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INTERNATIONAL WHALING COMMISSION

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Abstract

This study is the first to document the exposure of a range of cetaceans inhabiting Scottish waters to the neurotoxin, domoic acid (DA). Overall approximately 40% of the individuals screened (n=158) had detectable DA in their urine or faeces. This included 12 different species, such as harbour porpoise, long finned pilot whale, minke whale, white-beaked dolphin and sei whale. In general levels were low (median ~2 ng/g or ng/ml) but concentrations in excreta are difficult to interpret as time since exposure is not known. One harbour porpoise had a very high level in its urine (~2500 ng/ml) suggesting in at least one case exposure was at acutely toxic levels. However, these results were from stranded animals that, at post mortem examination, died of various causes and were not associated with any obvious signs of neurotoxicity, suggesting the overall exposure is likely to be low level but possibly chronic. *Pseudo nitzschia* spp. diatoms are now highly prevalent in Scotland throughout the year and are may produce large toxic blooms during the summer months. The consequences of prolonged DA ingestion for the health of individuals and cetacean populations in this region remains unknown.

Introduction

Toxins from harmful algal blooms (HABs) are becoming an increasingly widespread health issue for a variety of marine mammal species (Lefebvre *et al.* 2002). These molecules can be highly neurotoxic (Pulido 2008), teratogenic (Kimm-Brinson & Ramsdell 2001) hepatotoxic or cause respiratory paralysis (Franz & LeClaire 1989). High concentrations may be taken up directly or through the food chain when animals are foraging during large bloom or 'red-tide' events (Flewelling *et al.* 2005) and such high exposures are often associated with increases in the occurrence of sick, live stranded animals (Scholin *et al.* 2000) and mass mortalities. However, chronic health sequelae are also manifest following non-lethal acute exposure, particularly to the potent neurotoxin domoic acid (Goldstein *et al.* 2008). In addition, cases of DA toxicosis are often reported in stranded animals when no large bloom events are occurring, possibly due to the persistence of DA long after the bloom event has ended and sequestration into the sediments (Burns & Ferry 2007). In addition, there is increasing evidence for longer term, low level (chronic) exposure among marine mammals (Lefebvre *et al.* 2016), exacerbated by the maintenance of DA in the food chain and habitat. Whilst some affected cetacean species feed seasonally prior to periods of fasting during breeding or migration others, such as harbour porpoise (*Phocoena phocoena*), forage more or less continuously in order to meet their energy demands (Wisniewska *et al.* 2016). The health risks and exposure scenarios for each of these groups may therefore differ based on their varying life history and foraging strategies, as well as where they feed in the food chain and their preferred prey species.

Of the toxins produced by harmful phytoplankton, DA is of particular concern for marine mammal health. It seems to be developing a ubiquitous distribution, occurring from the tropics (Takata *et al.* 2009) to the poles (Lefebvre *et al.* 2016) and marine mammals are judicious indicators of their distribution and global spread (Hallegraeff 1993). Samples (excreta and tissues) collected during the post mortem examination of marine mammals that strand alive or dead along the coastline can be analysed for the presence of DA and concentrations are indicative of minimal exposure. Due to the hydrophilic nature and very short systemic half-life of DA (Suzuki & Hierlihy 1993), interpretation of the absolute concentrations measured is difficult, as low levels in blood and excreta may indicate historic higher exposure or alternatively, recent lower exposure.

HAB toxins were reported as significantly impacting marine mammal populations in the 1980s and 1990s following mass stranding events in manatees *(Trichechus manatus*), humpback whales (*Megaptera novaeangliae*) and California sea lions (*Zalophus californianus*) (Geraci *et al.* 1989; O'Shea *et al.* 1991; Gulland *et al.* 2002). Cetaceans are exposed primarily through the consumption of planktivorous fish but secondary prey such as copepods and other zooplankton are also key determinants of exposure (Maneiro *et al.* 2005). In addition to the primary planktivirous and invertebrate vectors, demersal benthivore fish also appear to be an important group (Vigilant & Silver 2007). Studies of DA in fish are limited but results suggest that it may be largely restricted to the viscera, with concentrations being much lower in fish flesh, reducing the risk to humans but not to marine mammals.

