, DSP (Hamano *et al.* 1985, Dickey *et al.* 1990). Some researchers tested okadaic acid on rats and mice, and noticed that they have some harmful effects. For example, okadaic acid caused histopathological changes in intestinal villi and also mid-zonal necrosis in livers (Hamano *et al.* 1985, Terao *et al.* 1993). Also, there have been observations that these toxins cause tumor promotion and protein phosphatase inhibition in mice (Cohen *et al.* 1990). Recent studies with rats have also shown some connection between okadaic acid and Alzheimer disease (Arendt *et al.* 1995).

The presence of DSP toxins is generally associated with the presence of species of the genus
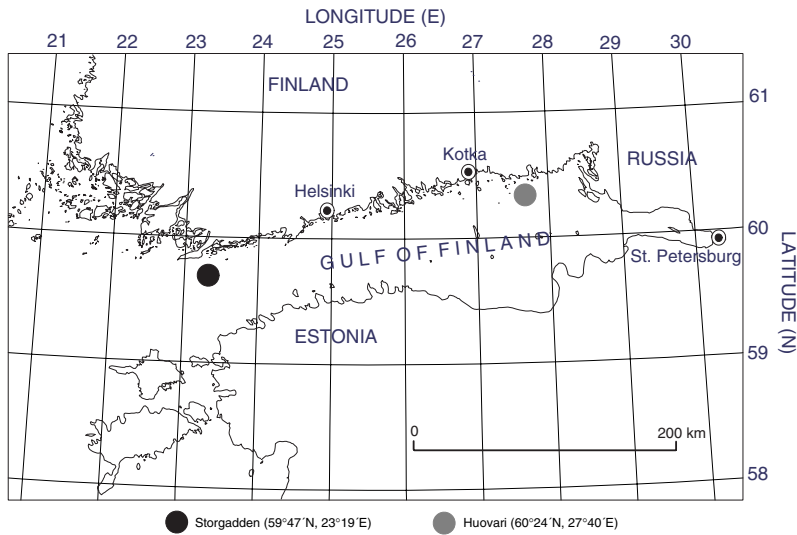
Fig. 1. Experiment sites.

Dinophysis (Murata *et al.* 1982, Kat 1985, Dickey *et al.* 1990, Haamer *et al.* 1990, Pleasance *et al.* 1990, Boni *et al.* 1993). Okadaic acid is observed frequently in the presence of the dinoflagellate *Dinophysis acuminata* (Yasumoto *et al.* 1985, Cohen *et al.* 1990, Edler and Hageltorn 1990). Other toxins that may be produced by dinoflagellates are referred to as dinophysistoxins (also called DTX toxins) and, being closely related to okadaic acid, may also cause diarrhetic shellfish poisoning (Stabell *et al.* 1991, Terao *et al.* 1993).

Several potentially toxic dinoflagellates occur regularly in the Baltic Sea, and cysts of these species have been found in the Baltic Sea sediments (Dale 1976, 1983, Wall *et al.* 1977, Nehring 1994). *D. acuminata* and *D. norvegica* are the dominant dinoflagellates in the Baltic Sea (Kononen and Niemi 1985, 1986). *D. acuminata* is one of the species prevalent during late summer algal blooms (Kononen and Niemi 1986) in the Gulf of Finland, and may be an okadaic acid producer (Yasumoto *et al.* 1985).

Little research has been made on dinoflagellate toxins in the Baltic Sea area, until now, there has been no clear evidence of the occurence of any of these toxins in the Gulf of Finland.

The purpose of this study was to find out the possible existence of okadaic acid in mussel samples.

Material and methods

Sampling and storage

Specimens of *Mytilus edulis* were collected from Långskär, near the Tvärminne Zoological Station, western Gulf of Finland, and kept exposed in plastic baskets (20×15 cm; holes $\varnothing$ 1 cm) at Storgadden off Tvärminne and at Huovari in the western and eastern Gulf of Finland, respectively (Fig. 1), between April and September 1993 (Table 1). Time between the emersion and exposure of mussels was

Table 1. Okadaic acid concentration (mean ± method error) in soft tissues of mussels incubated at two experimental sites in the Gulf of Finland (n.d.= not detected, d.w. = dry weight).

