Assessment of emerging biotoxins (pinnatoxin G and spirolides) at Europe’s first marine reserve: Lough Hyne

Moira McCarthy a, c, *, Vaishali Bane b, María García-Altares c, Frank N.A.M. van Pelt d, e, Ambrose Furey b, John O’Halloran a, c

a School of Biological, Earth and Environmental Sciences, University College Cork, The Distillery Fields, North Mall, Cork, Ireland
b Mass Spectrometry Research Centre (MSRC) Incorporating the PROTEOBIO and Team Elucidate Research Groups, Department of Chemistry, Cork Institute of Technology (CIT), Bishopstown, Cork, Ireland
c IRTA, Carretera de Poble Nou, km 5.5, 43540 Sant Carles de la Ràpita, Spain
d Department of Pharmacology and Therapeutics, University College Cork, Western Gateway Building, Western Road, Cork, Ireland
e Environmental Research Institute, University College Cork, Lee Road, Cork, Ireland

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Abstract
Active and passive sampling methods were employed over a four-month period, at a site off the South-West coast of Ireland, to characterise the occurrence of cyclic imines in the water column. The marine toxins 13-desmethyl-SPXC, 20-methyl SPXG toxins and pinnatoxin G were detected using active sampling from Diaion HP-20 resin. Seven water depths were sampled to determine stratification of the toxins in the water column using Solid Phase Adsorption and Toxin Tracking (SPATT). Both 13-desmethyl-SPXC and pinnatoxin G were detected using two different resin types; Diaion HP-20 and Amberlite XAD761. HP-20 proved more effective at accumulating the toxins, with a higher percentage of positive samples and a higher ratio of toxin adsorbed relative to XAD761. No temporal variation in toxin-quantities was detected, indicating that there was no change in density of causative algal species in the water column. Pinnatoxin G was detected more frequently from surface to 30 m depth, with a similar pattern observed for 13-desmethyl-SPXC occurrence using XAD761. No difference in the occurrence of 13-desmethyl-SPXC was observed between depths using HP-20 resin. This is the first reported incidence of pinnatoxin G in Irish waters and highlights cyclic imines as emerging toxins in European waters.

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1. Introduction
A small proportion of Harmful Algal Blooms (HABs) can produce toxins that accumulate in the tissues of other organisms, particularly filter-feeding bivalves. A number of these filter-feeders are important aquaculture species for human consumption worldwide. These toxins have harmful impacts on human health and cause the closure of shellfish farms, particularly during the summer months, leading to negative socio-economic effects (Anderson et al., 2000; Hoagland et al., 2002). Rising global ocean temperatures, increased occurrence of extreme weather events (such as El Niño), as well as growing coastal eutrophication, have all been linked to an increase in the incidence of HABs worldwide (Anderson et al., 2002; James et al., 2010). The growing geographical spread of these harmful species has been attributed to ballast waters transporting encysted algae to new environments and similarly, spread of algae by practises in aquaculture (Anderson et al., 2002; Masó and García, 2006; Smyda, 2007; van Dolah, 2000). Our greater ability to monitor and identify these toxins at very low levels using analytical methods could also be an explanation for their increased detection (Draisci et al., 2000; Furey et al., 2005).

