International Council for the Exploration of the Sea

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Mariculture Committee

DSP Toxins in Irish mussels and the Contribution of a new toxin DTX-2.

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Abstract

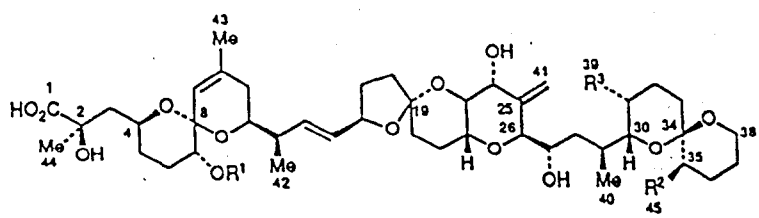
During 1992, mussel (*M. edulis)* samples from Bantry Bay, Kenmare Bay and Dunmanus Bay, on the S.W. coast of Ireland, were analysed for the DSP toxins okadaic acid and DTX-2. Toxins were detected from May to September, with highest levels occurring in all areas from June to August. Maximum concentrations of okadaic acid (78 µmol/100g edible tissue) were detected in Dunmanus Bay while maximum concentrations of DTX-2 (52 µmol/100g edible tissue) were detected in Bantry Bay. Generally, DTX-2 was the major toxin present at the beginning and end of the "toxic season", while okadaic acid was more prevalent during the period of maximum toxicity.

Introduction:

In Europe the major diarrhetic shellfish poison (DSP) toxin found in mussels has been okadaic acid (OA), whereas the structurally similar dinophysistoxin-1 and -3 have dominated in Japanese shellfish (Edebo *et al.* 1988). During routine monitoring for DSP toxins in mussels from Bantry Bay, Ireland, in 1990, a further toxin was detected using HPLC-fluorescence techniques. Detailed chemical analyses of the extract identified this compound as an isomer of okadaic acid and in keeping with the established nomenclature for these marine toxins it was named dinophysistoxin-2 (Tingmo *et al.* 1992). The chemical structures of these DSP toxins is shown in Figure 1.

Figure 1: The structures dinophysistoxins.

	R_1	R_2	R_3
Okadaic acid	H	H	CH_3
DTX-1	H	CH_3	H
DTX-2	H	CH_3	H
DTX-3	Acyl	CH_3	CH_3



Few data are available on the occurrence and levels of DTX-2 in shellfish. This paper presents the results of analysis of okadaic acid and DTX-2 in mussels from 3 bays on the south west coast of Ireland.

Methods:

During 1992 a total of 78 samples of mussel (*M. edulis*) were obtained from Bantry Bay, Kenmare Bay and Dunmanus Bay on the south west coast of Ireland (Figure 2). The occurrence of DSP toxins in these samples were tested using reversed-phase HPLC-fluorescence detection analysis of the ADAM (9-anthryldiazomethane)-derivatised mussel extracts, modified from that described by Lee *et al.* (1987). Briefly, the mussel digestive glands were removed and homogenised. One gram of homogenate was extracted with 80% methanol, centrifuged and the soluble lipid extracted with hexane. The toxins were then extracted from the methanol into chloroform and derivatized with 9-anthryldiazomethane (ADAM) for one hour in the dark at 40°C. The ADAM was prepared *in situ* as described by Yoshida *et al.* 1988. After derivatisation the solution was cleaned up using Sep-Pak silica cartridges prior to analysis by HPLC. Separations were performed at 30 °C on a 250 x 4.6 mm Econosphere C18 column, particle size 5µm. The mobile phase consisted of acetonitrile : water : methanol (80 : 15 : 5) with a flow rate of 1.0 ml/min. Under these conditions O.A. was eluted at 10.1 min and DTX-2 at 11.0 mins, Figure 3. Deoxycholic acid (15.5 min) was used as an internal standard to compensate for the efficiency of the derivatision, the variation in volume injected and retention time drift. As pure DTX-2 standard is not available, quantification of this toxin is based on the response obtained from the okadaic acid standard (Wako Chemicals GmbH, Neuss, Germany).

Figure 2: Sampling areas Bantry Bay, Kenmare Bay and Dunmanus Bay on the south west coast of Ireland

