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High resolution mass spectrometry-based screening reveals lipophilic toxins in multiple trophic levels from the North Sea

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ABSTRACT

Lipophilic marine biotoxins, which are mainly produced by small dinoflagellates, are increasingly detected in coastal waters across the globe. As these producers are consumed by zooplankton and shellfish, the toxins are introduced, bioaccumulated and possibly biomagnified throughout marine food chains. Recent research has demonstrated that ultra-high performance liquid chromatography coupled to high-resolution mass spectrometry (UHPLC–HRMS) is an excellent tool to detect marine toxins in algae and seafood. In this study, UHPLC–HRMS was used to screen lipophilic marine biotoxins in organisms from different trophic levels of the Belgian coastal zone ecosystem. A total of 20 tentatively identified lipophilic compounds was detected. Hereby, the trophic transfer of lipophilic marine biotoxins to the upper trophic level was considered to be rather limited. Furthermore, 36% of the compounds was clearly transferred between different organisms. A significant biotransformation of compounds from the okadaic acid and spirolide toxin groups was observed (64%), mainly in filter feeders. Through a multi-targeted approach, this study showed that marine organisms in the Belgian coastal zone are exposed to a multi-toxin mixture. Further research on both single compound and interactive toxic effects of the frequently detected lipophilic marine toxin ester metabolites throughout the food chain is therefore needed. As a future perspective, confirmatory identification of potential toxins by studying their fragmentation spectra (using new tools such as hybrid quadrupole Q-Exactive™ Orbitrap-MS) is designated.

1. Introduction

The occurrence of marine harmful algae is increasing around the globe (Valdiglesias et al., 2013; Ciminiello et al., 2014; Díaz et al., 2015). Natural dispersal as well as anthropogenic activities (e.g. shellfish translocation, global shipping, and ballast water discharge) have introduced these algae into non-native regions (Hallegraeff, 1998; Miller et al., 2010; Liebich et al., 2012). The discharge of nutrients from domestic, industrial and agricultural waste further contributes to the increased harmful algal bloom (HAB) frequency (Miller et al., 2010). Since filter-feeding bivalves consume algae, the accumulation of one or more lipophilic marine biotoxins from harmful phytoplankton is a well-known food safety threat in the shellfish industry (Marcaillou et al., 2005, 2010; Rundberget et al., 2011; Garcia et al., 2012). As these organisms accumulate marine biotoxins, even more so during HABs, increased concentration levels may be found at higher trophic levels in the food chain (Reguera et al., 2004; Franchini et al., 2010; Costa et al., 2013; Lage and Costa, 2013; Lopes et al., 2013). Lower trophic levels, such as zooplankton that graze on harmful microalgae, may experience considerable adverse effects (Hégaret et al., 2009; Vasconcelos et al., 2010). Moreover, through bioaccumulation, the intoxication of higher trophic level feeders such as fish, marine mammals, seabirds, and humans can occur (Álvarez et al., 2010; Silva et al., 2013; Turner, 2014). Additionally, harmful algae may also directly impact higher trophic levels (e.g. fish) through direct contact or anoxia when large blooms of algae decompose (Hoppenrath et al., 2007; Peperzak and Poelman, 2008; van der Woerd et al., 2011; Turner, 2014).

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Bioaccumulation is a key criterion used in European legislation (Gobas et al., 2009; Schäfer et al., 2015) to assess and manage the safety of chemicals and pollutants in aquatic systems and food webs. Bioaccumulation can be defined as a net accumulation of a chemical by an organism as a result of uptake from all environmental sources (Drexler et al., 2003). Screening estimates of bioaccumulation may include biomagnification, whereby the substance concentration in an organism is greater than in its diet (and the diet being the main exposure pathway), and trophic dilution, whereby the substance concentration in an organism is lower than in its diet. The processes of bioaccumulation of lipophilic marine biotoxins in marine environments are often very dynamic since marine biotoxins are continuously accumulated from the available phytoplankton species and transferred to different organisms through the food chain. Furthermore, bioaccumulation may depend on abiotic factors such as temperature, suspended organic matter and biotic factors such as age, sex and lipid content of an organism. While substantial work has been carried out on lipophilic marine biotoxins production from algal toxin producers and to a lesser extent on the environmental conditions that affect toxin production, very little research has been conducted to investigate trophic transfer of lipophilic marine biotoxins in the marine environment.

The North Sea is a rather shallow semi-enclosed basin of continental shelf water, surrounded by the European continent, the Scandinavian Peninsula and Great Britain (Vanden Eede et al., 2004; Speybroeck et al., 2007). In the past, harmful algae such as dinoflagellates were thought to follow the global trend and increase in the North Sea environment (Edwards et al., 2006; Peperzak, 2003; Hallegraeff, 2010). Recently, however, Hinder et al. (2011) reported a significant decrease in dinoflagellates and increase in diatom abundance, which may indicate an opposing shift in the plankton composition in the North Sea. To date, naturally occurring toxin producers (Klöpper et al., 2003; Krock et al., 2008, 2009; Hinder et al., 2011; Tillmann et al., 2012), shellfish accumulation (James et al., 2002; van der Fels-Klerx et al., 2012), and poisoning reports from around the North Sea have been well documented (Hinder et al., 2011; Whyte et al., 2014). However, research on lipophilic toxin profiles along diverse marine trophic levels is lacking.

