Horizontal and vertical food web structure drives trace element trophic transfer in Terra Nova Bay, Antarctica

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Abstract

Despite in the last decades a vast amount of literature has focused on trace element (TE) contamination in Antarctica, the assessment of the main pathways driving TE transfer to the biota is still an overlooked issue. This limits the ability to predict how variations in sea-ice dynamics and productivity due to climate change will affect TE allocation in food webs. Here, food web structure of Tethys Bay (Terra Nova Bay, Ross Sea, Antarctica) was first characterised using carbon and nitrogen stable isotopes ($\delta^{13}C$, $\delta^{15}N$) of organic matter sources (sediment and planktonic, benthic and sympagic primary producers) and consumers (zooplankton, benthic invertebrates and vertebrates). Then, relationships between TEs (Cd, Cr, Co, Cu, Hg, Ni, Pb and V) and stable isotopes were assessed in order to evaluate if and how horizontal (organic matter pathways) and vertical (trophic position) food web features influence TE transfer to the biota. Regressions between log[TE] and $\delta^{13}C$ revealed that the sympagic pathway drives accumulation of V in primary

consumers and of Cd and Hg in secondary consumers, and that a coupled benthic/planktonic pathway drives Pb transfer to all consumers. Regressions between log[TE] and $\delta^{15}N$ showed that only Hg biomagnifies across trophic levels, while all the others TEs showed a biodilution pattern, consistent with patterns observed in temperate food webs. Although the Cd behavior needs further investigations, the present findings provide new insights about the role of basal sources in the transfer of TEs in polar systems, especially important nowadays in light of the forecasted trophic changes potentially resulting from future climate change-induced modification of sea-ice dynamics.

Capsule : Depiction of trace element transfer in the Antarctic food web highlighted an important role of both sympagic and phytoplanktonic pathways, suggesting that forecasted modification of sea-ice dynamics due to climate change may alter contaminant accumulation and biomagnification patterns.

Keywords

metal; stable isotopes; sympagic algae; biomagnification; polar

1. Introduction

Despite Antarctica is a remote area, commonly seen as an undisturbed and pristine environment, many scientific researches revealed that contamination is an important issue, due to a combination of natural and anthropogenic processes. In the last decades, much research on trace element (TE) contamination has been carried out in Antarctica. Atmospheric deposition of airborne particles from other areas in the Southern Hemisphere was identified as the main process that contributes to the high levels of many trace elements (i.e. Cd, Cr, Cu, Hg, Ni, Pb, V and Zn) found in the surface ocean, snow and pack-ice (Bargagli, 2008; Bargagli et al., 2005; Sañudo-Wilhelmy et al., 2002; Tuohy et al., 2015). Continental runoff, soil leaching and ice-melting further increase dissolved and

particulate contaminants into the coastal areas (Negri et al., 2006; Prendez and Carrasco, 2003). As sediment is a sink for contaminants, its resuspension together with upwelling of TE-enriched waters have been recognized as important processes responsible for TE recycling in Antarctic continental shelf (Corami et al., 2005). Seasonal dynamics of se-ice melting and phytoplankton production are the main factors controlling the element concentration in seawater: as the sea-ice formation sequesters dissolved and particulate nutrients and TEs in winter, the following sea-ice melting, during the austral summer, releases them again in the surface water, fuelling pelagic production (Frache et al., 2001; Grotti et al., 2005; Illuminati et al., 2017). At the same time, pack-ice is a seasonal habitat for many microscopic organisms (hereafter called “sympagic organisms”) which represent additional sources of organic matter and TEs for pelagic consumers as soon as ice melts, and also for benthic organisms once settled on the seafloor (Grotti et al., 2005; Morata et al., 2011). High TE levels have been reported also in other terrestrial and marine primary produces, like moss, lichens (Bargagli et al., 2005) and macroalgae (Runcie and Riddle, 2004), as well as in many pelagic and benthic consumers at different trophic levels, from zooplankton (Kahle and Zauke, 2003), to benthic ascidiaceans, anthozoans, sponges, echinoderms, polychaetes and crustaceans (e.g. Cipro et al., 2017; Grotti et al., 2008; Negri et al., 2006; Trevizani et al., 2016) up to pelagic and benthic fish (Bustamante et al., 2003). Moreover, most scientific literature regarding TE accumulation focused on penguins, which have been identified as suitable Hg bioindicators in the polar areas (Becker et al., 2016; Calle et al., 2015; Carravieri et al., 2013; Nygård et al., 2001).

Despite the great effort made so far, the knowledge of TE transfer processes is still scanty and represents a critical missing piece in the framework of TE contamination in the Antarctic area. In particular, despite the acknowledged key ecological and trophic role of phytoplankton and sea ice-algae in the Southern Ocean (Petrou et al., 2016), as well as their tendency to bioaccumulate a wide range of TEs, very little is still known about their role as potential element transfer to upper trophic levels. Moreover, the majority of research on contaminant biomagnification focused on single elements (e.g. Hg,Bargagli et al., 1998; Cipro et al., 2017) or only a few taxa (Carravieri et al., 2013; Majer et al., 2014). In turn, the importance of organic matter pathways in the relocation of TEs along food web is poorly investigated, limiting the ability to predict how variations in sea-ice

79 dynamics and primary production due to climate change will affect TE transfer across species and
80 trophic levels
81 Owing to the robustness of $\delta^{13}C$ in the identification of basal sources of dietary carbon, and the
82 good correlation between $\delta^{13}N$ and trophic level of the organisms in food webs (Post, 2002; Vizzini
83 et al., 2016), the use of stable isotopes is highly suitable to evaluate the role of horizontal (i.e.
84 reliance of consumers on different basal sources and trophic pathways) and vertical food web
85 structure (i.e. trophic positions) in the TE transfer. In particular, as organic matter sources are
86 typically well distinct based on $\delta^{13}C$ signatures (Fry and Sherr, 1989) especially in Antarctica
87 (Norkko et al., 2007, Calizza et al. 2018), and consumers reflect the $\delta^{13}C$ of prey plus only a low
88 fractionation value (0-1‰) (De Niro and Epstein, 1978), positive or negative relationship between
89 TEs and $\delta^{13}C$ of consumers will reveal the importance of the underlying ^{13}C-enriched vs. ^{13}C-
90 depleted organic matter pathways in TE transfer from basal sources to consumers. In contrast, the
91 positive or negative relationship between TEs and $\delta^{15}N$ of all the organisms belonging to the food
92 web indicates TE biomagnification or biodilution along food webs (e.g. Nfon et al., 2009; Signa et
93 al., 2013).
94 In recent years, significant changes in sea ice extent and seasonal dynamics have been observed
95 at the poles, leading to dramatic ecological consequences in productivity and trophic patterns (Post
96 et al., 2013; Constable et al. 2014). As the sea ice thickness and seasonal melting are critical to
97 ensure the subsequent timing of ice algal and phytoplankton blooms and their vertical distribution,
98 the predicted changes in sea ice cover and thickness and the consequent shift in frequency,
99 magnitude and availability of sympagic/planktonic production may certainly propagate across
100 trophic level through bottom-up processes (Constable et al., 2014). Moreover, benthic macroalgae
101 are known to benefit from higher temperature, light (due to ice thinning) and increased nutrient load
102 from meltwater, hence higher macroalgae abundance in the shallow area of the Antarctic coasts is
103 also expected from climate change predictions (Clark et al., 2013), with repercussions on trophic
104 web structure.
105 As timing and quantity of primary production in Antarctica are expected to change in the future,
106 with consequence on food web structure and functioning (Constable et al., 2014; Post et al., 2013),