In Scotland, DA produced by diatoms of the *Pseudo-nitzschia* spp group is of particular concern and invertebrate consumers and blooms are monitored by the Scottish Association for Marine Science (Swan & Davidson 2010) and by Marine Scotland and the Food Standards Agency, particularly in relation to the shellfish monitoring programme under EU Regulation (EC) 854/2004. Blooms of *Pseudo-nitzschia* spp vary annually and regionally as well as seasonally, with major blooms being regularly annually reported in Shetland and Orkney, although it is also widespread around the west coast in summer (ICES 2016).

The aim of this study was to determine the exposure and prevalence of DA in a variety of cetacean species that stranded around the Scottish coast between 2008 and 2016. Whilst DA has not caused any mass mortality events in this region, the impact of chronic, low level exposure on cetacean populations is unknown; an emerging issue that requires attention. Although the health effects caused by exposures below the recognised threshold for observable effects requires further research, a few studies documenting physiological and behavioural effects have been published (Truelove *et al.* 1996). For example, DA at sub-seizure exposure concentrations was shown to cause cognitive disruption in mice (Lefebvre *et al.* 2017) and persistent changes in behavioural and molecular indicators of stress response (Gill *et al.* 2012). Here we report the first study to evaluate the exposure of stranded Scottish cetaceans to DA and establish the range of both inshore and offshore species that are subjected.

Materials and Methods

Cetacean samples

Samples of faeces and urine were collected from cetaceans that stranded live or dead around the coasts of Scotland between 2008 and 2016 by the Scottish Marine Animal Strandings Scheme (SMASS) and analysed for the presence of DA. The number of samples by species are shown in Table 1. All cases were examined post mortem by the SMASS veterinary pathologists and, where possible, a cause of death was assigned. However, detailed histology was not available to investigate potential pathological relationships with toxin exposure.

Toxin extraction and analyses

DA concentrations were determined using a direct competitive ELISA (ASP assay kits, Biosense, Norway). Samples were analysed at the Sea Mammal Research Unit using the same approach as reported in Hall *et al.* (2010). Samples were diluted to 1:100 for urine and 1:400 for faecal samples in 50% methanol. The DA in faecal samples was extracted using 50% methanol and were further cleaned up using solid phase extraction (SPE), strong anion exchange (SAX) columns (Supelco, UK) following the method of Lefebvre *et al.* (1999). All samples were analysed in duplicate.

Results

Concentrations of DA

The concentrations of DA in all stranded cetaceans by sample type are given in Table 1. Most of the samples were at the limit of detection, with a very wide range of concentrations up to a maximum of ~2490 ng/g detected in a urine sample from a single harbour porpoise. This outlier was therefore excluded from the majority of the analyses.

Due to the skewed distribution of the data (Figs. 1a and 1b) results were reported as medians, with minimum and maximum concentrations found by species. For most species the median concentrations were low however, sample sizes by species were extremely small with only one or two representatives of many species being analysed (Table 1). Over all species and sample types (n=158) the median concentration was 2.19 ng/g or ng/ml including the samples at the limit of detection. Excluding those at the LOD (n=119) increased the median concentration to 4.07 (range 0.54 – 119.87 ng/g or ng/ml). Thus 75% of the all samples analysed contained DA above the LOD.

