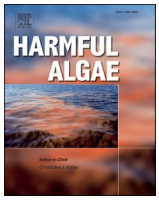
Harmful Algae 136 (2024) 102653

Contents lists available at ScienceDirect

Harmful Algae

journal homepage: www.elsevier.com/locate/hal



Estimating the risks of exposure to harmful algal toxins among Scottish harbour seals

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ARTICLE INFO

Edited by Dr. C. Gobler

Keywords:
Biotoxin
Domoic acid
Saxitoxins
Paralytic shellfish toxins
Phoca vitulina
Risk assessment

ABSTRACT

Harmful algal bloom (HAB) toxins consumed by marine predators through fish prey can be lethal but studies on the resulting population consequences are lacking. Over the past approximately 20 years there have been large regional declines in some harbour seal populations around Scotland. Analyses of excreta (faeces and urine from live and dead seals and faecal samples from seal haulout sites) suggest widespread exposure to toxins through the ingestion of contaminated prey. A risk assessment model, incorporating concentrations of the two major HAB toxins found in seal prey around Scotland (domoic acid (DA), and saxitoxins (STX)), the seasonal persistence of the toxins in the fish and the foraging patterns of harbour seals were used to estimate the proportion of adults and juveniles likely to have ingested doses above various estimated toxicity thresholds. The results were highly dependent on toxin type, persistence, and foraging regime as well as age class, all of which affected the proportion of exposed animals exceeding toxicity thresholds. In this preliminary model STX exposure was unlikely to result in mortalities. Modelled DA exposure resulted in doses above an estimated lethal threshold of 1900 μg/kg body mass affecting up to 3.8 % of exposed juveniles and 5.3 % of exposed adults. Given the uncertainty in the model parameters and the limitations of the data these conclusions should be treated with caution, but they indicate that DA remains a potential factor involved in the regional declines of harbour seals. Similar risks may be experienced by other top predators, including small cetaceans and seabirds that feed on similar prey in Scottish waters.

1. Introduction

The occurrence of harmful algal blooms (HABs), often persistent aggregations of marine phytoplankton that have toxic effects, is temporally and spatially variable and a significant issue in the North Atlantic (Bresnan et al., 2021; Hallegraeff 2010). The toxins HABs produce pose significant risks to the health and survival of marine predators (Landsberg 2002; Shearn-Bochsler et al., 2014). Mass mortalities and strandings among marine mammals due to HAB toxicosis have been regularly reported since the late 1990s (Flewelling et al., 2005; Landsberg 2002; Scholin et al., 2000) and the impact of a wide variety of toxins has been described across all the marine mammal taxonomic groups (van Dolah et al., 2003; McCabe et al., 2016; Turner et al., 2021). However, although studies have investigated exposure risk in marine mammals (Bejarano et al., 2007) and considered how foraging behaviours and strategies will influence uptake (Hendrix et al., 2021; Lefebvre et al., 2022) to our knowledge, no published studies have taken the next step and assessed what the risks of exposure mean for the dynamics of the population. . Here the potential impact of HAB toxin exposure on harbour seal (*P. vitulina*) health and survival, using empirical data on concentrations of toxins in their fish prey and a bioenergetic model, is described.

Mortalities and stranding events frequently follow acute exposure to high levels of toxin during persistent and widespread bloom events. However, acute events can occur at unexpected times of year, or be of prolonged duration, due to anomalous weather patterns or storms (McCabe et al., 2016; Turner et al., 2018) as well as the persistence of the toxins in the food chain (Lopes et al., 2018; Terrazas et al., 2017). In

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https://doi.org/10.1016/j.hal.2024.102653
Received 21 February 2024; Received in revised form 20 May 2024; Accepted 20 May 2024
Available online 31 May 2024
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addition to these acute events, there is increasing concern about the impact that low-level, chronic toxin exposure may have on the health and survival of marine organisms (Moyer et al., 2018). Many species of marine toxin persist long after the blooms have subsided (D. M. Anderson et al., 2012), posing a potential chronic risk. Indeed, a recent study by Akmajian et al. (2017) detected saxitoxin (STX, produced by dinoflagellates of the genus *Alexandrium* (D.A. Anderson et al., 2012) and domoic acid (DA, produced by diatoms of the genus *Pseudo-nitzschia* (Fehling et al., 2004)) in California sea lion (*Zalophus californianus*) scats throughout the year. In addition, the dose of toxin experienced by marine mammals will be determined by their foraging behaviour. Many species, particularly phocid seals, feed in bouts (Sharples et al., 2012), the duration of which will have a major impact on the short-term ingestion rate and therefore the potential for individual exposures to exceed toxic thresholds.

Following the observed major decline in harbour seal abundance in some regions of Scotland (Thompson et al., 2019), investigations into the potential role that DA (Hall and Frame 2010) and other HAB toxins may have played in this abundance decrease were undertaken (Jensen et al., 2015; Jensen 2014). The results indicated widespread exposure to DA and STX among harbour seals throughout Scotland and a potential role for HAB toxins in the decline. Exposure to DA at various levels, appears to be extensive and was the most prevalent of the two toxins, but particularly in the harbour seal declining areas (Hall and Frame 2010; Jensen et al., 2015). DA is a potent neurotoxin, causing amnesia, disorientation, and in extreme cases seizures and death in humans (Lelong et al., 2012), However, live seals experiencing signs of acute exposure to DA, as seen during major bloom events elsewhere, have not been reported. For example, California sea lions ingesting high levels of toxin in their prey (Lefebvre et al., 1999) suffer from seizures, head weaving and have high activities of serum creatine kinase (Gulland et al., 2002), signs also reported in affected Pacific harbour seals (McHuron et al., 2013). Nonetheless, the evidence that exposure, through analysis of faeces and urine samples from dead stranded and live-captured harbour seals and in faeces collected from seal haulout sites, is widespread throughout Scotland, suggests chronic uptake could be impacting these populations, with some individuals experiencing lethal doses. A recent study in monkeys exposed to DA near the human regulatory limit found an association with intention tremors (i.e. tremors produced during purposeful movements towards a target) which were linked to structural changes in the white matter of the brain (Petroff et al., 2019). Studies in adult mice revealed that low dose exposure increased the CA1 vesicular glutamate transporter levels in the hippocampus which could lead to increased hippocampal excitability (Moyer et al., 2018). This might explain the findings of Lefebvre et al. (2017) who reported spatial learning and memory deficits in low-level, chronically exposed mice. Spatial learning and memory deficits have also been reported in exposed California sea lions, linked to reduced hippocampal size and connectivity with other brain regions (Cook et al., 2015). Neurobehavioural development also appears to be affected by low-level exposure of DA in pregnant mice. Motor coordination, gait and exploratory activity of offspring from gestationally exposed females were affected at levels that did not induce toxicity in the dams (Shiotani et al., 2017).

