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Detection and effects of harmful algal toxins in Scottish harbour seals and potential links to population decline

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ABSTRACT

Over the past 15 years or so, several Scottish harbour seal (*Phoca vitulina*) populations have declined in abundance and several factors have been considered as possible causes, including toxins from harmful algae. Here we explore whether a link could be established between two groups of toxins, domoic acid (DA) and saxitoxins (STXs), and the decline in the harbour seal populations in Scotland. We document the first evidence that harbour seals are exposed to both DA and STXs from consuming contaminated fish. Both groups of toxins were found in urine and faeces sampled from live captured (n = 162) and stranded animals (n = 23) and in faecal samples collected from seal haul-out sites (n = 214) between 2008 and 2013. The proportion of positive samples and the toxins levels measured in the excreta were significantly higher in areas where harbour seal abundance is in decline. There is also evidence that DA has immunomodulatory effects in harbour seals, including lymphocytopenia and monocytosis. Scottish harbour seals are exposed to DA and STXs through contaminated prey at potentially lethal levels and with this evidence we suggest that exposure to these toxins are likely to be important factors driving the harbour seal decline in some regions of Scotland.

1. Introduction

From spring to autumn marine phytoplankton proliferate and give rise to blooms, some of which result in the release of toxins and are known as harmful algal blooms or HABs. HABs have increased in distribution, frequency and intensity globally since the 1980s (Anderson, 1989; Hallegraeff, 1993) and are now a major factor contributing to mass mortalities among marine mammals worldwide (Geraci et al., 1989; Scholin et al., 2000; Van Dolah, 2000). In Scottish waters two of the most important toxin-producing species are the dinoflagellates from the genus *Alexandrium* and the diatoms from the genus *Pseudo-nitzschia*. Some species from these two phytoplankton genera have the ability to produce marine toxins such as domoic acid and saxitoxins. These toxins can induce significant health effects in mammalian systems (Collins et al., 2009; Fehling et al., 2004; Hall and Frame, 2010; Hart et al., 2007; Landsberg, 2002; Van Dolah, 2005). Marine mammals such as seals are exposed to these neurotoxins by consuming contaminated prey for example planktivorous fish or squid (Bargu et al., 2002; Flewelling et al., 2005; Landsberg, 2002; Lefebvre et al., 1999; Robertson et al., 2004). Exposure to these neurotoxins can have direct consequences on the health and survival rate of this marine mammal species.

1.1. Scottish harbour seal decline

Since around 2000 many of the harbour seal (*Phoca vitulina*) populations inhabiting Scottish coastal waters have been in rapid decline, but the causes remain unknown. For example, harbour seal populations on the east coast and in the Northern Isles (Orkney and Shetland) in particular have decreased by up to 85% between 2000 and 2010, representing an average rate of decline of up to 18% per annum (SCOS, 2013). One factor potentially involved in the decline is the effect of marine toxins on harbour seal health and survival

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(Hall and Frame, 2010). Here, we investigate the exposure and health effects of DA and STXs in Scottish harbour seals; examine accumulated toxin levels in different prey species and explore possible links between toxin concentrations and degree of exposure between the declining and the stable harbour seal populations.

1.2. Domoic acid in marine mammals

Pseudo-nitzschia diatoms are a common part of the phytoplankton community in Scottish marine waters, with some of these species known to produce DA (Fehling et al., 2006; Trainer et al., 2012). Official monitoring for DA in shellfish from inshore and offshore areas in Scotland started in 1998 and it has since been detected in shellfish above the regulatory limit (20 mg DA/kg of shellfish meat) (Tett and Edwards, 2002) on a regular basis. In humans DA ingestion triggers the condition known as amnesic shellfish poisoning (ASP) and can cause neuronal degeneration and necrosis in specific parts of the brain. After an outbreak of illness in humans caused by the consumption of DA contaminated blue mussels in Canada in 1987, a regulatory level was established for human consumption (Bates et al., 1989). In the US, acutely (rapid ingestion of large amount of DA) DA-exposed California sea lions (*Zalophus californianus*, CSLs) showed neurological signs such as ataxia, head weaving, seizures or coma (Gulland et al., 2002). Haematological parameter changes have also been documented in CSLs following DA toxicosis, but the physiological mechanism underlying this is not well understood (Gulland et al., 2002). For example, Gulland et al. (2012) found that eosinophil counts were significantly higher in CSLs showing clinical signs of DA toxicity, and this was also reported for bottlenose dolphins (*Tursiops truncatus*) in the northern Gulf of Mexico (Schwacke et al., 2010). Studies have suggested that pregnant CSL females may have higher exposure due to sequestration of the toxins in the amniotic fluid and that DA causes reproductive failure (Brodie et al., 2006; Goldstein et al., 2008). In addition, endocrinological changes have also been documented when CSLs are exposed to DA as it also affects the adrenal gland, lowering the production of cortisol (Gulland et al., 2012, 2002).

