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Title: Trace element concentrations in common dolphins (*Delphinus delphis*) in the Celtic Seas ecoregion: interelement relationships and effects of life history and health status

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Highlights:

Strong inter-elemental relationships detected between Hg and Se, MeHg and Se, and Cu and Zn

THg:Se molar ratio values increased with age and body length approaching equimolarity

Juveniles reported higher MeHg:Hg ratio values, declining with age and body length

Zn and Cu could be used for monitoring the common dolphins' health status

Common dolphin recommended as a trace element higher tropic level indicator species

Abstract

Given the increased extraction of trace elements for use by new and emerging technologies, monitoring the environmental fate and potential effects of these compounds within the aquatic environment has never been more critical. Here, hepatic trace element concentrations were assessed in a key sentinel predator, the common dolphin (*Delphinus delphis*), using a long-term dataset. Variation in concentrations were assessed in relation to other elements, time period, decomposition state, sex, age, total body length, sexual maturity and nutritional status, and cause of death. Additionally, mercury toxicity thresholds for evaluating risk were reviewed and employed. Concentrations of elements which bioaccumulate, THg, MeHg, Cd, and Pb, in addition to Se and V, were strongly correlated with age, and/or body length. An association was observed between Zn concentrations and disease status, with significantly higher concentrations measured in individuals that died from infectious disease, compared to other causes. Strong inter-elemental relationships were detected, namely between Hg and Se, MeHg and Se, Cd and Se, and Cu and Zn. While THg:Se molar ratio values were observed to increase with age and body length, approaching equimolarity. THg was largely comprised of inorganic Hg in older individuals, potentially bound to Se, therefore the effects from THg toxicity may possibly be less important than originally assumed. In contrast, higher MeHg:Hg ratio values were reported in juveniles, suggesting a poorer efficiency in demethylation and a higher sensitivity. The generation of data on proportions of hepatic MeHg and inorganic Hg is highly informative to both future toxicity threshold assessments within pollutant indicator assessments, and to understanding the ultimate fate of mercury in the marine web.

Key words: Dolphin, hepatic tissue, Trace elements, Se:Hg molar ratio, Methylmercury, Inter-elemental relationships

1. Introduction

Marine mammals are useful sentinels for monitoring both marine ecosystems and human health risk (Bossart 2011; Fossi and Panti 2017; Kershaw and Hall 2019; Moore 2008; O'Brien et al. 1993). Aspects of the status of marine mammal populations are used as indicators to assess good environmental status (GES) under the EU Marine Strategy Framework Directive (MSFD, 2008/56/EC) (ICES WKDIVAGG 2018), aiming to ensure that ‘the collective pressures of human activities are kept within levels compatible with GES’ (Article 1). Under MSFD Descriptor 1, maintaining biodiversity is paramount and this necessitates the implementation of effective ‘programmes of measures’ to achieve GES (Murphy et al. 2021; Williams et al. 2023). The European Commission recommends integrating state and pressure indicators at the species level for marine mammals (European Commission 2022), advocating a holistic approach to species assessments. In the context of this framework, assessments of pressure indicators are crucial for interpreting any change in marine mammal population status. To assist this approach, OSPAR’s Marine Mammal Expert Group (OMMEG) undertook a ‘pilot assessment of the status and trends of persistent chemicals in marine mammals’ for OSPAR’s Quality Status Report 2023 (Pinzone et al. 2022). Though the initial focus has largely been on polychlorinated biphenyls (PCBs), including toxicity thresholds and trends analyses, future assessments will encompass a wider range of persistent chemicals, including persistent trace elements (Pinzone et al. 2022; Williams et al. 2023).

The common dolphin (*Delphinus delphis*) population in the North-east Atlantic faces numerous anthropogenic threats and environmental pressures. As a top predator, the species is susceptible to the bioaccumulation and biomagnification of pollutants due to their trophic position and prolonged lifespan of at least 30 years (though most individuals do not survive beyond 20 years of age) (Murphy et al. 2018, Murphy et al. 2021) and thus, it has been employed as a principal species in the North-east Atlantic for monitoring trace elements (e.g. (Das et al. 2003a; Lahaye et al. 2005; Méndez-Fernandez et al. 2014; Méndez-Fernandez et al. 2022; Monteiro et al. 2016; Pierce et al. 2008). Essential elements such as copper and zinc and non-essential toxic elements like mercury and cadmium have been all reported in common dolphin tissues (Méndez-Fernandez et al. 2014; Méndez-Fernandez et al. 2022), resulting from prey consumption, as well as placental and/or lactational transfer (Zhou et al. 2001). While some trace elements have a relatively short biological half-life, for example 50 to 70 days for methylmercury, the toxic, organic form of mercury (Evans et al. 2016; Jo et al. 2015; Rand et al. 2019), others, such as cadmium, can persist for over a decade in soft tissues (Lahaye et al. 2005; Stoppler 1991). Both methylmercury and cadmium can bioaccumulate in dolphin tissues, and concentrations of both elements were observed to increase in common dolphins sampled in French waters between 2001 and 2017 (Méndez-Fernandez et al. 2014). For cadmium, the authors suggested the increasing temporal trend resulted from a shift in diet, arising from common dolphins consuming more cadmium-enriched species and/or changing their foraging areas during the study period.

Mercury is among the most toxic trace elements, particularly after conversion to organic methylmercury by sulphate-reducing bacteria present in sediments and/or the water column (Bolea-Fernandez et al. 2019; Wang et al. 2022). Interestingly, marine mammals have developed a detoxification mechanism against mercury toxicity through the formation of mercury-selenium compounds including insoluble and non-toxic mercuric selenide crystals (Bolea-Fernandez et al. 2019; García-Alvarez et al. 2015; Kershaw and Hall 2019; Koeman et al. 1973; McCormack et al. 2020; Méndez-Fernandez et al. 2014; Martoja and Berry 1980). Furthermore, the presence of selenium can mitigate mercury toxicity through the redistribution of mercury to less sensitive organs (Bolea-Fernandez et al. 2019; García-Alvarez et al. 2015). Previous studies on marine mammals have focused on employing a (total) mercury:selenium molar ratio to quantify the risk associated with biologically available mercury, where a molar ratio >1 indicates a potential toxicity (García-Alvarez et al. 2015; McCormack et al. 2020a,b). Notably, selenium is not only crucial for mercury detoxification but also essential for various biological functions in marine mammals. Thus a deficiency in, potentially caused by mercury binding, poses an additional risk (García-Alvarez et al. 2015; Kershaw and Hall 2019).

Interactions between other trace elements, such as zinc and copper, have also garnered interest, with studies showing positive correlations in concentrations between the two elements (Das et al. 2004; Fosmire 1990; Spencer et al. 1985). Increased zinc concentrations have being associated with the occurrence of disease (Bennett et al. 2001), and as zinc absorption across the intestine increases during periods of malnutrition, this may have an impact on copper metabolism (and absorption) (Das et al. 2003b; Das et al. 2004; Das et al. 2006; Fosmire 1990; Spencer et al. 1985).

While, historically, toxic and persistent elements or minerals found their way into the environment through both natural and anthropogenic causes, our current demands for electronic devices, renewable energy resources (wind turbines, solar panels, electric cars and hydrogen fuel cells) and other emerging technologies have markedly increased the extraction of critical minerals required for their production. This has led to the creation of e-waste as well as waste from renewable technologies through their development, use and disposal (Dockrell et al. 2023; EEA 2023; Miller et al. 2018). Furthermore, our appetite for minerals has focused attention on deep sea mining for their retrieval, due to decreasing terrestrial deposits (Kaikkonen et al. 2021; Miller et al. 2018). Thus, monitoring trends and environmental fate of trace elements within the aquatic environment is of utmost importance.

Understanding the fate and potential effects of pollutants on top predators necessitates a comprehensive exploration of their nature and interactions. The current study focuses on the assessment of trace element concentrations in hepatic tissues of common dolphins sampled in Irish waters using a robust long-term dataset. We investigated interelemental relationships, and variation in trace elements distribution taking into account temporal factors, decomposition state, sex, age, body length, maturity and nutritional status, and cause of death. To date, various toxicity thresholds have been established to evaluate the risk of contaminants exposure for individuals /populations of marine mammals in European waters, with a significant focus on polychlorinated biphenyls (PCBs) (Jepson et al. 2016; Williams et al. 2023). The selection of appropriate concentration threshold values for other pollutants is imperative to support protective measures, and to facilitate 'status' assessments for pollutant indicators like the Marine Strategy Framework Directive (MSFD) and OSPAR convention (Williams et al. 2023). The current study reviewed information on toxicity thresholds for another persistent pollutant, mercury, and employed where appropriate.

2. Materials and methods

2.1 Sampling

The distribution of stranded and bycaught common dolphins sampled in Irish waters in the North-east Atlantic, including both contemporary and historical datasets, is shown in Figure 1. All samples were obtained through short-term funded projects. The subset of common dolphins that stranded along Irish coastlines between 2017 and 2019, and were subsequently necropsied, are detailed in Levesque et al. (2022). Necropsies were performed according to standard post-mortem procedures for cetaceans (see IJsseldijk et al. 2019). From this group, 24 individuals were specifically selected for pollutant analysis, with selection criteria based on sex and decomposition state.

Additionally, this study incorporates historical pollutant data on common dolphins from Irish waters, derived from two key sources. The first study is the EU-funded project ‘bioaccumulation of persistent organic pollutants in small cetaceans in European waters; transport pathways and impact on reproduction’ (BIOCET) reported in Pierce et al. (2008). As the BIOCET project primarily focused on bioaccumulation of persistent organic pollutants in female small cetaceans, it inherently exhibited a sampling bias towards females. The second data set, from Das et al. (2003a) encompasses data from stranded and bycaught common dolphins sampled between 1991 and 1993. The combined historical datasets from BIOCET and Das et al. account for an additional 46 common dolphins, with 31 and 15 individuals respectively.