Cyclic Imines (CIs) are a family of marine biotoxins which includes spirolides (SPXs) and Gymnodimines (GYMs) produced by algal species from the genus Alexandrium and Karenia. Based on the chemical structure spiridoile, spirro-protorecentrinemine, pinnatoxin, pteratoxin and gymnodimine are grouped as cyclic imines. The producer of pinnatoxin F and G in Australia, New Zealand and Japan has been identified as the dinoflagellate Vulcanodinium rugosum,
previously found in Mediterranean field samples (Rhodes et al., 2011) (Table A1). These toxins can accumulate in shellfish tissues (Hu et al., 2001; McCarron et al., 2012). Spiruloids were first identified in Canadian Shellfish and since then, have spread to many countries worldwide (Botana, 2014) (Table A2). Pinnatoxins, which are structurally similar, have only recently been identified in European waters in Norwegian blue mussels and seawater (Rundberget et al., 2011), since that time they have been detected in France and Spain (Garcia-Alfaraes et al., 2014; Hess et al., 2013). Many CIs have been discovered to be toxic to mice by intraperitoneal injection in doses ranging from 12.7 to 57 μg/kg body weight for pinnatoxin E, F and G; and concentrations of 6.9–99 μg/kg body weight for spiruloids, A, B, C, 13-desmethyl-C and 20-methyl spiriolide G (Munday et al., 2012a, 2012b). However, when administered with food CIs have proven less toxic, in some cases by an order of magnitude. An exception to this was recorded with pinnatoxin F also exhibiting a low LD₅₀ when the toxin was given with food indicating a high oral bioavailability (Munday et al., 2012b). Based on the toxicological studies to date, it has been concluded that some CIs have a neurotoxic effect and can bind and block acetylcholine receptors in central and peripheral nervous systems (EFSA, 2010). At present there are no regulatory limits for CIs in shellfish tissue in Europe, or other regions of the world and there are no long-term studies examining the chronic impacts of these toxins to determine a tolerable daily intake.

Passive monitoring using Solid Phase Adsorbent and Toxin Tracking (SPATT) and active sampling methods for the detection of marine biotoxins directly from water have been developed (MacKenzie et al., 2004; Rundberget et al., 2007). Both methods rely on using an adsorptive resin which can accumulate lipophilic toxins which ‘leak’ from the algal cell and persist in the water (MacKenzie et al., 2003). SPATT has been successfully used in a number of studies as an early-warning system to detect lipophilic marine biotoxins prior to the occurrence of a bloom event (Rodríguez et al., 2011; Turrell et al., 2007). Active toxin sampling involves the pumping of water through a series of filtration devices and through a cartridge containing the resin (Diatom HP-20) based on a design by Rundberget et al. (2007). In that study large quantities of okadaic acid (OA) and dinophysistoxin-2 (DTX-2) were successfully accumulated. Use of an adsorptive resin for the accumulation of biotoxins has some advantages over the use of shellfish tissues for the direct detection and characterisation of toxins present in the marine environment. The adsorption of the bionxin is direct and there is no biotransformation of the toxins, such as fatty acid esterification found in shellfish (O’Driscoll et al., 2011; Vale et al., 1999). Lack of biotransformation coupled with relatively ‘clean’ sample matrices simplifies the extraction and analysis of toxins accumulated using this method (MacKenzie, 2010). For the additional assessment of human health implications of algal biontoxins accumulated through SPATT, analysis of the metabolites produced by shellfish species that accumulate these toxins is also valuable, as some of these metabolites may be harmful to human consumers of toxic shellfish.

A comprehensive profile of the phytoplankton assemblage present in the water column at Lough Hyne Marine Reserve from January 2008 to June 2009 detected a number of toxin-producing algal species (Jessopp et al., 2011), thus it was chosen as the study site for this research. Passive and active sampling methods have been successfully applied to profile the Diarrhetic Shellfish Poisoning (DSP) toxins present, both spatially and temporally at this site (McCarthy et al., 2014), highlighting the use of these methods in profiling and monitoring phytoplankton distribution in the water column. In the current study, samples were analysed to determine the presence of cyclic imines at Lough Hyne, as these toxins are becoming more prevalent in European waters. In addition the distribution of these toxins was characterised over a four-month period at the study site.

2. Materials and methods

2.1. Construction and deployment of the active toxin sampler

The active toxin sampler was based on the design of Rundberget et al. (2007). The sampler was deployed for 7 consecutive days at Lough Hyne Marine Reserve, Cork, Ireland (51°29’ 58"N 9°17’ 49"W), from August 24th – August 31st, 2010 and was operated continuously over the period, apart from 30 min on the 25th August, 2010 when the 50 μm bag filter was replaced to ensure no clogging occurred. Water flow was 6.67 l min⁻¹ and the seawater pump was submerged at 1 m below the surface.