Of the other toxins occurring in Scottish waters, STXs, also referred to as the paralytic shellfish toxins (PSTs), are among the most lethal, causing paralytic shellfish poisoning in humans. These are highly potent sodium channel toxins and lethal doses can kill a victim within a few hours due to respiratory failure (Llewellyn 2006). PSTs have long been identified as the causative agent of mass mortality events in marine mammals, most notably the 1997 mass mortality of Mediterranean monk seals (*Monachus monachus*) in Mauritania (Hernandez et al., 1998; Reyero et al., 1999) but also a mass stranding of humpback whales (*Megaptera novaeangliae*) in the late 1980s (Geraci et al., 1989). More recently STXs were identified as causing mortality among dogs on the southeast coast of the UK that had eaten contaminated fish and invertebrates (particularly highly contaminated starfish and crabs) that washed ashore following a storm (Turner et al., 2018). Dead seals were also reported on the same beaches at the same time but unfortunately none were analysed for STX ingestion.

HAB toxins are also immunotoxic (Ferreiro et al., 2017; Levin et al., 2010; Martin-Lopez et al., 2012) and the low level exposure of harbour seal lymphocytes to STX and phocine distemper virus in vitro increased the amount of virus present in the lymphocytes (Bogomolni et al., 2016). Indeed, immunomodulatory effects of DA were also seen in live captured harbour seals from Scotland (Jensen et al., 2015). In addition, there is increasing evidence that marine mammals, including harbour seals in Scotland, are often exposed to multiple toxins whose health effects, both singly and combined are not well understood (Capper et al., 2013; Fire et al., 2011; Jensen et al., 2015; Twiner et al., 2011).

Whilst simulations from a bioenergetic model to estimate the exposure of California sea lions to DA have been carried out (Bejarano et al., 2007), the subsequent population consequences were not included in the study. The aim of this study was thus to estimate the risk of toxicity from the HAB toxins, DA and STX to Scottish harbour seal populations through the ingestion of contaminated prey.

2. Methods

2.1. Toxin level in prey samples

Samples of fish collected from Orkney, Shetland and East Scotland (sampling areas are given in Fig. 1) between 2010 and 2023 were analysed for the presence of DA and STXs. The samples were either collected from commercial fishing boat by-catch (principally the East Scotland samples), were made available during the Shetland inshore fish surveys carried out by University of Highlands and Islands Shetland (formerly NAFC Marine Centre, specific sampling locations are given in Fraser et al. (2023)), or were captured specifically for this study using either rod and line or longline techniques. The methods of capture and subsequent fish euthanasia were approved by the University of St Andrews, Animal Ethics Committee. All fish were weighed, and their total length measured. Further details are given in Kershaw et al. (2021). Only species that appeared in the diet of harbour seals from the east and north of Scotland (Wilson and Hammond 2019) were included here.

Previous studies have shown that the toxins remain in the fish viscera (Mazzillo et al., 2010), so the viscera were excised, weighed and analysed separately. The number of sandeels and poor cod collected at each fishing bout were too few and too small to analyse the viscera separately, so these were homogenised and analysed as whole fish. The analytical methods and detailed results are given in Jensen et al. (2015) and Kershaw et al. (2021) respectively. Briefly, DA concentrations in fish viscera were estimated using the Biosense Laboratories ASP ELISA kit (Bergen, Norway, AOAC Official Methods 2006.02) or the EuroProxima B.V. DA ELISA kit (R-Biopharm, Arnhem, Netherlands) following the kit instructions and extracting the DA according to the methods for shellfish samples. Fish viscera (pools of up to 10 fish, captured during the same fishing bouts, i.e. where the same species were caught at the same time and location) were finely blended. Fish viscera were pooled to obtain sufficient mass for analysis and to account for potential heterogeneity in toxin uptake by individual fish. Subsamples were extracted with methanol according to each kit instructions, centrifuged, filtered and diluted at between 1:100 and 1:200 with sample diluent provided. All samples were analysed in duplicate. The individual kit protocol was then followed and the concentrations of DA in the extracts estimated from the logistic standard curve of optical densities obtained from the plate reader at 450 nm. The limit of detection (LOD) was estimated to be 0.01 μg DA/g estimated from blank ($n = 22$) and low concentration ($n = 22$) replicates.

A comparison between the results from the two DA kits (analysed by the Sea Mammal Research Unit) and the results from LC-MS/MS (analysed by Cefas, Weymouth) was carried out. The results from the two DA

Fig. 1. Fish sampling locations.

kits were highly comparable and positively linearly related ($R^2 = 0.9626$ Supplementary Material). Accuracy of each method was assessed through the analysis of a shellfish tissue homogenate used as a routine Laboratory Reference Material (LRM) and with a homogenised, mixed species viscera (dab, whiting, cod, mackerel and sandeel) spiked at 200 ug/kg DA. Both obtained good recovery following extraction (methanol as indicated by the manufacturer for the extraction of shellfish tissue in the ELISA, mean recovery 105 %, Supplementary Material) and using UT SPE clean up prior to LC-MS/MS analysis. The accuracy of the LC-MS/MS method was found to be excellent for both shellfish and tissues when methanolic extracts were subjected to SPE clean-up prior to analysis (Supplementary Material).