2.2 Trace metals analysis

Trace elements were assessed in hepatic tissues, recognized as the most predominant tissue for monitoring elements in wildlife (Scheuhammer et al. 2015). Hepatic tissue samples from the contemporary sample set, collected during necropsies, were stored at -20°C before processing. Chemical analysis was completed under subcontract to recognised field leaders in trace element analysis. Concentrations of the following trace elements were determined on a mg/kg wet weight (ww) basis: the essential trace elements copper (Cu), nickel (Ni), selenium (Se), vanadium (V) and zinc (Zn), and the non-essential trace elements arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb) and mercury (Hg). For the latter, total mercury (THg), and methylmercury (CH3Hg+; MeHg) were determined. For THg and all other trace elements, aliquots of homogenised hepatic tissue samples (0.2 g) were digested in a mixture of nitric acid and hydrochloric acid using a high-pressure microwave system. Methylmercury analysis was completed on aliquots of homogenised test sample (0.5 g) which were solubilised in hydrobromic acid. The MeHg was extracted into toluene then back-extracted into 1% L-cysteine solution. Quantification was then carried out by inductively coupled plasma-mass spectrometry (ICP-MS) with collision cell. Each sample was analysed in duplicate.

The analytical progamme was underpinned by a rigorous quality control regime, including the use of blanks, spikes, and reference materials; such as NIST 1566b (Oyster Tissue), NRCC DORM-4 (Fish Protein), NRCC TORT-2 (Lobster Hepatopancreas) for THg, while FAPAS 07281, Canned Fish and FAPAS 07305 both canned fish were used to support MeHg analysis. Certified reference material (CRM) recovery was within ± 20 % of the certificate value in the majority of cases. Results were accepted if the CRM corrected results agree with certificate. All reference material data were within acceptable ranges. Data were corrected for reagent blank and spike recovery with the reporting limit determined from 10 times the standard deviation of reagent blank values, adjusted for dilution and sample weight. For THg, recovery was typically 80% with criteria set to within 80 to 120 %. For MeHg, typical recovery observed was between 20 to 50%. Duplicate precision for both elements was set as ±20 % for levels above the limit of quantification (LOQ). In cases where trace element concentrations were below the LOQ, concentrations were reported as half the limit (after Law et al. 2012).

Historical datasets that determined trace element concentrations in common dolphin hepatic tissue samples included analyses for Cu, THg, Se and Zn. The analytical methodologies for these historical datasets are detailed in Pierce et al. (2008) for the BIOCET study, and Das et al. (2003a). While the BIOCET study reported concentrations on a ww basis, Das et al. (2003a) provided data in dry weight terms, hence for that dataset, only total Hg, MeHg and Se data were utilized for the molar ratio analyses.

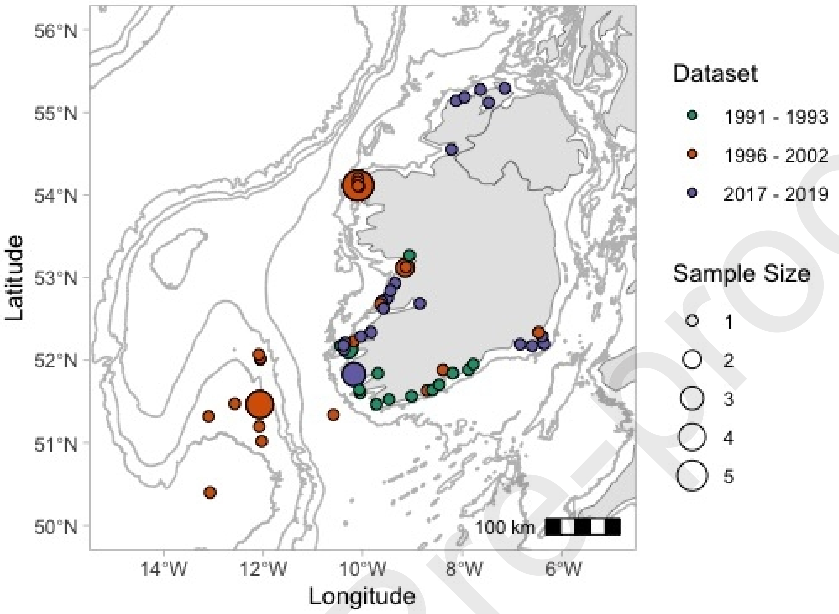

Figure 1. Location of common dolphin strandings and bycatches of individuals sampled for pollutant analysis [1991 – 2019], by three samples periods 1991 – 1993 (Das et al. 2003a), n = 15), 1996 – 2002 ((Pierce et al. 2008), BIOCET, n = 31) and 2017 – 2019 (this study, n = 24).

2.3 Pathological and life history analysis

During necropsies, the following associated biological data were systematically collected: sex, total body length (cm), decomposition code (DCC: categorized into extremely fresh, slight decomposition, or moderate decomposition), nutritional status (classified as good, moderate, or poor, as outlined in Levesque et al. (2022)). For all individuals, five to ten teeth were collected during the necropsy and stored frozen (-20°C) until further analysis. Age was determined through analysing growth layer groups in dental tissue using methods adapted from Lockyer (1995), as detailed in Murphy and Rogan (2006). Under the assumption that the outermost hypercalcified layer (neonatal line) equates to 0 years (Lockyer 1995) and each subsequent growth layer group equates to one year of life (Gurevich et al. 1980), an individual's age was determined. All readings were initially made blind, i.e. without available biological information on the individual, and replicate counts were made by at least two readers.

During necropsies, gonadal material were collected, fixed and stored in 10% neutral buffered formalin until further analysis. Ovaries from females were assessed for evidence of corpora scars (corpus luteum and/or corpora albicantia), indicating sexual maturity (as per Murphy et al. 2010; Murphy et al. 2018). Male testicular tissues were processed for histological examination, with sexually maturity determined based on the evidence of spermiogenesis in the seminiferous tubules following the criteria established by Murphy et al. (2005). Additionally, ventral blubber thickness (VBT) (mm), was measured anterior to the dorsal fin along the ventral midline. The cause of death (COD) for each individual was established using specific diagnostic criteria (Deaville and Jepson 2011; Jepson et al. 2005; Levesque et al. 2022). For analytical purposes, individuals were categorised into three COD groups based on the classification system of Murphy et al. (2015); infectious disease, trauma (including bycatch, boat/ship strike, bottlenose dolphin attacks, and dystocia) and others (live stranding, starvation, neoplasia and not established).

2.4 Data treatment

Standard diagnostic testing was uniformly applied to all data. The assessment of data normality involved a two-pronged approach: a statistical evaluation using the Kolmogorov-Smirnov test, and a visual inspection via Q-Q plots. Additionally, the Levene's test was employed to evaluate homogeneity of variances.

To explore interelemental relationships (i.e., co-linearity) among trace metals and their associations with age (measured in years) and total body length (in cm), Spearman's rank correlation coefficients were calculated. This choice of test was predicated on the non-normal nature of some of the data sets, which could also not be normalised following transformation. To avoid a type I error, p-values were adjusted using the Bonferroni correction method, where multiple tests were performed for each trace element.

The risk of adverse health effects from Hg exposure was assessed by considering the potential for demethylation and Se binding. This assessment was conducted using both the THg to Se molar ratio and the MeHg to Se molar ratio. Additionally, the MeHg to THg ratio was also calculated to further understand the proportion of MeHg in THg content.

$$Total\ Hg{:}Se\ Molar\ Ratio$$
$$= \frac{Total\ mercury\ \left(mg.kg^{-1}\right)}{Selenium(mg.kg^{-1})} \times \frac{78.96\ (\frac{g}{mol};atomic\ weight\ of\ Hg}{200.59\ (\frac{g}{mol};atomic\ weight\ of\ Se)}$$

$$MeHg{:}Se\ Molar\ Ratio = \frac{Methylmercury\ (mg.kg^{-1})}{Selenium(mg.kg^{-1})} \times \frac{78.96\ (\frac{g}{mol};atomic\ weight\ of\ Hg)}{215.63\ (\frac{g}{mol},molecular\ weight\ of\ MeHg)}$$

Generalised linear models (GLM) were used to assess what variables, including interelemental interactions, could predict log transformed trace element concentrations. Predictors included sex, total body length, maturity group, COD group, DCC, and ventral blubber thickness (VBT), where such data were available. VBT was used as a proxy for nutritional condition (Albrecht et al. in review). Further, total body length was employed as a proxy for age, as age data were not available for all individuals. For the purposes of the GLM analyses, calves and juveniles were combined as a single maturity group due to small sample sizes, and individuals were then classified as either sexually immature or sexually mature. As all predictors were of biological relevance, they were employed within a global model (glm function in R Studio) separately for each trace element. The dredge function in the R package MuMIN (Barton 2019) was employed for automated model selection and the best model was identified by the lowest small-sample corrected Akaike Information Criterion (AICc). Furthermore, the top three models within ΔAICc < 2 of the best model were considered. Top models were retrieved using the get.models function with the MuMIN package. Upon model validation, including residual diagnostic testing as well as performing the Rosner's test for outliers (EnvStats package), it was found that THg, Se and Zn data violated assumptions due to large residual outliers. In these cases, a robust linear model (RLM) fitted using the rlm function in the R package MASS (Venables and Ripley 2002) was employed for the global model. In such cases, p-values were calculated manually with the aid of the Student t-distribution (pt function) in R.

For all models, the coefplot package (Lander 2022) in R was utilised as an aid to visualise the coefficients within each global model and their corresponding top models from the dredge selection. For models in which categorical variables with more than two categories (i.e. COD and DCC) were found to have significant effects, the model was run a second time, changing the reference value for either COD or DCC to ensure all categories were compared within the GLMs or RLMs.

All data analyses were carried out using the statistical software RStudio version 4.2.0 (R Core Team 2019) and IBM SPSS Statistics 28, and the significance threshold for all tests was set at $p \leq 0.05$. Means and standard deviations are presented, unless stated otherwise. Given different accumulation rates for foetuses for some trace elements, as observed within this (see results section) and other studies, foetal data were not included within the Spearman's correlations, GLMs or RLMs.