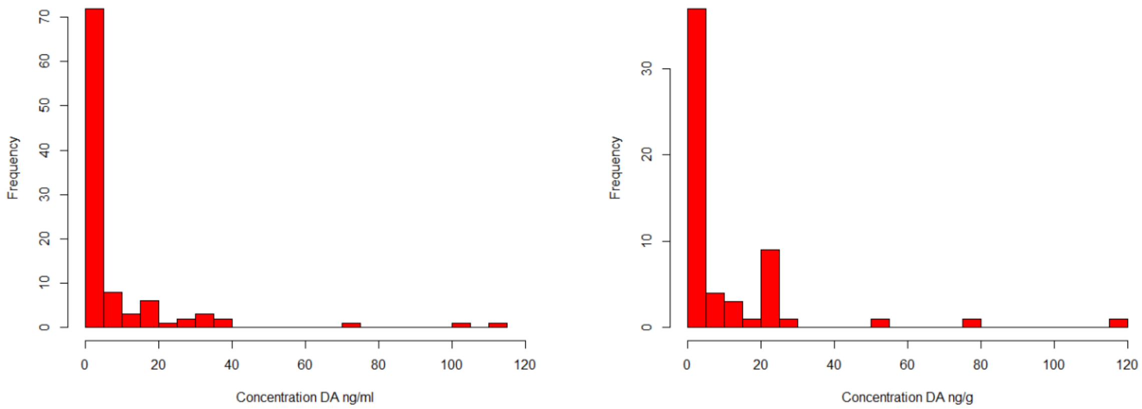

Figure 1. Frequency distribution of concentration of DA in (a) urine and (b) faecal samples from all stranded cetaceans (excluding very high result >2000 ng/ml in urine of harbour porpoise).

Median concentrations were slightly higher in faeces than urine, as has been seen in other marine mammals (Jensen *et al.* 2015), although this difference was not statistically significant (glm with Gamma distribution, p=0.39) (Fig. 2a). There was also no difference between samples from animals that stranded during the autumn/winter compared to the spring/summer (glm with Gamma distribution, p=0.55, Fig 2b).

Table 1. Domoic acid concentrations in urine and faecal samples from stranded cetaceans around Scotland, 2008-2016 (concentrations at LOD included as LOD/2 = 0.5 ng/ml or ng/g).

Species	Samples	Urine	Faeces	Urine ng/ml	Faeces
	n	n	n	median (min, max)	median (min, max)
Atlantic white-sided dolphin	2	2	0	28.36 (26.17, 30.54)	-
Bottlenose dolphin	2	1	1	2.96 (NA)	5.9 (NA)
Harbour porpoise	107	62	45	1.63 (0.5, 2487.16)	2.49 (0.5, 119.87)
Minke whale	2	2	0	0.92 (0.92, 0.92)	-
Northern bottlenose whale	1	1	0	1.26 (NA)	-
Long finned pilot whale	31	21	10	0.61 (0.5, 30.62)	3.55 (0.5, 79.75)
Pygmy sperm whale	1	1	0	113.13 (NA)	-
Sei whale	1	1	0	4.74 (NA)	-
Short-beaked common dolphin	1	1	0	27.48 (NA)	-
Sowerby’s beaked whale	2	1	1	0.8 (NA)	9.87 (NA)
Striped dolphin	3	3	0	3.19 (3.19, 19.54)	-
White-beaked dolphin	6	5	1	1.14 (0.84, 31.04)	0.5 (NA)

However, due to the short half-life of the toxins in the mammalian system (Suzuki & Hierlihy 1993), absolute concentrations represent only the minimum exposure and levels are not necessarily comparable between samples or species. Concentrations will depend not only on the diet of the animals as well as the occurrence of the toxins in their prey, but whether they feed more or less continuously or undergo periods of fasting or bout feeding.

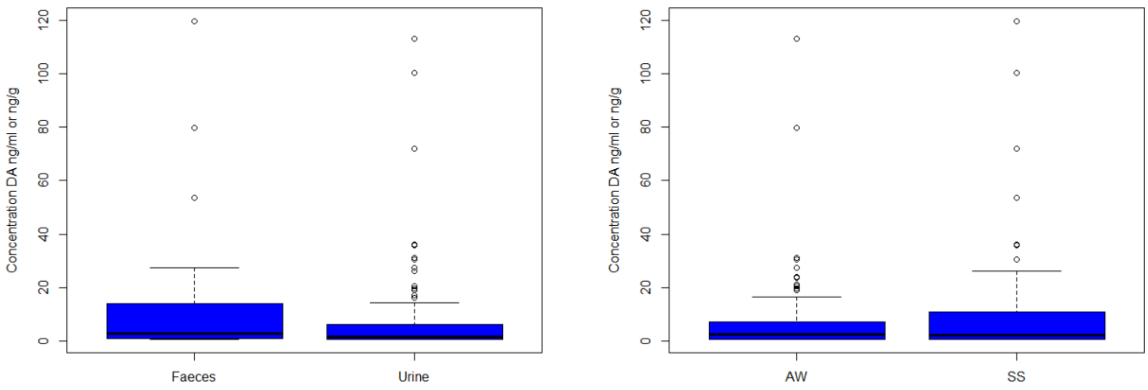

Figure 2. Boxplot of concentrations of DA in samples from all stranded cetaceans by (a) sample type and (b) season (AW=Autumn/Winter, SS=Spring/Summer)

The only two species for which a reasonable sample sizes were available were the long finned pilot whales (*Globicephala melas,* sampled following two mass stranding events) and the harbour porpoise. These species dominate the above frequency histograms.