Concentrations of STX were also evaluated using the EuroProxima B. V. ELISA method developed for the quantification of toxins in shellfish flesh (R-Biopharm, Arnhem, The Netherlands). Extractions were carried out according to the manufacturers' instructions in which between 1 g and 4 g homogenised fish viscera was extracted with sodium acetate buffer. Following centrifugation, the supernatant was diluted at 1:50 with sample dilution buffer. Samples were assayed in duplicate and the concentrations calculated from the four-parameter logistic standard curve. Samples with high concentrations of STX were then diluted with sample buffer in two-fold dilution steps and parallelism was compared with the standard curve. The antibody in the kit also cross reacts with a number of STX analogues. An assessment of its performance compared with the pre-column oxidation HPLC-FLD method used for regulatory control of bivalve molluscs throughout Europe, indicated good correlation in bivalve mollusc samples (Harrison et al., 2016) and in a comparison with the fish viscera samples in this study showed a good linear relationship ($R^2 = 0.8133$, Supplementary Material).

The number of fish-pools analysed by species group are shown in Table 1, representing a total of 1333 individual fish. The species analysed are given in Supplementary Materials (Table S1). Details of the concentrations in the fish samples are summarised in Fig. 2 (a and b) see also Kershaw et al., 2021).

2.2. Model parameters

This analysis focusses on three seal management areas (http://marine.gov.scot/information/seal-management-areas), Shetland, North coast and Orkney and East Scotland, all of which have experienced

Table 1
Pooled fish viscera samples (harbour seal prey) analysed for the presence of HAB toxins by region.

Fish Group	East Scotland	North coast and Orkney	Shetland	Total
Flatfish	177	2	30	209
Gadid	94	69	36	199
Pelagic	24	15	10	49
Sandeel	12	1	13	26
Sandy Benthic	3	1	1	5
Scorpion Fish	35	2	8	45
Other	14	1	7	21
Total	359	91	105	555

Sandeels and poor cod were analysed as whole fish (see supplementary materials for numbers of fish by species and group).

declines in harbour seal abundance since around 2000. Harbour seal diet data for the three areas was obtained from Wilson (2014) and Wilson and Hammond (2019). In East Scotland the diet is dominated by sandeel (45 %) and flatfish (39 %) in the Spring/Summer and by flatfish (50 %) and gadids (17 %) in the Autumn/Winter. In Orkney the diet is made up of gadids (31 %) and sandeel (53 %)in the Spring/Summer and by gadids (39 %) and sandeel (18 %) in the Autumn/Winter. In Shetland the diet comprises pelagic fish (31 %), sandeel (23 %) and gadids (23 %) in the Spring/Summer and gadids (28 %) and sandeel (32 %) in the Autumn/Winter. The mass of an average adult and juvenile seal and its daily energy requirements were taken from Härkönen and Heide-Jorgensen (1991) in which the mean mass of an adult was estimated as 58.5 kg requiring 5589 kcal/day and a juvenile was 33.5 kg requiring 4680 kcal/day. Calorific densities of fish by species were estimated from Murray and Burt (1977) and Pedersen and Hislop (2001).

2.3. Toxicity thresholds

Various toxicity thresholds have been estimated for the two toxins of interest in different mammalian species but there are no published thresholds for marine mammals. Thresholds from studies on humans, in which oral doses through the consumption of contaminated shellfish have been established, were therefore used here (Table 2). As harbour seals have large body fat stores in the form of blubber, it is more appropriate to use lean body mass when using comparative toxicological thresholds from model species with low fat stores. The proportion of total body mass in harbour seals that is lean (calculated for animals outside the breeding season) is estimated to be approximately 0.7 (Polasek et al., 2015) and this was used to convert total body mass to lean body mass for dose (μg/kg lean body weight/day) estimation.

Three toxicity levels were available for the three toxins; a no observable adverse effect level (NOAEL), a lowest observable adverse effect level (LOAEL) and a neurotoxic dose or lethal dose sufficient to kill 50 % of the study animals (LD_{50}, collectively termed here the lethal dose level, LDL). The LOAEL and LDL were then used to estimate the proportion of the feeding days above that level.

2.4. Simulations

The model was written in R (R Development Core Team 2018) and is simple individual-based stochastic model (Railsback and Grimm 2012). Firstly, a fish species was chosen at random from the diet database as if taken by an individual seal, weighted by the numerical occurrence of that species and its mass in the diet for a given region (Shetland, North coast and Orkney or East Scotland) by month in order to reflect the proportion of that species found in the harbour seal diet. The chosen fish was then assigned a random species-group/mass/region specific concentration of toxin from the fish toxin database. Fish species groups were based on Wilson and Hammond (2019) (Table 1). The concentrations of toxins in the fish were measured in the viscera (except for the small species such as *Ammodytes* and *Trisopterus*) as previous studies have shown that negligible concentrations of toxin are found in the fish flesh (Mazzillo et al., 2010). Concentrations were therefore adjusted to whole fish using fish-group specific visceral ratios (Kershaw et al., 2021, Table S2 Supplementary Material). Where a fish species in the diet was not available from the toxin database, a mean concentration for the given toxin measured across all species was used.

Further fish were chosen in this way until the seal had satisfied its daily calorific energy requirement. Each fish species chosen was independent of the species that was previously chosen (see discussion). The total dose of toxin was then calculated for each feeding-day simulation as μg/kg lean body mass/day. This dose was set to zero at the start of the next feeding day. Algal toxins are hydrophilic and are excreted rapidly by humans and mammals, with an estimated half-life of 9 h for DA in California sea lions (Bejarano et al., 2007).