understanding the extent of TE transfer is extremely important nowadays. In particular, it is extremely important to disentangle if and how diet and organic matter pathways (benthic, planktonic or sympagic) drive the transfer of trace elements to the biota. This will also help to clarify if species across trophic levels are able to buffer (i.e. biodilute) TE transfer to top predators, as observed in lower latitude food webs (Signa et al., 2017a). Therefore, the main aim of this paper was to assess and identify the pathways of trace elements (Cd, Cr, Co, Cu, Hg, Ni, Pb, V) in the food web of Tethys Bay (Terra Nova Bay, Ross Sea, Antarctica). To do this, the food web structure was first characterized through stable isotopes ($\delta^{13}C$ and $\delta^{15}N$). After that, the influence of the horizontal food web structure (i.e. reliance of consumers on different basal sources and trophic pathways) in TE transfer to consumers was assessed through linear regressions between TEs and $\delta^{13}C$, while the role of the vertical food web structure (i.e. trophic positions) and the occurrence of biomagnification was evaluated through linear regressions between TEs and $\delta^{15}N$ and computation of the trophic magnification factor (TMF). We hypothesised that i) pelagic primary producers, namely phytoplankton and sympagic algae, play an important role in the TE transfer to primary consumers, with potential repercussions in the contamination level of top predators ii) diet and trophic position are also important drivers for TE transfer to the upper trophic level of the food web of Tethys Bay, in the Ross Sea.

2. Materials and methods

2.1 Study area and sampling

Sampling was performed at Tethys Bay, (Terra Nova Bay, Ross Sea, Antarctica) (74°41’40”S 164°03’22”E), at the end of January 2013 (austral summer). The bay extends 3 km from the inner to the outer limit and is connected to the open waters of the polynya of Terra Nova Bay. The seafloor is characterized by rocky and muddy patches, and benthic vegetation coverage is generally scarce.

Evident sea-ice melting and cracking in the bay started ten days before sampling and sea-ice broke up three days before sampling. Sea-ice coverage and primary productivity in the bay are

characterised by marked seasonality, with periods of complete absence of ice coverage and phytoplankton blooms typically observed in January. Further information on the study area can be found in Faranda et al., (2000) and Norkko et al., (2007).

Methods for basal source and invertebrate collection are reported in Calizza et al. (2018). Briefly, benthic invertebrates were sampled along linear transects by dredging in medium-depth waters (40-200m) and by scuba diving in shallow waters (15-25m). Fish were collected through fish lines and creels which allowed to sample both the benthic habitat and the water column. Bird feathers of adult Adelie penguins and skua were collected by hand from recently died organisms. Resources potentially contributing to the diet of secondary consumers were also collected, including (i) benthic organic matter sources [sediment and coarse (> 2mm) organic detritus] and primary producers (the red macroalgae *Iridaea cordata* and *Phyllophora antarctica*) were also collected by dredging and scuba diving in shallow and deep waters; (ii) planktonic primary producers and consumers (respectively phytoplankton and zooplankton) were collected with a plankton net (20 µm mesh size) at a depth of 100 m. Zooplankton was carefully separated from the rest of the bulk sample by hand under a stereoscope. To obtain phytoplankton, the remaining sample was filtered at 100 µm and collected on pre-combusted Whatmann GF/F filters; (iii) sympagic primary producers (microscopic and filamentous algae growing both within the ice and at the interface between sea-ice and water) were collected in November 2012, before sea-ice broke up, by coring the pack-ice at two sites in the inner and outer part of the bay. Interface algae (i.e. the 2 cm bottom layer of the core) were considered separately from those growing within the core (i.e. between 2 cm and 1 m from the bottom, hereafter “core algae”).

2.2 Laboratory activities

For both isotopic and trace element (TE) analysis, soft tissues of all invertebrate and vertebrate species/taxa ranging across trophic levels and trophic guilds were considered (Table S1). All samples were stored at -80 °C at the “Mario Zucchelli” Italian Research Station, and at -20°C during transportation to Italy, where, after freeze-drying, they were ground using a ball mill (Mini-Mill Fritsch Pulverisette 23: Fritsch Instruments, Idar-Oberstein, Germany).

2.2.1 Isotopic analysis

When necessary, samples were pre-acidified (HCl 1 M) to eliminate inorganic carbon, which can interfere with the $\delta^{13}C$ signature (Carabel et al., 2006). Un-acidified powder from each sample was also analysed in order to obtain the $\delta^{15}N$ signature, which is known to be affected by HCl exposure (Carabel et al., 2006). Then, samples underwent stable isotope analysis (SIA) by means of a continuous flow mass spectrometer (IsoPrime100, Isoprime Ltd., Cheadle Hulme, UK) coupled with an elemental analyser (Elementar Vario Micro-Cube, Elementar Analysensysteme GmbH, Germany). Each sample was analysed in two replicates, and isotopic signatures were expressed in δ units ($\delta^{13}C$; $\delta^{15}N$) as the per mil (‰) difference with respect to standards: δX (‰) = $[(R_{sample} - R_{standard})/R_{standard}] \times 10^3$, where X is ^{13}C or ^{15}N and R is the corresponding ratio of heavy to light isotopes ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) (Post, 2002). The reference materials used were the international Vienna PeeDee Belemnite (PDB) as a standard for carbon, and atmospheric nitrogen (N_2) for nitrogen. Measurement errors were found to be typically smaller than 0.05‰. For $\delta^{13}C$, outputs were corrected for lipid content (Post et al., 2007) based on the C/N ratio (not reported) of each sample.

2.2.2 Trace element analysis

For quantification of trace elements (TEs: Cd, Co, Cr, Cu, Hg, Ni, Pb and V), ground samples were mineralised in an automatic microwave digestion system (MARS 5, CEM): sediment was analysed using a solution of 67–70% HNO_3, 30% HF, 30% H_2O_2 and Milli-Q water at a ratio of 6:2:0.4:1.6, while biological tissues using 67–70% HNO_3, 30% H_2O_2, and Milli-Q water at a ratio of 5:1:4. Then, mineralized samples were analysed by inductively coupled plasma optical emission spectrometry (ICP-OES, Optima 8000, PerkinElmer). Concentrations of Hg were determined using a hydride generation system linked to the ICP-OES with a reductant, consisting of 0.2% sodium (Na) borohydride and 0.05% Na hydroxide.