3. Results and Discussion

3.1 Trace element concentrations

Common dolphins were sampled across various years, spanning from 1991 to 2002, (excluding 1994, 1995 and 1997), and from 2017 to 2019. Notably, no samples from the east coast of Ireland were processed for pollutant analysis, underscoring this region as a priority for future research due to its proximity to larger urban areas in both Ireland and the UK (Figure 1). Within the entire sample set, the age range of individuals spanned from neonates (0 years) to 25 years, and total body lengths varied from 93 to 223 cm, respectively (detailed in Figure S1 Supplementary Material). The dataset included one male foetus, measuring 50.5 cm in body length. The average total body length and age across the complete dataset was 178.4 cm (n = 70) and 9.3 years (n = 53), respectively. Notably, 36% of the aged sample comprised individuals younger than four years. Comparing the two sampling periods, both the historic (1991-2002) and contemporary (2017-2019) samples exhibited similar mean ages of 8.5 and 8.4 years, respectively, with a median age of 6 years (n = 25, 1996-2002 vs. n = 17, 2017-2019, see Table 1). Mean and median total body lengths of common dolphins were slightly larger in the contemporary sample set (mean: 186.1 cm, median: 198 cm, n = 23), compared to the historic sample (mean: 165.7 cm, median: 186.8, n = 32). This disparity may partly be attributed to an artefact of sample selection considering the species is moderately sexually dimorphic in nature (Murphy and Rogan 2006), and the higher number of males sampled in the later period (2 vs. 12 individuals). However, the overall sex ratio for the entire sample set was biased towards females with a ratio of 1:0.3 (n = 72).

Of the trace elements analysed, the highest mean hepatic concentration was reported for Zn (62.4 mg/kg ww), with other elements decreasing in mean concentration from Zn > Hg > Se > Cu > Cd > MeHg > As > V > Cr > Pb > Ni (see Table 1). As Ni was only detected at trace levels, below the minimum detection limit (<0.01 mg/kg), it was not considered further in the statistical analyses. Previously, Ni was measured at higher concentrations in other tissue types in Dall's porpoises (*Phocoenoides dalli*), for example, including skin and bone (Fujise et al. 1988). Both THg and Se were among the trace elements reported in highest concentrations within the current study, with mean hepatic concentrations of 28.4 mg/kg and 15.7 mg/kg, respectively. Higher than what has been previously reported for the species off the Atlantic Iberian coast by Méndez-Fernandez et al. (2014) and Monteiro et al. (2016) (see Table 2). Further, hepatic concentrations of essential elements Cu and Zn were also higher in common dolphins sampled in Irish waters (7.9 mg/kg ± 4.2 and 62.4 mg/kg ± 42.3, respectively) compared to Iberian waters (Table 2).

The male foetus (ID: Mayo 3-01) that died during the month of February 2001 presented with the highest concentration of Cu (31 mg/kg) within the current study. While the foetal hepatic Hg concentration was relatively low (at 0.5 mg/kg) and the individual also presented with the lowest Se concentration (1.7 mg/kg) within the sample. The mother (Mayo 2-01) of this foetus was involved in a mass live stranded event and presented with pollutant burdens slightly higher than the mean values for Cu (9.4 mg/kg) and Zn (45.1 mg/kg) for the historic sample, and relatively high THg and Se concentrations (75.5 mg/kg and 32.4 mg/kg, respectively). Results are consistent with previous studies on common dolphins and harbour porpoises (*Phocoena phocoena*) where limited placenta transfer of THg, Se and Cd were also reported (Lahaye et al. 2007a; Lahaye et al. 2007b). Though Lahaye et al. (2007a) did observe that hepatic Hg concentrations increased as a function of foetal body length in the common dolphin, concentrations that were proportionate to maternal hepatic, renal and muscular Hg levels. High foetal Cu concentrations previously observed in the common dolphin, harbour porpoise and Dall's porpoise were attributed to bioaccumulation and limited excretion via the bile and/or elevated renal metallothionein activity for storage of essential elements for growth (Fujise et al. 1988; Lahaye et al. 2007a; Lahaye et al. 2007b).

On average, THg, Cu and Zn concentrations were higher for the contemporary (2017-2019) Irish dataset (31.1 ± 46.4 mg/kg, 8.7 ± 2.3 mg/kg and 86.0 ± 53.0 mg/kg, respectively), compared to the historic dataset (26.4 ± 29.8 mg/kg, 7.21 ± 5.1 mg/kg, 44.8 ± 19.1 mg/kg, respectively). Whereas mean hepatic Se concentrations were higher in the historic dataset (18.3 ± 15.2 mg/kg), compared to the contemporary (14.4 ± 19.6 mg/kg). In all cases, large standard deviations were estimated, potentially due to varying concentrations with age and suggests a lack of a significant difference between mean values (see section 3.4 for further assessment). Further to this, within the contemporary dataset, one female presented with very high concentrations of THg (226 mg/kg), MeHg (2.5 mg/kg), Se (96.3 mg/kg), Cu (9.7 mg/kg) and Zn (50.7 mg/kg), which may have skewed the mean results. This individual (ID: IWDG 2017-182) had a body length of 202 cm and while the age of the animal was not determined, the female was sexually mature, and resting, i.e. not pregnant or lactating. Cause of death was not established, but the individual presented with enlarged adrenal glands and spleen (with capsular haemorrhage), as well as dental loss. As trace elements As, Cd, Cr, MeHg, Pb and V were only measured for the contemporary dataset, comparisons between time periods could not be made.

Assessment of temporal trends in trace elements within common dolphin tissues within French waters showed that both hepatic THg and renal Cd concentrations increased between 2001 and 2017, while hepatic Pb concentrations declined (Méndez-Fernandez et al. 2022). For Pb, the decline was associated with the implementation of the EC Lead Content of Petrol Regulations 1985, which came into force in the year 2000 and thereafter (Méndez-Fernandez et al. 2022). Increased inputs and/or changes in feeding and foraging habits of the common dolphin, particularly with respect to Cd accumulation, was suggested to have accounted for the observed trends, potentially due to increased consumption of oceanic cephalopods, or other Cd-enriched species (Méndez-Fernandez et al. 2022). Isotopic analysis of these individuals revealed that δ^{15}N in muscle tissues (indicating trophic level) was the most important influencing variable for the observed trend in Cd concentrations, followed in importance by year (indicating a temporal trend), sex and age. As Cd concentrations in kidney tissues of common dolphins are over five times the concentrations observed in hepatic tissues (Méndez-Fernandez et al. 2014; Monteiro et al. 2016), direct comparisons can’t be made with the results of the French study – which also reported on a dry weight basis. Though, on average, hepatic Cd concentrations determined for animals sampled in Irish waters (2.07 ± 2.25 mg/kg) were higher than those more recently reported for Iberian waters in the North-east Atlantic (Table 2).

Table 1. Age (years), total body length (cm) and trace metal concentrations (mg/kg ww) in common dolphin hepatic tissues, sampled in Irish waters (1991 – 2019). Includes data on a wet weight basis from the BIOCET study (1996-2019), and the contemporary sample set (2017-2019). SD = standard deviation, n = sample size.

Time period	**Age**	**Total body length**	**As**	**Cd**	**Cr**	**Cu**	**Pb**	**THg**	**MeHg**	**Se**	**V**	**Zn**
1991 – 2019												
Mean	9.3	178.4	0.690	2.0724	0.0511	7.86	0.0053	28.385	1.345	15.74	0.1081	62.4
Median	7.0	193.0	0.632	1.2600	0.0400	7.30	0.0050	21.180	1.170	12.50	0.0980	46.5
SD	7.6	37.0	0.327	2.6549	0.0276	4.18	0.0028	37.431	0.791	18.06	0.0882	42.3
Range	0.0–25.0	50.5–222.5	0.310–1.490	0.0030–10.1000	0.0200–0.1000	2.32-30.99	0.0020–0.0100	0.230–226.000	0.390–4.000	1.14–96.30	0.0090–0.3500	21.1–280.0
N	53	70	23	23	9	54	23	54	23	35	23	54

1996 – 2002

Mean	8.5	165.7				7.21		26.409		18.34		44.8
Median	6.0	186.8				6.04		10.620		19.89		42.9
SD	8.1	43.2				5.10		29.795		15.23		19.1
Range	0.0–25.0	50.5–212.0				2.32-30.99		0.230–105.710		1.72-49.81		21.1–127.8
n	25	32				31		31		12		31

2017 – 2019

Mean	8.4	186.1	0.690	2.0724	0.0511	8.74	0.0053	31.048	1.345	14.39	0.1081	86.0
Median	6.0	198.0	0.632	1.2600	0.0400	7.90	0.0050	23.000	1.170	11.00	0.0980	72.2
SD	6.8	31.8	0.327	2.6549	0.0276	2.27	0.0028	46.387	0.791	19.55	0.0882	53.0

Range	0.0–20.0	117.5-222.5	0.310-1.490	0.0030–10.1000	0.0200–0.1000	5.20–14.00	0.0020–0.0100	0.600–226.000	0.390–4.000	1.14–96.30	0.0090–0.3500	38.6–280.0
n	17	23	23	23	9	23	23	23	23	23	23	23

Table 2. Comparison of hepatic trace element concentrations mean ± SD/SE mg/kg ww in common dolphins in the North-east Atlantic.