2.2. Construction and deployment of SPATT bags

SPATT bags were based on the design of MacKenzie et al. (2004), but modified slightly to include a Velcro re-sealable opening which allowed removal of the resin and the bags to be recycled. The 95 μm polyester mesh was purchased from John Staniar and Co., Whitefield, Manchester, UK. Bag proportions were 100 mm × 100 mm and a loop was sewed on one top corner to enable attachment to the submerged mooring line using zip ties. Two types of resin were used in this study. The first, Diatom HP-20 (Supelco, 13607) has a broad application base, it is a polyaromatic adsorbent resin for hydrophobic compounds such as biomolecules and antibiotics. The second, Amberlite ® XAD761 (Supelco, 10356) an adsorbent resin useful for the purification of pharmaceuticals, the removal of proteins, organic impurities and high MW colourants. The resins were activated as per the manufacturer’s instructions, by soaking in methanol and rinsed using deionised water, 5 g dry weight (8.8 g wet weight) of HP-20; and 5 g dry weight (6.1 g wet weight) of activated XAD761 were added to each SPATT bag. The bags were placed in ziplock plastic bags at 4 °C and kept damp until use as per Rundberget et al. (2009). SPATT bags were deployed for two week time intervals from May–August 2010. The bags were kept in airtight ziplock bags for transportation back to the laboratory and were stored at −20 °C until extraction.

2.3. Field site location

Lough Hyne Marine Reserve, Co. Cork, Ireland was chosen as the study location for this investigation. Three sites were chosen within the Lough, two sites of 20 m depth, the North Basin (NB) (51°50’32"N 9°30’15"W) and the South Basin (SB) (51°50’01"N 9°29’94"W) and one 50 m deep site in the Western Trough (WT) (51°50’08"N 9°30’42"W). The SPATT bags were attached at the surface, 5 m, 10 m and from then on at 10 m intervals from the surface until the sea-bed and replaced every two weeks. The active sampler was submersed in the South Basin (51°49’88"N 9°29’86"W).

In addition to deploying bags, vertical phytoplankton hauls were also taken with a net every two weeks. Identification of the phytoplankton was performed to determine the causative algal species. This was done for a subset of samples from August 2010, where a significant increase in some phytoxotoxin quantities was detected in the passive filters.

2.4. Solvents and reagents

Chemicals used for liquid chromatography coupled to mass spectroscopy (LC-MS) (HPLC Grade acetonitrile; ammonium acetate; trifluoroacetic acid; HPLC Grade Water), were purchased from


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Fisher Scientific, Dublin, Ireland. Methanol, HPLC-grade, for extraction of SPATT was procured from Sigma–Aldrich, Germany. Water was filtered using a MilliQ dispenser. Certified reference material (CRM) standard of 13-desmethyl SPX-C (SPX-1) was purchased from the National Research Council (NRC), Halifax, Canada. Solvents used for chromatography; methanol (HPLC grade), water (HPLC grade) and acetonitrile (HPLC grade) were purchased from LAB-SCAN (Dublin, Ireland) and ammonium hydroxide (28% in water; ≥99.99% trace metals basis) was purchased from Sigma–Aldrich (Dublin, Ireland).

2.5. Sample extraction methods

Extraction of toxins from absorbent resins was based on the method of Fux et al. (2008). Briefly, the samples were rinsed twice in 500 ml of deionised water to remove salts. Resins were removed from the bags and placed into empty glass SPE cartridges (Supelco, 500 ml of deionised water to remove salts. Resins were removed from the bags and placed into empty glass SPE cartridges (Supelco, Waters, Milford, MA, USA operated at 30 °C). The extracts were stored in amber glass jars at −20 °C until LC-MS/MS analysis. The extraction process was the same for both resins with one exception; for XAD761, the resin was eluted with 80:20 methanol: water. This was done to aid in the removal of more polar toxins (e.g. domoic acid) for potential future analysis (Vale and Sampayo, 2002). Samples from each site were pooled together by depth for analysis from May, June and July 2010 sampling months for the three sites. Samples from August 2010 were examined individually for each site and each depth.