Analytical quality control was performed using Certified Reference Materials (CRMs): Marine sediment NIST 2702 (National Institute of Standards and Technology) for sediments, *Lagarosiphon major* BCR®–060 (Institute for Reference Materials and Measurements) for primary producers,

189 Fish protein DORM-4 (National Research Council of Canada) for vertebrates and Oyster tissue
190 1566b NIST® (National Institute of Standards and Technology) for invertebrates. The recovery was
191 84 to 101%. The detection limit was calculated as three times the standard deviation for digestion
192 blanks (n > 20) and was similar for all analysed TEs, corresponding to 0.003 mg kg^{-1} dw. All results
193 are given in mg kg^{-1} dw.
194

195 **2.3 Data elaboration and statistics**

196 The biotic samples analysed were grouped into eight categories (Table S1). In detail, organic
197 matter sources were grouped in: sediment (SED) including sedimentary organic matter and
198 detritus; sympagic algae (SYMP) including core and interface sea-ice algae; phytoplankton
199 (PHYTO-P); phytobenthos (PHYTO-B) including the two rodophyta *I. cordata* and *P. antarctica*.
200 Consumers were grouped in: zooplankton (ZOO-P) including the *Clione limacina* (Gastropoda),
201 copepods and euphasiids; zoobenthos (ZOO-B) including the invertebrate benthic species
202 *Adamussium colbecki* (Bivalvia), *Odontaster validus* (Asteroidea), *Sterechinus neumayeri*
203 (Echinoidea), *Ophiontus victoriae* (Ophiuroidea) plus unidentified specimens of the genus *Halicona*
204 (Demospongiae) and others belonging to Cucumaridae (Holoturoidea), Alcyonacea (Anthozoa),
205 and Polychaeta; fish species (FISH) including *Artedidraco orianae*, *A. skottsbergi*, *Chionodraco*
206 *hamatus, Lepidonotothen nudifrons, Trematomus bernacchii, T. hansoni*, *T. newnessi* and *T.*
207 *pennellii*; and birds (BIRDS) including the penguin *Pygoscelis adeliae* and the Antarctic Skua
208 *Stercorarius antarcticus*. Analyses focused on these targets because they include the most
209 common species within their respective trophic guilds in the study area, as well as in Antarctic
210 coastal communities.
211 Differences in the isotopic values among categories were tested by means of a non parametric
212 MANOVA (NPMANOVA) based on Euclidean distances (Calizza et al., 2017, 2013) followed by
213 post-hoc tests. The significance is computed by permutation of group membership, with 10000
214 replicates.

215 Differences in trace elements (TE) concentration between the eight categories were tested through
216 permutational analysis of variance (PERMANOVA) carried out for each TE based on the Euclidean
217 distance matrix obtained by normalised TE data, followed by pairwise tests.
218 Identification of principal OM pathways of the food web was carried out using a qualitative
219 approach. Differences in $\delta^{13}C$ values have been shown to provide information on the contribution
220 of sympagic vs. benthic and pelagic sources to the diet of Antarctic consumers (Calizza et al.,
221 2018; Norkko et al., 2007), reliably reflecting differences in OM pathways between organisms
222 (Careddu et al., 2015; Rossi et al., 2015; Signa et al., 2017b). Similarly, $\delta^{15}N$ values are known to
223 increase unequivocally across trophic levels, and therefore, provide a robust base to identify
224 trophic position of consumers within food webs (Mancinelli et al., 2013; Post, 2002)
225 The effect of the horizontal and vertical food web structure (i.e. organic matter pathways and
226 trophic position) in shaping the TE pathways within the Antarctic trophic web was assessed
227 through linear regressions between the logarithm of TEs as the dependent variable and $\delta^{13}C$ and
228 $\delta^{15}N$ respectively as independent variables. In the first regressions (log[TE] vs. $\delta^{13}C$), the role of
229 primary producers and sediment as TE sources was tested separately for primary and secondary
230 consumers to distinguish potential different patterns. Classification of consumers in primary and
231 secondary consumers was carried out in advance through identification of suitable $\delta^{15}N$ thresholds
232 coupled with literature comparison of feeding habits: mean $\delta^{15}N$ of primary consumers ranged
233 between 4.8 ± 0.8 and 6.2 ± 1.5 ‰ and $\delta^{15}N$ of secondary consumers ranged between 6.0 ± 0.5
234 and 9.6 ± 1.2 ‰ (Table S1). In contrast, in the second regressions (log[TE] vs. $\delta^{15}N$), all the biotic
235 samples were included to assess the biomagnification/biodilution patterns along the whole food
236 web. Residual analysis was performed in both cases to ensure that assumptions were not violated.
237 If outliers were identified, they were removed and the regression analysis was performed again.
238 Trophic magnification factors (TMF) was also calculated based on log[TE] vs. $\delta^{15}N$ linear
239 regressions to quantify the biomagnification (TMF>1) or biodilution (TMF <1) power of the trace
240 elements studied along the food web. TMF was computed as follows, according to Borgå et al.
241 (2011): $Log[TE] = a + b(\delta^{15}N)$; $TMF = 10^{b}$.
242

3. Results

3.1 Isotopic signatures

Isotopic distribution differed between all groups analysed (NPMANOVA F= 30.2, p< 0.0001, and associated post-hoc tests, p always < 0.05) (Table S2), with the exception of fish and birds, which do not differed significantly (p> 0.05). Specifically, isotopic distributions of the four main basal resource guilds (i.e. phytoplankton, phytobenthos, sedimentary organic matter and sympagic algae) differed either for their $\delta^{13}C$ and/or the $\delta^{15}N$ values (Fig. 1). Basal resources occupied a $\delta^{13}C$ range much wider than that occupied by invertebrates and vertebrate consumers which, in turn, mainly differed for their $\delta^{15}N$ values.

3.2 Trace elements

Trace element (TE) analysis revealed different patterns both among sample categories and TEs (Fig. 2). Cr, Pb, Ni and V showed similar trends, with overall significantly higher concentrations in organic matter sources than in consumers. In particular, Cr peaked in phytoplankton, followed by sediment and sympagic algae, while benthic macroalgae (PHYTO-B) reported similar concentrations to zooplankton and birds. In contrast, Ni, Pb and V showed the highest concentration in both phytoplankton and sediment, followed by sympagic and benthic macroalgae, which showed values overall comparable to birds and zooplankton (Ni, Pb) and zoobenthos (V). Cu concentration in phytoplankton was also high, but the highest values were recorded in sympagic algae. The other source and consumer categories highlighted similar lower values with the lowest ones recorded in fish. Co concentration peaked in sediment, followed by all the other categories with evenly lower values. In contrast, Hg levels were significantly higher in birds than in all the other categories, among which fish showed the highest concentration, followed by benthic invertebrates, sympagic algae and phytoplankton as an homogeneous group, and the others with the lowest values. Finally, Cd did not show a clear trend and the highest values were observed in benthic invertebrates, phytoplankton and phytobenthos, followed by zooplankton and sympagic algae.

In primary consumers, only Hg, Pb and V were significantly correlated with $\delta^{13}C$, with the first two elements negatively, and only the latter positively correlated, while in secondary consumers, the correlation was significant and positive for Cd and Hg, and negative for Ni and Pb (Fig. 3 and Tab.1). Pb decreased along $\delta^{13}C$ in both primary and secondary consumers, while Cd and Hg had an opposite trend in the two groups, decreasing in primary consumers and increasing in secondary consumers (Fig. 3).

