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## Antarctic toothfish *Dissostichus mawsoni* as a bioindicator of trace and rare earth elements in the Southern Ocean

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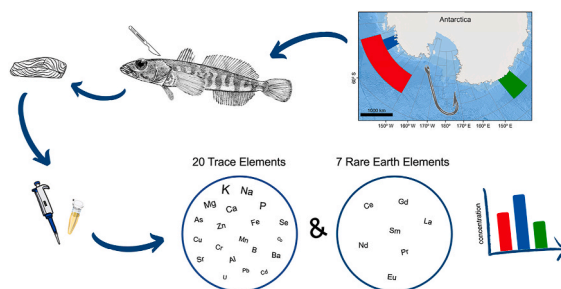
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### HIGHLIGHTS

- 27 Trace and Rare Earth Elements were measure in the muscle of *Dissostichus mawsoni*.
- Concentrations of TE and REE in *D. mawsoni* vary spatially in the Southern Ocean.
- No bioaccumulation nor biomagnification was found for any study TE and REE.
- *D. mawsoni* can be a good bioindicator for TE and REE in the Southern Ocean.
- *D. mawsoni* is a good source of essential elements for consumers, including humans.

### GRAPHICAL ABSTRACT



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### ABSTRACT

The Antarctic toothfish *Dissostichus mawsoni* is a Southern Ocean long-lived top predator which is regularly captured on an annual fishery operating in the region. By its biological and ecological characteristics, it is a potential bioindicator for the concentrations of trace and rare earth elements in the Antarctic. As these elements are mainly transferred through the diet and a deficiency or excess of these elements can lead to diverse health problems, it is important to measure their concentrations on the organisms. This study provides, for the first time, the concentration of 27 trace (major essential, minor essential and non-essential) and rare earth elements in the muscle of *D. mawsoni* captured in three areas of the Amundsen and Dumont D'Urville Seas (Antarctica). Major essential elements had the highest concentrations, with potassium (K) as the most concentrated, and rare earth elements the lowest. Significant differences between areas were found for most of the studied elements. No bioaccumulation nor biomagnification potential was found for the studied elements, with several elements

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decreasing concentrations towards larger individuals. Decreasing trends are related with the different habitats occupied by *D. mawsoni* through their life, suggesting that elements' concentrations in the water is determinant for the concentrations in this top predator, and/or there is a dilution effect as the fish grows. Our results also support that Se presents a detoxification potential for Hg in *D. mawsoni*, but only when Hg concentrations are higher to unhealthy levels. This study supports *D. mawsoni* as a potential bioindicator for the concentrations of the different trace and rare earth elements in the Southern Ocean, though only when comparing individuals of similar size/age, but also to evaluate annual changes on their concentrations. Furthermore, *D. mawsoni* can be a good source of major essential elements to humans with concentrations of major essential elements above some of other marine fish worldwide.

## 1. Introduction

Trace and rare earth elements are present in the marine environment and can be released through natural (e.g. hydrothermal vents), or anthropogenic activities (e.g. mining) (Deheyn et al., 2005; Sen and Peucker-Ehrenbrink, 2012; Tchounwou et al., 2012). When in the environment, these elements enter the food webs with organisms incorporating them mostly by the diet, but also directly from the water (Pernice et al., 2009; Wang, 2002). Some of these trace elements are essential to organisms, in higher (major essential elements, e.g. calcium (Ca)) or lower concentrations (minor essential elements, e.g. zinc (Zn)). Yet, some do not have a known function in organisms' metabolism and can be toxic even at low concentrations (non-essential elements, e.g. cadmium (Cd)) (Coelho, 2021; Jakimska et al., 2011; Tchounwou et al., 2012; Zoroddu et al., 2019). Trace elements concentrations on biodiversity have been extensively studied (e. g. Bustamante et al., 2000; Furtado et al., 2020; Lischka et al., 2021a). However, studies on rare earth elements remain scarce (e. g. MacMillan et al., 2017; Squadrone et al., 2020; Yang et al., 2016). These previous studies showed that some trace elements have a bioaccumulation and biomagnification potential, with long-lived top predators being the most susceptible to having the highest concentrations (Szyrkowska et al., 2018). In contrast, rare earth elements might not bioaccumulate throughout the life of individuals, though it may be species-specific, nor magnify through the food chain (MacMillan et al., 2017; Squadrone et al., 2020).

Previous studies on the concentration of trace and rare earth elements in the waters of the Southern Ocean showed that trace elements vary regionally and with depth, while concentrations of rare earth elements tend to increase with depth, except for cerium (Ce) whose concentrations remain similar throughout the water column (Castrillejo et al., 2013; Hathorne et al., 2015; Nozaki and Alibo, 2003; Sañudo-Wilhelmy et al., 2002). Concerning the concentrations of these elements in the Southern Ocean wildlife, various studies analysed the levels of trace elements in different groups of organisms, such as fish (Bustamante et al., 2003), cephalopods (Lischka et al., 2020), seabirds (Cherel et al., 2018) and marine mammals (Szefer et al., 1993). These studies showed that concentrations of some elements can be as high as in urbanized coasts (e. g. Cd (Lischka et al., 2021b)) and the potential of bioaccumulation and biomagnification in the food webs of the Southern Ocean (e. g. Mercury (Hg) (Queirós et al., 2020a; Seco et al., 2021)). To the best of our knowledge, no studies have measured rare earth elements concentrations in the Southern Ocean biota. However, as rare earth elements can present some levels of toxicity for organisms (Oral et al., 2010), and their levels are increasing worldwide due to intensification of its use in industries (Pinto et al., 2019), it is important to assess if these elements are already present in the Southern Ocean biota and if they present a bioaccumulation and/or biomagnification potential in these food webs.

Within the Southern Ocean food web, the Antarctic toothfish *Disostichus mawsoni* is a deep-sea notothenioid fish with a circumpolar distribution south of the Antarctic Polar Front (Duhamel et al., 2014). It is a long-lived species, living up to 40 years, and reach great sizes and weight, over 1.5 m and 100 kg respectively (Brooks et al., 2011; Hanchet et al., 2015). *D. mawsoni* is a demersal top predator that feeds mainly on

fish and cephalopods, but other taxa such as crustaceans, echinoderms, are also present in its diet (Fenaughty et al., 2003; Jo et al., 2013; Park et al., 2015; Queirós et al., 2022; Roberts et al., 2011; Stevens et al., 2014). Such biological and ecological features suggest that *D. mawsoni* is susceptible to accumulate high concentrations of trace elements. Indeed, previous studies already confirmed this potential of *D. mawsoni* to accumulate Hg (Hanchet et al., 2012; Queirós et al., 2020b; Son et al., 2014). Apart from its ecological role, *D. mawsoni* is also target of a fishery managed by the Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) within its convention area (CCAMLR, 1980; Grilly et al., 2015; Hanchet et al., 2003). Because seafood is one of the major sources of essential nutrients to humans, yet when in high concentrations can also be harmful, the concentrations of these elements should be constantly monitored (Weichselbaum et al., 2013; Zoroddu et al., 2019). As its annual fishery around the continent grants a constant access to samples, its low movement capacity, as well its ecological role, *D. mawsoni* meets all characteristics to be considered a potential indicator for the concentrations of trace and rare earth elements in the Southern Ocean deep-sea ecosystem (Coelho, 2021; Metian et al., 2013).