Simulations were carried out separately for adult and juvenile harbour seals. In the wild, harbour seal feeding is divided into bouts of varying duration, separated by resting periods hauled out on land. Thus, three foraging strategies were considered in separate simulations. Feeding trips comprising feeding every day, every second day and every third day were factored into the model by multiplying the daily 'feeding day' energy requirement by a factor of one (no change), two or three and reducing the number of feeding days in a year (*nFeedDays*) pro rata: 365/1, 365/2 and 365/3 respectively.

The resulting annual mortality (M) for the higher thresholds or likelihood that animals would experience adverse health effects for the lower thresholds (LOAEL) due to toxin intake was calculated, for each simulation as follows:

$$1-(1-pThresh)^{(nFeedDay\ *(monthsYearHAB/12))}$$

Where:

pThresh is the proportion of feeding-days where one of the toxin thresholds in Table 2 were exceeded.

nFeedDays is the number feeding-days in a year.

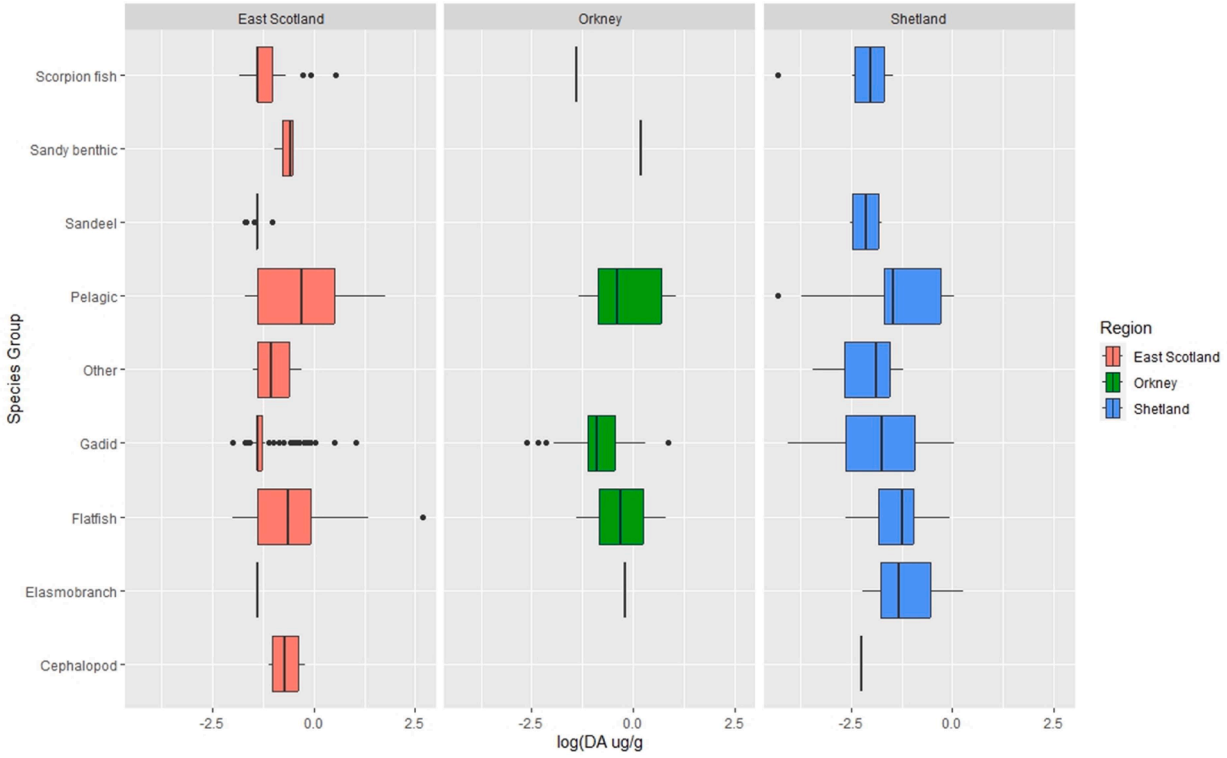
monthsYearHAB is the number of months of the year when HAB toxins are expected to be elevated in the environment and therefore in the prey. Data from the Food Standards Agency Scotland, Shellfish Official Control Monitoring for Shetland (Parks et al., 2019) was used to guide the choice of scenarios. As this may vary regionally, three scenarios were explored: for any given region, toxins remaining in prey for four (March to June), six (March to August) or nine (March to November) months of the year. Toxins can persist in the environment even after a bloom has subsided due, for example, to sediment adsorption (Burns et al., 2009) and toxins are recorded in shellfish samples from January through to October (Parks et al., 2019). As the diet of harbour seals also varies seasonally, prey items were randomly sampled from the diet database but within the relevant regions and seasons.