Harbour porpoise

For the harbour porpoise the concentrations (excluding the outlier) were again slightly but not significantly higher in the faeces than the urine samples (glm with Gamma distribution, p=0.35, Fig 3a). However, there was a significant positive relationship between the concentrations in the two excreta (lm, p=0.013, R^2=0.32) for 16 individuals in which both results were available. In addition concentrations were significantly higher in the spring/summer than the autumn/winter (glm with Gamma distribution, p=0.05).

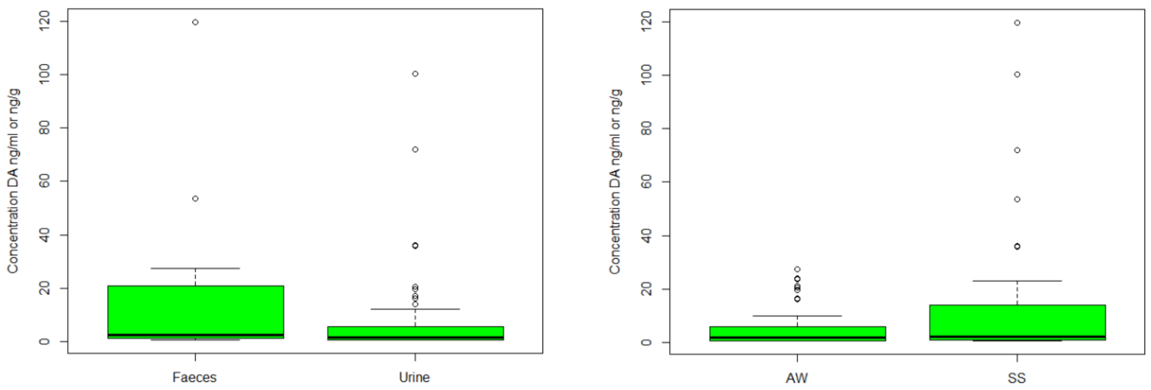

Figure 3. Boxplot of concentrations of DA in samples from stranded harbour porpoise by (a) sample type and (b) season (AW=Autumn/Winter, SS=Spring/Summer)

When the data were investigated by month (Fig 4) it was apparent that the higher concentrations were all reported in porpoises that stranded during March.

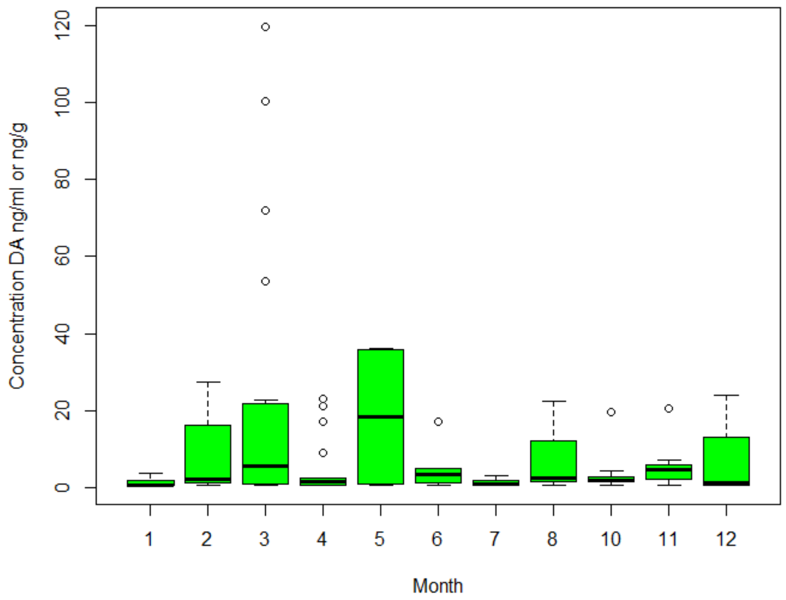

Figure 4. Boxplot of concentrations of DA in samples from stranded harbour porpoise by month.