2.6. LC-MS analysis

2.6.1. Agilent 1200 3200QTrap

The presence of cyclic imines in the active pump samples was quantified according to the protocol described in Garcia-Altares et al., 2014 by LC-ESI-MS/MS, using an Agilent 1200 LC system (Agilent Technologies, Santa Clara, CA, USA) coupled to a hybrid triple quadrupole linear ion trap 3200QTrap® mass spectrometer (MS) equipped with a TurboV™ electrospray ion source (Applied Biosystems, Foster City, CA, USA). Separation under alkaline conditions (pH 11) were achieved using a Waters X-Bridge™ C8 column (guard column 2.1 × 10 mm, 3.5 μm particle size, column 2.1 × 50 mm, 3.5 μm particle size; Waters, Milford, MA, USA). Quantification of 20-methyl SPXG was performed against NRC CRM 13-desmethyl-SPXC standard.

2.6.2. LTQ-Orbitrap

LC-MS analysis of samples obtained using both active and passive sampling methods was carried out on LTQ-Orbitrap Discovery Fourier transform mass spectrometer (Thermo Scientific; Hemel Hempstead, UK) equipped with heated electrospray ionization (HESI) source using the method of Garcia-Altares et al. (2014). The following source parameters were used in positive ion mode: heater temperature 300 °C; capillary temperature 250 °C; sheath gas flow rate 50 arbitrary units; auxiliary gas flow rate 10 arbitrary units; sweep gas flow rate 0 arbitrary units; capillary voltage 47.5 V; tube lens voltage 90 V and ion spray voltage 4 kV. The fragmentation of compounds was carried out at 45% HCD. The contaminant ion from mobile phase filter (nylon) was locked at m/z 453.34352 to improve the sensitivity of analysis.

Waters X-Bridge™ C8 column (2.1 × 50 mm, 3.5 μm particle size) along with a guard column (2.1 × 10 mm, 3.5 μm particle size); Waters, Milford, MA, USA operated at 30 °C was used to separate two compounds. Mobile phase A was 6.7 mM of ammonia in water (pH 11) and mobile phase B was 6.7 mM of ammonia in 90:10 ACN: Water (pH 11). The elution gradient was started at 20% mobile phase B, reached 100% mobile phase B in 8 min, was held for 1 min, then back to 20% mobile phase B in 0.5 min and equilibrated for 2.5 min before the next run started; at flow rate of 500 μL/min. The injection volume was 10 μL. Data processing was carried out using X-calibur 2.0.7 software.

This method was used to quantify the presence of 13-desmethyl SPX-C and Pinnatoxin G in SPATT bags. Quantitation of both toxins was performed against NRC CRM 13-desmethyl-SPXC standard assuming equimolar response.

2.7. Data analysis

Data were tested for normality using the Kolmogorov–Smirnov test. All data were non-normally distributed, consequently data were analysed using Kruskal Wallis non parametric ANOVA. Where there were significant differences between sites/depths, a Mann–Whitney U test was performed to determine where these differences occurred. Data were analysed using IBM SPSS (Statistical Package for the Social Sciences) Version 20.

3. Results

3.1. Detection of cyclic imines in passive and active samplers

Ion chromatograms and spectra confirm the presence of 13-desmethyl-SPXC (Fig. 1) and pinnatoxin G (Fig. 2) from the passive SPATT samplers detected using the LTQ Orbitrap. Sub-samples of HP-20 were also analysed from the active sampler and cyclic imines 13-desmethyl-SPXG, 20-methyl SPXG and pinnatoxin G toxins were detected using the 3200QTrap (Table 1 and Fig. 3) and the LTQ Orbitrap with high mass accuracy. The occurrence of 20-methyl SPXG was not quantified in the SPATT samplers due to lack of CRM for confirmation and validation.

3.2. Comparison of resins in SPATT

Overall Diaion HP-20 was more effective than Amberlite XAD761 in adsorbing 13-desmethyl SPX-C and pinnatoxin G. For HP-20, 85% of the samples tested positive for 13-desmethyl SPX-C, compared to 19% of samples from XAD761, and HP-20 also accumulated a higher quantity of the toxin (ng/g) at a ratio of 4.7: 1 (HP-20: XAD761). 81% of the HP-20 samples, versus 51% of the XAD761 tested positive for pinnatoxin G, with HP-20 also showing a greater efficiency at accumulating this toxin, with a ratio of 1.45: 1 (HP-20: XAD761).