In this study, using muscle of *D. mawsoni* captured in the Amundsen and Dumont D'Urville Seas, we: 1) measured the concentrations of 27 trace and rare earth elements to provide a baseline data for future studies in the Southern Ocean, 2) evaluated the bioaccumulation and biomagnification potential of these elements in an Antarctic deep-sea food web, and 3) assessed the detoxification potential Hg by Selenium (Se) in *D. mawsoni*. To analyse the biomagnification and detoxification potential of some elements, we considered both the  $\delta^{15}\text{N}$  values (proxy for trophic position) and Hg concentrations measured in previous studies (Queirós et al., 2020b, 2022). Because of the commercial interest of *D. mawsoni*, we discussed throughout the manuscript how this species can be a good source of nutrients to consumers but also if the different trace elements can be used to determine the origin of the fish.

## 2. Materials and methods

### 2.1. Data collection

*D. mawsoni* were captured using an autoline longline system (Fenaughty, 2008) baited with ommastrephid squid *Illex argentinus* onboard a fishing vessel licensed by CCAMLR (CCAMLR, 2019). Individuals were captured during the Austral Summer of 2016/2017, in three different fishing areas: CCAMLR Small Scale Research Unit (SSRU) 88.2H (Amundsen Sea seamount; n = 38), CCAMLR SSRU 88.2 F (Amundsen Sea slope; n = 36) and CCAMLR SSRU 58.4.1G (Dumont D'Urville Sea slope; n = 7) (Fig. 1). Because of the sensitivity of commercial fishery data, the exact position of the lines is not disclosed but they were used in the analyses. Muscle samples (~50 g) and sagittal otoliths (hereafter otoliths) of *D. mawsoni* were collected from fish heads after beheaded by fishermen, and frozen at  $-40\text{ }^{\circ}\text{C}$ . Due to limitations onboard it was not possible to measure or weight the fish before beheaded and processed by fishermen.

## 2.2. Analytical procedures

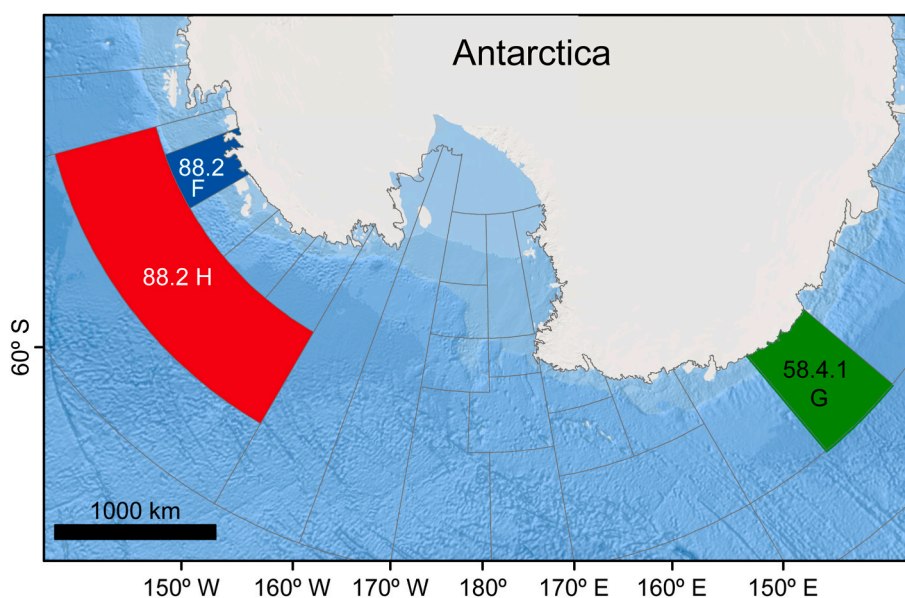
At the laboratory, the otolith length was measured with a digital Vernier calliper ( $\pm 0.01$  mm). When both otoliths were measured, an average value was considered. Otoliths were used as proxy for fish size instead of standard length estimations calculated using published allometric equations (e. g. Williams and McEldowney, 1990) because these equations tend to underestimate the fish's size (José Queirós pers. obs.), and otoliths were measured while standard length would be just an estimative. Muscle samples were freeze-dried and homogenized using a mortar and pestle. To determine trace and rare earth elements concentrations, a microwave-assisted acid-digestion was performed following Viana et al. (2021). Briefly, 200 mg of muscle were weighted into an acid-washed Teflon® vessel using an analytical balance ( $\pm 0.01$  mg). Afterwards, 1 ml of  $\text{HNO}_3$  68%, 2 ml of  $\text{H}_2\text{O}_2$  30% and 1 ml of ultrapure  $\text{H}_2\text{O}$  were sequentially added to each vessel. After being sealed, each vessel was submitted to increasing pressure and temperature conditions in a CEM MARS 5 (model 240/50) microwave for 25 min following the pattern: 15 min of ramping heating to 150 °C; 5 min of ramping heating to 170 °C; 5 min of constant temperature at 170 °C. This process was followed by a cooling down at room temperature. The final solution was collected to 25 ml polyethylene tubes and the final volume reached using ultrapure  $\text{H}_2\text{O}$ .

Concentrations of the major essential elements: Ca - calcium, K - potassium, Mg - magnesium, Na - sodium, P - phosphorous; the minor essential elements: As - arsenic, Co - cobalt, Cr - chromium, Cu - copper, Fe - iron, Mn - manganese, Se - selenium, Zn - zinc; the non-essential elements: Al - aluminium, B - boron, Ba - barium, Cd - cadmium, Pb - lead, Sr - strontium, U - uranium; and the rare earth elements: Ce - cerium, Eu - europium, Gd - gadolinium, La - lanthanum, Nd - neodymium, Pr - praseodymium, Sm - samarium were measured either by inductively coupled plasma-optical emission spectrometry (ICP-OES, Horiba Jobin Yvon Activa M) or by inductively coupled plasma-mass spectrometry (ICP-MS, Thermo Elemental, X-Serie) (Table 1). For analytical quality control we used the certified reference material DORM-4 (fish muscle). The recovery efficiencies (%) for the studied elements varied between 87 and 153% (see Appendix A for detailed information on the different elements). Due to the unavailability, at the time of the analyses, of reference material that included all the analysed elements and the absence of reference value in DORM-4 for B, Ba and rare earth elements no corrections were made to the values

measured by the ICP. Thus, we must be aware that values presented for these elements can be over- or underestimated. Some studied elements, i.e. Cr, Se, Sr and U, had recovery efficiencies outside the usual 90–110%. This is related with a known issue of ICP-MS measuring some elements, e.g. Cr and Se, in biological matrixes. Even though all presented values were corrected considering the respective recovery efficiencies, these results suggest that the equipment was undermeasure the concentrations of Sr, and overmeasuring the values of Cr, Sr and U. Method detection limits, calculated using the 3-sigma approach of 5 blanks prepared following the same protocol as for the samples, were 0.00001 (Eu, Gd, Sm), 0.00002 (La, Nd, Pr), 0.0004 (Ce), 0.0001 (Co, Cd, Mn, Pb, U), 0.0002 (Ba), 0.0003 (Sr), 0.002 (As, Cu, Se), 0.005 (B), 0.006 (Cr, Zn), 0.02 (Al), 0.03 (Fe), 0.5 (Ca, K, Mg), 1.5 (Na) and 3 (P) ( $\text{mg L}^{-1}$  dry weight (dw)). The absence of blanks below the detection limit of the equipment (i.e. all blanks presented a measurable concentration of Cr in  $\text{mg L}^{-1}$ ) might suggest any sort of contamination throughout this process, thus we need to be careful when analysing these results. Concentrations of trace and rare earth elements are presented in  $\text{mg kg}^{-1}$  dw.