In order to investigate the prevalence of exposure a more conservative threshold concentration of 8 ng/ml in urine was taken rather than the LOD of 0.5 ng/ml. This is shown in the cumulative plot in Fig 5a and gave an overall prevalence of 48% of animals as positive for DA in urine. A similar plot and threshold for faecal samples (8 ng/g) gave an overall prevalence of 33% of animals positive for DA in faeces.

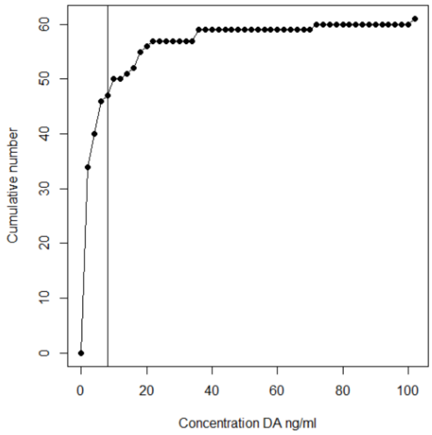

Figure 5a. Cumulative frequency plot of DA concentrations in harbour porpoise urine samples (vertical line = 8 ng/ml).

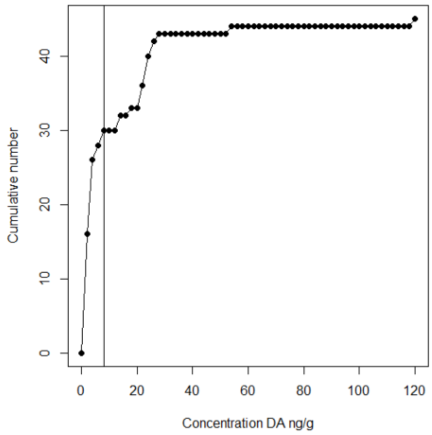

Figure 5b. Cumulative frequency plot of DA concentrations in harbour porpoise faecal samples (vertical line = 8 ng/g).

Long finned pilot whales

For the pilot whales (n=31), there was no difference in concentrations between faeces and urine (glm, Gamma distribution, p=0.325, see Table 1) and no seasonal difference (glm, Gamma distribution, p=0.57). Only 5 individuals had both faecal and urine samples analysed but there was a positive relationship between the two types of sample, with the faecal results being higher than the urinary concentrations, although the relationship was not significant at the 5% level (lm, p=0.08, R^2=0.59).

In terms of exposure prevalence, using the same urinary and faecal thresholds, 9% of the pilot whales were positive in urine and 20% were positive in faeces.

Spatial distribution

The concentration of DA in all the samples by location are shown in Fig 6 with the colours from blue to red indicating the concentration from low to high. The shape of the symbols also relates to the different species, with the harbour porpoise dominating. The two mass strandings of pilot whales were on the north and east coasts in 2011 and 2012.

The strandings are dominated by those reported on the east coast of Scotland. The number of strandings reported and responded to on the west coast is more limited due to the sparse population, convoluted coastline and the logistical constraints. With those caveats in mind, there were no obvious spatial clusters with the higher levels being reported in stranded cetaceans from both coasts, including the outer Hebrides (up to 120 ng/g in faeces). The harbour porpoise with the very high level (~2500 ng/ml in urine) stranded on the east coast of Scotland in August, 2012.