Linear regressions between log[TE] and $\delta^{15}N$ of the whole food web components, organic matter sources and consumers, highlighted a similar behaviour of all TEs, except for log[Hg] (Fig. 4 and Tab.1). Indeed, the log values of the concentration of TEs decreased as the $\delta^{15}N$ increased, indicating TE biodilution along the food web, while only log[Hg] increased along $\delta^{15}N$ values, suggesting Hg biomagnification across trophic levels (Fig. 4). These patterns were confirmed by the trophic magnification factor (TMF), which was lower than 1 for all TEs except for Hg whose TMF was 1.23 (Fig. 4). Both negative and positive correlations were all highly significant (Table 1).

4. Discussion

4.1 Food web structure

Isotopic values of the organic matter sources from Tethys Bay, Ross Sea (Antarctica) covered a wide $\delta^{13}C$ range ($\Delta^{13}C$= 31.2‰) varying from the highly depleted values of the benthic red macroalga *Phyllophora antarctica*, to the highly enriched ones of sympagic algae. The other red macroalga *Iridaea cordata*, phytoplankton and sediment organic matter showed intermediate and overlapped values. The large differences in $\delta^{13}C$ signatures among primary producers is not new in the Antarctic region and current results are consistent with previous studies (Gillies et al., 2012; Norkko et al., 2007, Calizza et al. 2018). In particular, the high variability found within the sympagic algae group is due to the different $\delta^{13}C$ signature of interface and core ice-algae, probably associated to diverse taxonomic composition (mainly diatoms vs. filamentous algae respectively). In addition, high seasonality in the isotopic values of the particulate organic matter (POM) is typical of the Antarctic region because of the different environmental conditions, and especially the strong

297 seasonality of ice coverage and primary production (Cozzi and Cantoni, 2011). Accordingly, the
298 more depleted values of the core algae may be linked to the highly negative $\delta^{13}C$ signature of POM
299 in the winter period (-30 to -21%, Kennedy et al., 2002) during sea-ice formation. In contrast, the
300 more enriched values of the interface algae may also reflect the isotopic enrichments occurring
301 during the spring ice-algae bloom and then with the presence of freshly produced algal biomass
302 (Cozzi and Cantoni, 2011; Norkko et al., 2007). The horizontal food web structure of Tethys Bay
303 was characterised by two main trophic pathways, the first based on phytoplankton, sediment and
304 the benthic macroalga *I. cordata,* and the second one based on sympagic algae. The highly ^{13}C-
305 depleted values of the other macroalga, *P. antarctica,* was out from the isotopic space
306 encompassing the other primary producers and the consumers. Consequently, we may infer that
307 *P. antarctica* does not enter in the Tethys Bay food web, unless in a detrital form, because of the
308 abundance of polyphenols that protect it from grazers (Norkko et al., 2007; Calizza et al. 2018).
309 Unlike organic matter sources, consumers grouped mostly based on their $\delta^{15}N$ signatures and
310 showed a rather narrow $\delta^{13}C$ range, except for zoobenthos, which spanned a wide range as effect
311 of a variable contribution of benthic/planktonic sources and sympagic algae in their diet (Calizza et
312 al., 2018). Despite narrower, the $\delta^{15}N$ range of the zoobenthos suggests that benthic invertebrates
313 belong to two trophic levels, including both primary and secondary consumers. Indeed, Antarctic
314 benthic invertebrates vary greatly in the feeding habits, from grazers, filter feeders and
315 depositivores to predators and scavengers (Corbisier et al., 2004), and exhibit also a high degree
316 of trophic plasticity and omnivory (Norkko et al., 2007). In contrast, the narrow $\delta^{13}C$ range coupled
317 with the wide $\delta^{15}N$ range reveal that zooplankton relies mostly on other planktivorous species
318 although a certain influence of phytoplankton and core sympagic algae in the diet cannot be ruled
319 out, therefore showing an omnivorous feeding strategy (Tamelander et al., 2008). Lastly, the
320 highest $\delta^{15}N$ signatures of fish and birds and their intermediate position within the $\delta^{13}C$ range,
321 highlight the role as top predators that couple the underlying resource pathways, as previously
322 documented in Arctic (McMeans et al., 2013) and temperate marine systems (Vizzini et al., 2016).
323

324 **4.2 Trace element levels in food web components**

Sediment trace element (TE) concentration measured in this study was overall comparable to previous studies carried out in the Ross Sea (Grotti et al., 2008; Ianni et al., 2010) with the exception of Pb and Hg, which showed higher values compared with existing literature (Pb: Grotti et al., 2008; Ianni et al., 2010; Hg: Bargagli et al., 1998; Negri et al., 2006). The elevated pelagic primary productivity of the Antarctic system (Petrou et al., 2016), together with the high TE levels in the dissolved compartment (Corami et al., 2005; Sañudo-Wilhelmy et al., 2002), highlight the important role of this component within the food web and also its enrichment in TEs, thus representing a potential source of TEs for both pelagic and benthic consumers (once settled on the seafloor) (Cabrita et al., 2017; Deheyn et al., 2005). Similarly, sympagic algae are known to be a good bioaccumulator of nutrients and TEs, which are entrapped within the pack-ice during winter, becoming then bioavailable in summer, as soon as the pack-ice starts to melt (Grotti et al., 2005, Pusceddu et al. 2009, Calizza et al. 2018). Present results confirm previously recorded high TE levels, as phytoplankton and core sympagic algae (sampled in spring before the beginning of the ice melting) showed overall the highest TE concentration among primary producers, giving an indication of their potential in the TE transfer pathway to both pelagic and benthic consumers. In more detail, phytoplankton reached TEs levels from 2-fold (Hg) to 4- (Cd) and even 10-fold (Pb) higher than those previously recorded in the same area (Bargagli et al., 1998, 1996; Cabrita et al., 2017; Dalla Riva et al., 2003).

As regards consumers, only Cd, Cu and Hg revealed remarkable TE concentration. High Cd levels in both primary producers and consumers (except for vertebrates) were already observed in Terra Nova Bay by Bargagli et al. (1996) who defined it as “the Cd anomaly”. The authors explained this phenomenon as the effect of the upwelling of Cd-enriched deep waters, which makes this element available to primary producers and then to primary consumers through trophic transfer. Copper showed accumulation patterns similar to Cd, consistently with the findings by Grotti et al. (2008), while Hg concentration and pattern suggest biomagnification, as previously documented in the same area (Bargagli et al., 1998) and other polar and temperate areas (e.g. Nfon et al., 2009; Signa et al., 2017), with the highest concentration in top predators and the lowest ones at the base of the food web. The highest Hg levels were found in bird feathers, especially in those of the brown

353 skua (*Stercorarius antarcticus*) consistent with the values observed in various skua populations
354 from many Antarctic areas (Becker et al., 2016).
355
356 **4.3 Horizontal and vertical food web structure drives trace element transfer**