Trace Element	*Current study* *Mean ± SD*	*Mendez-Fernandez et al. (2014)* *Mean ± SD*	*Monteiro et al. (2016)* *Mean ± SE*
Cd	2.1 ± 2.7	0.4 ± 0.5	0.4 ± 0.1
Cu	7.9 ± 4.2	5.1 ± 2.1	7.1 ± 0.5
THg	28.4 ± 37.4	10.4 ± 31.8	16.7 ± 2.9
Se	15.7 ± 18.1	5.0 ± 5.8	7.4 ± 1.0
Zn	62.4 ± 42.4	40.5 ± 19.5	46.7 ± 2.5

3.2 Biological variability in trace element concentrations

Concentrations of hepatic THg, Se, Cd, Pb and V were all significantly correlated with either body length and/or age of individuals (see Table 3). A strong significant association between hepatic MeHg and age was also observed within the current study (R_S = 0.826) and, while not tested for significance, it was also previously observed for common dolphins sampled in French waters (Holsbeek et al. 1998). While some trace elements have been reported to bioaccumulate in marine mammals, including Hg, Cd and Pb (Bustamante et al. 2004; Lahaye et al. 2005; Lahaye et al. 2006; Lahaye et al. 2007b; Mackey et al. 1996; Méndez-Fernandez et al. 2014; Méndez-Fernandez et al. 2022; Monteiro et al. 2020; Zhou et al. 2001), as well as V (Mackey et al. 1996; Saeki et al. 1999), the half-life of these elements (or species of these elements) within hepatic tissues can vary immensely. For example, in ringed seals (*Phoca hispida*), a toxicokinetic model for Hg species estimated comparatively shorter half-lives for hepatic THg (20 days) and MeHg (50 days), compared to mercury selenide (HgSe, 500 days) (Ewald et al. 2019). It was reported that hepatic THg and MeHg concentrations in ringed seals were not reflective of lifetime accumulation, but recent dietary input (Ewald et al. 2019). As larger sized individuals would consume more, this may result in a pseudo-persistence effect for THg in marine mammals, particularly when in its HgSe form. In addition to transport of Hg across membranes and partitioning of the trace element to other tissues, where it accumulates (e.g. muscle tissue in the case of MeHg) (Bolea-Fernandez et al. 2019).

Essential trace elements are regulated through homeostasis, by absorption, transport and excretion (Méndez-Fernandez et al. 2014; Underwood 2012). However, within the current study, increasing concentrations of essential trace elements Se and V as a function of age suggests a homeostatic imbalance for these elements in hepatic tissues in older individuals and/or increased daily consumption of prey containing such elements with their partition in hepatic tissues. For Se, this may also be due to its role in detoxification and the production of Se-metal complexes resulting in its slow accumulation and elimination (Ewald et al. 2019; Kershaw and Hall 2019; Monteiro et al. 2020). Whereas for V, retention in nuclei and mitochondria of hepatic cells in marine mammals has been reported (Saeki et al. 1999).

Age showed a much stronger correlation than body length for all trace elements where significance was detected. Further to this , while a significant correlation with age was observed for Cd (R_S = 0.633), no significant association (R_S = 0.495) was observed with body length. Whilst the effects of growth dilution during periods of rapid growth cannot be ruled out (Chételat et al. 2020), age being a stronger predictor of Cd concentrations must also be considered. Common dolphin growth is best described by a Gompertz growth model (Murphy and Rogan 2006), or a similar type of sigmoid function model (Murphy et al. 2009), with individuals exhibiting relatively fast growth in the first few years and also a secondary growth spurt (Danil and Chivers 2007). Following which, males continue to grow at a slower rate and for a longer period than females, thus achieving a larger asymptotic size (Murphy and Rogan 2006). Males attain physical maturity around 12 years of age and 206 cms in body length (Murphy et al. 2005), and the maximum age reported in the North-east Atlantic population is 30 years (Murphy et al. 2010; Murphy et al. 2014). This further highlights the importance in determining age in future monitoring programmes for such trace elements, reducing variability in the results for those individuals that have already achieved asymptotic size, for example. In contrast, essential trace elements such as Cu, Zn and Cr, in addition to non-essential trace element As, did not show a significant association with either age or body length (see Table 3).

3.3 Interelemental relationships between trace elements

Knowledge on trace element accumulation, as well as developing an understanding of the complex inter-relationships between trace elements is important, particularly where one trace element has an antagonistic or synergistic influence on another element’s potential toxicity. Strong significant correlations were found between some trace elements, notably THg and Se (R_S = 0.984) (Figure 2), and MeHg and Se (R_S = 0.810). Similar results were reported by other authors where these antagonistic relationships were assessed (e.g. García-Alvarez et al, 2015, Méndez-Fernandez et al, 2014, Durante, 2020), and discussed further in section 3.3. Hepatic Cu and Zn concentrations were also significantly correlated (R_S = 0.543, Figure 2), a relationship that may be due to the binding mechanisms of metallothionein’s, proteins involved with metal detoxification and homeostasis (Anan et al. 2002; Das et al. 2000; Das et al. 2006; Holsbeek et al. 1998; Lahaye et al. 2007a). Such a relationship may also be an indication of antagonistic behaviour between these two elements, due to the adverse highly complex biochemical effects that a high intake of Zn may have on the metabolism of Cu, as reported previously by Spencer et al. (1985) and Fosmire (1990). While the involvement of both Cu and Zn in the detoxification of Cd has previously been reported in marine mammals, through metallothionein induction and binding and their roles in such, including the biosynthesis of these proteins (Lahaye et al. 2007a; Lahaye et al. 2007b; Pinzone et al. 2022), neither showed a significant association with Cd within the current study. Previously, hepatic Cd and Cu (Méndez-Fernandez et al. 2014), and renal Cd and Zn were found to be strongly correlated in common dolphins (Lahaye et al. 2007a). A significant correlation was observed however within the current study between Se and Cd (R_S = 0.544), which may be due to the production of Se-complexes, reducing Cd toxicity (Monteiro et al. 2020).

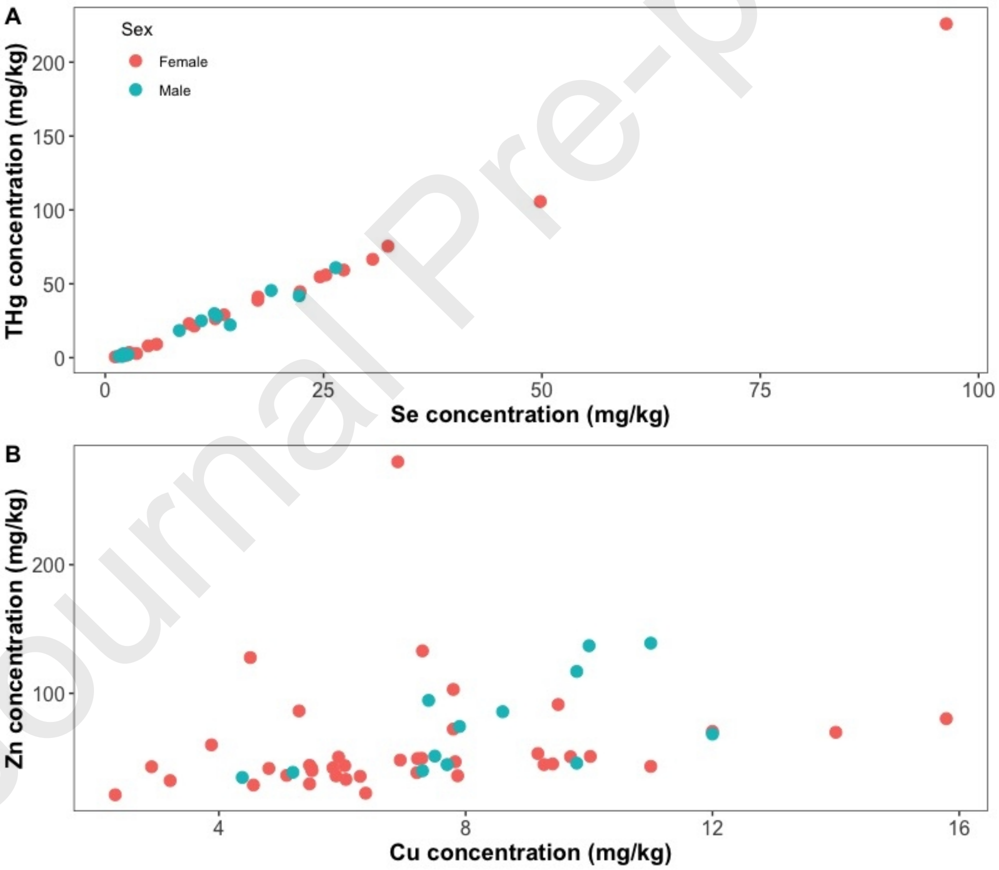

Figure 2. Concentrations (mg / kg ww) of trace elements in hepatic tissues of common dolphins sampled in Irish waters: (A) THg vs Se (females n = 21, males n=14), and (**B**) Zn vs Cu (females n = 40, males n = 14).

Table 3. Spearman correlation coefficients (R_S) between hepatic trace element concentrations (mg/kg ww), age (years) and total body length (cm) of common dolphins. *Statically significant after Bonferroni correction, n = sample size, grey shaded cells = test not carried out.

	Age	***Total body length***	***Se***	***Cd***	***Zn***	***Cu***
THg ***at 0.0167 sig.***	0.934 *	0.694 *	0.984 *			
n	40	52	34			
MeHg ***at 0.0167 sig.***	0.826 *	0.714 *	0.810 *			
n	16	22	23			
Se ***at 0.0125 sig.***	0.937 *	0.593 *		0.554*		
n	26	33		23		
Cd ***at 0.0125 sig.***	0.633 *	0.495	0.554*		0.29	0.044
n	16	22	23		23	23
Cu ***at 0.0125 sig.***	-0.139	-0.057		0.044	0.543 *	
n	40	52		23	53	

Zn	-0.203	0.065		0.29		0.543 *
at 0.0125 sig.						
n	40	52		23		53
Cr	0.205	0.59				
at 0.025 sig.						
n	5	9				
As	-0.4	-0.243				
at 0.025 sig.						
n	16	22				
Pb	0.857 *	0.687 *				
at 0.025 sig.						
n	16	22				
V	0.884 *	0.758 *				
at 0.025 sig.						
n	16	22				

3.4 Hg toxicity thresholds

Few studies have linked adverse effects in marine mammals to exposure to trace elements (e.g. (Bowles 1999; Das et al. 2004; Lavery et al. 2009; Rawson et al. 1993; Rawson et al. 1995; Ronald et al. 1977). While marine mammals are at the top of the food chain, and thus susceptible to the bioaccumulation of lipophilic pollutants with a long-biological half-life, they have a limited ability to detoxify, sequester and excrete some highly toxic elements, such as Hg (Caurant et al. 1996; Das et al. 2003b; Kershaw and Hall 2019; Wagemann et al. 1998). Thus, marine mammals can report THg concentrations between 10 to 100 times those of other top predators, of similar life spans and dietary intake (Caurant et al. 1996; Kershaw and Hall 2019; Nigro and Leonzio 1993). A THg tolerance limit ranging from 100 to 400 mg/kg ww in hepatic tissues was originally proposed by Wagemann and Muir (1984), though the evidence for such was unclear (Bowles 1999). Within the current study, only two individuals presented with THg concentrations in excess of 100 mg/kg, both sexually mature individuals.