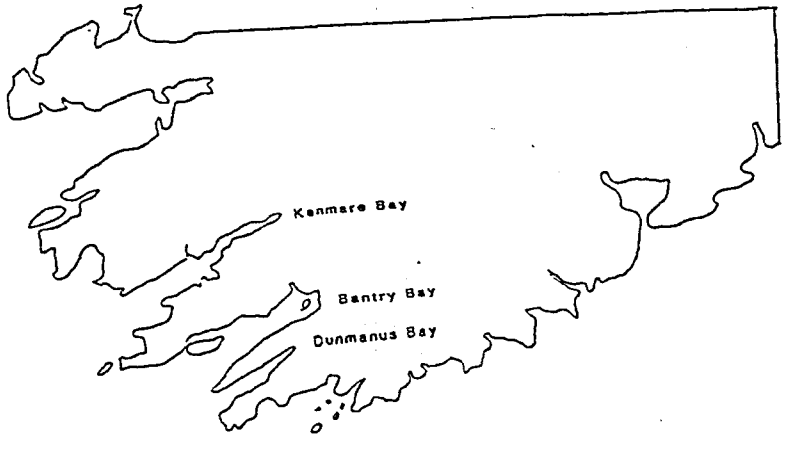
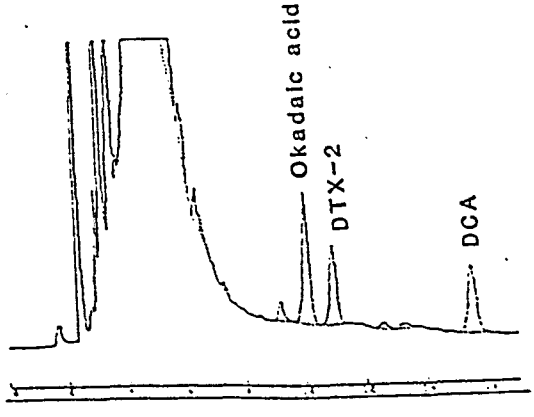


Figure 3: Analysis of digestive gland extract of Bantry Bay for DSP toxins by HPLC.



Results and Discussion:

The levels of DSP toxins detected during the sampling programme are shown in Figure 4. Toxins were detected in mussel digestive glands from May to September, with highest levels occurring in all areas from June to August. Okadaic acid levels ranged from below the limit of detection to 49, 41 and 78 µg/100g edible tissue in mussels from Bantry Bay, Kenmare Bay and Dunmanus Bay respectively, while DTX-2 levels ranged from not detected to 52, 21 and 12 µg/100g at the same sites. The highest concentration of okadaic acid (78 µg/100g) was detected in Dunmanus Bay on July 14 while the highest DTX-2 level occurred on June 23 at North Chapel, Bantry Bay.

At all sites the level of DTX-2 reached a maximum at the end of June and early July while the maximum okadaic acid concentration was detected 2 to 3 weeks later in mid July. Generally, DTX-2 was prevalent at the beginning and end of the "toxic season", while okadaic acid was the major toxin present during the period of maximum toxicity. This pattern was apparent for both Bantry Bay sites, where the contribution to the total toxin level appears greater for DTX-2 than for O.A. up to mid July, from mid July to late August the major toxin detected was O.A. and during September the only toxin detected was DTX-2. Both O.A. and DTX-2 were detected in shellfish from Kilmakillogue, but the major contribution to the total toxin level at this site resulted from O.A. Up to the end of July 1993 the DSP toxicity in Bantry Bay and Kilmakillogue had not reached the levels measured during the same period in 1992, but relative proportions of O.A. and DTX-2 were similar to those found at the beginning of the 1992 "toxic season".
The current legal tolerance level for DSP in Europe is 20 µg/100g of edible tissue (Pleasance *et al.* 1992). The detection limit for these toxins (O.A and DTX-2) using HPLC techniques is approximately 2.0 µg/100g of edible tissue, roughly 10 times lower than the rat bioassay response. This permits the use of HPLC in the monitoring of DSP toxins at levels less than the tolerance level and as such can be a useful early warning system for the onset of DSP toxicity. Further research into the toxicity of DTX-2 and factors which influence the production of O.A. and DTX-2 is ongoing.

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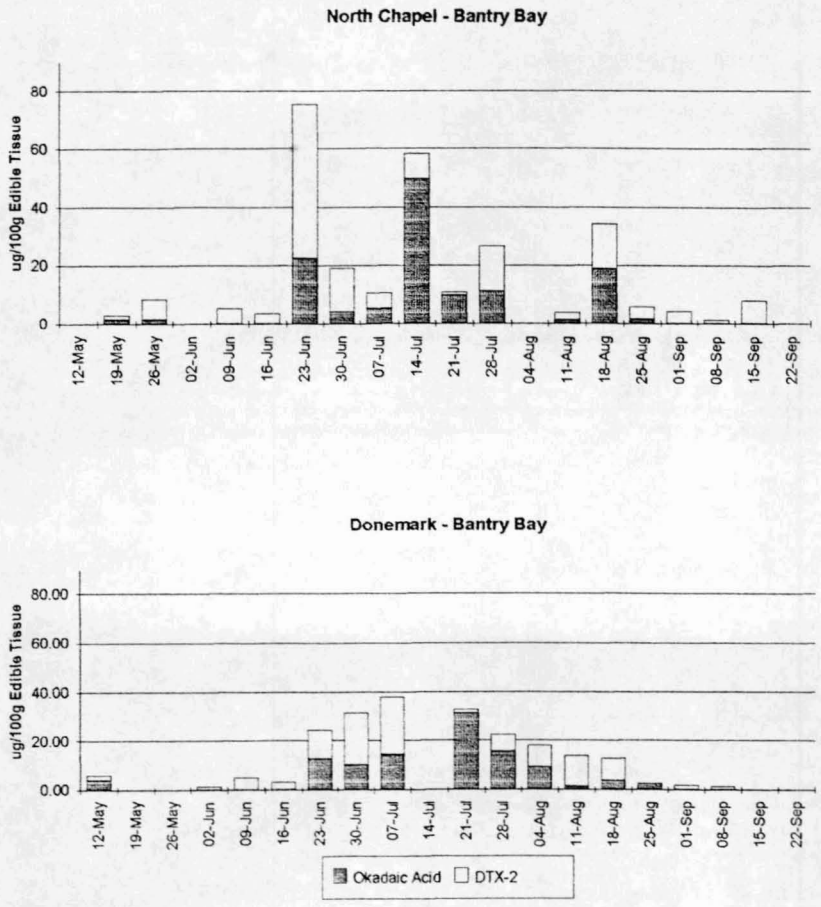

