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Application of passive (SPATT) and active sampling methods in the profiling and monitoring of marine biotoxins

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ABSTRACT

Solid phase adsorbent and toxin tracking (SPATT) enables temporally and spatially integrated monitoring of biotoxins in aquatic environments. Monitoring using two adsorbent resins was performed over a four-month period at Lough Hyne Marine Reserve, Ireland. A range of Diarhettic Shellfish Poisoning (DSP) toxins were detected from SPATT extracts throughout the study period. The majority of biotoxins were detected in the top 20–30 m of the water column and a spike in toxin accumulation was measured during August 2010. Phytoplankton analysis confirmed the presence of toxin-producing species *Dinophysis acuta* and *Dinophysis acuminata* during the bloom. SPATT has the potential to provide useful information on phycotoxin distribution in the water column; enabling evidence-based decisions regarding appropriate depths for obtaining phytoplankton and shellfish samples in marine biotoxin monitoring programmes.

Active sampling was performed continuously over 7-days and high quantities of toxins were successfully accumulated in the HP-20 resin, okadaic acid (~13 mg), dinophysis toxin-2 (~29 mg), pectenotoxin-2 (~20 mg) and pectenotoxin-2-seco acid (~6 mg) proving this an effective method for accumulating DSP toxins from the marine environment. The method has potential application as a tool for assessing toxin profiles at proposed shellfish harvesting sites.

1. Introduction

Harmful algal blooms (HABs) are occurring with increasing frequency and their incidence is spreading geographically, primarily through anthropogenic influences such as eutrophication, ballast waters of ships and climate change; see reviews: Anderson et al. (2002); James et al. (2010); Smayda (2007). The contamination of filter-feeding shellfish with phycotoxins produced from HABs is becoming a significant health risk for human shellfish consumers worldwide. Regulation EC No 853/2004 governs, for the protection of consumers, the total amount of marine biotoxins that may be present in shellfish within the European Union (E.U) (European Commission, 2004).

Liquid Chromatography-Mass Spectroscopy (LC-MS), in conjunction with phytoplankton monitoring and assessment, has become the primary technique for detection of

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algal toxins in shellfish throughout the E.U. for the majority of marine phycotoxins, excluding Paralytic Shellfish Poisoning (PSP) toxins and Domoic acid (DA) (European Commission, 2004). However, despite the efficacy and precision of phytoplankton monitoring coupled with the LC-MS analysis of shellfish tissues (Puente et al., 2004a, 2004b), there are some disadvantages related to both techniques (Lane et al., 2010; MacKenzie et al., 2004). Phytoplankton monitoring provides a 'snapshot' of the algal species present in the marine environment and it is limited in its detection of toxic species both spatially and temporally. Furthermore, the presence of toxin-producing algae does not provide definitive evidence that toxin accumulation is occurring in shellfish, as toxin production has been found to vary in algal species depending on the stage of growth (Anderson, 1994; Caillaud et al., 2011). Identification of phytoplankton to species level can be difficult and time-consuming, and for some algae, discrimination between species requires detailed morphological observations requiring specialised tools for positive identification (Lane et al., 2010; Miller and Scholin, 1998). LC-MS analysis is a precise tool for the detection of biotoxins which accumulate in shellfish tissues. However, analytical interference from biological matrix effects (Furey et al., 2013), and biotransformation of the toxin within the shellfish can create analogues and fatty acid esters (Fast et al., 2006; Suzuki et al., 2004; Vale et al., 1999) which can complicate the analysis.