Experimental period (Sampling time in boldface)	Site	Dry weight of soft tissues taken for analysis	Concentration of okadaic acid ng g^{-1} d.w.
28 Apr.–**18 May**	Storgadden	3.0	21.7 ± 3.6
9 Jun.–**5 Jul**.	Huovari	1.6	82.5 ± 16.5
11 Aug.–**6 Sep**.	Huovari	1.2	n.d.
15 Jul.–**8 Sep**.	Storgadden	2.0	n.d.
8 Sep.–**29 Sep**.	Storgadden	1.0	n.d.

about two weeks. After collection by diving mussels were put, waiting for the next exposure, into the sea-aquarium. In aquarium there was a good circulation of oxygen and sea water.

Fifty mussels were placed in each basket. The baskets were kept at 5 m and 30 m depths. For each sampling at both station mussels from both depths were pooled for toxin analysis. The sampling, every 3–4 week, was carried out twice at Huovari and three times at Storgadden (Table 1). After rejection of damaged specimens, 90 specimens from each pooled sample were stored at – 20 °C before being freeze-dried at – 50 °C. The soft mussel tissues were separated for chemical analysis. Differences between the sample weights were a result of size differences between mussels, in feeding activities during each exposure period and the amount of water in the soft tissues of each specimen. The amount of soft tissue varied between 1.0 to 3.0 g (dry weight, Table 1). All the soft tissue parts (including also hepatopancreas) obtained was used for analysis. During each sampling time plankton was sampled with the help of hoop net (mesh size 10 μm) at the same place between 0–10 m and preserved with Lugol+ Ac–solution. Samples were analysed quantitatively (cells per litre) for the dinoflagellate species *Dinophysis acuminata* and *D. norvegica* by using Utermoehl-technique (Utermoehl 1958).

Analysis

The soft tissue of *Mytilus edulis* was analyzed for the presence of okadaic acid using an HPLC method according to Lee *et al.* (1986). The sensitivity range of this method is about 1–80 ng okadaic acid per injection of 0.1 ml of fractionated tissue extract. We used the same method for the mussel reference material (all soft tissues of mussels) obtained from the European Community's Reference Laboratory on Marine Biotoxins, Vigo, Spain.

Briefly, the method was as follows: Soft tissue was homogenized (Fritsch Planetary mill pulverisette 5) and then extracted with methanol (Merck p.a.). The organic phase was extracted with petroleum ether (May & Baker LTD p.a.) and chloroform (Merck p.a.). The chloroform solution was made up to 10 ml, and 1.8 ml of the solution was esterified with ADAM (9-anthryl-diatzomethane). The chloroform extract was cleaned using a Sep-pak silica column cartbridge (Millipore, Waters Associates, part no. 51900). The fraction containing okadaic acid was carefully eluted from the silica and dissolved with 0.1 ml of methanol. 3 parallel samples of the dissolved fractionate (10, 20 and 30 ml) were injected to the HPLC system (pump: Waters M-45; column type: Nova-Pak C-18 60 Å 4 μM, 3.9 × 150 mm, detector: Perkin-Elmer LS-5B Luminescence Spectrometer; wavelengths: excitation 365, emission 412 nm). The amount of toxin found was calculated with the help of ADAM- esterified okadaic acid standard solutions. The amount of toxin (ng g^{-1}) was normalised using the dry weight (1.0–3.0 g) of the mussel soft tissue.

The method error of the analysis is based on triplicate HPLC-runs made from the same sample. After each sample run the height of a toxin peak was measured. Finally the toxin concentration was calculated with the help of okadaic acid standards calibration curve (X-axis = concentration of toxin, ng/injection; Y-axis = peak height, mm). Standards (OACS-1) were received from NRC (National Research Council, Canada). There were some differences between the peak heights of triplicate runs. Therefore, the calculated okadaic acid concentrations (ng OA/ injection) also varied. The variation between calculated OA concentrations was indicated as mean value ± method error.

Results and discussion

Okadaic acid was detected in two sample sets of *Mytilus edulis*. The amount of okadaic acid detected in blue mussel samples, 21.7 ± 3.6 (mean ± method error) ng g^{-1} dw in May and 82.5 ± 16.5 (mean ± method error) ng g^{-1} dw in July, was low compared to results obtained by others (Edler and Hageltorn 1990, Haamer *et al.* 1990). In September–October, the maximum okadaic acid concentrations determined by Edler and Hageltorn (1990) on the Swedish west coast were about 0.4–17 μg g^{-1} hepatopancreas in *Mytilus edulis*. For the each exposure were used 40 mussels at depths 1 and 10 m. In the observations along the Swedish west coast Haamer *et al.* (1990) detected lower okadaic acid levels between March and August (below 0.2 μg okadaic acid g^{-1} mussel tissue) and higher levels in late November (2.18 μg g^{-1} mussel tissue). Mussels, sampled near mainland, contained usually lower toxin levels

than samples from the open sea.