3.3. Spatial and temporal distribution of toxins

The occurrence of 13-desmethyl SPXG did not vary temporally over the study period and there were no differences between sites for HP-20 or XAD761. There were no significant differences in the occurrence of 13-desmethyl SPXG over the 7 depths sampled for the HP-20 resin. However, there were significant differences in the quantities of toxin detected at each depth in the samples obtained from XAD761 (p = 0.013; H0 = 16.24) with 40 m and 50 m depths significantly lower than those detected from surface to 30 m. It can be observed in Fig. 4(b) that there were almost no positive samples detected at these depths, which accounts for the difference detected.

Pinnatoxin G showed no temporal variation over the sampling period. However significant differences between sites and depths were observed. Significant differences in distribution within the water column were observed for pinnatoxin G from XAD761 (p < 0.001; H0 = 39.37) and HP-20 (p < 0.001, H0 = 24.29). With the
concentration in samples from surface to 20 m varying significantly from those analysed from 30 to 50 m. This corresponds with Fig. 4 (c) and (d), where there are few positive samples detected from 30 to 50 m. Differences between sites were also observed in both resins for pinnatoxin G. Toxin levels analysed in HP-20 resin from the SB and WT were significantly different from one another, with a higher concentration of toxin accumulated at the SB (p = 0.007; H2 = 10.065). XAD761 samples from the SB were significantly different from those analysed from the NB and the WT (p = 0.004, H2 = 11.023). See Table B1 for quantities of toxins from each sample at each depth over the study period.

**Table 1**
Concentration of 13-desmethyl-SPXC, 20-methyl SPXG and pinnatoxin G (ng/mL) present in the active pump samples (n = 2). 20-methyl SPXG was quantified using NRC-CRM-13-desmethyl-SPXC calibration curve on the 3200QTrap. Analytes were quantified below the first level of the calibration curve (2.5 ng/ml).

<table>
<thead>
<tr>
<th></th>
<th>13-desmethyl-SPXC</th>
<th>20-methyl-SPX-G</th>
<th>Pinnatoxin-G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1A</td>
<td>0.66</td>
<td>2.24</td>
<td>0.41</td>
</tr>
<tr>
<td>Sample 1B</td>
<td>0.78</td>
<td>2.16</td>
<td>0.36</td>
</tr>
<tr>
<td>Sample 2A</td>
<td>0.32</td>
<td>1.04</td>
<td>0.1</td>
</tr>
<tr>
<td>Sample 2B</td>
<td>0.76</td>
<td>2.5</td>
<td>0.45</td>
</tr>
</tbody>
</table>
3.4. Algal species detected

Algal samples from August were analysed to identify the phytoplankton species present at Lough Hyne. Table C1 provides a full list of algal species identified. Among the species detected was *Alexandrium* sp., which was suspected as a possible producer of 13-desmethyl SPXC.

4. Discussion

Pinnatoxins were first isolated from the Japanese mollusc *Pinna muricata* in the mid 1990’s (Chou et al., 1996; Takada et al., 2001). Since that time, these toxins have been detected in Australia, New Zealand, Canada, and in European waters, specifically in Norway, France and Spain (Garcia-Altares et al., 2014; Hess et al., 2013; McCarron et al., 2012; Rhodes et al., 2010; Rundberget et al., 2011; Selwood et al., 2010). This study is the first to detect pinnatoxin G in Irish waters, highlighting the ongoing spread of this algal toxin. This toxin, as well as 13-desmethyl-SPXC and 20-methyl SPXG, were detected with high mass accuracy (<2 ppm) using the LTQ-Orbitrap mass spectrometer. The performance criteria of the two methods used in this study, qualitative low resolution LC-MS/MS (3200QTrap) and qualitative high resolution LC-MS/MS (LTQ Orbitrap Discovery) have been described in detail in Garcia-Altares et al. (2014) for shellfish extracts. Tables A1 and A2 summarise the world-wide occurrence of cyclic imines reported in the literature.