Bottlenose dolphins (*Tursiops truncates*) that stranded along the Florida coastline, with hepatic THg concentrations exceeding 60 mg/kg ww, presented with liver abnormalities that included central necrosis, lymphocytic in-filtration, fatty degeneration and lipofuscin deposits, abnormalities observed in nine (out of eighteen) individuals (Rawson et al. 1995; Siebert et al. 1999). While endpoints of trace element toxicity were not routinely checked during necropsies within the current study, three additional common dolphins presented with THg concentrations in excess of 60 mg/kg, again all sexually mature individuals. Of the eight common dolphins with concentrations above 50 mg/kg, five died as a result of a live stranding event, four of which were involved in a single mass stranding in County Mayo in 2001. Other studies assessing free-ranging harbour porpoises from the North and Baltic Seas noted an absence of lesions characteristic of acute or chronic Hg exposure (observed in domestic animals, humans and marine mammals), even for porpoises with relatively high hepatic and renal Hg concentrations (upper range 449 mg/kg and 160 mg/kg ww, respectively) (Siebert et al. 1999). Though, increased THg and MeHg concentrations were associated with a prevalence of pathological lung disease such as pneumonia and parasitic infections, the effects of which were statistically stronger for MeHg than THg (Siebert et al. 1999). While other studies on marine mammals have reported much lower MeHg and THg immunotoxicity thresholds for lymphocyte proliferation and suppression of phagocytosis, for example (see Desforges et al. 2016).

For terrestrial species, reviews of literature based on experimental studies that focused on MeHg neurotoxicity, feed through their diet, suggested that Hg residues that exceeded c. 25–30 mg/kg ww in hepatic and renal tissues may be associated with lethal toxicity in carnivorous mammals (Thompson 1996; Shore et al. 2011; Scheuhammer et al. 2015). Such thresholds for Hg are three orders of magnitude higher than the environmental quality standard (EQS) for freshwater biota, addressing risks due to secondary poisoning (Qsbiota, secpois). An EQS of 0.02 mg/kg ww for THg and its compounds was proposed for large fish and based on chronic toxicity data for top predators such as birds and mammals, a value employed within the priority substances Directive 2013/39/EU[1] for the EU's Common Implementation Strategy for the Water Framework Directive.

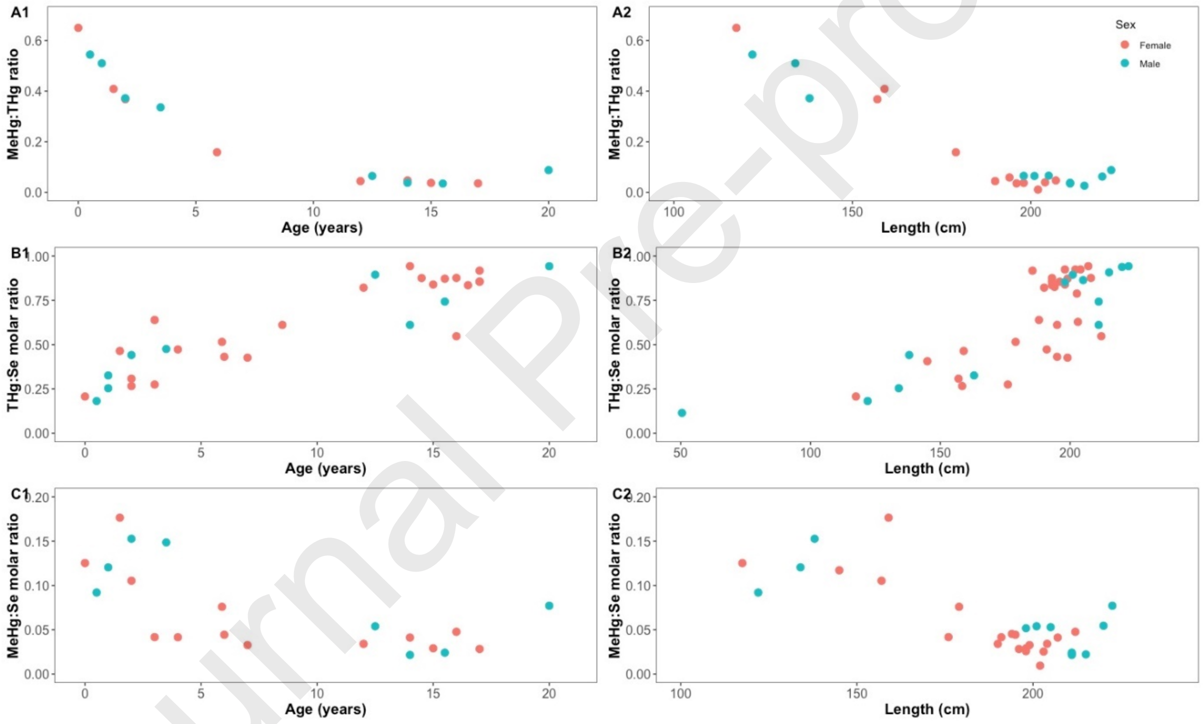

Figure 3. (A) MeHg:THg ratio (A1: female n = 13; male n = 8, A2: female n = 19, male n = 11), (B) THg:Se molar ratio (B1: female n = 22, male n = 9; B2: female n = 29, male n = 13) and, (C) MeHg:Se molar ratio (C1: female n = 13, male n = 8; C2: female n = 19, male n = 11) against age (years) and total body length (cm) by sex in the hepatic tissues of common dolphins.

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[1] https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2013:226:0001:0017:en:PDF

Dietz et al. (2021) undertook a risk assessment for THg exposure in marine mammals, where in absence of available data on MeHg and Se, THg was employed as a proxy for MeHg. In the current study it was observed that while THg may be an indication of MeHg levels in younger common dolphins, this was not the case for older individuals. Overall, the MeHg:THg ratio ranged from 0.01 to 0.65, declining with age and total body length of individuals (Figure 3a). Common dolphins less than one year old reported a ratio between 0.54 to 0.65 (n = 2), while sexually mature individuals older than 12 years in age reported a range of 0.03 to 0.09 (n = 9). Similar results were observed in previous studies on marine mammals, suggesting an inferior efficiency in demethylation of MeHg in younger individuals (e.g. Caurant et al. 1996; Bolea-Fernandez et al. 2019; Das et al. 2003b; Méndez-Fernandez et al. 2022; Siebert et al. 1999). Dietz et al. (2021) reported that marine mammals with hepatic THg less than 16 mg/kg ww were deemed no risk, 16-64 mg/kg were deemed low risk, 64-83 mg/kg as moderate risk and 83-123 mg/kg at severe risk of health effects owing to Hg exposure. Risk categories were based on earlier experimental work undertaken by Ronald et al. (1977) on four captive harp seals (*Pagophilus groenlandicus*). Though there has been some discussion on the high level of Hg administered to seals within that study, given the poor levels of detoxification observed and the lack of supplementary Se in their diet, which potentially resulted in it being a limiting factor for detoxification (Das et al. 2003b).

Given the potential confounding effects of other variables such as dietary consumption, age, overall health status etc., as well as the interactive effects from exposure to multiple pollutants, and limited feeding trial data for determining dose response relationships, many cetacean studies to date have focused on employing the THg:Se molar ratio for quantifying the risk of Hg exposure. Where a value close to or greater than 1 indicates a risk of Hg toxicity, as available Se is already bound to Hg (or other elements), potentially resulting in oxidative stress and poor health status from Hg toxicity (Dehn et al. 2006; Dietz et al. 2000; Kershaw and Hall 2019; Méndez-Fernandez et al. 2022; Monteiro et al. 2020). Within the current study, all molar ratio values were observed to be less than 1, though approaching 1 in older individuals, over 10 years in age (see Figure 4a). Thus, the risk of Hg toxicity was found to increase with age, due to bioaccumulation. Such molar parity in older individuals has been reported in other cetacean species (e.g. García-Alvarez et al, 2015). However, as a large proportion of THg in older individuals in the current study was comprised of inorganic Hg, potentially bound to Se (which also was found to increase significantly with age, through dietary intake and potentially retention), the risk to Hg exposure may be less than assumed based on the molar ratio. To assess the potential risk from MeHg, the MeHg:Se molar ratio was calculated. All molar ratio values obtained were again less than 1, ranging from 0.03 to 0.18 in sexually immature individuals to 0.01 to 0.08 in sexually mature individuals, and in contrast to the THg:Se molar ratio results, a decline was observed with age and body length of individuals (see Figure 3b). Other studies undertaken on common dolphins in the North-east Atlantic noted that 9 (out of 201) individuals were found to present with a THg:Se ratio value greater than 1 and overall, molar ratio values were observed to decline significantly between 2001 and 2017 in French waters (Méndez-Fernandez et al. 2022).

3.5 Influence of biological and health parameters on trace element concentrations

Generalized linear models and robust linear models employed to predict hepatic trace element concentrations included variables total body length, sex, sexual maturity status, DCC, COD as a proxy for health status, and VBT as a proxy for nutritional status. As observed in previous studies (e.g. Ferreira et al. 2016; Stockin et al. 2023), hepatic trace element concentrations did not show sex dependency (see Table 4). This is in contrast to other pollutants such as PCBs and DDT, where a high proportion of these lipophilic pollutants are offloaded in mature females through gestational and (primarily) lactational transfer, thus leading to higher levels accumulating in mature males (Murphy et al. 2015; Murphy et al. 2018; Williams et al. 2023). However, sex was determined as an important influencing variable for Cd and THg concentrations by Méndez-Fernandez et al. (2022) when modelling temporal trends in common dolphins sampled in French waters. Though within that study, Cd was assessed in renal tissue, a potential storage site for the persistent element (Méndez-Fernandez et al. 2014; Monteiro et al. 2016). As hepatic tissue is the main storage site for Hg (Kershaw and Hall 2019; Méndez-Fernandez et al. 2022), the results of the current study may be due to the inherent sampling bias towards females, which requires further investigation. Considering that trace element loads among the sexes in small cetaceans may be explained by foraging behaviour and preferences (Méndez-Fernandez et al. 2022). As well as potential sex and hormonal differences in modulating the synthesis of metallothioneins (Blazka and Shaikh 1991; Das et al. 2003b).