1.3. Paralytic Shellfish Poisoning toxins in marine mammals

Paralytic Shellfish Poisoning (PSP) toxins (saxitoxins and derivatives) pose the greatest concern for seals in Scotland due to the high lethal effect of some of the analogues (Deeds et al., 2008). Due to a lack of information on chronic PSP toxicity data, the European Food and Safety Authority (EFSA) established an oral acute reference dose (ARfD) in humans of 0.5 μg STX equivalents/kg body weight in humans (Alexander et al., 2009). Canids, which are considered to be evolutionarily and physiologically similar to seals, have a lethal dose of 180–200 μg STX/kg b.w. (McFarren et al., 1961). Mice have an acute oral LD50 dose of 263 μg STX/kg and whilst humans have a minimum oral dose of 7–16 μg of STX/kg body weight (Levin, 1992; Schantz et al., 1975). Mortal cases in humans linked to PSP poisoning have been observed at oral doses reaching 500–12,400 μg STX/kg body weight (Meyer, 1953). Since the outbreak of PSP in the UK in 1968 where 80% of the Shags (*Phalacrocorax aristotelis*) on the Farne Islands on the northeast coast of England, were killed (Coulson et al., 1968), an official monitoring programme has been set up to monitor the Scottish coast for the presence of *Alexandrium* spp. in the vicinity of shellfish aquaculture sites. Concurrently, shellfish were also analysed for the presence of PSP toxins using the mouse bioassay (MBA), until its replacement in 2008 with a chemical analysis method using Liquid Chromatography with Fluorescence Detection (LC-FLD) (Turner et al., 2009). Although toxic PSP events in shellfish have been very sporadic in Scottish waters, no known human cases of PSP have been reported (Swan and Davidson, 2011). PSP toxins have also been found in a range of organisms such as fish and benthic invertebrates (Landsberg, 2002) even though *Alexandrium* cells were absent from the water column (Sakamoto et al., 1992). Toxic *Alexandrium* spp. normally produce more than one PSP toxin derivative including STX, gonyautoxin I ~ VIII, neosaxitoxin (NEO), C-toxins and decarbamoyl toxins where some of these derivative are very toxic whilst others are only mildly toxic or potentially non-toxic (Anon, 2005; Oshima, 1995). PSP toxins bind to the voltage-gated sodium channels in the brain blocking the flow of ions across the cell membrane. This process inhibits nerve and muscle cells to send electrical signals, which prevents normal cellular function, and could lead to paralysis. PSP toxins can also bind to the potassium channel where it modifies the channel gating and reduces the potassium conductance (Cusick and Sayler, 2013; Narahashi et al., 1967).

Respiratory arrest is the most severe symptom of PSP exposure, which could rapidly be followed by death. Evidence of STX exposure in the endangered North Atlantic right whale (*Eubalaena glacialis*) (Doucette et al., 2006) occurred in 2001 where STX was considered to be a contributing factor in the failure of the population to recover from decline. Saxitoxin was also considered to be involved in the sudden unusual mortality event of the Mediterranean monk seal (*Monachus monachus*) off western Sahara in 1997 (Hernández et al., 1998) and humpback whales (*Megaptera novaeangliae*) off Cape Cod Bay, USA in 2001 (Geraci et al., 1989).

2. Methods

2.1. Sample collection from live captured harbour seals

Urine, faeces and blood samples were collected from live-captured harbour seals (Table 1) between 2008 and 2013, from various locations around Scotland with the aim to detect and quantify DA and PSP toxins. These locations were arbitrary located in three assigned zones, East, North and West for the purpose of this study (Fig. 1). The harbour seal populations have different abundance trends. While the west coast population is stable or increasing in certain areas, in the Northern Isles and the east coast the population of harbour seals is declining. The harbour seal samples were collected in relation to capture-release studies carried out by the Sea Mammal Research Unit (SMRU) under the UK Animal (Scientific Procedures) Act 1986, Project and Personal Licenses. Once captured, all the seals were weighed and anaesthetised using 0.05 mg kg^{-1} Zoletil100 (Virbac, France) intravenously using the extradural vein. Blood samples were collected from the extradural vein and stored in plain and heparinised blood

Table 1
Number of live captured harbour seals used for this study.

Region	Season	Faeces (*Sex*)	Urine (*Sex*)	Serum
East coast	Spring	7 (*2 F, 5 M*)	6 (*1 F, 5 M*)	7
	Summer	4 (*1 F, 3 M*)	10 (*4 F, 6 M*)	10
	Autumn	9 (*5 F, 4 M*)	19 (*11 F, 8 M*)	19
Northern Isles	Spring	n.a	n.a	n.a
	Summer	10 (*9 F, 1 M*)	21 (*16 F, 5 M*)	21
	Autumn	15 (*8 F, 7 M*)	17 (*7 F, 10 M*)	17
West coast	Spring	16 (*9 F, 7 M*)	23 (*10 F, 13 M*)	23
	Summer	4 (*4 F, 0 M*)	14 (*10 F, 4 M*)	14
	Autumn	n.a	n.a	n.a

The table displays the number of live captured harbour seals by region, season (spring = Mar, Apr, May; summer = Jun, Jul, Aug; autumn = Sept, Oct) and matrix (faeces, urine or serum).