2.5. Population level

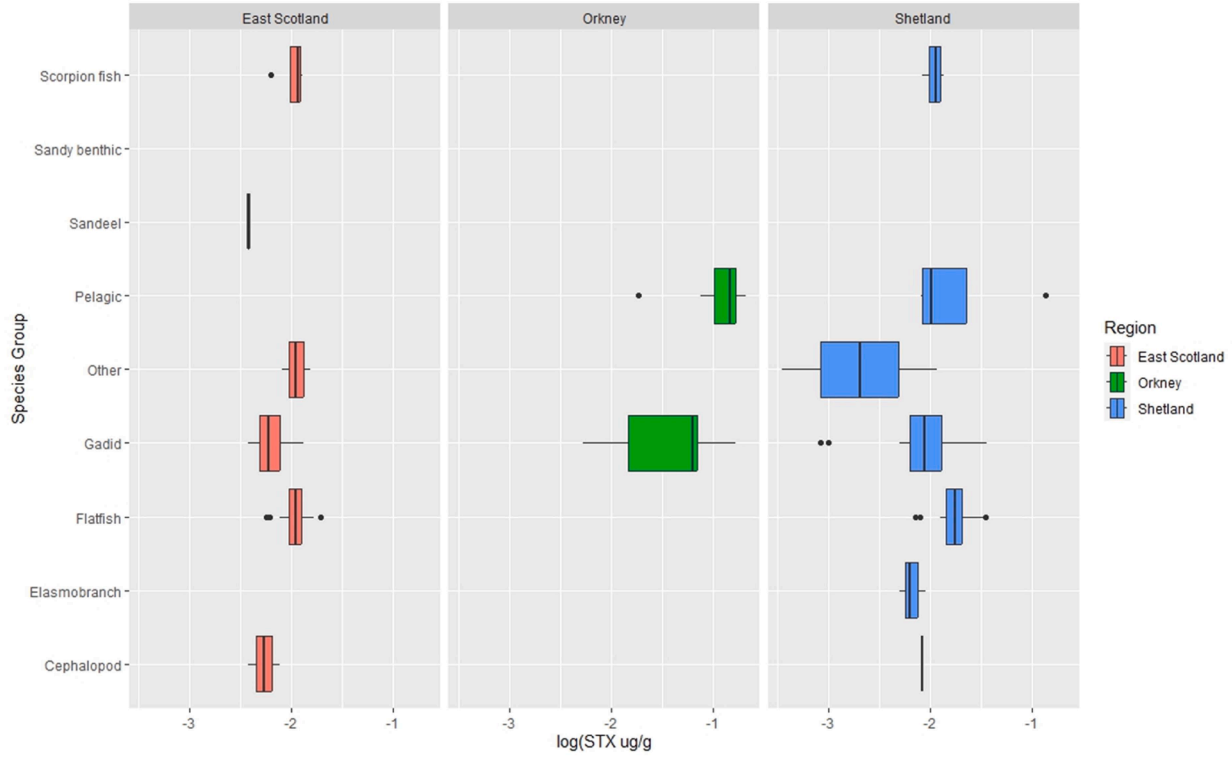
In order to understand the potential impact that such annual mortality could have at the population level, we constructed a simple Leslie matrix population model using the range of fecundity and age class specific survival rates published by Arso Civil et al. (2019). Within these ranges, combination of rates were restricted to those that produced a hypothetical stable population (i.e. with an annual population rate of change of $< +/-0.025$). We then investigated the impact that any additional mortality due to toxin ingestion might have compared to an unexposed stable population (see Supplementary Material).

3. Results

The proportion of simulations for adults and juveniles that exceeded a given toxicity threshold (*pThresh*) for each of the toxins are given in Table 3.



(a) DA



(b) STX

Fig. 2. Concentrations of toxins in pools of fish viscera (log transformed to account for the skewness in the data).

Table 2
Toxicity thresholds as μg/kg body weight.

Toxin	NOAEL	LOAEL	LDL (NTD / LD_{50})	Reference
DA	200	900	1900	(Costa, Giordano & Faustman 2010), (European Food Safety Authority 2009a)
STX equivalents	0.5	5.3	200	(European Food Safety Authority 2009b)

NOAEL = no observable adverse effect level; LOAEL = lowest observable adverse effects level; LDL= lethal dose level (NTD = neurotoxic dose).

Table 3
Simulation (n = 10,000 feeding days) results.

Toxin	Feeding Regime	LOAEL				LDL			
		monthYearHAB				*monthYearHAB*			
Adult									
		pThresh	4 months	6 months	9 months	*pThresh*	4 months	6 months	9 months
DA	1	0.001	0.024	0.036	0.053	0	0	0	0
	2	0.001	0.059	0.087	0.128	0	0	0	0
	3	0.002	0.064	0.093	0.136	0.0006	0.024	0.036	0.053
STX	1	0	0	0	0	0	0	0	0
	2	0.0040	0.217	0.306	0.423	0	0	0	0
	3	0.1439	0.998	0.999	0.999	0	0	0	0
Juvenile									
DA	1	0.0002	0.024	0.036	0.053	0	0	0	0
	2	0.0009	0.053	0.079	0.116	0.00001	0.006	0.009	0.013
	3	0.0037	0.141	0.202	0.286	0.00004	0.016	0.024	0.038
STX	1	0.0005	0.059	0.087	0.128	0	0	0	0
	2	0.1483	0.999	0.999	1	0	0	0	0
	3	0.6294	1	1	1	0	0	0	0

Proportion of simulations above toxin threshold (*pThresh*, see Table 2), and estimated annual proportion experiencing adverse health effects or lethal effects (*monthYearHAB* Eqn 1).

3.1. Domoic acid

Thresholds (Table 2) were exceeded in both age-classes for DA intake where the feeding regime was once every three days. Juveniles feeding at all regimes were at risk of both sublethal and lethal effects due to DA intake, suggesting they may be at more risk than adults.

Fig. 3 shows the density distribution of the DA doses for all simulations by feeding regime for the four months (*monthYearHAB*) at-risk scenario. The no-observable lethal effects level (NOAEL), lowest-observed-adverse-effect level (LOAEL), and neurotoxic dose (NTD) are shown. A small proportion of feeding-days exceeded the thresholds – some exceeding the lethal dose thresholds. The tail of this distribution was longer for the juveniles than the adults, reaching above 3000 μg DA/kg lean body weight/day.

The annual likelihood for sublethal effects among the juveniles of between 2.4 % and 14.1 % for *monthYearHAB* set to four increased to between 5.3 % and 28.6 % for *monthYearHAB* set to nine, depending on feeding regime (Table 3). In terms of lethal effects, annual likelihood percentages ranged from between 0.001 % and 0.004 % *monthYearHAB* set to four rising to between 1.3 % and 3.8 % for *monthYearHAB* set to nine. Among adults, the likelihood of sublethal effects were between 2.4 % and 6.4 % for *monthYearHAB* set to four to between 5.3 % to 13.6 % for *monthYearHAB* set to nine. The only regime where lethal effects were seen in adults were for the three day scenario, ranging from affecting between 2.4 % and 5.3 % (Table 3). This contrasts with the juveniles where risks of lethal effects were seen when animals were feeding every two days (up to 1.3 %) as well as every three days.