Some trace elements within the current study did show age dependency correlations. Total body length (as a proxy for age) and/or sexual maturity status were found to be important significant predictors within models for THg, MeHg, Se, and Cd, exhibiting a positive association due to bioaccumulation and the long half-lives of some of these trace elements. As well as the potential differences in prey selection and consumption between sexually immature and mature individuals (Brophy et al. 2009; Stockin et al. 2023) for those elements with shorter half-lives. Neither decomposition state, nutritional nor health status had a significant effect within the models for THg, MeHg, Se, and Cd. The inclusion of Se within the THg models, and thus controlling for the strong correlation between Se and THg, slightly reduced the influence of both total body length and sexual maturity status on THg concentrations and resulted in Se being the most significant predictor in all three top THg models (Table 4). In contrast, the addition of Se to the Cd model was not observed to be of significance. Time period, as a categorical variable (1996-2002 and 2017-2019), was only found to influence Se concentrations within the above listed elements, being negatively related, though was still less influential than the parameter sexual maturity status (Supplementary material Table S1).

Cu is an essential element required for normal growth and metabolism in marine mammals, including glucose metabolism (Bilandžić et al. 2016). Total body length and nutritional status (VBT) were important predictor variables for this element, both showing significant negative associations, i.e. in relation to nutritional status, higher concentrations were observed in individuals in a poorer nutritional condition (Table 4). In the case of body length, this may be due to either juveniles (in addition to foetuses) retaining Cu for growth and development, because of growth dilution and/or enhanced metabolic regulation with age (e.g. Bilandžić et al. 2016; Wagemann et al. 1988; Woshner et al. 2001). It should be noted that for common dolphins, VBT was reported to be negatively correlated with total body length, and VBT was also observed to vary significantly with season (Albrecht et al. in review). Controlling for the strong Cu to Zn relationship reduced the influence of nutritional status on Cu concentrations and it was no longer found to be significant – and resulted in Zn then being the most significant predictor for Cu concentrations (Table 4). Time period was also a significant influencing factor, exhibiting a positive association, being higher in the contemporary dataset. Once time period and Zn concentrations were statistically controlled for, an individual's cause of death was then found to be of importance, within the second and third top models (Supplementary material Table S1). Individuals that died as a result of infectious disease had significantly higher Cu concentrations than animals that died from 'other' causes, such as live stranding, starvation, gas embolism, or was not established.

Previously, Zn has been suggested to being a good indicator of nutritional status, as elevated Zn concentrations have been linked to emaciation in other species, such as the harbour porpoise, potentially due to increased absorption during periods of malnourishment (Das et al. 2003b; Das et al. 2006). However, within the current study, hepatic Zn concentrations were not observed to be dependent on either nutritional status, sexual maturity status nor total body length (see Table 4). Important significant influencing variables included both COD and DCC, with infectious disease cases observed to have higher Zn concentrations than animals that died as a result of trauma and 'other' causes of death (Figure 4a). Zn concentrations in the best model (only) were also observed to decrease with decomposition state, i.e. higher concentrations reported in animals that were 'extremely fresh' upon necropsy (DCC 1) (see Figure 4b). Once the strong Cu Zn relationship was controlled for however, decomposition state was no longer of significance within the best model – and again Cu was the most significant predictor of Zn concentrations (Table 4). Time period was also deemed to be a significant factor, showing a positive association, when controlling for COD. Though once Cu concentrations were taken into consideration, time period was only observed to be significant within the second-best model (Supplementary Material Table S1). Within the historical dataset, the absence of individuals categorised as 'extremely fresh' and dying from 'infectious disease' may account for some of the observed results.

Within the current study, 28% of individuals presented with Zn concentrations exceeding the suggested limit of homeostatic control for marine mammals (20-100 mg/kg ww) (Law 1996). However, this may potentially be due to a response to poorer health status, rather than a contributory cause (Bennett et al. 2001; Ferreira et al. 2016). The strong association between Zn concentrations and disease status was apparent within the current study. Zn is an important essential element for the immune system, and earlier work on 86 harbour porpoises (*Phocoena phocoena*) sampled in UK waters also reported significantly higher Zn concentrations in animals that died as a result of infectious disease compared to healthy porpoises that died from physical trauma (Bennett et al. 2001). Results that were attributed to Zn redistribution to tissues following infection, as observed in humans, as well as elevated hepatic concentrations arising as a result of acute-phase protein synthesis (Bennett et al. 2001; Das et al. 2006).

4. Conclusions and recommendations

The current study shows the complexity of the inter dependencies and relationships among pollutants on a temporal level, for chemicals that are well regulated. While assessments for contaminants of emerging concern are only in their initial stages, the more supporting factors developed for assessments of pollutants, the better our understanding of their fate and toxicity.

Previously, the common dolphin was recommended as a higher tropic level indicator species for monitoring trace elements under descriptor 8 (concentrations of contaminants are at levels not giving rise to pollutant effects) of the EU MSFD (Méndez-Fernandez et al. 2022; Monteiro et al. 2016). Recommendations supported by the results of the current study for monitoring persistent chemicals such as Cd, Pb, and Hg and its species. In addition to those elements that may be used as indicators of an individual's health status, e.g. Zn and Cu. Age was among the most important variables correlated with most trace elements measured, though this parameter could not be included within all analyses due a lack of age data for many individuals. Thus, priority should be given towards processing samples for age determination.

Future studies on the common dolphin need to consider the antagonistic and synergistic interactive effects of trace elements, namely, interactions between Hg and Se, Hg and MeHg, MeHg and Se, Cd and Se and Cu and Zn – and potentially Cd with both Cu and

Zn. Further, without the provision of information on the proportions of hepatic organic MeHg and inorganic Hg, as well as Se, toxicity thresholds for marine mammals based solely on THg are limiting and reduce confidence in those toxicological assessments (Das et al. 2003b; Rawson et al. 1995; Scheuhammer et al. 2015). Though, as reported by Ewald et al. (2019), the formation of HgSe may not substantially reduce transportation of MeHg across the blood-brain barrier of ringed seals, due to HgSe slow kinetics of formation, which would also need to be considered further.

While the employment of toxicity thresholds for hepatic trace element concentrations have been discussed prior (e.g. Das et al. 2003b; Desforges et al. 2016; Hansen et al. 2016; Law 1996), further work on such is required if employed within indicator assessments, which gives due consideration to the type of toxicity endpoint applied - for example, immunological, physiological, or reproductive. Preliminary work on a marine mammal PCB related indicator for reporting under the MSFD, and potentially OSPAR, focused primarily on reproductive endpoints, as any impairment in reproductive capabilities would impact, in time, population size (Williams et al. 2023). Thus, results of the pressure-related indicator could be evaluated against a state-related abundance and distribution indicator, through the DPSIR (driver, pressure, state, impact, and response) analytical framework (Williams et al. 2023). Future work, focusing also on other pollutants, in other tissue types, could develop general guidelines for reviews of marine mammal toxicity thresholds, including scoring criteria for parameters in assessments, such as the type of study undertaken (e.g. live or dead animals, in vivo, in vitro, in silico), study design including the employment of appropriate sample sizes, if original data were used, whether single or multiple chemical exposures were assessed, range of doses/exposure levels employed, use of appropriate endpoint(s), measurement of relevant health effects, inclusion of other confounding variables, and if the study provided enough data to assess quality and for reproducibility, etc., (see Table 5 in Schaefer and Myers (2017) to ensure thresholds are robust for use. Species specific thresholds could also be considered for indicator assessments.

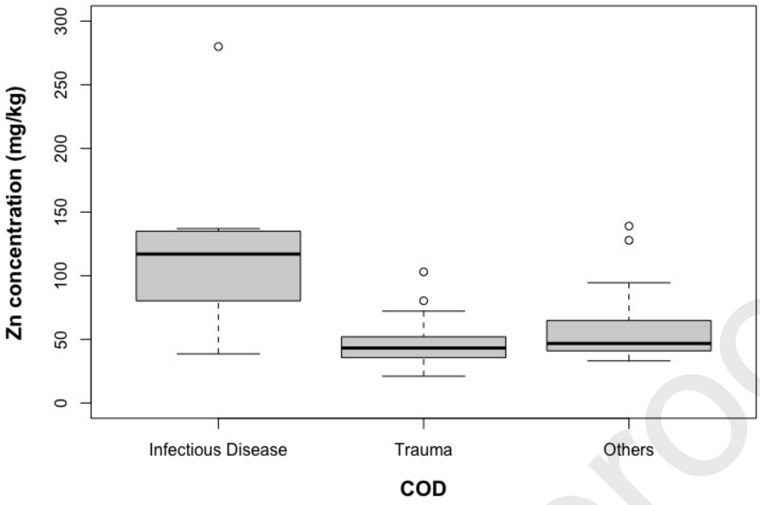
*Table 4: Coefficient estimates for parameters explaining trace metal concentrations within the top three GLMs and RLMs. COD (1) = infectious Disease (reference), COD (2) = trauma, COD (3) = other; DCC (1) = extremely fresh (reference), DCC (2) = slight decomposition, DCC (3) = moderated decomposition; Sex (1) = Female (reference), Sex (2) = Male; Maturity (1) = sexually immature (reference), Maturity (2) = sexually mature; VBT = ventral blubber thickness. *** p ≤0.001, ** p ≤ 0.01, * p ≤ 0.05. Where coefficients are not present, but parameters were included in the original model, such parameters were not selected by dredge.* Only the top three models within ΔAIC < 2 of the best model are shown.