357 Isotopic results from this study indicated that two pathways, sympagic and benthic/planktonic
358 (indistinguishable based on the samples analysed here) flow through the food web of Tethys Bay
359 and are coupled by top predators, similar to the food web studied by McMeans et al. (2013) in the
360 Arctic system. The clear $\delta^{13}C$ trend of the organic matter sources, varying from the highly depleted
361 red macroalga *P. antarctica* to a homogenous more ^{13}C-enriched group made of the red macroalga
362 *I. cordata*, sediment and phytoplankton, up to the most ^{13}C-enriched sympagic algae, allowed the
363 identification of relevant patterns in TE transfer. Indeed, linear regressions between consumer
364 log[TE] and $\delta^{13}C$ highlighted that the trophic pathway based on sympagic algae was not relevant in
365 driving TE transfer to primary consumers, unless for V. In contrast, the significant and positive
366 correlation between both log[Cd] and log[Hg] vs. $\delta^{13}C$ revealed the importance of the sympagic
367 pathway in the transfer of these two elements up to secondary consumers. This not consistent Cd
368 and Hg pattern between primary and secondary consumers may depend on the low number of
369 primary consumers relying on sympagic algae sampled in this study. Indeed, among all, only some
370 specimens of Alcyonacea, filter feeder polychaetes and the sea urchin *Sterechinus neumayeri*
371 showed the reliance on sympagic algae as a food source, while also other benthic species, such
372 as the detritivores and suspension feeders *Flabelligera mundata, Laternula elliptica, Paramoera*
373 *walkeri* rely on this resource in the Antarctic region (Wing et al., 2012). This is due to vertical pulse
374 of ice algae-derived organic matter to the deep layers of polar areas that is an important process
375 which contribute to a tight pelagic-benthic coupling (Morata et al., 2011). Looking at secondary
376 consumers, only the sea star *Odontaster validus* showed high Cd and Hg levels derived from the
377 sympagic trophic pathway. Being a benthic active predator, feeding mainly on invertebrates (Dalla
378 Riva et al., 2004) but including also other items in the diet, due to a marked omnivorous feeding
379 behaviour (Norkko et al., 2007), the direct reliance on sympagic algae, when available, cannot be
380 ruled out.

Primary consumers, for which incorporation of sediment and phytoplankton advected from the upper water column layers was more likely, showed generally higher but also more variable Cd and Hg accumulation. In particular, as mainly filter feeders (filter feeder holothuroidea, polychaeta and sponges) grouped in the upper left side of the graphs due to the high Cd and Hg concentration, we may infer that phytoplankton is the main driver of Cd and Hg in the benthic habitat of Tethys Bay. Moreover, phytoplankton in the study are has been shown to present lower $\delta^{13}C$ (Calizza et al., 2018) than those found here, further strengthening this hypothesis.

Lead was the only element with an opposite trend, significantly decreasing as the $\delta^{13}C$ increased in both primary and secondary consumers, suggesting that settled phytoplankton, macroalgae and sediment represent also important Pb sources for invertebrates in Tethys Bay. Lead is defined as a "scavenging type" element that typically shows increasing concentration at higher depths (Frache et al., 2001; Illuminati et al., 2017), hence this behaviour may have contributed to the observed patterns. Indeed, Illuminati et al., (2017) found that the effect of the sea ice melting on Pb seawater content is not relevant in summer, being the surface water layer (~15 m depth) mostly interested by atmospheric deposition and wet deposition from continental land (Dalla Riva et al., 2003), while the sinking of biogenic particles following the phytoplankton bloom affects the Pb level in the deeper layers (~100m - same maximum depth where the phytoplankton was sampled in this study). Our isotopic results, in agreement with literature findings (Elias-Piera et al., 2013; Norkko et al., 2007), support the hypothesis that benthic macroalgae, sediment and phytodetritus are important organic matter sources in the trophic web of Tethys Bay, hence representing relevant sources of Pb for many organisms with potential detrimental effects.

As regards the other TEs for which any significant pattern was identified, especially Co, Cr and Cu, it could be argued that a conjoint contribution of all sources in the TE transfer to consumers takes place, rather than having no role in TE transfer. Indeed, as previously mentioned, omnivory and trophic plasticity are widely diffused in the polar food webs, mainly due to the high influence of seasonality in primary production that requires continuous adaptation in the selection of food by the fauna (Norkko et al., 2007; Tamelander et al., 2008) and may have contributed to the observed patterns. Moreover, it should be noticed that TE bioavailability and transfer to the biota are highly

element- and species-specific resulting from many factors, from sediment physicochemical properties, to the specie-specific routes of exposure and detoxification processes (Signa et al., 2017a). Although the study of such processes was not in the scope of this research, their occurrence cannot be ruled out when the patterns between TEs and carbon isotopic signature were not clear.

Linear regression between trace elements and $\delta^{15}N$, used as a proxy for trophic position (Post, 2002), were highly significant for all the elements analysed. However, only total mercury (Hg) biomagnified along the food web of Tethys Bay, as indicated by the positive slope together with the trophic magnification factor (TMF) > 1, which is a proxy for the biomagnification power. In particular, the values of the slope (0.09) and TMF (1.23) were consistent with the lower values of the range published, due to the use of total Hg, instead of Me-Hg, together with the large variety of trophic levels within the food web studied (Signa et al., 2017c), while most biomagnification studies often focuses on only partial food webs and trophic levels. Indeed, the pivotal study of Bargagli et al. (1998) was the first and the only one describing such Hg behaviour in Terra Nova Bay, by comparing Hg concentration in many organisms belonging to different trophic levels, from phytoplankton to penguins. After that, subsequent studies confirmed this trend (Cipro et al., 2017), although some of them focused on only a part of the food web, namely benthic organisms (Majer et al., 2014) and penguins (Calle et al., 2015; Carravieri et al., 2013; Nygård et al., 2001). Present results confirm that trophic position plays the key role in the Hg levels and relocation in the food web of Tethys Bay, although other factors also contribute in determining the differing Hg concentration observed in organisms with similar trophic position. Indeed, benthic fauna showed higher Hg concentrations than zooplankton, although their overlapped isotopic signatures, and it may be explained by the higher Hg exposure in the benthic vs. pelagic compartment, as Hg methylation and photodemethylation occur respectively in sediments and seawater resulting respectively in an increase and decrease of Hg bioavailability (Goutte et al., 2015). Similarly, seabirds and fish showed an overlapped isotopic niche but different Hg concentration, probably mirroring different diet or foraging habitat. In particular, Adélie penguins, which feed preferentially on ice-krill and pelagic fish (Ainley, 2002), share $\delta^{15}N$ and Hg ranges with benthic predatory fish

(i.e. *Artedidraco orianae*, *A. skottsbergi* and *Trematomus hansoni),* whose main prey are benthic invertebrates belonging to various trophic levels (La Mesa et al., 2004). Hence, the effect of a different diet seems to counterbalance the effect of the different feeding habitat resulting in an overlapped $\delta^{15}N$ and Hg signature. In contrast, the brown skua *S. antarcticus* is known as predator and scavenger feeding on bird carrions (Becker et al., 2016). This diet is consistent with the role of top predator, justifying the high Hg level found, but is not consistent with the concurrent low $\delta^{15}N$ values in feathers, which are probably the result of the reliance of a wide range of prey in the previous inter-breeding season spent in the Sub-Antarctic and Subtropical zones (Cherel et al., 2017).

Unlike Hg, all the other TEs (i.e. Cd, Cr, Co, Cu, Ni, Pb, V) showed significant opposite patterns that, concurrent with TMF < 1, indicated a biodiluting behaviour along the food web. This is consistent with previous studies carried out both in polar (Nfon et al., 2009) and temperate areas (Signa et al., 2017c), which showed also similar TMF values, suggesting a univocal pattern, regardless of geographical area or seasonality. Indeed, biomagnification of other trace elements besides Hg has been reported infrequently and this common biodilution trend has been attributed to the efficient TE sequestration and/or excretion abilities of the organisms during the trophic transfer, concurrent with the growth dilution in the large-bodied species at high trophic levels.