Figure 4: Concentrations of okadaic acid and DTX-2 (µg/100g edible tissue) in mussles (*M.edulis)* from 2 sites in Bantry Bay 1992.

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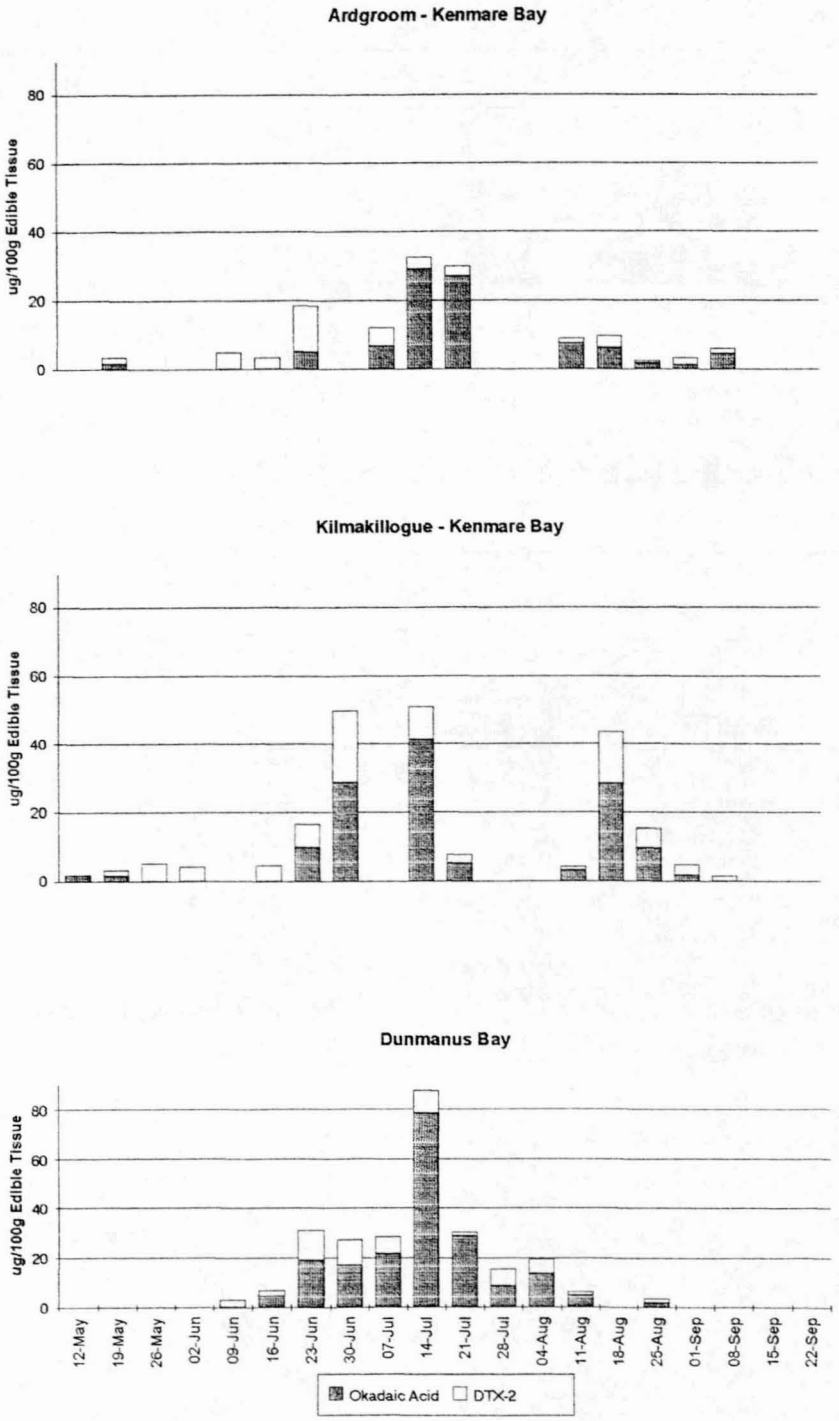

Figure 4: con't/... Concentrations of okadaic acid and DTX-2 (µg/100g edible tissue) in mussles *(M.edulis)* from Kenmare Bay (2 sites) and Dunmanus Bay 1992.