Solid Phase Adsorption and Toxin Tracking (SPATT) analysis was developed by MacKenzie et al. (2004) and it involves the passive accumulation of the toxins directly from the water-body through deployment of adsorptive resins. This method provides temporally and spatially integrated monitoring of the water column. Based on this original design, a number of additional trials have been completed using the SPATT technology; it has been recommended as having potential as an early warning system for PSP toxins (Rodríguez et al., 2011), Diarrhetic Shellfish Poisoning (DSP) toxins and Azaspiracids (Turrell et al., 2007). Toxin accumulation in SPATT has also been examined in conjunction with accretion rates in bivalves, and phytoplankton abundance in the water column to determine whether the toxin quantities accumulated in SPATT can be placed in a biologically relevant context (Fux et al., 2009; Lane et al., 2010; Rundberget et al., 2009; Turrell et al., 2007). For a comprehensive review of current advances and research performed using SPATT analysis see MacKenzie et al. (2010).

Using the information obtained from the passive SPATT samplers designed by MacKenzie et al. (2004) a method for the active sampling of biotoxins from the marine environment was developed by Rundberget et al. (2007). Large-scale, active sampling of the water body and successful accumulation of large quantities of the lipophilic toxins okadaic acid (OA) and dinophysis toxin-2 (DTX-2) was performed using the previously optimised Diaion HP-20 resin (Fux et al., 2008; MacKenzie et al., 2004; Turrell et al., 2007). The use of adsorptive resin to accumulate marine biotoxins, either through passive (SPATT) or active methods, provides direct accumulation of the toxic compounds within the water column. The sample matrices have been demonstrated to provide a 'cleaner' extract for the isolation and confirmation of the toxins than do shellfish tissue (MacKenzie et al., 2004).

Lough Hyne marine reserve was chosen as the study site, as a comprehensive investigation of the phytoplankton profile of the marine reserve was performed from January 2008–June 2009 (Jessopp et al., 2011). During that study a number of toxin-producing algal species were detected. Lough Hyne was also selected as it provided an opportunity to examine the occurrence of these algal toxins in an enclosed and highly sheltered system (Maughan and Barnes, 2000). This enabled investigation of the use of SPATT technology as a biomonitoring tool to determine stratification and migration of toxin-producing algal species in the water column, in addition to its previously explored role as an early-warning system.

The main objectives of this study were firstly, a temporal and spatial analysis of the marine biotoxins present at Lough Hyne Marine Nature Reserve, West Cork, Ireland over a 4-month period using SPATT analysis. The second objective of this study was the construction and testing of a seawater pumping machine modelled after the unit devised by Rundberget et al. (2007). This was performed to determine whether this method could effectively accumulate high quantities of lipophilic toxins from the water column.

2. Materials and methods

2.1. Construction and deployment of SPATT bags

SPATT bags were constructed from 95 μm polyester mesh purchased from John Staniar and Co., Whitefield, Manchester, UK. The bags had dimensions of 100 mm × 100 mm and were sealed at the top with velcro to allow removal of the resin and bags to be reused. A loop made of nylon fishing line was sewed on the top corner of the bag to enable attachment to the mooring line using cable ties (Fig. A1). Two types of resin were placed in the bags, Amberlite® XAD761 (Supelco, 10356) an adsorbent resin for the removal of proteins, high MW colourants, organic impurities, etc; and Diaion HP-20 (Supelco, 13607) a polyaromatic resin for absorption of hydrophobic compounds; antibiotics and biomolecules; also used for desalting and has a broad application base. The resins were activated by soaking in methanol according to manufacturer's instructions and rinsed using deionised water, 5 g dry weight (6.1 g wet weight) of activated XAD761; and 5 g dry weight (8.8 g wet weight) of HP-20 were added to each SPATT bag. The bags were stored in airtight ziplock bags at 4 °C and kept from drying out until deployment, as per Rundberget et al. (2009). The bags were attached to mooring lines at different depth intervals for two weeks at a time over a 4-month period from May 2010 to August 2010. After removal from the marine environment, the bags were transported back to the laboratory in airtight ziplock bags and frozen at −20 °C until extraction.

2.2. Construction and deployment of the active toxin sampler

Construction of the active toxin sampler was modelled on that of Rundberget et al. (2007) (A1). The submersible pumping machine was deployed for 7 consecutive days at