As proposed in the Marine Spatial Plan (2014), aquaculture activities are to be developed in the Belgian Part of the North Sea (BPNS) (Maes et al., 2013; MARE, 2015). The 67 km of the near-straight Belgian coastline is characterized by the presence of (sand) beaches, stone groins and concrete dykes (Vanden Eede et al., 2004; Speybroeck et al., 2007). Along this coastline, key edible species can be found such as mussels, oysters and crabs. However, both the broad public and the scientific community are not aware of the occurrence and accumulation of marine biotoxins as no routine HAB monitoring is in place. Therefore, it is important to understand the marine toxin status within the different marine key species of this environment.

The main goal of this study was to investigate the prevalence of various lipophilic toxins in key edible organisms collected at different trophic levels of the BPNS. During the last decade, liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) was the method of choice to detect *a priori* defined lipophilic marine biotoxins in seafood and marine matrices (Krock et al., 2008; Rodríguez et al., 2015). More recently, high-resolution mass spectrometry (HRMS) has been confirmed as an even better tool to conduct the synchronous detection of targeted and multi-targeted lipophilic marine biotoxins in different matrices because of its highly accurate mass measurements and full-scan properties (Blay et al., 2011; de la Iglesia et al., 2013; García-Altares et al., 2014; Domènech et al., 2014; Orellana et al., 2014, 2015). Here, HRMS analysis was used to study the occurrence and trophic transfer of toxins in marine organisms of the BPNS. Toxin extracts of living marine organisms, sampled both inshore and offshore, were used to study the bioaccumulation of lipophilic marine biotoxins.

2. Material and methods

2.1. Chemicals and analytical standards

Certified calibration solutions for okadaic acid (Certified Reference Material (CRM)-OA-c $14.3 \pm 1.5\ \mu g\ mL^{-1}$), dinophysistoxin-1 (CRM-DTX-1 $15.1 \pm 1.1\ \mu g\ mL^{-1}$), pectenotoxin-2 (CRM-PTX-2 $8.6 \pm 0.3\ \mu g\ mL^{-1}$), azaspiracid-1 (CRM-AZA-1 $1.24 \pm 0.07\ \mu g\ mL^{-1}$), spirolid-1 (CRM-SPX-1 $7.0 \pm 0.4\ \mu g\ mL^{-1}$), and yessotoxin (CRM-YTX $5.6 \pm 0.3\ \mu g\ mL^{-1}$) were obtained from the National Research Council, Institute for Marine Bioscience (Halifax, Canada). Reference material, i.e. shellfish tissue containing OA, DTX-1, AZA-1, AZA-2 and AZA-3, were kindly donated by Dr. M. Andjelkovic. Analytical grade solvents were used for extraction purposes while LC–MS grade solvents were used for UHPLC–MS applications. They were obtained from Fisher Scientific (Loughborough, UK). Ultrapure water was obtained using a purified-water system (Sartorius AG, Goettingen, Germany). Millex-GV syringe filters (PVDF 0.22 μm) were obtained from Millipore (Darmstadt, Germany) and glass beads of 0.5 mm were purchased from Thistle Scientific Ltd. (Glasgow, UK).

2.2. Study area and sample collection

Multiple locations within the BPNS were sampled between July and September 2014. An overview of the study area and the sampling stations is provided in Fig. 1. Six sites were chosen around the Ostend harbor and the adjacent sluice dock, i.e. an artificial seawater basin of 85 ha (station 1- station 6). Another six coastal (open water) sites (Fig. 1, 130, 330, 230, 700, 710, 780) were sampled using the research vessel Simon Stevin. The sampled area is characterized by natural sand banks, with water depths varying between 10 and 24 m. Water samples were taken by Go-Flow® bottles and a CTD (Conductivity, Temperature, Depth) carrousel. Phytoplankton and zooplankton were then isolated by filtering the seawater through a plankton net with a mesh size of 15 μm or 80 μm, respectively. A minimum of 50 L of seawater was filtered at each station. The concentrates were stored into 1-L flasks at 4 °C for further analysis of phytoplankton and zooplankton composition, and toxin screening. The phytoplankton and zooplankton community compositions were determined using a stereo and/or an inverted microscope. Organisms were identified to the lowest taxonomic level possible. Different marine organisms were sampled if present in the environment. Specimens (i.e. shrimp (*Crangon crangon*), shellfish (*Mytilus edulis*, *Crassostrea gigas* and *Patella* sp.), shore crab (*Carcinus maenas*) and fish (*Trachurus trachurus* L. Carangidae)) were either sampled by hand (harbor and sluice dock stations) or using a beam trawl operated from the research vessel. Each sample was stored in a 2-L zipper bag and transported to the laboratory for subsequent analysis.

2.3. Instrumentation

UHPLC analysis was carried out using an Accela UHPLC pumping system coupled to an Accela Autosampler and Degasser (Thermo Fisher Scientific, San Jose, CA, USA). Chromatographic separation of compounds was achieved on a Nucleodur C18 Gravity column according to Orellana et al. (2014, 2015).

Mass spectrometric analysis was carried out on an Exactive™ benchtop Orbitrap mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA), equipped with a heated electrospray ionization probe (HESI-II) that operated in switching polarity mode. The mass