## 2.3. Data analysis

The normal distribution and homogeneity of variances of concentrations of trace and rare earth elements in *D. mawsoni*'s muscle were tested using a Shapiro-Wilk test and a Bartlett's test, respectively. Differences in the concentrations of trace and rare earth elements between the three fishing areas were tested using a Kruskal-Wallis test followed by a Dunn's multiple comparison test, with exception for K whose differences were tested with an ANOVA followed by a Tukey's multiple comparison test. Because the number of samples with concentrations of Co, Pb, Gd and La above the method detection limit at the Dumont D'Urville Sea slope precluded statistical analysis, differences in concentrations between fish from both Amundsen Sea areas for these elements were compared using a Mann-Whitney test. Due to the low number of samples with concentrations of Cd, Eu, Nd, Pr, Sm, and U above the method detection limit, no differences between areas were tested. To evaluate if trace and rare earth elements allow to distinguish the origin of fish between the three studied areas, a principal component analysis (PCA) was performed using the function *prcomp* in R software (R core team, 2020). Despite the distribution of the data do not follow a normal distribution, the PCA was performed as this analysis do not



**Fig. 1.** Map of the study area. Coloured squares show the three different study areas. Red: Amundsen Sea seamount (CCAMLR SSRU 88.2 H); Blue: Amundsen Sea slope (CCAMLR SSRU 88.2 F); Green: Dumont D'Urville Sea slope (CCAMLR SSRU 58.4.1 G).

**Table 1**

Concentrations of trace and rare earth elements (mg kg<sup>-1</sup> dw) in the muscle of *Dissostichus mawsoni*, otolith length (mm) and δ<sup>15</sup>N values (‰) in the three sampling areas. Statistics was only applied to the three studied areas. Bold indicate significant differences. Values with different superscript letters are significant different (p < 0.05).