3.2. Saxitoxins

Concentrations of STXs in the fish samples were low (<0.2 μg STX eq./kg) resulting in no seal feeding-days reaching the lethal toxic thresholds (Table 2). However, between 21 % and 99 % of adult animals and 6 % and 100 % of juveniles reached the LOAEL depending on the number of months at risk (Table 3).

3.3. Population level

Using the Leslie matrix approach to understand the magnitude of the potential impact at the population level we compared the effect that an additional 0.053 annual adult mortality would have on a population of harbour seals compared to when it is in a stable condition. We found that this would produce an annual decline of between 3 and 5% (see Supplementary Material).

4. Discussion

Here we estimate the risk to Scottish harbour seals from the independent ingestion of two HAB toxins, DA and STX found in their prey. Using empirical data on the concentrations of the two toxin groups in fish samples from the east and north coasts of Scotland, the results of harbour seal diet studies in these regions (Wilson 2014) and published oral dose toxic thresholds (Table 2), the risk of lethal effects from STX ingestion was zero for both adults and juveniles. However, the annual probability of exceeding the neurotoxic dose from DA uptake was between 0 and 5.3 % for adults depending on the duration of exposure and persistence of toxin in the food chain (*monthYearHAB*). Among juveniles the risks ranged from 0 to 3.8 %, again depending on DA persistence and feeding regime, being exceeded at the lower feeding regime of every two days as well as every three days. The likelihood of thresholds being exceeded were greater for the feeding every three day foraging strategies. This was because the animals would have to ingest prey at a higher rate (i.e. in a shorter space of time) in order to attain their daily calorific and energy requirements than if they fed every day or every other day, therefore increasing their chances of exceeding the toxicity threshold. Thus among the juveniles the risk might be higher as a proportion of simulated individuals exceeded the threshold when feeding more regularly (every two days as well as every three days) due to their higher mass specific daily energy requirements.

Improvements in the model framework could be obtained by increasing its complexity, as discussed below, but intrinsic temporal and spatial variability in the concentration of the toxins in the fish and in

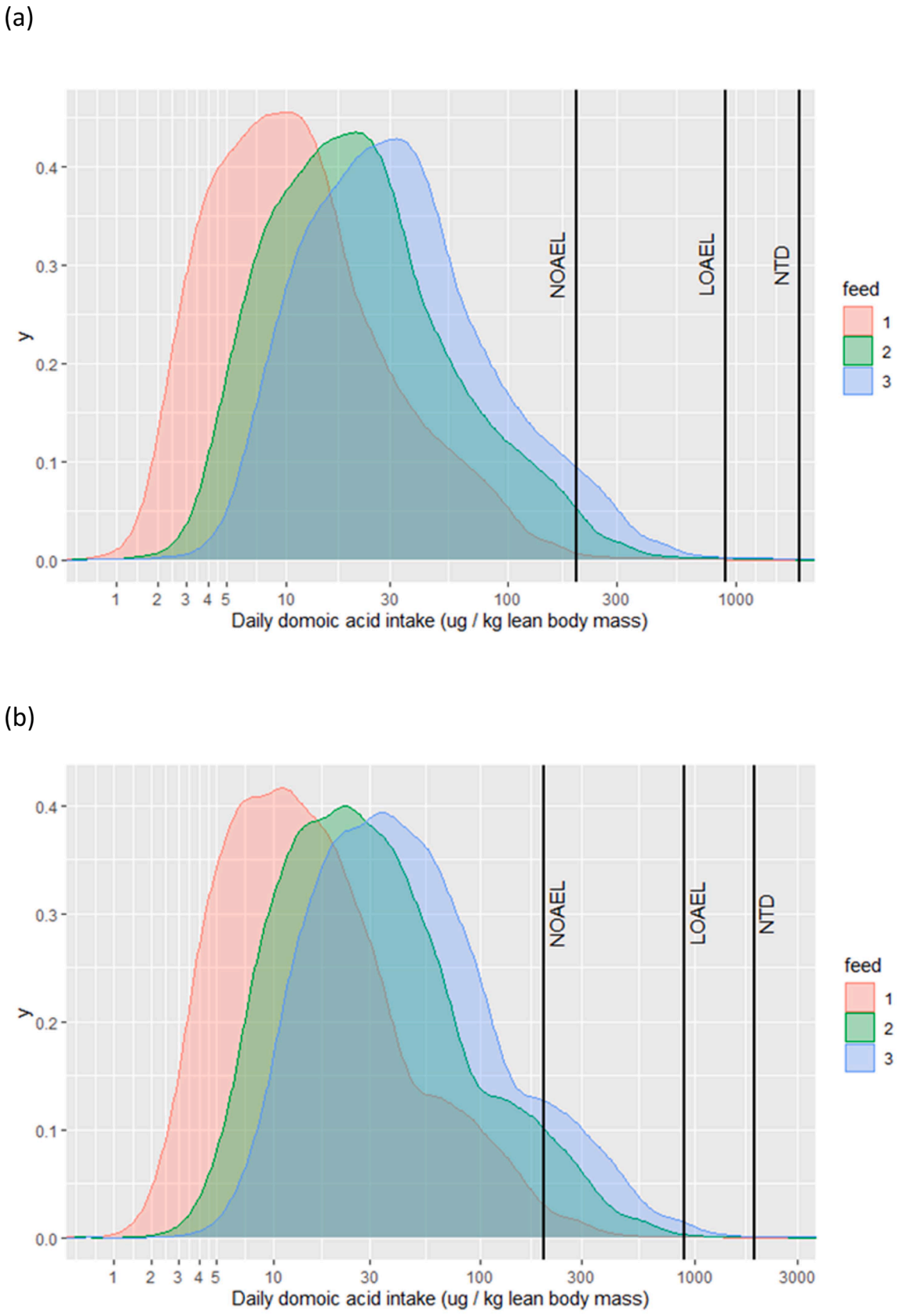


Fig. 3. Daily DA intake density plots from simulated seal feeding-days for adults (Fig 3a) and juveniles (Fig 3b) where *monthYearHAB* (the number of months of the year when HAB toxins persist) is set to four. Each graph is grouped by feeding frequency: every day = 1, every second day = 2, every third day = 3. The vertical lines indicate three toxicity thresholds (see Table 2): NOAEL = no observable adverse effects level; LOAEL = lowest-observed-adverse-effect level; NTD = neurotoxic dose.