Cd deserves a mention apart because of the well-known “Cd anomaly” in the Antarctic area (Bargagli et al., 1996), which leads to very high Cd concentrations, compared to other marine systems (Bargagli et al., 1996; Sañudo-Wilhelmy et al., 2002). Moreover, Cd is prone to bioaccumulate in benthic invertebrates and may biomagnify depending on the organisms involved (Majer et al., 2014; Signa et al., 2017c). Accordingly, Cd concentration in benthic invertebrates from Tethys Bay resulted markedly high, contrasting with the very low Cd concentration found in fish muscle and bird feathers. At a first glance, this result may be interpreted as a biodilution along trophic levels, but the occurrence of Cd sequestration processes in other tissues may have led to this result. Indeed, although the muscle tissue represents the stable pool of trace elements for fish (Barwick and Maher, 2003), Cd is more efficiently sequestered by fish in liver and kidney, than in muscle (Bargagli et al., 1996; Bustamante et al., 2003) . Therefore, while muscle is considered the

most appropriate tissue for isotopic description of feeding habit and trophic position in fish (Costantini et al. 2018), the low Cd concentration found here does not mean necessarily biodilution or lack of Cd exposure at the whole organism level, and multi-tissue analysis is needed to clarify this point.

5. Conclusions and future perspectives

Trace element (TE) contamination and transfer is a topic of paramount concern worldwide including the remote Antarctic region, because of the occurrence of natural and anthropogenic transport processes on local-to-global scales. Despite levels of many trace elements (TE) have been widely described in single components of the Antarctic food web, the pathways fueling TE transfer to the biota, taking into account all the food web components, are poorly characterized. Here, in this first effort to describe the Cd, Cr, Co, Cu, Hg, Ni, Pb and V transfer pathways in Terra Nova Bay, we provide evidence of the importance of both sympagic and phytoplanktonic pathways, and trophic position, in driving the TE transfer to the biota. In particular, linear regression analysis between the log-transformed concentration of TEs and $\delta^{13}C$ revealed a significant influence of the sympagic algae pathway on the Cd and Hg levels of secondary consumers. Phytoplanktonic pathway was more relevant in the Cd and Hg transfer to primary consumers and in the Pb transfer to both trophic levels. This is probably because of its higher concentration in the deeper layer due to the typical Pb “scavenging” behavior. A concurrent role of both patterns was highlighted, instead, for the other TEs.

Despite it turned out that both trophic pathways are coupled by top predators (fish and birds), Hg was the only chemical whose concentration increased from basal sources to top predators and that also biomagnified along the food web, as revealed by the significant positive correlation between log[Hg] and $\delta^{15}N$ and the trophic magnification factor TMF >1. In agreement with most literature, the behavior of all the other TEs was opposite to that of Hg, giving evidence of a biodilution along trophic levels. Nevertheless, the ability of top predators in sequester dietary elements in other tissues than muscle, leaves an open window to further investigations in the area.

Concluding, climate change scenarios forecast a dramatic change in sea-ice extent and dynamics in polar areas (Constable et al., 2014; Post et al., 2013). In turn, this will produce important changes in frequency and magnitude of sympagic/planktonic production, with potential shifts in their relative contribution to the benthic communities (Clark et al., 2013; Constable et al., 2014). In this context, these data represent a useful reference of present baseline conditions in Terra Nova Bay (Ross Sea), and revealed that change of trophic pathways could also lead to change of TE transfer through the Antarctic food web. Indeed, in light of the marked efficiency of benthic macrofauna in the exploitation of pulsed food sources according to their availability (Calizza et al., 2018; Mäkelä et al., 2017; Norkko et al., 2007), our findings suggest that changes in food inputs fuelling the food web may rebound in unprecedented exposure of Antarctic species to TEs, in terms of a different TE species and concentration, than what experienced in the past.

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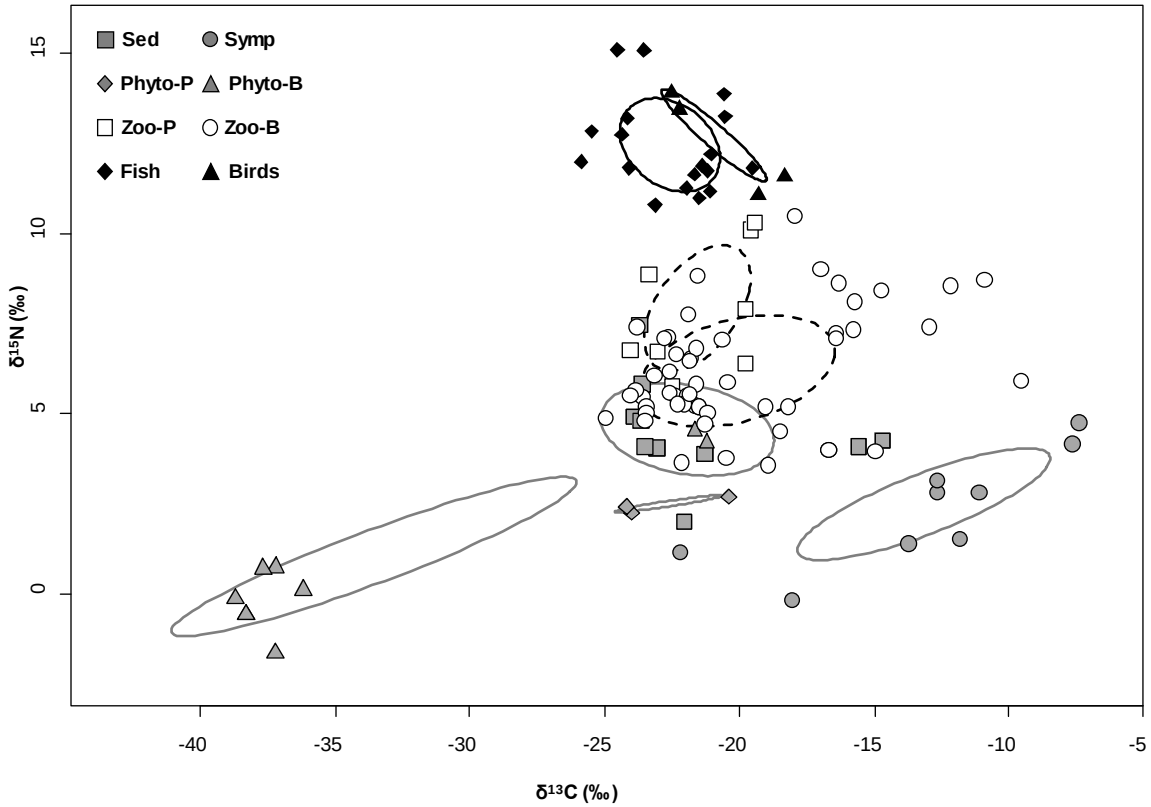
718 **Artworks**