Two resins were utilised in this study for the adsorption of toxins from the marine environment. Diaion HP-20 resin has previously been incorporated into SPATT trials (Rundberget et al., 2009; Turrell et al., 2007) and was proven to adsorb CIs from the Marine Environment (Fox et al., 2009). Amberlite XAD761 has been investigated as a potential resin for the detection of more polar toxins from the water column, such as domoic acid (Marine and Institute, 2009) and was also used during the sampling period to successfully harvest a suite of DSP toxins from Lough Hyne (McCarthy et al., 2014). HP-20 resin proved more effective at adsorbing the CIs present within the environment over the study period in the passive SPATT samplers; with a higher frequency of positive samples and higher ratio of toxin per gram of resin for pinnatoxin G and 13-SPX-C than XAD761.

Monitoring the water column was performed both temporally and spatially over a four-month period using SPATT. There was no significant temporal variation measured over the study period for pinnatoxin G or 13-desmethyl SPXC, thus no changes in densities of *V. rugosum* or *Alexandrium* sp occurred from May–September in 2010. Identification of algal species present at Lough Hyne was carried out in August 2010 and *Alexandrium* sp were detected in the samples (Table C1). *Alexandrium ostenfeldi* has been shown to produce SPX-C among other marine biotoxins, such as okadaic acid and 20-methyl SPX G (Cembella et al., 2001; Katikou et al., 2010). *V. rugosum* was not identified in the phytoplankton samples during this study, however a recent study examining the accumulation of pinnatoxin G in mussels (*Mytilus galloprovincialis*) and clams (*Venerupis decussata*) over a 3-year period, coupled with regular monitoring the water column was performed both temporally and spatially over a four-month period using SPATT. There was no significant temporal variation measured over the study period for pinnatoxin G or 13-desmethyl SPXC, thus no changes in densities of *V. rugosum* or *Alexandrium* sp occurred from May–September in 2010. Identification of algal species present at Lough Hyne was carried out in August 2010 and *Alexandrium* sp were detected in the samples (Table C1). *Alexandrium ostenfeldi* has been shown to produce SPX-C among other marine biotoxins, such as okadaic acid and 20-methyl SPX G (Cembella et al., 2001; Katikou et al., 2010). *V. rugosum* was not identified in the phytoplankton samples during this study, however a recent study examining the accumulation of pinnatoxin G in mussels (*Mytilus galloprovincialis*) and clams (*Venerupis decussata*) over a 3-year period, coupled with regular

Two sampling regimes were applied; SPATT was used to passively sample the water column providing temporal and spatial monitoring of the distribution of pinnatoxin G and 13-desmethyl-SPXC over the study area. Active sampling accumulated higher quantities of toxin on HP-20 resin than SPATT and proved an effective method for the characterisation of toxins present at Lough Hyne. Using this method, as well as pinnatoxin G and 13-desmethyl-SPXC, 20-methyl SPXG was also detected from the adsorbent resin. SPATT samples were not analysed for the spirolide 20-methyl SPXG due to lack of standard available for confirmation and validation of the method. Active sampling has the potential to provide detailed information on the toxin profile present at shellfish farming sites, as it can successfully accumulate and concentrate toxins present at very low quantities in the water column (McCarthy et al., 2014). Use of these resin-based methods, in conjunction with analysis of shellfish tissues, will provide regulators with early warning of the presence of marine biotoxins in the water column and supplement the information obtained from their detection in bivalve shellfish.

Fig. 3. Extracted ion chromatograms of the selected MRM transitions for 13-desmethyl-SPXC, 20-methyl SPXG and pinnatoxin G (PnTXG) present in sample “Active Pump 2A”; Turbo Spray ESI + MRM (27 pairs) (3200QTrap).
sampling of the phytoplankton present in the water column, noted that toxin was present in relatively high quantities within the tissues of both species, even when the causative algal species was absent for prolonged periods from the water column (Hess et al., 2013). *V. rugosum*, among other toxin-producing algal species, can encyst and remain in the sediment for long periods until conditions for growth become favourable (Satta et al., 2013). In this situation, application of SPATT for toxin monitoring would provide a semi-quantitative method for the measurement of these toxins, while providing a simple extraction matrix for subsequent analysis.