Model		**THg**	**THg**	**Se**	**MeHg**	**Cd**	**Cd**	**Cu**	***Cu***	***Zn***	***Zn with***
			with Se				**with Se**		***with Zn***		***Cu***
		RLM	**GLM**	**RLM**	**GLM**	**GLM**	**GLM**	**GLM**	***GLM***	***RLM***	***GLM***
		n = 52	***n = 33***	***n = 33***	***n = 22***	***n = 22***	**n =22**	***n = 48***	***n = 48***	***n = 48***	***n = 48***
1	Length (cm)	0.014***	0.003***	0.004	0.003***			-0.003**	-0.002*		
	Sex			(2)-0.136				(2)0.082			
	Maturity	(2)0.545**	(2)0.111*	(2)0.549**		(2)0.3.31**	(2)0.331**	(2)0.193**	(2)0.131		
	COD			(2)-0.016 (3)0.146						(2) -0.402*** (3) -0.365***	(2)-0.338*** (3)-0.286***
	DCC									(2) -0.248** (3) -0.273**	
	VBT (mm)							-0.010*			
	Se		1.121***								
	Zn								0.280**		

	Cu										0.601***
	Df	4	5	7	3	3	3	6	5	6	5
	AICc	56.2	-83.5	6.5	-38.0	8.1	8.1	-48.3	-52.2	-17.1	-26.2
	Delta AIC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	Length (cm)	0.014***	0.003***	0.004	0.005*	0.005*	0.005**	-0.002**	-0.002**		
	Sex	(2)-0.150							(2)0.065		
	Maturity	(2)0.558**	(2)0.111*	(2)0.497*	(2)-0.161			(2)0.184*	(2)0.143*		
	COD									(2) -0.303* (3) -0.258*	(2)-0.333*** (3)-0.277***
	DCC										(2)-0.127 (3)-0.167*
	VBT (mm)		-0.003					-0.011*		-0.013	
	Se		1.133***								

	Zn								0.250**		
	Cu										0.499**
	Df	5	6	4	4	3	3	5	6	5	7
	AICc	56.8	-82	6.6	-37.5	8.3	8.3	-47.5	-51.9	-16.8	-26
	Delta AIC	0.52	1.50	0.07	0.57	0.15	0.15	0.87	0.35	0.27	0.14
3	Length (cm)	0.013***	0.003***	0.004	0.003***						
	Sex		(2)-0.026	(2)-0.122							
	Maturity	(2)0.558**	(2)0.115*	(2)0.507**		(2)0.306*	(2)0.306*				
	COD	(2)0.057 (3)0.219								(2) -0.355** (3) -0.311*	(2)-0.311*** (3)-0.257**
	DCC					(2)0.232 (3)0.006	(2)0.232 (3)0.006				
	VBT (mm)				-0.005						-0.006

Se		1.106***							
Zn							0.288**		
Cu									0.556**
df	6	6	5	4	5	5	3	4	6
AICc	57.6	-82	6.8	-36.0	9.4	9.4	-51.3	-16.2	-24.5
Delta AIC	1.35	1.53	0.25	2.09	1.25	1.25	0.90	0.88	1.69



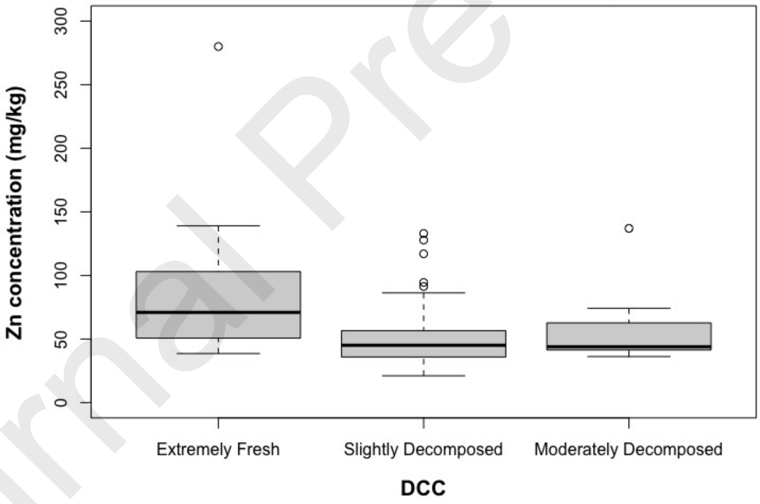


Figure 4. Influence of cause of death (COD) (n = 52) and decomposition state (DCC) (n = 52) on Zn concentrations (mg/kg ww). The dark horizontal line indicates the median, outliers are indicated by O and extreme values by *.

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Paper:

Title: Trace element concentrations in common dolphins (*Delphinus delphis*) in the Celtic Seas ecoregion: interelement relationships and effects of life history and health status

Highlights:

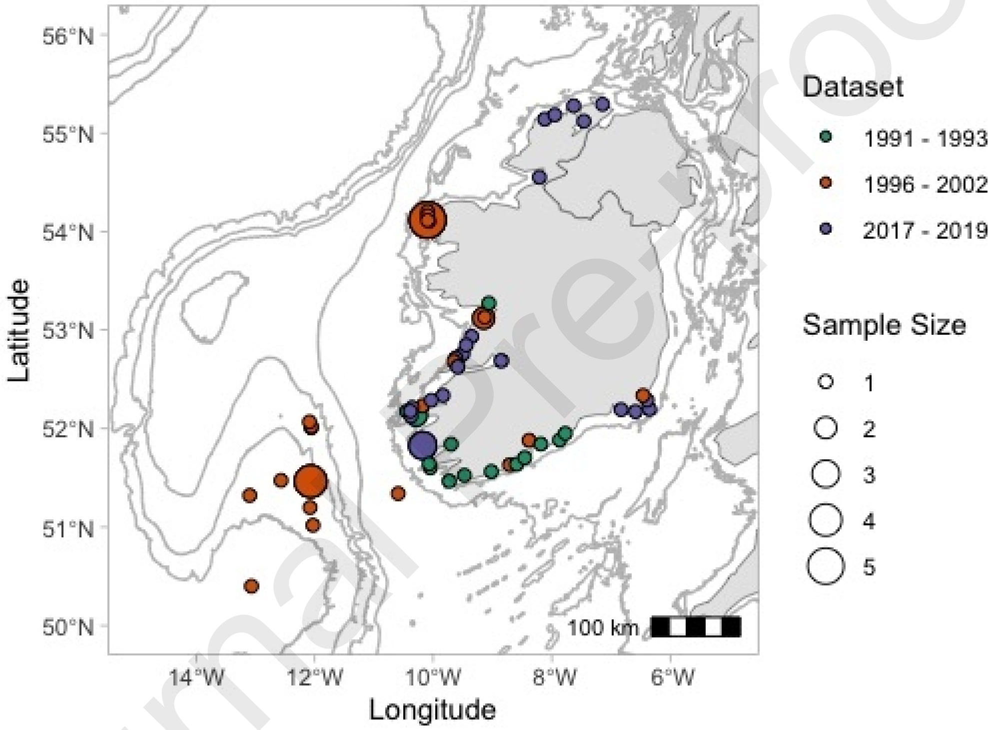
Strong inter-elemental relationships detected between Hg and Se, MeHg and Se, and Cu and Zn

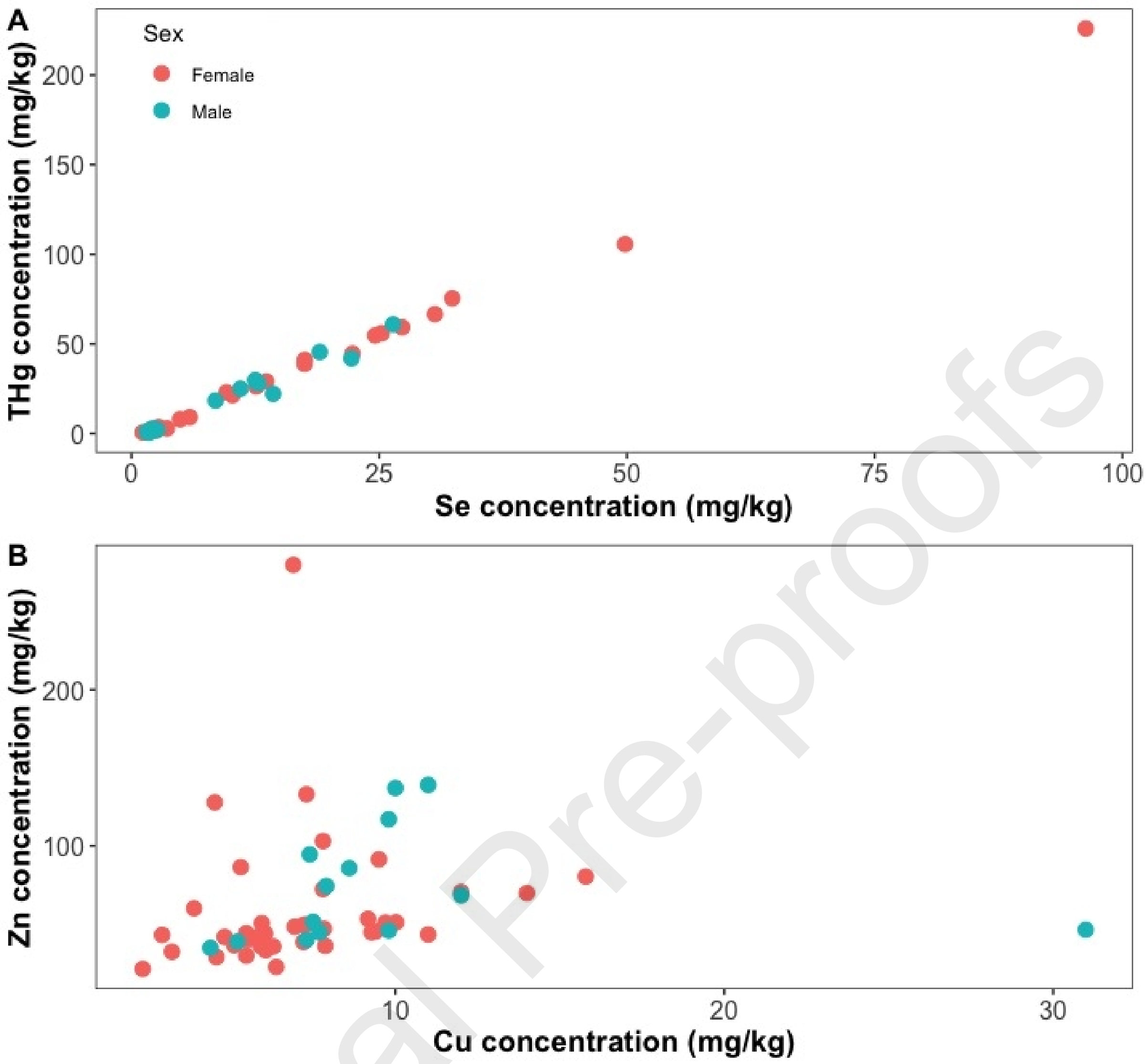
THg:Se molar ratio values increased with age and body length approaching equimolarity