Element	Overall		Amundsen Sea seamount		Amundsen Sea slope		Dumont D'Urville Sea slope		Statistics between areas
	n	Mean ± SD (Min; Max)	n	Mean ± SD (Min; Max)	n	Mean ± SD (Min; Max)	n	Mean ± SD (Min; Max)	
Major Essential Elements	Ca	81 1237 ± 2648 (153; 18,598)	38 1063 ± 3089 <sup>a</sup> (153; 18,598)	36 1574 ± 2381 <sup>b</sup> (235; 12,348)	7 454 ± 165 <sup>a,b</sup> (287; 708)	<b>p &lt; 0.001</b> H = 19.3			
	K	81 12,360 ± 3644 (3957 ± 22,975)	38 11,225 ± 2786 <sup>a</sup> (6131 ± 17,336)	36 14,188 ± 3730 <sup>b</sup> (6012; 22,975)	7 9124 ± 2750 <sup>a</sup> (3957 ± 12,911)	<b>p &lt; 0.001</b> F = 11.6			
	Mg	81 1139 ± 408 (565; 2261)	38 881 ± 250 <sup>a</sup> (565; 1623)	36 1454 ± 353 <sup>b</sup> (684; 2261)	7 915 ± 194 <sup>a</sup> (767; 1342)	<b>p &lt; 0.001</b> H = 37.0			
	Na	81 7612 ± 2817 (3215; 14,999)	38 6653 ± 2451 <sup>a</sup> (3215; 13,674)	36 8766 ± 2791 <sup>b</sup> (3286; 14,999)	7 6882 ± 2982 <sup>a,b</sup> (4136; 12,602)	<b>p = 0.003</b> H = 11.9			
	P	81 6749 ± 2111 (3512; 13,645)	38 6192 ± 1903 <sup>a</sup> (3512; 13,645)	36 7171 ± 2047 <sup>b</sup> (3706; 10,851)	7 7598 ± 2961 <sup>a</sup> (3861; 13,446)	<b>p = 0.076</b> H = 5.2			
Minor Essential Elements	As	81 29.17 ± 35.79 (1.67; 165.53)	38 23.91 ± 42.35 <sup>a</sup> (1.67; 165.53)	36 28.95 ± 21.65 <sup>b</sup> (6.91; 118.37)	7 58.86 ± 45.62 <sup>b</sup> (19.65; 155.91)	<b>p &lt; 0.001</b> H = 22.6			
	Co	13 0.021 ± 0.013 (0.012; 0.060)	7 0.025 ± 0.017 (0.012; 0.060)	6 0.017 ± 0.004 (0.012; 0.024)	0 n.a.	<b>p = 0.534</b> U = 16.0*			
	Cr	81 0.99 ± 0.40 (0.65; 3.94)	38 1.06 ± 0.55 (0.70; 3.94)	36 0.92 ± 0.17 (0.72; 1.50)	7 0.98 ± 0.25 (0.65 ± 1.35)	<b>p = 0.438</b> H = 1.6			
	Cu	80 0.58 ± 0.92 (0.27; 8.56)	38 0.69 ± 1.33 (0.27; 8.56)	36 0.50 ± 0.11 (0.31; 0.75)	6 0.41 ± 0.15 (0.31; 0.71)	<b>p = 0.053</b> H = 5.9			
	Fe	72 4.84 ± 3.80 (1.12; 20.66)	31 4.12 ± 3.97 <sup>a</sup> (1.26; 16.96)	36 5.70 ± 3.73 <sup>b</sup> (1.43; 20.66)	5 3.04 ± 1.23 <sup>a,b</sup> (1.12; 4.25)	<b>p = 0.005</b> H = 10.8			
	Mn	81 0.56 ± 0.76 (0.09; 5.40)	38 0.35 ± 0.58 <sup>a</sup> (0.09; 3.58)	36 0.84 ± 0.90 <sup>b</sup> (0.20; 5.40)	7 0.24 ± 0.06 <sup>a</sup> (0.17; 0.32)	<b>p &lt; 0.001</b> H = 37.4			
	Se	81 2.07 ± 0.47 (1.19; 3.90)	38 2.05 ± 0.54 (1.25; 3.90)	36 2.09 ± 0.42 (1.19; 3.09)	7 2.04 ± 0.35 (1.54; 2.67)	<b>p = 0.754</b> H = 0.6			
	Zn	81 16.87 ± 8.07 (7.08; 50.79)	38 15.89 ± 7.94 <sup>a</sup> (7.80; 50.79)	36 19.29 ± 8.01 <sup>a</sup> (8.31; 46.33)	7 9.69 ± 2.09 <sup>b</sup> (7.08; 13.30)	<b>p &lt; 0.001</b> H = 17.0			
	Non-Essential Elements	Al	71 3.70 ± 4.49 (0.54; 27.42)	30 3.22 ± 4.64 <sup>a</sup> (0.54; 22.91)	36 4.47 ± 4.55 <sup>b</sup> (0.90; 27.42)	5 1.04 ± 0.20 <sup>a</sup> (0.75; 1.28)	<b>p &lt; 0.001</b> H = 15.2		
		B	81 2.33 ± 0.93 (0.75; 5.59)	38 1.86 ± 0.67 <sup>a</sup> (0.75; 3.91)	36 2.83 ± 0.97 <sup>b</sup> (1.24; 5.59)	7 2.27 ± 0.63 <sup>a,b</sup> (1.53; 3.15)	<b>p &lt; 0.001</b> H = 23.5		
Ba		70 0.23 ± 0.32 (0.03; 1.47)	28 0.13 ± 0.21 <sup>a</sup> (0.03; 0.77)	36 0.33 ± 0.38 <sup>b</sup> (0.03; 1.47)	6 0.05 ± 0.01 <sup>a</sup> (0.03 ± 0.07)	<b>p &lt; 0.001</b> H = 28.6			
Cd		19 0.038 ± 0.040 (0.010; 0.142)	15 0.043 ± 0.044 (0.010; 0.142)	2 0.018 ± 0.009 (0.012; 0.024)	2 0.021 ± 0.003 (0.019; 0.022)	n.a.			
Pb		25 0.04 ± 0.08 (0.01; 0.42)	12 0.07 ± 0.12 (0.01; 0.42)	11 0.02 ± 0.01 (0.02; 0.03)	2 0.02 ± 0.01 (0.01; 0.02)	<b>p = 0.413</b> U = 52.0*			
Sr		81 11.00 ± 28.51 (1.90; 235.46)	38 12.72 ± 39.24 (1.90; 235.46)	36 10.43 ± 14.74 (2.17; 75.86)	7 4.58 ± 1.56 (2.79; 6.83)	<b>p = 0.201</b> H = 3.2			
U		8 0.011 ± 0.009 (0.006; 0.033)	3 0.018 ± 0.013 (0.009; 0.033)	3 0.008 ± 0.003 (0.006; 0.011)	2 0.006 ± 0.000 (0.006; 0.007)	n.a.			
Rare Earth Elements		Ce	53 0.011 ± 0.015 (0.003; 0.083)	15 0.008 ± 0.007 (0.003; 0.027)	34 0.012 ± 0.016 (0.003; 0.083)	4 0.016 ± 0.022 (0.004; 0.049)	<b>p = 0.756</b> H = 0.6		
		Eu	2 0.002 ± 0.000 (0.002; 0.002)	1 0.002	0 n.a.	1 0.002	n.a.		
		Gd	18 0.002 ± 0.001 (0.001; 0.005)	9 0.002 ± 0.001 (0.001; 0.005)	7 0.003 ± 0.001 (0.002; 0.005)	2 0.002 ± 0.001 (0.002; 0.003)	<b>p = 0.252</b> U = 20.0*		
	La	26 0.007 ± 0.008 (0.002; 0.040)	6 0.004 ± 0.003 (0.002; 0.010)	19 0.007 ± 0.009 (0.003; 0.040)	1 0.004	<b>p = 0.642</b> U = 49.0*			
	Nd	18 0.007 ± 0.009 (0.003; 0.039)	2 0.005 ± 0.003 (0.003; 0.008)	16 0.007 ± 0.009 (0.003; 0.039)	0 n.a.	n.a.			
	Pr	3 0.006 ± 0.003 (0.003; 0.009)	0 n.a.	3 0.006 ± 0.003 (0.003; 0.009)	0 n.a.	n.a.			
Sm	10 0.003 ± 0.002 (0.001; 0.008)	2 0.002 ± 0.001 (0.001; 0.002)	8 0.003 ± 0.002 (0.002; 0.008)	0 n.a.	n.a.				
Otolith length	81	8.2 ± 1.1 (6.1; 10.7)	38 8.9 ± 0.7 (7.1; 10.7)	36 7.3 ± 1.0 (6.1; 9.9)	7 8.5 ± 1.0 (7.1; 10.1)	n.a.			
	δ <sup>15</sup> N values <sup>1</sup>	81 14.4 ± 1.0 (12.3; 16.8)	38 14.7 ± 1.1 (12.3; 16.8)	36 14.1 ± 1.0 (12.4; 16.0)	7 14.6 ± 0.4 (14.1; 15.2)	n.a.			
Hg <sup>2</sup>	81 0.66 ± 0.50 (0.13; 2.62)	38 0.91 ± 0.61 (0.21; 2.62)	36 0.42 ± 0.17 (0.13; 0.89)	7 0.57 ± 0.37 (0.26; 1.33)	n.a.				

\*Comparison performed only between fish from the Amundsen Sea seamount and Amundsen Sea slope; <sup>1</sup>δ<sup>15</sup>N values obtained from Queirós et al. (2022); <sup>2</sup>Hg concentrations measured in Queirós et al. (2020b).

require the normality of the data (Jolliffe, 2002). As PCA do not run with empty values, when concentrations were below the limit of detection, we considered a value of half of the limit of the detection.

To study the bioaccumulation and biomagnification of the different elements, a Spearman's correlation was performed between the

elements' concentration and the respective otolith length (proxy for fish size (Williams and McEldowney, 1990)) and δ<sup>15</sup>N values (proxy for trophic position (Bearhop et al., 2004)), considering the values of the three studied areas pooled. The only exception was K for which we performed a Pearson's correlation. No correlations were performed for

Eu and Pr as less than 4 values were measured above the method detection limit in the three studied areas.  $\delta^{15}\text{N}$  values were measured in Queirós et al. (2022). To evaluate the detoxification potential of Se for Hg, we performed a Spearman correlation between the concentrations of both elements in the muscle for the three fishing areas pooled and separated. For this analysis, we considered the Hg concentrations measured in Queirós et al. (2020b) ( $\text{mg kg}^{-1}$  dw).

All tests were performed using GraphPad Prism® v9.2.0 and the R software (R core team, 2020) considering  $\alpha = 0.05$ .

