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Comparative accumulation and composition of lipophilic marine biotoxins in passive samplers and in mussels (*M. edulis*) on the West Coast of Ireland

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ABSTRACT

Monitoring of lipophilic marine toxins was carried out in three shellfish production sites on the West Coast of Ireland. The toxins were monitored using passive samplers (solid phase adsorption toxin tracking; SPATT) and toxin-free mussels that were replaced weekly in the selected sampling stations. The toxin profiles and concentrations obtained in the SPATT and in the transplanted mussels were compared with those observed in indigenous (native) mussels from each production site as well as with the phytoplankton that was detected in the water. Numerous lipophilic toxins were detected in the SPATT discs by ultra-performance liquid-chromatography–mass spectrometry/mass spectrometry (UPLC–MS/MS) and included okadaic acid (OA), dinophysistoxin-2 (DTX2), pectenotoxin-2 (PTX2), AZA1, AZA2, yessotoxin (YTX) and SPX-13-DesMe-C. The accumulation rate of toxins in the indigenous mussels and in the SPATT discs correlated well. Toxins were detected in all SPATT discs from all locations, even in the absence of toxin-producing phytoplankton, as observed previously by other research groups. It is quite clear from our data that the presence of okadaic acid in the water (even at high concentrations) did not induce toxicity in the transplanted mussels in the absence of phytoplankton. A severe toxic event of azaspiracids (AZAs) occurred in one of the sampling stations. The SPATT discs accumulated predominantly AZA1 and -2 suggesting that both toxins are biosynthesized by the AZA-producing organism. As opposed to the DSP event, the AZA event resulted in the contamination of the transplanted mussels for several consecutive weeks. This is the first study that reports the presence of YTX and SPX-13-DesMe-C in Irish waters. In our study conditions, the SPATT did not enable the forecasting of shellfish contamination as the increase in toxin concentration occurred at the same time in the shellfish and in the SPATT.

1. Introduction

The consumption of shellfish contaminated with biotoxins, produced by a number of naturally occurring phytoplankton species, can lead to human illness of different nature and severity depending on the toxin class. Due to the potential threat to human health from the consumption of contaminated shellfish many countries, particularly those with commercially important shellfish industries, have a monitoring and regulatory system in place to minimise the risk of placing toxic product on the market. The monitoring programmes typically include the analysis of shellfish flesh for the presence of biotoxins and the analysis of water samples for the presence of toxin-producing phytoplankton. In the EU regulatory limits for toxins belonging to the Amnesic Shellfish Poisoning (ASP) group, Paralytic Shellfish Poisoning (PSP) group and lipophilic toxin group are set out in Regulation (EC) 853/2004 (Anonymous, 2004a). The lipophilic group of toxins includes toxins belonging to the okadaic acid (OA) group, the azaspiracid (AZA) group, the yessotoxin (YTX) and the pectenotoxin (PTX) group.

Under EU Regulation (EC) 854/2004 classified production areas must be periodically monitored to check for the presence of biotoxins in live bivalve molluscs and of toxin-producing plankton in production and relaying waters (Anonymous, 2004b). Although phytoplankton monitoring has increased our understanding of toxic events it only provides a snapshot of the phytoplankton present at the time and location of sampling and it is often difficult to establish a clear correlation between the presence of toxic phytoplankton and shellfish toxicity. Additionally, phytoplankton sampling and subsequent cell identification and enumeration is

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time consuming, labour intensive and requires specialist taxonomic skills.

Shellfish testing continues to be the best tool for determining the fitness of product for human consumption, provided appropriate detection methods are employed. Adequate detection methods should provide high selectivity and sensitivity towards one or several classes of toxins as well as offering a quantitative response. Liquid-chromatography–mass spectrometry (LC–MS) is a well accepted fully quantitative multi-analyte method that provides high sensitivity and high selectivity, especially when tandem MS is used (MS/MS). This method is perceived as a method of choice for toxin quantitation in shellfish and is currently being evaluated as a replacement to the reference animal tests (Anonymous, 2005) currently in place in the legislation. Although shellfish flesh testing remains the most appropriate approach for food safety purposes, the use of shellfish for monitoring the distribution of toxins in the marine environment has several disadvantages. These include difficulties in sample collection, handling and transport to the laboratory, which may be some distance from the growing area; species specific differences in toxin uptake and depuration; bio-transformation in the shellfish as well as analytical interferences due to matrix effects (Ito and Tsukada, 2002; Stobo et al., 2005; Fux et al., 2008a).

The use of passive samplers for monitoring the distribution of marine toxins could overcome some of these issues as the technique offers a spatially and temporally integrated response. The use of passive sampling also avoids the problem of bio-transformation, as well as providing a cleaner extract for LC–MS compared to shellfish extracts. Although, quantification of toxins in shellfish is essential for food safety purposes because of toxicity differences in the toxin metabolites compared to the parent toxins, analysis of toxins in passive samplers can be convenient for screening purposes. MacKenzie et al. (2004) identified a passive sampler suitable for the accumulation of lipophilic toxins, referred to as solid phase adsorption toxin tracking (SPATT) and we have also shown the applicability of such SPATTs in mesocosm and field experiments for a wide range of lipophilic toxins (Fux et al., 2008b).