The use of soft tissues is not directly comparable to similar studies used hepatopancreas of *Mytilus edulis* for okadaic acid analysis. This factor might had some influence the results of our work. Our storage temperature (– 20°C) was low enough to prevent enzyme activity in the mussel soft tissues during short (few months) storage period (Edebo *et al.* 1988). The reference material, got from the National Research Council of Canada, is recommended to be stored at temperatures – 12 °C or lower. It was also observed that okadaic acid content can degrade only to half in mussel tissues after 163 min domestic cooking (Edebo *et al.* 1988). Therefore, we believe that the storage temperature of our samples did not influence the results. We analysed the Spanish reference material (all soft tissues of *Mytilus edulis*) with the same method and detected 1.28 μg ± 0.1 μg okadaic acid g^{-1} reference. Our results showed that the method was reliable enough for analysis of okadaic acid in our mussel samples. Some researchers have concluded that the total amount of okadaic acid in hepatopancreas and in the rest of the body tissues were similar although the hepatopancreas contained about 10 times more okadaic acid than the other body tissues. Explanation for this is that the hepatopancreas represents only 10% of the total body weight (Pillet *et al.* 1995).

The abundance of *D. acuminata* and *D. norvegica* in the surface layer (0–10 m) during the observation period (April–September) was rather low. The maximum values (about 1 700 algae cells l^{-1}) were measured at the end of September (29.IX.) and minimum values (about 150 algae cells l^{-1}) in the beginning of June (9.VI.). No evidence of a correlation between the abundance of algae (*acuminata* and *norvegica*) cells and okadaic acid content was found in mussels from May and July. The concentrations of *D. acuminata* and *D. norvegica* per litre were relatively low during the experimental period (April–September). There are though some studies that show a relation between the occurence of okadaic acid in *Mytilus edulis* and low concentrations of Dinophysis in the Gulf of Trieste (Della Loggia *et al.* 1993).

Conclusions

Okadaic acid was detected in the mussel samples, but toxic levels (toxin content ≥ 200 ng g^{-1} digestive gland of mussels) were not. By comparison to other studies the okadaic acid concentrations were low. In spite of that, these were the first signs of okadaic acid in the northern Baltic Sea. No clear positive correlation was found between the density of *D. acuminata* and *D. norvegica* and the occurrence of the okadaic acid.

References

Arendt T., Holzer M., Fruth R., Brockner K. & Gartner U. 1995. Paired helical filament-like phosphorylation of Tau 1 deposition of B/A4- Amyloid and memory impairment in rat induced by chronic inhibition of phosphatase 1 and 2 A. *Brainscan* 17. pp. 1–3.

Boni L., Milandri A., Poletti R. & Pompei M. 1993. DSP cases along the coast of Emilia Romagna (Northwestern Adriatic Sea). In: Smayda T.J. & Shimizu Y. (eds.), *Toxic Phytoplankton Blooms in the Sea*. Elsevier Science Publishers B.V. pp. 475–481.

Cohen P., Holmes C.F.B. & Tsukitani Y. 1990. Okadaic acid: a new probe for the study of cellular regulation. *Trends Biochem. Sci.* 15: 98–102.

Dale B. 1976. Cyst formation, sedimentation and preservation: Factors affecting dinoflagellate assemblages in recent sediments from Trondheimsfjord, Norway. *Review of Paleobotany and Palynology* 22: 39–60.

Dale B. 1983. Dinoflagellate resting cysts: "benthic plankton". In: Fryxell G.A. (ed.), *Survival strategies in the algae*. Cambridge University Press 1983. pp. 69–136.

Della Loggia R., Cabrini M., Del Negro P., Honsell G. & Tubaro A. 1993. Relationship between Dinophysis spp. in seawater and DSP toxins in mussels in the Northern Adriatic Sea. In: Smayda T.J. & Shimizu Y. (eds.), *Toxic Phytoplankton Blooms in the Sea*. Elsevier Science Publishers B.V. pp. 483–488.

Dickey R., Robzin S.C. Faulkner D.I. & Bencsath F.A. 1990. Identification of okadaic acid from a Caribbean dinoflagellate, Prorocentrum concavum. *Toxicon* 28: 371–377.