### 3. Results

#### 3.1. Trace and rare earth element concentrations in the muscle of *Dissostichus mawsoni*

Overall, the average trace and rare earth element concentration decreased in the following order:  $\text{K} > \text{Na} > \text{P} > \text{Ca} > \text{Mg} > \text{As} > \text{Zn} > \text{Sr} > \text{Fe} > \text{Al} > \text{B} > \text{Se} > \text{Cr} > \text{Cu} > \text{Mn} > \text{Ba} > \text{Pb} > \text{Cd} > \text{Co} > \text{Ce} > \text{U} > \text{La} > \text{Nd} > \text{Pr} > \text{Sm} > \text{Gd} > \text{Eu}$  (Table 1, Fig. 2). Concentrations in the three study areas followed a similar pattern, though in the Amundsen Sea seamount  $\text{Se} > \text{B}$  and  $\text{U} > \text{Ce}$ , in the Amundsen Sea slope  $\text{Mn} > \text{Cu}$ , and in the Dumont D'Urville Sea slope  $\text{P} > \text{Na}$ ,  $\text{Mg} > \text{Ca}$ ,  $\text{B} > \text{Se} > \text{Al}$  and  $\text{Cd} > \text{Pb}$  (Table 1, Fig. 2). Regarding elements for which no fish had concentrations above the method detection limit, Pr in the Amundsen Sea seamount, Eu in the Amundsen Sea slope and Co, Pr, Nd and Sm in the Dumont D'Urville Sea slope (Table 1, Fig. 2).

Significant differences were found for trace and rare earth elements between the three studied areas, except for Ce, Cr, Cu, P, Se, and Sr (Table 1). Post-hoc multiple comparison tests showed that most of the differences were between the Amundsen Sea seamount and the Amundsen Sea slope (11 elements), followed by differences between the Amundsen Sea slope and the Dumont D'Urville Sea slope (7 elements) and by Amundsen Sea seamount and Dumont D'Urville Sea slope (2 elements) (Table 1). No significant differences were found for Co, Gd, La and Pb when comparing both Amundsen Sea areas (Table 1). The highest concentrations of both trace and rare earth elements were mostly found in fish from the Amundsen Sea slope, apart for Cd, Co, Cr, Cu, Pb, Sr, and U whose highest concentrations were measured in fish from the Amundsen Sea seamount, and for P, As and Ce in the Dumont D'Urville Sea slope (Table 1, Fig. 2). In contrast, most of the elements had its lowest concentrations measured in the Dumont D'Urville Sea slope, excluding As, B, Ce, Mg, Na and P for whose lowest concentrations were found in the Amundsen Sea seamount, and Cd and Cr in the Amundsen Sea slope (Table 1, Fig. 2). The largest differences between areas were found for Al, As, Ba, Ca, Ce, Mn, Pb, Sr, and Zn, (Table 1, Fig. 2). The PCA analyses did not show any separation between samples from the three areas (Fig. 3).

#### 3.2. Correlation between trace and rare earth elements and both otolith length and $\delta^{15}\text{N}$ values

With exception for P, significant negative correlations were found between all the other major essential elements and the otolith length (Table 2). Regarding minor essential elements, significant negative correlations were found for most elements, with exception for Co and Cr (Table 2). For non-essential elements, negative correlations were found for Al, B, Ba and Sr (Table 2). Ce was the only rare earth element presenting a significant negative correlation with the otolith length (Table 2).

Concerning correlations with  $\delta^{15}\text{N}$  values, significant negative correlations were only found for the major essential elements Ca, K, Na and P, the minor essential elements Mn and Zn and the non-essential B (Table 2).

#### 3.3. Correlations between mercury and selenium

A significant negative correlation was found between Hg and Se concentrations when considering all the three studied areas together (Spearman correlation,  $n = 81$ ,  $p < 0.001$ ,  $r = -0.4377$ ). A significant positive correlation between Hg and Se concentrations was found for fish captured in the Amundsen Sea seamount (Spearman correlation,  $n = 38$ ,  $p = 0.001$ ,  $r = 0.521$ ). No significant correlations were found between the concentrations of Hg and Se in the Amundsen Sea slope (Spearman correlation,  $n = 36$ ,  $p = 0.639$ ,  $r = 0.080$ ) and in the Dumont D'Urville Sea slope (Spearman correlation,  $n = 7$ ,  $p = 0.498$ ,  $r = -0.321$ ).

### 4. Discussion

This is the first study to evaluate the concentrations of trace, apart from Hg (Hanchet et al., 2012; Queirós et al., 2020b; Son et al., 2014; Yoon et al., 2018), and rare earth elements in the muscle of the *D. mawsoni*. Our results show that concentrations of these elements in this species vary geographically and within adjacent fishing areas. For most of the studied elements, highest concentrations were found in fish from the Amundsen Sea slope (15 elements), whereas lowest concentrations were found in fish from the Dumont D'Urville Sea slope (17 elements, of which no fish had Co, Nd, Pr and Sm concentrations above the method detection limit). These differences can be related with either the diet, e.g. As, since it differs between slope and seamount areas and between life stages (Queirós et al., 2022; Stevens et al., 2014), with variability of elements' concentrations in Southern Ocean waters, e.g. K, (Castrillejo et al., 2013; Hathorne et al., 2015; Nozaki and Alibo, 2003; Sañudo-Wilhelmy et al., 2002), or even by contrasting trends in environmental changes, e.g. ice melting for Fe (Stammerjohn et al., 2012), that increase the input of some elements into the environment (Kim et al., 2015).

Using otolith length as proxy for the fish size and  $\delta^{15}\text{N}$  values, measured in Queirós et al. (2022), as a proxy for the trophic position, our results suggest no bioaccumulative effect in the muscle of *D. mawsoni* for the studied elements. In contrast, most concentrations decreased with the fish's size, which is possible related to a growth dilution effect, differences in metabolism and lipid content between younger and older fish, or even with the habitat. Absence of significant correlations for most elements with  $\delta^{15}\text{N}$  values also show no biomagnification potential in these food webs. Furthermore, we studied the potential of Se to detoxicate Hg (measured in Queirós et al. (2020b)), with our results suggesting a detoxification potential of Se for Hg but only when Hg concentrations are high.

#### 4.1. Trace and rare earth element concentrations in the muscle of *Dissostichus mawsoni* in the Amundsen and Dumont D'Urville Seas

Major essential elements and rare earth elements in the muscle of *D. mawsoni* have the highest and lowest concentrations of the studied elements, respectively (Table 2). With exception to P, highest concentrations of major essential elements were found in fish from the Amundsen Sea slope, which suggests higher concentrations of these elements entering in the food web from the environment which, ultimately, reflect in the top predators. K was the most concentrated element in the muscle of *D. mawsoni* in the three studied areas, though with differences between areas. These differences were between fish from the Amundsen Sea slope and the other two studied areas, with fish from the Amundsen Sea slope having almost 2-times more K than those from the Dumont D'Urville Sea slope. As no differences exist between the diet of *D. mawsoni* in the Amundsen Sea slope and the Dumont D'Urville Sea slope (Queirós et al., 2022), differences in K concentrations might be related with its concentration in the water. Values of K measured in the *D. mawsoni* can be 2-fold higher than some marine fish in the world, e.g. Turbot (*Psetta maxima* and *P. m. maotica*) in the Black