their seasonal persistence, as well as variability in seal foraging patterns, inevitably results in uncertainty in effect estimates. For example, the model assumes that all individuals in the population forage in the same way. Analysis of the telemetry tracking data for harbour seals in Orkney indicated that there is very wide variability in this simplified assumption (Carter et al., 2022) which will affect individual exposure and therefore the proportion of the population exceeding the lethal thresholds.

The decline in harbour seal abundance (estimated from series of aerial survey counts of animals hauled out during their annual moult) in some regions of Scotland over the past 20 years or so has been dramatic. The population most affected has been in the East Scotland Seal Management Area, which declined by 95 % in the 15 year period between 2002 and 2017 (Thompson et al., 2019). For the North Coast and Orkney Seal Management Area the decline was 85 % between the mid-1990s and 2016. In Shetland the trend was slightly different, with a step change in abundance, involving a drop of approximately 40 % that occurred sometime between 2001 and 2005 (Thompson et al., 2019). Our preliminary results suggest that toxin exposure remains a potential factor contributing to these declines.

Bejarano et al. (2007) similarly assessed the exposure and risks to California sea lions from DA exposure in their main prey, anchovies (*Engraulis mordax)* and sardines (*Sardinops sagax caerulea*). They also found the lethal risks were higher among pups and juveniles, due to their higher mass specific energy demands. Although we used an average adult and juvenile seal and their estimated daily ingested energy requirements, a future refinement would be to take a similar approach and vary the animals' mass and sex, scaling their energy requirements accordingly. Whilst population dynamics models are female based and therefore the number of males surviving in the population is usually unnecessary for predicting trends, in the case of harbour seal trends, the trajectories are based on counts of seals hauled out on land as an index of total population size which includes all age classes and sexes. Moreover, Bejarano et al. (2007) found that the uncertainty associated with the energy density of the vector species and their toxin concentrations, rather than uncertainty in the sea lion parameters, had most influence on the model results. We used empirical toxin concentrations in the viscera of harbour seal fish prey, scaled to whole fish concentrations, to estimate ingested doses. This enabled us to capture the variability in the measured concentrations, although we were not able to account for variability in fish energy density, using published mean values as has previously been the approach to estimating fish consumption by seals (Hammond and Grellier 2006).

Our model also assumes feeding resulted in one daily bolus toxin dose and that the toxins are eliminated by individual animals between feeding days. The estimated half-life for DA from an experimental study in captive harbour seals was 3.5 h (Jensen 2014) and for STX data in dogs and laboratory animals estimated the half-life to be approximately 1.5 h (Pearson et al., 2010). Jing et al. (2018) estimated the absorption kinetics of DA following oral exposure in monkeys. They found it had pronounced "flip-flop" kinetics with slow absorption from the gut, prolonging the terminal half-life to 11.3 $\pm$ 2.4 h. In addition, their model simulated the concentrations of DA in the brain, which together with the heart is the main target organ for effects and predicted a longer duration of exposure following oral dosing which has been suggested may be important for chronic toxicity following asymptomatic exposures (Lefebvre et al., 2017).

The simplification that the species of each prey captured is independent of the species of previously caught prey is unlikely to reflect reality for two reasons. First, fish species have specific habitat preferences and so a seal preferring to forage in a certain habitat type would encounter a restricted number of prey species. Second, at a smaller scale, many fish species tend to aggregate and so it is likely that within one dive, or even within one feeding lunge, there is a predominance of a single species. For both reasons the simulation would tend to overestimate prey diversity. Since, toxin loads vary according to fish species groups (Fig. 2), there would also be a consequent decrease in the frequency of ingesting extreme daily toxin loads for a species with high toxin concentrations because of the dissociation between individual prey encountered in the model. In summary, this simulation simplification would tend to underestimate the occurrence of lethal toxin doses.

The seasonal persistence of the toxins in the prey was also an important factor in determining the lethal risks. We investigated three scenarios (4, 6 and 9 months) whereby toxins were still present in the fish. These are reasonable time periods when compared to the occurrence of phytoplankton blooms in the regions (about May to October Bresnan et al., 2009) and the periods when toxins were detectable in shellfish flesh following a toxic bloom event. These scenarios are also in-keeping with evidence that fish species have measurable levels of DA throughout the year (Kershaw et al., 2021).

The toxicity thresholds used here were based on those for the protection of human health (European Food Safety Authority 2009b; European Food Safety Authority 2009a). However, there is evidence for differences in species susceptibilities which may affect the toxicity thresholds. Neurotoxic doses for DA ranged from 1900 ug/kg for humans to 80,000 μg/kg for rats (Costa et al., 2010; Iverson et al., 1989; Perl et al., 1990). For STX the LD_{50} doses ranged from approximately 100 μg/kg body weight for pigeons to between 400 and 800 μg/kg body weight for monkeys (European Food Safety Authority 2009b). We have used a similar approach to Bejarano et al. (2007), applying the thresholds for humans, bearing in mind that this is a source of uncertainty in assessing the risks for harbour seals.

