The Use of Immunoassay Technology in the Monitoring of Algal Biotoxins in Farmed Shellfish

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Abstract

The use of immunoassay technology as an adjunct method for monitoring biotoxins in shellfish was investigated at aquaculture sites in Killary Harbour, Ireland, during summer 2009. Sub-samples of mussels (*Mytilus edulis*) were taken from batches collected as part of the Irish National Phytoplankton and Biotoxin Monitoring Programme (NMP). Samples were analysed for Diarrhetic Shellfish Poisoning (DSP) toxins using a commercially available ELISA immunoassay kit. The results were compared with those obtained by chemical (liquid chromatography with mass spectrometry, LC-MS) and biological (mouse bioassay, MBA) methods from the monitoring programme. DSP levels increased in late June 2009 over the European Union maximum permitted level of 0.16 µg g^{-1} and positive MBA results led to harvest closures. This event was reflected in both the chemical and immunoassay results, where a positive relationship between them was found.

Introduction

Along most of the Atlantic seaboard of Europe, including Ireland, contamination of shellfish with Diarrhetic Shellfish Poisoning (DSP) toxins derived from *Dinophysis* spp. is the biggest problem for shellfish producers (Raine *et al*. 2010). The current standard method within Europe for the analysis of DSP toxins in shellfish is the mouse bioassay (Yasumoto *et al*. 1978). This technique, accepted by EC regulation (Regulation 2074/2005) for monitoring programmes, is now often used in tandem with chemical methods such as high performance liquid chromatography (HPLC) and liquid chromatography with mass spectrometry (LC-MS). The ethical issues and limitations of these methods, such as expense, lag time and use in a restricted number of laboratories, have prompted a requirement for new analytical technologies, particularly in peripheral regions. Immunoassay technology is now available for the analysis of amnesic (ASP), paralytic (PSP) and diarrhetic (DSP) toxins in shellfish (Hallegraeff *et al*. 2004). The present study is part of an investigation into the accuracy, reliablility and ease of use of the currently available DSP immunoassay a direct comparison to bioassay and chemical techniques.

Methods

Farmed blue mussels, *Mytilus edulis*, were collected from Killary Harbour (53° 37' N, 9° 48' W) between May and Sept 2009 (Fig 1). Sub-samples were taken fortnightly collected under the Irish National Phytoplankton and Biotoxin Monitoring Programme (NMP). On each occasion, 3 samples were obtained from inner, middle and outer Killary Harbour (Fig 1).

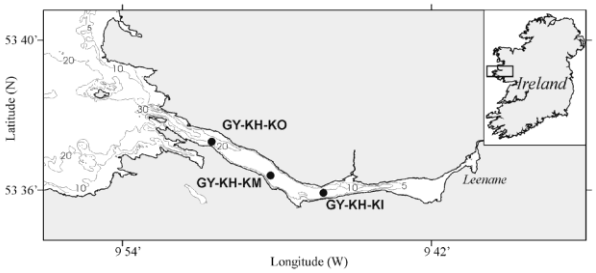

Fig. 1.Map of Killary Harbour showing sampling locations; inner: GY-KH-KI, middle: GY-KH-KM and outer: GY-KH-KO.

Environmental parameters were recorded on each occasion, including water temperature, which was also continuously monitored at the middle sample site using three data loggers (TidbiT, Onset Computer Corporation) suspended at spaced intervals (2, 5 and 13 m depth) on a moored line, and recording data at hourly intervals. Samples for phytoplankton analysis were collected using a 12 mm i.d. tube to achieve an integrated water sample

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over the depth range 0-10 m (Lindahl 1986) and samples were preserved with Lugols Iodine before analysis using an inverted microscope (McDermott and Raine 2010). Mussels were stored at -20 C until analysis. When thawed, mussel tissue was removed from the shell and homogenized. Toxins were extracted with methanol by vortex mixing and centrifuging 1 g mussel tissue with 9 ml 80% (v/v) methanol. The toxin extracts were serially diluted using buffer solution supplied in the analysis kit (DSP, Abraxis) and the analysis proceeded according to the manufacturer's instructions. DSP toxins were quantified both before and after hydrolysis (1.25N NaOH, 100°C, 20 min and then neutralised with HCl), which converts dinophysistoxin (DTX) esters into dinophysistoxins which can be detected by the kit.

The Abraxis DSP ELISA Kit is a rapid assay; it is a direct competitive ELISA, based on recognition of okadaic acid (OA) and DTX-1, DTX-2 by specific anti-bodies. The assay works on a colour reaction. Toxins in positive samples compete against a conjugate enzyme (to which a colour solution binds) for binding sites on antibodies which have been loaded onto a microtitre (96-well) plate. The intensity of the colour produced is inversely proportional to the concentration of toxin present and was read using a micro-plate reader (Biotek). Results were expressed as okadaic acid equivalents i.e. OA and its derivative dinophysistoxins DTX-1, DTX-2 and esters (DTX-3). A standard curve is prepared for each analysis and ttoxin content in each sample is determined by interpolation.

Results

Fig.2 shows levels of DSP toxin in mussel flesh from the three sites in Killary Harbour during tsummer 2009. Chemical (LC-MS) data show that toxin levels exceeded the EU maximum permitted level (MPL) of 0.16 µg g^{-1} on 22 June at outer and middle sites and 29 June at the inner site, suggesting that contamination was transported into the harbour from outside. These dates co-incided with the onset of positive MBA results and enforced the closure of the three areas for harvest (Fig.2).