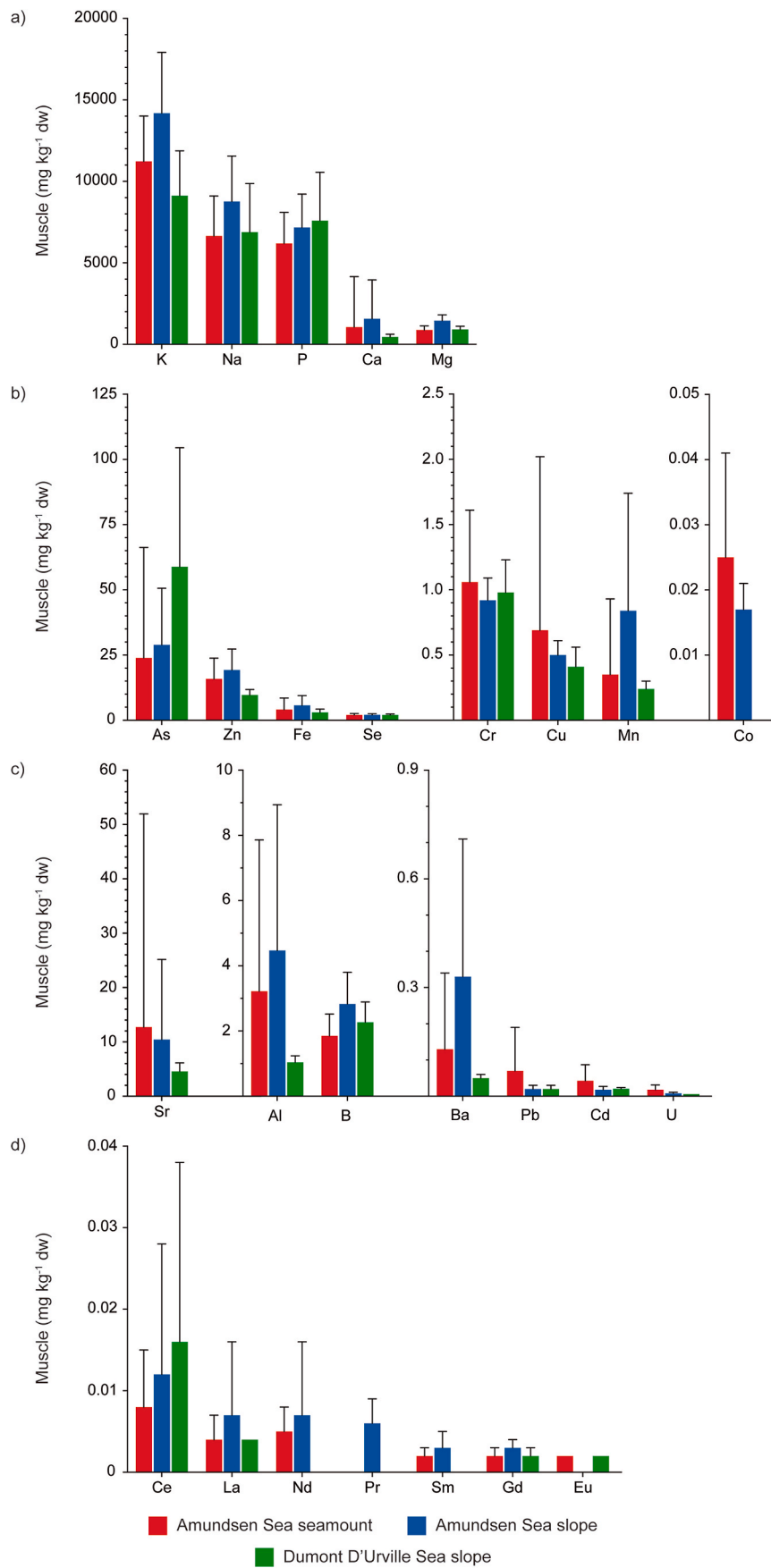


Fig. 2. Concentrations of trace and rare earth elements in the muscle of *Dissostichus mawsoni* from the Amundsen and Dumont D'Urville Seas. a) Major essential elements; b) Minor essential elements; c) Non-essential elements; d) Rare earth elements. Values are Mean +SD.

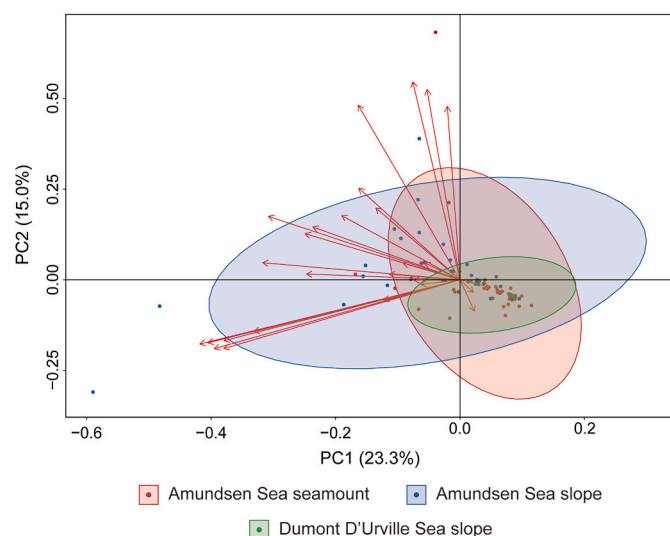


Fig. 3. Principal component analysis for the three studied areas.

Table 2

Spearman correlations between trace and rare earth elements concentrations and otolith length and  $\delta^{15}\text{N}$  values for all areas together. Bold indicate the significant correlations.

Element	n	Otolith length (mm)		$\delta^{15}\text{N}$ values (‰)		
		p-value	Spearman r	p-value	Spearman r	
Major Essential Elements	Ca	81	<0.001	-0.611	0.002	-0.339
	K	81	<0.001	-0.479*	0.013	-0.276*
	Mg	81	<0.001	-0.702	0.059	-0.211
	Na	81	<0.001	-0.465	<0.001	-0.389
	P	81	0.145	-0.163	0.007	-0.298
Minor Essential Elements	As	81	<0.001	-0.390	0.947	-0.008
	Co	13	0.778	0.088	0.554	-0.181
	Cr	81	0.200	0.144	0.512	0.074
	Cu	80	0.025	-0.251	0.313	-0.114
	Fe	72	0.004	-0.336	0.819	-0.028
	Mn	81	<0.001	-0.613	0.046	-0.223
	Se	81	0.003	-0.328	0.844	0.022
	Zn	81	0.003	-0.322	0.038	-0.231
Non-Essential Elements	Al	71	0.001	-0.371	0.794	0.032
	B	81	<0.001	-0.632	0.004	-0.316
	Ba	70	<0.001	-0.580	0.090	-0.204
	Cd	19	0.716	-0.089	0.519	-0.158
	Pb	25	0.419	0.169	0.528	-0.132
	Sr	81	0.005	-0.313	0.138	-0.166
	U	8	0.536	0.262	0.501	-0.286
	Rare Earth Elements	Ce	53	0.010	-0.351	0.208
	Eu	n.a.				
	Gd	18	0.363	-0.228	0.345	0.236
	La	26	0.232	-0.243	0.311	-0.207
	Nd	18	0.453	-0.189	0.510	-0.166
	Pr	n.a.				
	Sm	10	1.000	-0.006	1.000	0.006

\*Pearson r.