719



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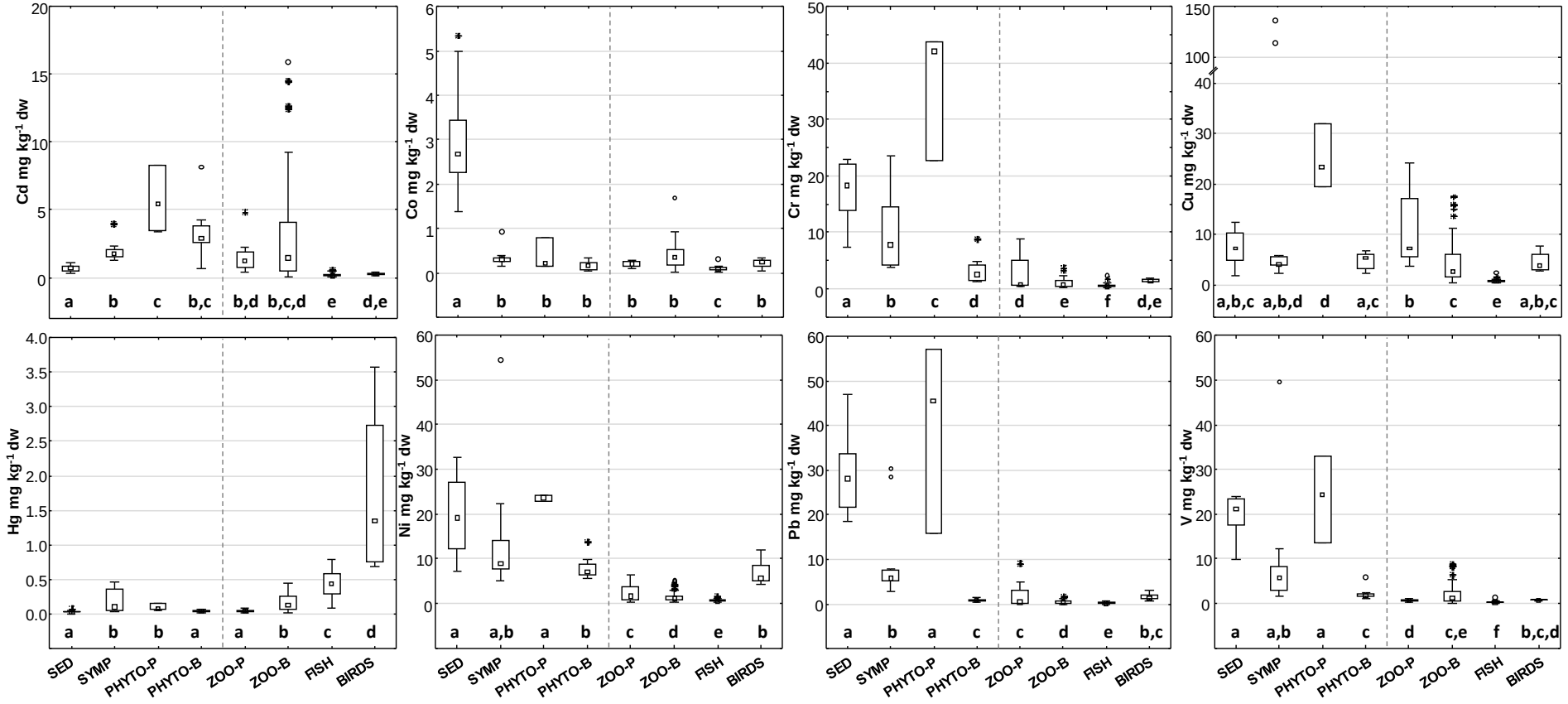
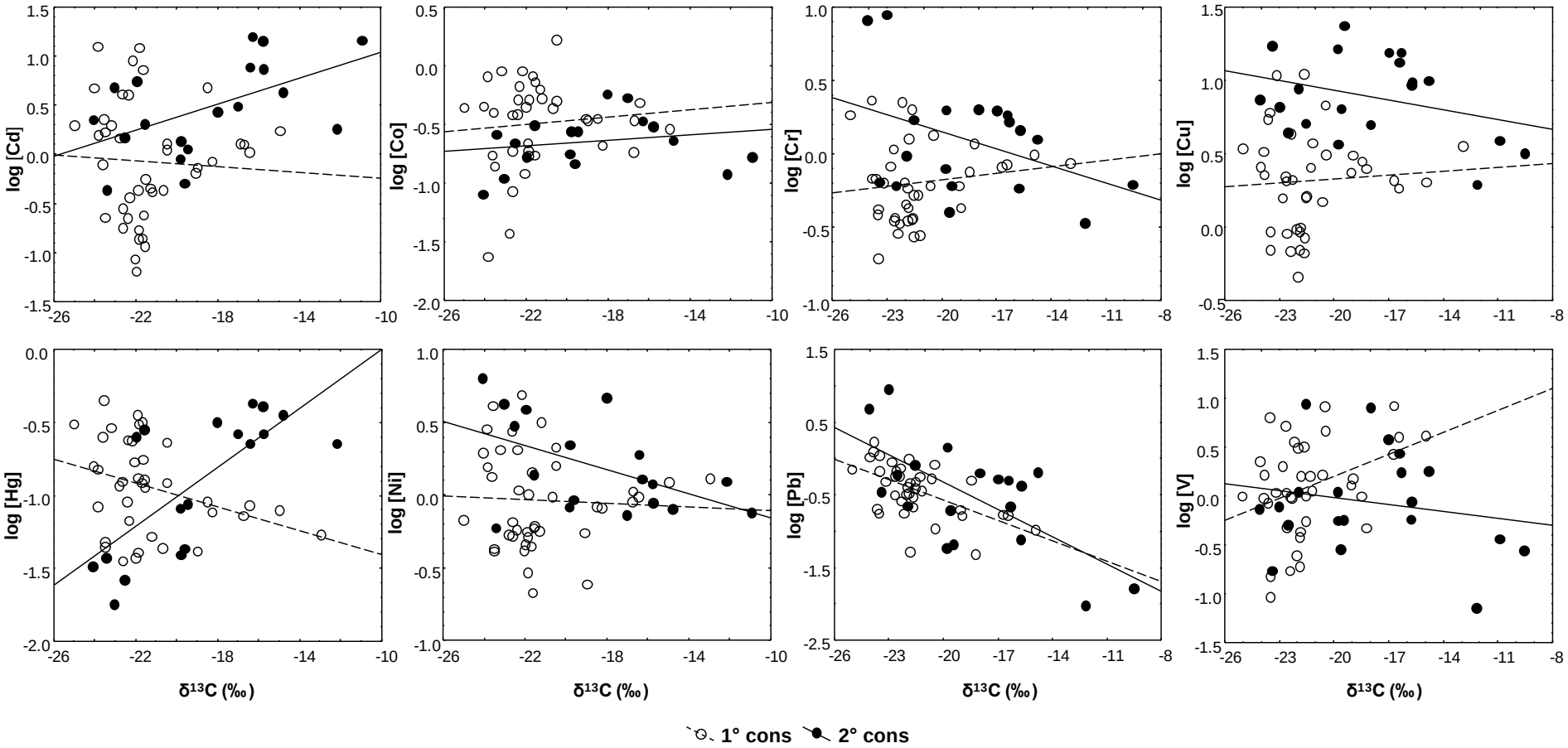
721 Fig. 1. Isotopic distributions of different basal sources (grey symbols), invertebrate (white symbols) and vertebrate (black symbols) taxa at Tethys
722 Bay, Ross Sea (Antarctica). Different symbols represent different categories and each symbol represents a specimen. Ellipses encompass the
723 core (i.e. 46%) of each categories.