Our model does not include samples of fish prey collected during bloom 'events' i.e. large toxic blooms that resulted in shellfish fishery and harvesting area closures. However, samples were collected during periods of high phytoplankton bloom and in locations where HABs were above the trigger levels (i.e. cell concentrations triggering additional monitoring if breached (Parks et al., 2019)). Further prey sampling during large HAB 'events', which do result in shellfish harvesting closures, should be undertaken to determine the maximum potential oral doses. In the UK, HAB toxins are monitored primarily to manage shellfish harvesting areas and to protect human health under the EC Regulations 'on the control of products of animal origin intended for human consumption'. The temporal and spatial distribution of sampling effort and the species monitored (shellfish) are therefore not well matched to the data requirements of risk studies in marine top-predators.

In our simulations we included the observation that harbour seals do not necessarily feed every day. We observed that any toxic effect was more pronounced as our feeding scenarios moved from feeding every day to feeding one in three days. Whilst this analysis was very simple, it demonstrates that there was large inter-seal variability in seal feeding behaviour (Carter et al., 2022). It also showed the difficulty of accurately identifying feeding bouts – especially in areas of rapid water movement. In summary, our simple analysis confirmed that our range of feeding scenarios was probably sufficient, but the analysis could not direct us more accurately. There have been significant developments in the use of state-space models to determine harbour seal feeding behaviour (Russell et al., 2015). But these methods are inherently problematic in areas of rapid water movement. The development of appropriate tagging technology (for example, using accelerometery to detect seal feeding lunges, or video sequences to quantify prey fields) may provide the information to accurately determine feeding behaviour at a temporal and spatial scale that can usefully inform toxicology simulation models.

Pacific harbour seals with clinical signs following DA exposure (McHuron et al., 2013) had similar concentrations of DA in their faeces and urine as harbour seals from Scotland (Hall and Frame 2010). However, to our knowledge, there have been no reports of Scottish harbour seals with similar clinical, neurological signs. This demonstrates the difficulties associated with inferring potential effects from concentrations of toxins in excreta. Our approach of investigating concentrations in prey provides an alternative estimate of doses and potential effects. However, concentrations of toxins in excreta continue to be

important in indicating exposure (Akmajian et al., 2017). Very few dead harbour seals wash ashore in the UK compared to the number that must die based on their population trajectories (SMASS, 2015), so other lethal impacts and toxicological endpoints, such as cardiomyopathy (Zabka et al., 2009), have not been investigated.

The estimated impact of toxin exposure from the preliminary model is within the observed range for the declining harbour seal populations in Scotland (SCOS, 2022). For example, in Orkney the annual rate of decline has been between 7 and 10 %. Whilst our risk assessment model requires more refinement, even at this simple level with the caveats reported, these results suggest that DA exposure may remain a potential contributory factor in the causes of the Scottish regional decline in harbour seals. Previous studies have shown the proportion of animals exposed (based on the proportion of DA positive excreta samples) is higher in regions of decline (particularly the East Scotland Seal Management Area) than in stable or increasing regions (Jensen et al., 2015). Exposure may be sufficiently high (even outside toxic HAB bloom 'events') for animals to receive doses above estimated toxic thresholds. In addition, hidden exposure may be occurring that is not captured in this risk assessment model. An example of this is the recent intoxication of dogs on the southeast coast of England due to the consumption of PSTs in invertebrates and fish that had washed ashore after a storm. This region also has a large population of harbour and grey seals (*Halichoerus grypus*) (Thompson et al., 2019) and although the number of dead seals washing ashore at the same time was not known, there were simultaneous reports of seal carcasses being found on the same beaches (Turner et al., 2018). If animals were consuming highly contaminated prey (particularly flatfish) at their offshore foraging grounds, they may well have died rapidly at sea without stranding, given the highly toxic nature of STX and other PSTs. Using the range of PST concentrations measured in the dabs (*Limanda limanda*) (concentrations ranged from between 148 and 566μg STX eq/kg depending on the method of analyses) reported by Turner et al. (2018) in a simple dose calculation, both adults and juveniles exceeded the lethal dose (200μg/ kg lean body mass /day) if they fed every other day (or less frequently), and if all their prey was similarly contaminated (as may be the case if they were flatfish specialists).

Moreover, this model provides a simple, flexible approach for exploring dose scenarios and assessing the consequences and risks to other top predators, such as small cetaceans and seabirds in Scottish waters who feed on similar prey to harbour seals. We have demonstrated that exposure to toxins, particularly DA, could be an important potential factor in seal and other marine mammal mortality, and continued low dose exposure may also be resulting in adverse health effects on the neurological (Lefebvre et al., 2017) and immune systems (Bogomolni et al., 2016). HABs are increasing in occurrence and their niche has expanded with rising ocean temperatures (Gobler et al., 2017). Thus, climate change is a significant factor in the future intensification of HAB events and the risk that they pose to both marine mammal and human health is likely to also increase. We would therefore recommend future monitoring of toxins in harbour seal prey be focussed on their foraging areas and major prey species.

CRediT authorship contribution statement

Ailsa J. Hall: Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Joanna L. Kershaw:** Writing – review & editing, Methodology, Investigation, Data curation. **Shaun Fraser:** Writing – review & editing, Resources, Investigation, Data curation, Conceptualization. **Keith Davidson:** Writing – review & editing, Supervision, Investigation, Conceptualization. **Stephanie Rowland-Pilgrim:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Andrew D. Turner:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Bernie McConnell:** Writing – original draft, Software, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

The authors would like to thank all those who helped obtain fish samples, particularly Arthur Johnson, skipper of the Atlantia II. Also, many thanks to Caroline Cummings and Alexandra Tranganida for their assistance with sample analysis and to Debbie Russell for her help in exploring the population level effects. This research was supported by funding from the Scottish Government, Marine Scotland, Marine Mammal Scientific Support Research Programme MMSS/002/15 and by NERC National Capability NPG funding, grant No. NE/R015007/1.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.hal.2024.102653.

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