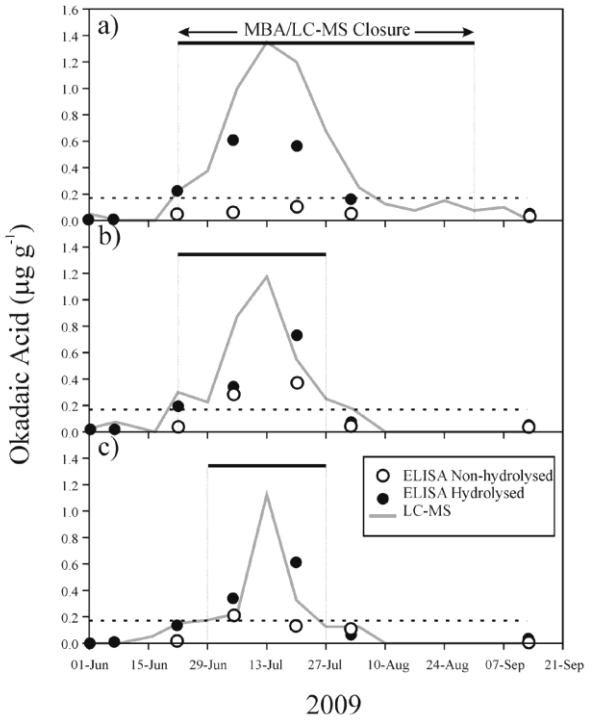

Fig 2. A comparison of DSP toxin levels in shellfish analysed using DSP ELISA immunoassay before and after hydrolysis, LC-MS and mouse bioassay in Killary Harbour 2009 for (a) outer, (b) middle, (c) inner sampling sites.

Subsequently DSP toxin levels rapidly increased at all three sampling sites to ca. 1.2 µg g^{-1}, with toxicity increasing faster at the outer and middle sites than inner site. Toxin levels decreased after mid-July and, with the exception of the outer site, fell to and remained below MPL from 10 Aug. The contamination of mussel tissue with DSP biotoxins coincided with an increase in *Dinophysis acuminata* and *D. acuta* spp. cell densities (Fig. 3a). *Dinophysis* spp. cell densities in integrated samples increased to 2100 l^{-1} on 5 July corresponding to the inital sharp increase in DSP toxin levels at this time.

Water temperatures near the seabed at the middle site increased from 10.8 on 7 June to 15°C on 24 Aug (Fig 3b). This increase was more or less gradual but was punctuated with two sudden peaks. On 16 June temperatures increased by almost 2°C in less than 24 hours.

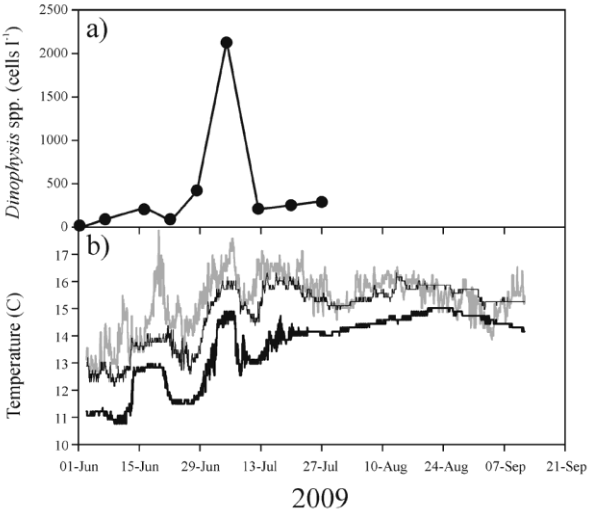

Fig. 3.a) *Dinophysis* cell densities in integrated water samples and b) water temperature data in Killary Harbour, middle site, 2009.

A similar event was observed on 5 July. Both occasions related to increases in *Dinophysis* cell densities, with the second event linked to the sharp increase to >2000 cells l^{-1}, and both followed by a drop in temperature of similar dimension 2-3 days afterwards. It is possible that the increase in *Dinophysis* cell numbers and increase in bottom water temperature were caused by exchange of water between Killary Harbour and near coastal shelf, which brought in the *Dinophysis* population. DSP toxin levels from Killary Harbour during summer 2009 by LCMS were compared with immunoassay (DSP ELISA). Both ELISA and LC-MS showed the same general trend (Fig. 2). Both data sets show an initial non-toxic phase followed by steady increase exceeding MPL, progressing to a steady decline. All hydrolysed samples analysed during the closure period produced positive results by ELISA (Fig.2). Outside this period, no 'false positives' were found in either hydrolysed or non-hydrolysed samples determined by ELISA. On the other hand, most non-hydrolysed samples gave results <MPL during the closure period. All positive (i.e.>EU MPL of 0.16 µg g^{-1}) results determined by LC-MS (and MBA) were also positive by ELISA when hydrolysis was used.

Discussion and Conclusion

The DSP ELISA kit readily detected and quantified presence of DSP toxins in farmed mussels during a toxic event in Killary Harbour in summer 2009. A positive relationship was observed between the MBA, toxin concentrations dby LC-MS and ELISA when samples were hydrolysed. The hydrolysis step thus appeared to be an essential part of the methodology of the immunoassay. The technique has clear potential as an alternative method for toxin analysis, and may prove useful if, for example, screening of shellfish for toxins is required in remote areas where delays in receiving analytical toxin testing results might occur. The immunoassay method proved rapid and easy to use and thus had a number of advantages over chemical methods.

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