The present work describes the use of the SPATT approach in three locations on the West Coast of Ireland during the summer 2005. The toxin profiles and concentrations of the regulated toxins belonging to the OA, AZA, PTX and YTX groups, as well as the unregulated spirolide (SPX) group obtained on the SPATT samples were compared to the toxins accumulated in both indigenous and transplanted mussel samples. The indigenous mussel samples were commercially cultivated on site while the transplanted mussels originated from areas shown to be free of toxins at the time of study. The transplanted bivalves were immersed next to the SPATT samples for the same period of time. Toxin quantitation in all matrices was performed by either traditional LC–MS/MS or ultra-performance LC–MS/MS (UPLC–MS/MS).

2. Materials and methods

2.1. Solvents and reagents

Certified standard solutions of OA, PTX2, YTX, GYM and SPX-13-DesMe-C were obtained from the National Research Council in Halifax, Canada. AZA1, obtained from mussel isolation, was a generous gift from Satake and Ofuji (Tohoku University, Japan) to the Marine Institute. Sodium hydroxide (NaOH) and hydrochloric acid (HCl) were purchased from Sigma–Aldrich, Ireland. Methanol and acetonitrile (ACN) used for LC–MS mobile phases were purchased as pestiscan grade from Labscan Ltd., Dublin, Ireland. Formic acid (98%) and ammonium formate (97%) used for LC–MS mobile phases were obtained from Sigma–Aldrich, UK. MilliQ water was obtained from a reversed osmosis system. For UPLC analysis, water and ACN were purchased as HPLC grade from Fisher Scientific, Loughborough, UK. Ammonium formate (97%) was purchased from Sigma–Aldrich, Steinheim, Germany and formic acid (98%) from BDH laboratory, Poole, UK.

2.2. SPATT design and handling

The design of the SPATTs was a disc, as reported in our previous study (Fux et al., 2008b). The frames (diameter 8.8 cm) were embroidery hoops, purchased from Singer Sewing Centre, Galway, Ireland. The 95 μm nylon mesh was purchased from John Staniar & Co., Whitefield Manchester, UK. The Diaion® HP-20 resin was purchased from Sigma–Aldrich, UK.

The SPATT discs were prepared as follows: Diaion® HP-20 resin was weighed (3.00 ± 0.05 g) and methanol (100 mL) was subsequently added. The resin was activated by shaking for 40 min in a multitube vortexer prior to filtration on 95 μm mesh ($\approx 21 \times 12$ cm). The resin was wrapped with the mesh and clipped in the frame allowing exposure on both sides of the frame. Methanol residues were removed by sonicating for 10 min in 500 mL water. The SPATTs were stored in MilliQ water until deployment.

2.3. Sampling

2.3.1. SPATT and mussel sampling

Three SPATT discs were fixed to PVC tubes (ca. 30 cm length) attached to a 15-m polypropylene rope at three different depths: just below the surface, 5 and 10 m as shown in Fig. 1. Nets containing 300 g of uncontaminated, transplanted mussels (*Mytilus edulis*) were placed at the same depth as the SPATT discs.

Three locations on the West Coast of Ireland, Bantry Bay, Killary Harbour and McSwynes Bay (Fig. 2) were selected for the study, based on the occurrence of biotoxins in these areas in the past number of years. In McSwynes Bay and Killary Harbour SPATT discs and transplanted mussels were deployed in shellfish production areas (station depths were 15 and 17 m, respectively) and indigenous mussels were collected from the same areas as part of the Irish National Biotoxin Monitoring programme. For logistic reasons, in Bantry Bay, SPATT discs and transplanted mussels were deployed at a salmon farm (15 m depth), and indigenous mussels

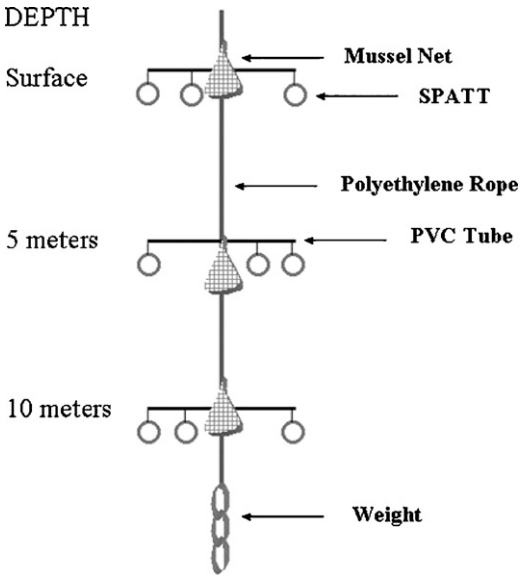

Fig. 1. System used for SPATT discs and transplanted mussels deployment.