Spatial monitoring of toxins was carried out at three different study sites within Lough Hyne; the North Basin (NB) and South Basin (SB) were both monitored from surface to 20 m, while the Western Trough (WT) was monitored from the surface to 50 m depth. Different depths were assessed during the study to determine whether stratification of toxins occurs within the water column, as *Dinophysis* have been observed to migrate vertically in the water (Villarino et al., 1995). Occurrence of 13-desmethyl SPXC showed no significant differences between sites over the study period for both resin types, and for HP-20 there were no significant differences between the 7 depths sampled, with the toxin detected from surface to 50 m depth. However, XAD761 showed significant differences in the quantities of toxin detected at 40 and 50 m, with only a few positive samples detected at this depth. For pinnatoxin G a similar pattern was observed to that of 13-desmethyl SPX-C sampled from XAD761, with a significant difference in the occurrence of toxin at surface to 20 m, from toxin levels detected at 30–50 m depth.

This lack of toxin detected at greater depths using XAD761, and for pinnatoxin G using HP-20, could be attributed to the formation of an oxy-thermocline at Lough Hyne in April 2010, from approximately 30 m to the seabed, which remained until October 2010 after sampling had ceased (McCarthy et al., 2014). The formation of this oxy-thermocline has been well established at Lough Hyne during the spring and summer months (Jessopp et al., 2011; McAllen et al., 2009). As yet, the impacts of different

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**Fig. 4.** Quantities of 13-desmethyl-SPXC and Pinnatoxin G detected from SPATT bags containing Diaion HP-20 and Amberlite XAD761 (ng/g) over the 4-month sampling period at Lough Hyne for each of the depths sampled (surface – 50 m). Data for August 2010 is separated according to site: SB – South Basin; NB – North Basin; WT – Western Trough. (a) 13-desmethyl SPX-C: Diaion; (b) 13-desmethyl SPXC: Amberlite; (c) Pinnatoxin G: Diaion; (d) Pinnatoxin G: Amberlite (LTQ Orbitrap).
environmental parameters such as temperature and oxygen on the binding kinetics of adsorbent resins used for SPATT trials have been little explored. A recent study was performed examining the impacts of differing estuarine salinities on HP-20 resin (Fan et al., 2014), with the adsorption of okadaic acid and dinophysis toxin-1 (DTX1) on this resin differing depending upon the salinity. At higher salinities (27‰), intra-particle diffusion influenced the toxin adsorption, while at medium salinity (13.5‰) film diffusion was the primary adsorption process. Salinity at Lough Hyne has been measured at approximately 34–35‰ from surface to seabed with little variation (Ballard and Myers, 1996), however the formation of an oxy-thermoline with a decrease in both temperature and oxygen may impact on the toxin adsorption in either one or both resins used in this study. HP-20 resin successfully accumulated 13-desmethyl SPX-C from surface to 50 m, with occasional accumulation of pinnatoxin G at greater depths also observed, while this pattern was not observed in XAD761.

Differences in the occurrence of pinnatoxin G between sites for both resin types were observed. Overall, a higher quantity of toxin was accumulated at the SB than either of the other two sites (Table B1). The SB site was located nearest to the entrance of tidal influences to Lough Hyne and to the inflow of oceanic waters, with the other two sites located in more ‘sheltered’ areas of the Lough. This may account for the differences observed between sites, a similar pattern to that observed for DSP toxin occurrence at Lough Hyne (McCarthy et al., 2014). Cyclic Imines have been implicated as the cause of positive Mouse Bioassay results when investigating toxic shellfish, showing a high toxic effect to mice when administered through intraperitoneal injection (Munday et al., 2012b). However, as yet there have been no recorded acute symptoms of these toxins in humans. The toxicity of spiroloides and pinnatoxin G is still being explored in health impacts from the consumption of contaminated shellfish. This study has provided information on stratiﬁcation of the cyclic imine pinnatoxin G in Irish waters. The active and passive sampling methods can also provide information on stratification and seasonal changes of marine biotoxins and in assessing potential sites for aquaculture, which could assist relevant stakeholders in developing management strategies for shellfish farms. Vigilance in monitoring coastal waters and shellfish species is paramount, to ensure that newly emerging toxic algal species with serious human health implications are detected prior to any negative impacts on shellfish consumers.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.toxicon.2015.10.007.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.toxicon.2015.09.041.

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