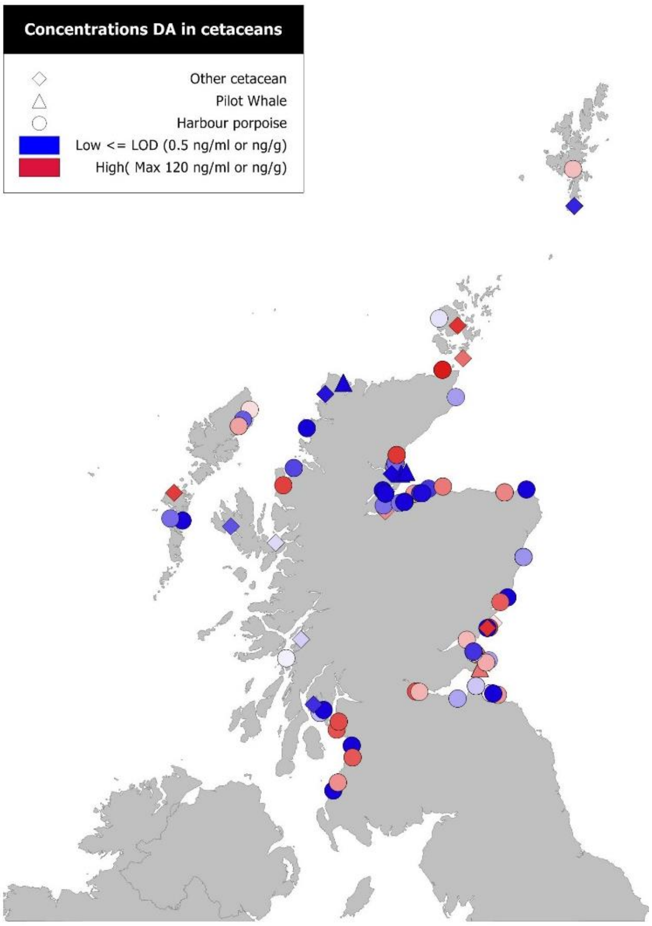

Figure 6. DA in cetaceans by stranding location, concentration and species.

Discussion

This is the first study to show that a variety of stranded cetacean species around the Scottish coast have been exposed to DA. They range from the most abundant small cetacean species in the North Atlantic, the harbour porpoise to the deeper diving offshore species such as the pygmy sperm whale (*Kogia breviceps)*. Interestingly whilst only a single pygmy sperm whale was investigated in this study, it has one of the highest concentrations in its urine sample. The primary prey of this species are cephalopods which have been reported to be important accumulators of HAB toxins (Lopes *et al.* 2013). Prey choice is likely to be one of the main drivers for exposure variation among the different species, including both primary and secondary prey, as will the persistence of DA in the sediment long after the cessation of any HAB event.

In general concentrations found in excreta were low and similar to those reported recently in various species from the Arctic (Lefebvre *et al.* 2016). However, it is difficult determine the relevance of the absolute concentrations in the samples as systemic half-lives are short (approximately 9-12 hours, ref). The presence of DA in faecal samples may be longer and be a more reliable indicator of exposure. Urinary levels will give at the least a minimum exposure level.

For the most abundant species investigated, the harbour porpoise, it was interesting to note that almost half the animals sampled had detectable DA in their excreta. Using the same higher detection threshold of 8 ng/g or ml as a conservative estimate for exposure, 42% of all cetaceans screened were positive. This threshold is similar to the lowest concentration recorded in the acutely exposed California sea lions (Goldstein *et al.* 2008) and avoids some of the measurement error associated with the LOD. However, further analysis of repeat measures for determining the LOD and its variability as well as the range of quantitation for the ELISA method used (Hayashi *et al.* 2004) will be carried out in future.

The higher concentrations in harbour porpoise in March likely coincided with early blooms of *Pseudo nitzschia* spp but there was no consistent spatial pattern in these strandings with animals from both the west and east coasts having higher levels in this month. The highest levels in porpoises were however, found in 2013, a year in which high concentrations of *Pseudo nitzschia* were observed all around the Scottish coast during the year, with one shellfish harvesting area on the west coast being closed. The very highest level reported in the urine from a single porpoise is similar to concentrations reported from acute cases in California sea lions and it is highly likely that this animal suffered from neurotoxic effects associated with high exposure. However, in general the concentrations found suggest that exposure is probably low level but could be chronic in nature, given the persistence of DA in the environment following a bloom.

In conclusion, we have shown that DA exposure is widespread in cetaceans that inhabit Scottish waters but that the individual and population health consequences of this remains unknown.

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