Sea (Simionov et al., 2019), Sunfish (*Mola*) and Atlantic Menhaden (*Brevoortia tyrannus*) in the Northwest Atlantic (reviewed in Perrault et al., 2014). Larger concentrations in several orders of magnitude of other major essential elements can also be found in the muscle of *D. mawsoni* in comparison to other marine fish worldwide, e.g. Ca, Mg and Na in Sea Cat fish (*Osteogeneiosus militaris*) and Sole (*Solea senegalensis*) (Galindo et al., 2012; Perrault et al., 2014; Sharif et al., 1993; Simionov et al., 2019). Similar to K, lower concentrations of Ca in fish from the Dumont D'Urville Sea slope when compared to Amundsen Sea

slope (3-fold difference) and seamount (2-fold difference) might indicate that these waters are impoverished in Ca in relation to those from the Amundsen Sea. Because of the differences in the diet of *D. mawsoni* between slope and seamount areas (Queirós et al., 2022; Stevens et al., 2014), the lower concentrations of Mg and Na in the Amundsen Sea seamount cannot be attributed directly to water concentrations and/or diet. High concentrations of major essential elements suggests that *D. mawsoni* can be a good source of these elements to their predators, including humans (FAO, 2016; Wang, 2002; Zoroddu et al., 2019). Indeed, considering the daily reference intake of an adult of ~70 kg, if 100% of the trace elements were absorbed from the food (Zoroddu et al., 2019), Mg would be the limiting trace element with a maximum ingestion of ~90 g of *D. mawsoni* per day, with all the other elements (including minor essential elements (discussed below)) allowing a consumption of more than 300 g. This shows that *D. mawsoni* do not pose any threat to human consumption in terms of toxication for excess of essential elements.

As expected, after major essential elements, the minor essential elements had the highest concentrations. Concentrations of minor essential varied in a wide range with As, the most concentrated, being more than 1000 times concentrated than Co, the less concentrated and lower than most non-essential elements. As was the minor essential element with the highest concentrations in the muscle of *D. mawsoni* in all studied areas, though with lower concentrations in fish from Amundsen Sea seamount. This difference is explained by the larger proportion of pelagic prey in the diet of fish from this area (Queirós et al., 2022), because As has a low biomagnification potential and its concentrations in organisms are dependent on the food web type, i.e. benthic dominated food webs have higher As concentrations (Du et al., 2021; Kato et al., 2020). As was traditionally considered a toxic element to animals (Braconi et al., 2011; Sevcikova et al., 2011). However, recent studies show that As is only toxic in its ionic forms (e.g.  $\text{As}^{3+}$ ) and not when in organic forms (e.g. Arsenobetaine), with the later comprising up to 90% of the total As found in seafood (Bodin et al., 2017; Kato et al., 2020; Metian et al., 2013). Indeed, studies found its presence in phosphatidylcholines which have a key role in the cell membranes of fish (Viczek et al., 2016). High concentrations found in the muscle of *D. mawsoni* confirm As importance also for this species. Nevertheless, values of As in *D. mawsoni* are smaller than other Antarctic fish species that usually feed in lower trophic levels and mostly in benthic prey, e.g. *Trematomus hansonii* from Pointe Géologie and *T. bernachii* from Cape Evans (though higher than *T. bernachii* from Terra Nova Bay) (Goutte et al., 2015; Grotti et al., 2008; Mesa et al., 1997). Regarding the low concentrations of Co, these are common in the muscle of other marine fish in the Southern Ocean and elsewhere (Grotti et al., 2008; Squadrone et al., 2020), supporting that this is not the main tissue that fish accumulate this element (Metian et al., 2013). This suggest that fish, and in particular *D. mawsoni*, is not a good bio-indicator for Co concentrations in the Southern Ocean. Concentrations of minor essential elements such as Cu, Mn and Zn in *D. mawsoni* are lower than other Antarctic fish (Beltcheva et al., 2011; Bustamante et al., 2003; Grotti et al., 2008). The only exception was Cr whose higher concentrations measured here are higher than those of *T. bernachii* from Terra Nova Bay and Cape Evans (Grotti et al., 2008). Notwithstanding, the high recovery efficiency suggesting an over-measuring of this element and some evidence of a small contamination during the methodological process (see materials and methods), requests that higher Cr concentrations in *D. mawsoni* in our study region are confirmed in future studies. Differences found in elements' concentrations to other Antarctic fish in other areas of the Southern Ocean, as well the differences found between fish from Amundsen Sea areas and the Dumont D'Urville Sea slope, can be related with the different diet between areas, though for some elements might be related with differences in the water concentrations. These can be related with oceanographic conditions, but also with climate change since it is known that climate change may influence the concentration of trace elements in biodiversity (Schartup et al., 2019). As example, higher concentrations



of Fe and Mn in fish from both Amundsen Sea areas compared to those from Dumont D'Urville Sea slope can be related with the contrasting trends in sea ice changes between both seas (Kim et al., 2015; Stammerjohn et al., 2012). A similar pattern was already found for Hg in *D. mawsoni* muscle (Queirós et al., 2020b).

Non-essential elements do not have a known biological function for organisms and some are usually toxic at low concentrations (Tchounwou et al., 2012). Thus, it is surprising that Al, B and Sr in fish from all the studied areas had higher concentrations than some minor essential elements. These high concentrations may indicate high bioavailability of these elements in the water and/or these elements may have some function in fish. Indeed, a previous work showed that B is important in the embryogenesis in Zebra fish *Danio rerio* (Rowe and Eckhart, 1999). Nevertheless, we must be careful as the absence of recovery efficiency for B precluded the confirmation of the measured values. Regarding Sr, despite higher concentrations when compared to minor essential elements, concentrations are within the range expected for the muscle of marine fish (Chowdhury and Blust, 2011). Cd is also a toxic trace element for animals, and especially important when analysing fishing products (Braconi et al., 2011; EC, 2006; Sevcikova et al., 2011). Our results show that values of Cd in the muscle of *D. mawsoni* are below the threshold of 0.05 mg kg<sup>-1</sup> (wet weight) for safety consumption, confirming that *D. mawsoni* does not present any threat to human health regarding Cd concentrations (EC, 2006).

To the best of our knowledge, this is the first study measuring the concentrations of rare earth elements in the Southern Ocean biota. Rare earth elements were, in general, the elements with the lowest concentrations. However, in both Amundsen and Dumont D'Urville Seas slopes, Ce had higher concentrations than U. This difference can be explained by the higher concentration of Ce in coastal waters of the Southern Ocean (Hathorne et al., 2015). Ce was also the rare earth element with the highest concentration in the muscle of *D. mawsoni* in all the study areas. Previous studies around the world, e.g. South China Sea and Canadian Arctic, also found Ce as the most concentrated rare earth element in muscle of marine fish (MacMillan et al., 2017; Wang et al., 2022). Nevertheless, values measured in *D. mawsoni*'s muscle are lower than those measured in several species from the South China Sea (Wang et al., 2022). High Ce concentrations in the muscle of several species might suggest that marine fish is capable of accumulate this element in the muscle.