724

725 Fig. 2. Trace element concentrations (mg kg^{-1} dw) in organic matter sources: sediment organic matter (SED); sympagic algae (SYMP);
726 phytoplankton (PHYTO-P) and phytobenthos (PHYTO-B), and in consumers: zooplankton (ZOO-P); zoobenthos (ZOO-B); fish (FISH) and birds
727 (BIRDS) from Tethys Bay, Ross Sea (Antarctica). Whiskers indicate the non-outlier range of variation; boxes: 25th to 75th percentiles. Significant
728 differences among sample categories are indicated with different letters.

729



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731 Fig. 3. Linear regressions for log-transformed trace element (TE) concentrations (mg kg^{-1} dw) vs. $\delta^{13}C$ (‰) signatures of primary consumers in
732 white, and secondary consumers in black from Tethys Bay, Ross Sea (Antarctica). Regression equations and parameters (r and p-values) are
733 reported in Table 1.

734

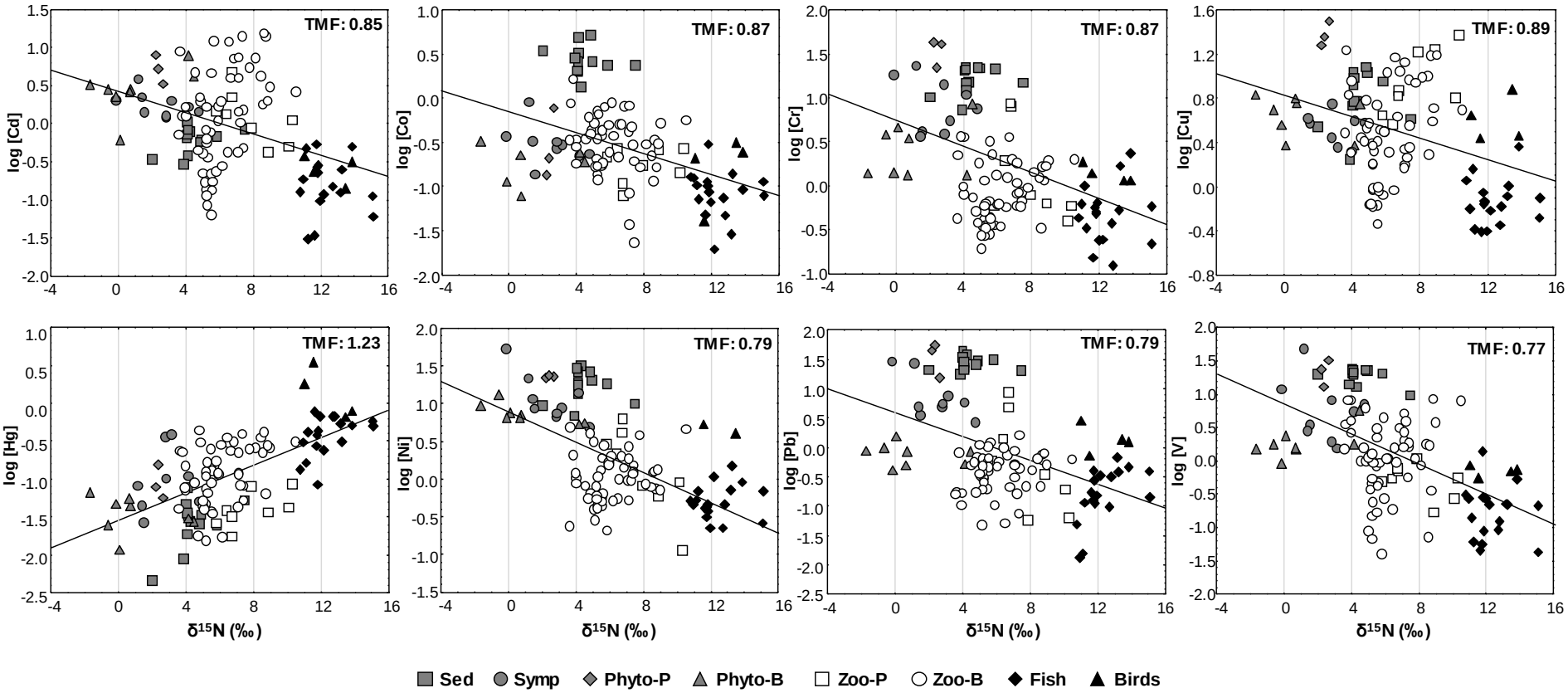

Fig. 4. Linear regressions for log-transformed trace element (TE) concentrations (mg kg^{-1} dw) vs. $\delta^{15}N$ (‰) signatures of the whole food web of Tethys Bay, Ross Sea (Antarctica). Trophic magnification factors (TMFs) are reported on each panel. Regression equations and parameters (r and p-values) are reported in Table 1.

Tables

Table 1. Results of linear regressions (equation, coefficient of regression r and p value) between log-transformed concentration of trace elements and stable isotopes: $\delta^{13}C$ (primary and secondary consumers) and $\delta^{15}N$ (whole food web).

a) $\delta^{13}C$ vs. [log TE]			
Primary consumers	**equation**	**r**	**p**
Cd	y = -0.3849 - 0.0145*x	-0.05	0.76
Co	y = -0.1569 + 0.0156*x	0.10	0.56
Cr	y = 0.1152 + 0.0148*x	0.14	0.40
Cu	y = 0.5123 + 0.0091*x	0.07	0.67
Hg	y = -1.8228 - 0.0412*x	-0.34	**0.04**
Ni	y = -0.1618 - 0.0059*x	-0.05	0.77
Pb	y = -2.4367 - 0.0933*x	-0.57	**0.00**
V	y = 1.6903 + 0.0745*x	0.36	**0.03**
Secondary consumers			
Cd	y = 1.6931+0.0658*x	0.53	**0.02**
Co	y = -0.419 + 0.012*x	0.20	0.42
Cr	y = -0.6323 - 0.0391*x	-0.40	0.09
Cu	y = 0.4886 - 0.0223*x	-0.32	0.16
Hg	y = 1.0146 + 0.1012*x	0.72	**0.00**
Ni	y = -0.5732 - 0.0414*x	-0.50	**0.03**
Pb	y = -2.8261 - 0.1254*x	-0.66	**0.00**
V	y = -0.4854 - 0.0233*x	-0.19	0.43
b) $\delta^{15}N$ vs. [log TE]			
whole food web	**equation**	**r**	**p**
Cd	y = 0.4271 - 0.0721*x	-0.43	**0.00**
Co	y = -0.419 +0.012*x	-0.46	**0.00**
Cr	y = 0.7157 - 0.0814*x	-0.48	**0.00**
Cu	y = 0.8223 - 0.0493*x	-0.38	**0.00**
Hg	y = -1.5331 + 0.0897*x	0.63	**0.00**
Ni	y = 0.9326 - 0.1009*x	-0.58	**0.00**
Pb	y = 0.592 - 0.1016*x	-0.46	**0.00**
V	y = 0.8594 - 0.1139*x	0.56	**0.00**