Edebo L., Lang E.S., Li X.P. & Allen Mark S. 1988. Toxic mussels and okadaic acid induce rapid hypersecretion in the rat small intestine. *Acta Pathologica, Microbiologica*

Scandinavia C. Immunol. 96: 1029–1035.

Edler L. & Hageltorn M. 1990. Identification of the causative organism of a DSP outbreak on the Swedish west coast. In: Graneli E., Sundström B., Edler L. & Anderson D. (eds.), *Toxic Marine Phytoplankton*. Elsevier Science Publishing Co., Inc. pp. 345–349.

Haamer J., Anderson P.O., Lindahl O., Lange S., Li X.P. & Ledebo L. 1990. Geographic and seasonal variation into okadaic acid content in farmed mussels, Mytilus edulis Linnaeus, 1758, along the Swedish west coast. *J. Shellfish. Res.* 9: 103–108.

Hamano Y., Kinoshita Y. & Yasumoto T. 1985. Suckling mice assay for diarrhetic shellfish toxins. In: Anderson D., White A. & Baden D. (eds.), *Toxic Dinoflagellates*. Elsevier Publishing Co., Inc. pp. 381–388.

Kat M. 1985. Dinophysis acuminata blooms, the distinct cause of dutch mussel poisoning. In: Anderson D., White A. & Baden D. (eds.), *Toxic Dinoflagellates*. Elsevier Science Publishing Co., Inc. pp. 73–77.

Kononen K. & Niemi Å. 1985. Long-term variation of the phytoplankton composition at the entrance to the Gulf of Finland. *Ophelia* 3: 101–110.

Kononen K. & Niemi Å. 1986. Variation in phytoplankton and hydrography in the outer archipelago at the entrance to the Gulf of Finland in 1968–1975. *Finnish Mar. Res.* 253: 35–51.

Lee J.S., Yanagi T., Kenma R. & Yasumoto T. 1986. Fluorometric determination of diarrhetic shellfish toxins by high-performance liquid chromatography. *Agric. Biol. Chem.*. 51,3: 877–881.

Murata M., Shimatani M., Sugitani H., Oshima Y. & Yasumoto T. 1982. Isolation and structural elucidation of the causative toxin of the diarrhetic shellfish poisoning. *Bull. Jpn. Soc. Sci.Fish.* 48: 549–552.

Nehring S. 1994. Spatial distribution of dinoflagellate resting cysts in recent sediments of Kiel Bight, Germany (Baltic Sea). *Ophelia* 39: 137–158.

Pillet S., Pereira A., Braekman J.-C. & Houvenaghel G. 1995. Patterns in long term accumulation of okadaic acid and DTX1 in blue mussels, Mytilus edulis, experimentally fed with the DSP- containing alga Prorocentrum lima. In: Lassus P., Arzul G., Erard E., Gentlen C. & Marcaillou C. (eds.), *Harmful Marine Algal Blooms*. Lavoisier, Intercept Ltd. pp. 487–492.

Pleasance O.B., Quillam M.A., de Freitas A., Marr J.C. & Cembella A.D. 1990. Ion-spray mass spectrometry of marine toxins II. Analysis of diarrhetic shellfish toxins in plankton by liquid chromatography/mass spectrometry. *Rapid Commun. Mass. Spectrom.* 4: 206.

Stabell O.B., Stefenak J., Pedersen K. & Underdal B. 1991. Diversity of shellfish toxins of "diarrhetic" type revealed by biological and chemical assays. *J. Toxicol. Environ. Health* 33: 273–282.

Terao K., Ito E., Ohkusu M. & Yasumoto T. 1993. A comparative study of the effects of DSP toxins on mice and rats. In: Smayda T.J., Shimizu Y. (eds.), *Toxic Phytoplankton Blooms in the Sea*. Elsevier Science Publishers B.V. pp. 581–588.

Utermoehl H. 1958. Zur Vervollkommnung der quantitativen Phytoplankton Methodik. Mitt.int.Verein.Theor. *Angew Limnologie* 9: 1–38.

Wall D., Dale B., Lohmann G.P. & Smith W.K. 1977. The environmental and climatic distribution of dinoflagellate cysts in modern marine sediments from regions in the North and South Atlantic Oceans and adjacent seas. *Marine Micropaleontology* 2: 121–200. Elsevier Scientific Publishing Company, Amsterdam.

Yasumoto T., Murata M., Oshima Y., Sano M., Matsumoto G. & Clardy T. 1985. Diarrhetic shellfish toxins. *Tetrahedron* 41. pp. 1019–1023.