Despite the differences found between study areas for most of the elements, the PCA could not separate the fish from the three areas into discrete groups. This suggests that, looking for these trace and rare earth elements together in the muscle, it is not possible to determine the origin of the fish between these two seas, as commonly used in marine fish worldwide, e.g. Atlantic cod *Gadus morhua* (Campana et al., 1994) and Patagonian toothfish *Dissostichus eleginoides* (Ashford et al., 2005). Nevertheless, these studies used the otolith and not the muscle.

#### 4.2. Bioaccumulation and biomagnification of trace and rare earth elements in the Amundsen and Dumont D'Urville Seas

Previous studies show that, in contrast to rare earth elements, some trace elements tend to bioaccumulate through the life of the organisms (Squadrone et al., 2020; Szykowska et al., 2018). Others, like Hg, are also able to biomagnify through the food web, though the number of elements with this capacity are scarce (Szykowska et al., 2018; Wang, 2002). The negative correlations between most of the studied elements and the otolith length, a proxy for fish length and, consequently, for the fish's age (Hanchet et al., 2015; Williams and McEldowney, 1990), suggests no bioaccumulation through the life of the *D. mawsoni*, but a decrease in the concentration as the fish grows. This was expected as the muscle is not the main tissues where fish bioaccumulate trace elements (Metian et al., 2013), and similar results were already found for Cd in two other Antarctic fish species, though using the entire organism (Cipro et al., 2018; McGeer et al., 2011). However, it contrasts with previous

studies that showed a bioaccumulation of Hg in both *D. mawsoni* and *D. eleginoides* (McArthur et al., 2003; Queirós et al., 2020b). Several explanations can be found for these results. Firstly, the bioaccumulation of elements depends on the organism capacity to incorporate and excrete such element (Coelho, 2021), thus this decreasing concentration with size may suggest that *D. mawsoni* either has lower capacity to accumulate these elements or it easily excretes them. Secondly, differences in the diet between larger individuals found in the Amundsen Sea seamount and smaller individuals found in both slope areas, including a change from benthic- to pelagic-dominated food-web, can also explain such pattern (Queirós et al., 2022). Thirdly, it can be related with a growth dilution effect (Cipro et al., 2018) or, fourthly, with different lipid contents between younger and adult *D. mawsoni* that can influence elements' concentration (Farkas et al., 2003; Merciai et al., 2014). Nevertheless, this fourth hypothesis depends on the lipid content which remains poorly studied in this species (Clarke et al., 1984). Lastly, higher metabolic rate of younger fish demands higher food intake and, consequently, higher accumulation (Newman and Doubet, 1989), or different energy allocation between younger and older fish, with the younger allocating more energy to growing processes rather than detoxification processes (Merciai et al., 2014). Besides all these hypothesis potentially explain differences between fish without considering environmental variability, as some differences between elements in fish from the different areas are better explained by environmental variability, these negative relations might also be related with the different areas rather than a lack of bioaccumulation effect in *D. mawsoni*. Similar, decreasing Ce concentrations with the otolith length may suggest that this rare earth element follows the same pattern of the other trace element.

Regarding biomagnification, only seven of the 27 studied elements showed a significant relation between concentration and  $\delta^{15}\text{N}$  values. Negative correlations suggest that fish feeding higher in the food web present lower concentrations of these elements. Similar to bioaccumulation, contrasting results were found for Hg (Queirós et al., 2020b; Seco et al., 2021). This is likely related with the higher presence of pelagic prey in the diet of *D. mawsoni* in the Amundsen Sea seamount that may decrease the intake of these elements, despite its higher  $\delta^{15}\text{N}$  values (Queirós et al., 2022), and the lower concentrations of several trace elements in offshore waters where larger individuals, in higher trophic position, are found (Castrillejo et al., 2013; CCAMLR, 2017a, 2017b; Hathorne et al., 2015; Queirós et al., 2022; Sañudo-Wilhelmy et al., 2002). Future analyses including fish of different sizes and prey species from the same area are needed to evaluate the bioaccumulation and biomagnification potential of these elements in this ecosystem.

#### 4.3. Detoxification potential of selenium for mercury

Se is known to detoxicate Hg from organisms (Jakimska et al., 2011; Yang et al., 2010). The negative correlation between Se and Hg may suggest that Se is not involved in the detoxification of Hg in the muscle of *D. mawsoni*. However, significant relationship in fish from the Amundsen Sea seamount, an area where high levels of Hg in the muscle of *D. mawsoni* were previously found (Queirós et al., 2020b), may suggest that Se play a detoxification role for Hg in the *D. mawsoni* but only above a concentration where it becomes toxic for the individual. The negative trend when analysing all studied areas together is related with the high Se concentrations found in the Amundsen Sea seamount, contrasting with the low Hg values found in the same study area (Queirós et al., 2020b). Nevertheless, we must be aware that these higher Se values in this study area can also be related with the overmeasuring of Se by the equipment (see material and methods). Similar results were found in other areas of the world for fish and other taxa, such as cephalopods and crustaceans (Annasawmy et al., 2022; Bodin et al., 2017).

## 5. Conclusions

This study measured the concentration of 27 trace and rare earth

elements in the muscle of Antarctic toothfish *D. mawsoni* from three areas in the Southern Ocean. Our results show that, as expected, major essential elements are the most concentrated, whilst rare earth elements present the lowest concentrations in the muscle of *D. mawsoni*. Differences between the concentration of these elements in *D. mawsoni*'s muscle and a similar pattern found by previous in the water, may suggest that this species as a good bioindicator for the concentrations for most of the trace and rare earth elements in the region. Nevertheless, because we did not analyse the concentrations of these elements in the water, future studies should confirm this relation. Our results also showed no bioaccumulation and no biomagnification potential for these elements, with concentrations of several elements decreasing towards larger individuals. The decreasing concentrations of several elements in the muscle of *D. mawsoni* show that, to use this species as bioindicator, we need to compare values of individuals with similar size/age. Otherwise, studies can also normalize trace elements concentrations by size allowing the use of *D. mawsoni* to evaluate differences in trace elements between Southern Ocean areas without restrictions. We also showed that Se might be involved in the detoxification of Hg in *D. mawsoni*, but only when concentrations of Hg are in levels potential harmful for the organism.

#### Credit author statement

**José P. Queirós:** Conceptualization; Methodology; Formal analysis; Writing - original draft; Writing - review and editing; Visualization; Funding acquisition. **João F. Machado:** Methodology; Formal analysis; Writing - original draft; Visualization. **Eduarda Pereira:** Resources; Writing - original draft; Funding acquisition. **Paco Bustamante:** Writing - original draft; Writing - review and editing. **Lina Carvalho:** Methodology; Writing - original draft. **Eugénio Soares:** Methodology; Writing - original draft. **Darren W. Stevens:** Writing - original draft. **José C. Xavier:** Conceptualization; Writing - original draft; Supervision; Funding acquisition.

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#### 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.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.chemosphere.2023.138134>.

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