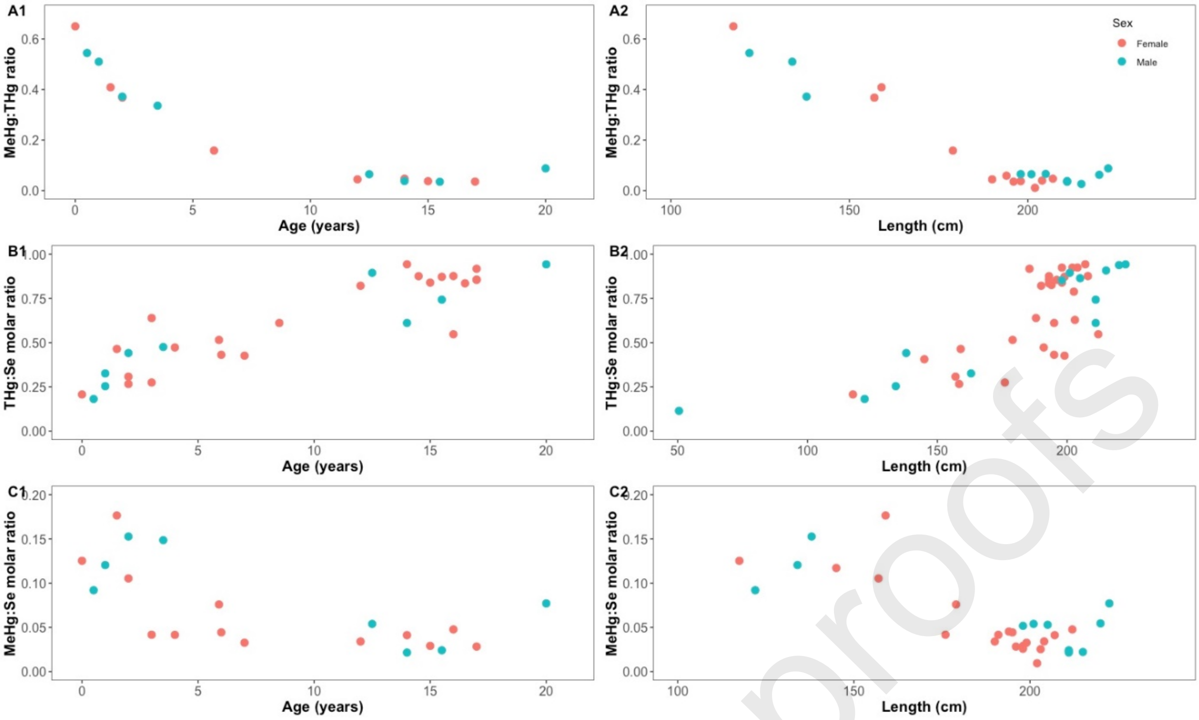
Juveniles reported higher MeHg:Hg ratio values, declining with age and body length

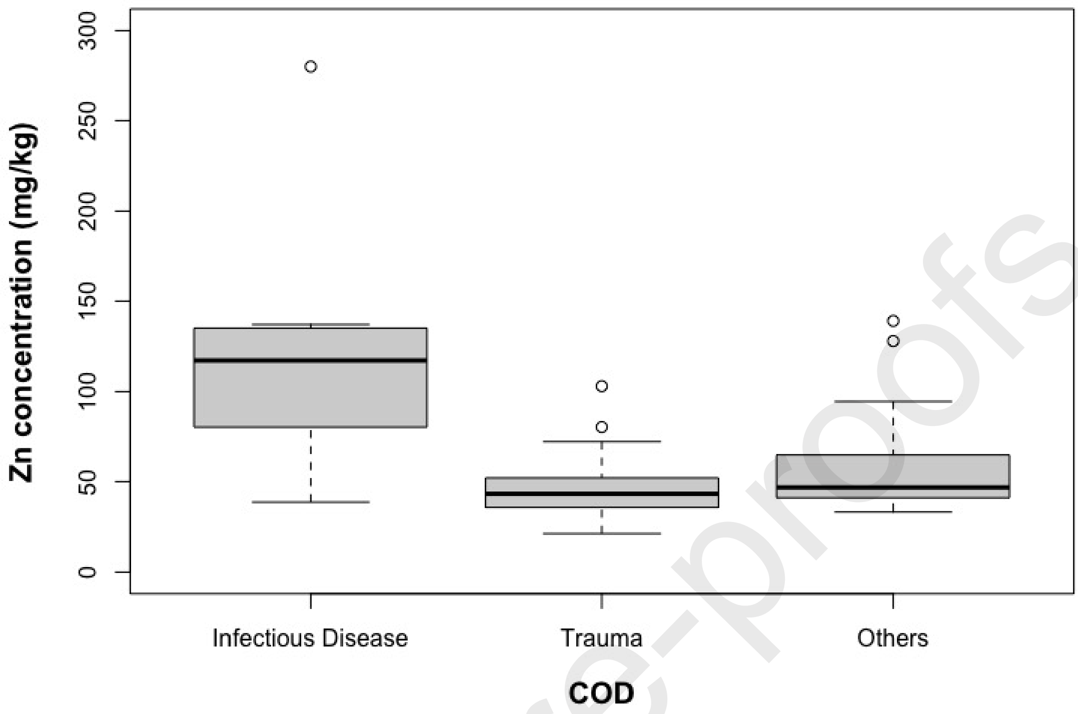
Zn and Cu could be used for monitoring the common dolphins’ health status

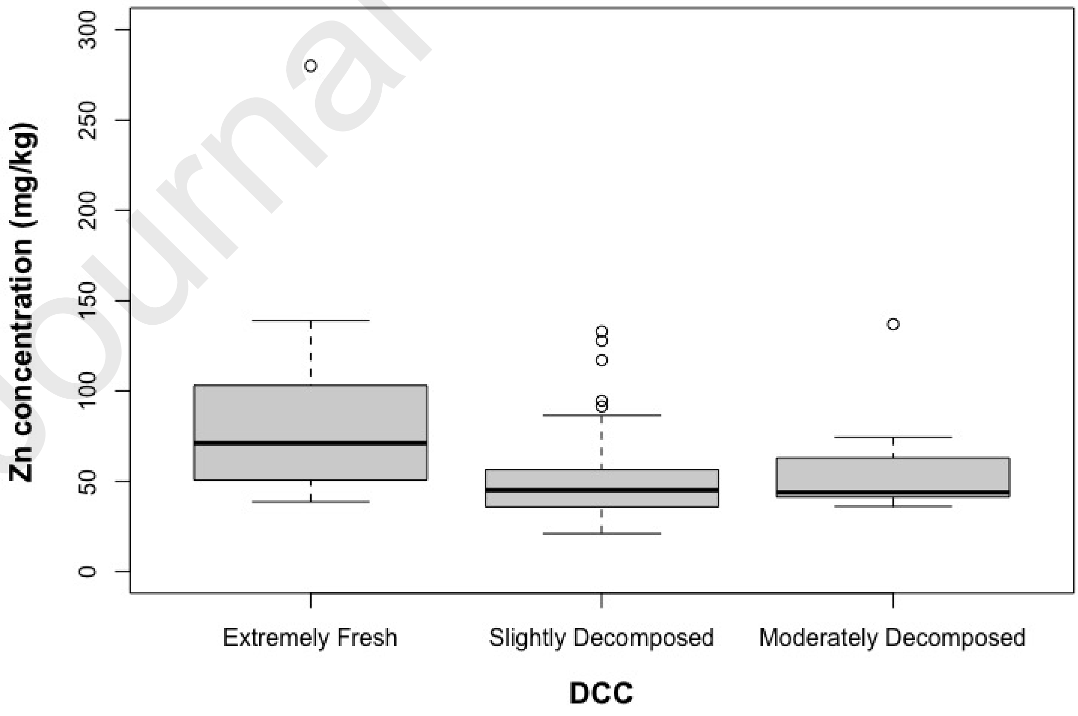
Common dolphin recommended as a trace element higher tropic level indicator species




A
Sex
Female
Male
THg concentration (mg/kg)
0
50
100
150
200
0
25
50
75
100
Se concentration (mg/kg)
B
Zn concentration (mg/kg)
100
200
10
20
30
Cu concentration (mg/kg)


A1
A2
MeHg:THg ratio
0.0
0.2
0.4
0.6
Age (years)
0
5
10
15
20
Length (cm)
100
150
200
Sex
Female
Male
B1
B2
THg:Se molar ratio
0.00
0.25
0.50
0.75
1.00
50
C1
C2
MeHg:Se molar ratio
0.00
0.05
0.10
0.15
0.20


Zn concentration (mg/kg)
0
50
100
150
200
250
300
Infectious Disease
Trauma
Others
COD


Zn concentration (mg/kg)
0
50
100
150
200
250
300
Extremely Fresh
Slightly Decomposed
Moderately Decomposed
DCC

Title: Trace element concentrations in common dolphins in the Celtic Seas ecoregion: interelement relationships and effects of life history and health status

Authorship statement:

Conceptualization: SM, BMcH, CM, EMcG. Investigation: OG, SM, BMcH, CM, EMcG, ER, FC, GJP, KD, JO'D, AE. Formal analysis: OG, SM, CM, BMcH. Data curation: OG, SM, BMcH, CM, EMcG, ER, FC, GJP, KD, JO'D, AE. Roles/Writing – original draft: SM, OG, BMcH. Writing – review & editing: CM, EMcG, ER, FC, GJP, KD, JO'D.

Declaration of interests

☒ 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.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: