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Significance and Interspecific Variability of Accumulated Trace Metal Concentrations in Antarctic Benthic Crustaceans[†]

key words: background concentrations, “Cd-anomaly”, Southern Ocean, Wedell Sea

Abstract

Trace metals (Cd, Cu, Pb and Zn) were analysed in crustaceans collected on Polarstern cruises ANT XVI/2 (1999) and ANT XXI/2 (2003/04, BENDEX) to the Weddell Sea. Our study provides further evidence for the frequently reported “Cd anomaly” in polar crustaceans, with data ranging from 1.2 (*Ceratoserolis trilobitoides*) to 6.2 mg Cd kg⁻¹ DW (*Notocrangon antarcticus*) in 1999 and from 1.2 (*Waldeckia obesa*) to 20.3 mg Cd kg⁻¹ (*Tryphosella murrayi*) in 2003. Pb concentrations well below 1 mg kg⁻¹ in most of the samples analysed might serve as a regional or even global background value for comparison in biomonitoring studies. Increasing Cu concentrations from eggs of decapods (*e.g.*, 5 vs. 51 mg kg⁻¹ in *N. antarcticus*) or juveniles in the brood pouch of an amphipod species to adult females indicate that the enzymatic requirements and haemocyanin component demand for Cu in early life-history stages is probably not met without a distinct bioaccumulation of this essential element after hatching. Most interestingly, Cd also increases (< 0.1 vs. 6.2 mg kg⁻¹ in *N. antarcticus*). This could be the consequence of efficient uptake mechanisms for Cu that cannot discriminate between this element and Cd. Cu and Zn concentrations in decapods of this study are largely within the range reported worldwide (40–90 mg Cu kg⁻¹ and 40–80 mg Zn kg⁻¹), indicating that these elements are regulated. The enormous heterogeneity of Cd and Zn in many amphipod species investigated (*e.g.*, from 0.6 in *Gnathiphimedia mandibularis* to 34.4 mg Cd kg⁻¹ in *Orchomenopsis acanthura* and from 41 in *Eusirus antarcticus* to 1244 mg Zn kg⁻¹ in *Iphimediella bransfieldi*) supports the hypothesis of the “Cd anomaly” and suggests that there is probably no consistent metabolic demand for the essential element Zn in this taxonomic group. The heterogeneity of Cu in amphipods is less pronounced.

1. Introduction

Antarctica has very rich marine invertebrate benthic communities, showing great diversity and abundance, high levels of gigantism and longevity, slow growth rates, delayed maturation and absence of pelagic larval stages. The presence of ice, the unusual depth of the shelf (up to 500 m and more), the extensive area of deep water around the continent, and the lack of connection with temperate shelves made the Antarctic shelf an insular evolutionary site equivalent to Lake Baikal or the Galapagos (BARGAGLI, 2005, p. 116, EASTMAN and CLARKE, 1998). Ecological research in this area is promoted by international environmental programmes managed by the Scientific Committee on Antarctic Research (SCAR)

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[†]*In memoriam* EKKEHARD VARESCHI, participant in Cruise ANT XXI/2 of RV “Polarstern”, who died together with his wife ANGELIKA in Tanzania on a scientific field trip to East African lakes in September 2005

or the Commission of the Convention on the Conservation of Antarctic Marine Living Resources (CCAMLR) as part of the Antarctic Treaty System. Generally, Antarctica and the Southern Ocean are scarcely affected by environmental contamination, except in localised areas adjacent to abandoned or inhabited human settlements. Nevertheless, natural and anthropogenic contamination of the Antarctic marine ecosystems with trace metals and other xenobiotics receives continued attention in the scientific literature, as has been recently reviewed by BARGAGLI (2005).

To assess the environmental quality regarding heavy metals in the oceans, the bioavailable fraction from food and water is of great importance. Both sources are integrated into accumulated concentrations in organisms. There is increasing evidence that trophic transfer may be even more important than dissolved uptake in various invertebrates (WANG, 2002). Further support of this view is provided by the fact that bioconcentration factors (BCFs) derived, for example, from field samples of benthic amphipods are often much higher than BCFs estimated from experiments regarding dissolved uptake (CLASON and ZAUKE, 2000). Thus, the total bioavailable fraction is only accessible by determining the amount of metals incorporated in organisms (ZAUKE *et al.*, 1996b). The accumulation patterns and subsequent accumulated concentrations in marine organisms often vary specifically as can be explained and predicted by toxicokinetic modelling (CLASON *et al.*, 2004b; LUOMA and RAINBOW, 2005). As a result, we can frequently find different species of benthic marine invertebrates with greatly varying metal concentrations in the same body of ocean water (PETRI and ZAUKE, 1993; ZAUKE *et al.*, 2003).

In order to differentiate human impact from natural variability, knowledge of background concentrations of metals and their fluctuations in biomonitor organisms is essential, as well as a thorough understanding of accumulation and detoxification strategies (LUOMA and RAINBOW, 2005). The latter depend on various aspects, including the biological species and element considered, the applied exposure regime, cation homeostasis mechanisms, life-history status, spatial and temporal scales and other factors.

Fronts, convergences and divergences are permanent features of the Southern Ocean, separating water masses that have different physico-chemical and biological characteristics. Southern Ocean surface waters are exceptional because Cd and phosphate concentrations are much higher than in the surface waters of other oceans (BARGAGLI, 2005, p. 220). Furthermore, studies on various organisms from the Southern Ocean have revealed particularly high Cd concentrations, referred to as the "Cd anomaly" (BARGAGLI *et al.*, 1996; PETRI and ZAUKE, 1993). The bioaccumulation of Cd in Antarctic marine organisms could be due to the enhanced levels of Cd in the ocean water but also to their slower growth rates, later sexual maturity and especially to the longer moult cycles of crustaceans (since moulting is an efficient way of eliminating metals) at low temperatures. Alternatively, high concentrations of Cd have been reported in particular taxa irrespective of temperature (*i.e.*, at both high and low latitudes) – for example in particular cephalopods (BUSTAMANTE *et al.*, 1998), hyperiid amphipods and all pectinid bivalves (scallops). It was furthermore hypothesized that the increased bioaccumulation of Cd may be related to effective but unselective uptake mechanisms which had evolved due to potential metabolic deficiencies in Cu in the Antarctic Ocean (PETRI and ZAUKE, 1993). Additionally, the composition of phytoplankton assemblages and species-specific requirements of algae for trace elements could play a very important role in the Cd transfer to primary consumers and into marine food webs. These findings suggest that probably no global background values will apply to organisms from this remote area. Instead, regional background values should be established in order to follow possible future trends of metals in biomonitors.

The ecological implications of the "Cd anomaly" are enormous irrespective of the possible causes (see above). In the ice-free pelagic zones of the Southern Ocean high Cd concentrations in hyperiid amphipods of genus *Themisto* and in the digestive glands of cephalopods as well as in the liver and kidney of myctophid fish may be one reason for the

hyper-accumulation of Cd in many pelagic seabirds, the highest reported Cd concentrations in vertebrates being found in some albatrosses and petrels. An analogous transfer of Cd can be inferred for the benthic coastal food chain, where high Cd concentrations in crustaceans and especially in bivalves such as *Adamussium colbecki* are related to high Cd concentrations in fish, seabirds and seals (see BARGAGLI, 2005, p. 259ff. and the literature cited therein).

The present study was part of the Benthos Disturbance Experiment (BENDEX) performed on the expedition ANT XXI/2 of RV "Polarstern" to the Weddell Sea from November 2003 until January 2004 (ARNTZ and BREY, 2005). The distribution of benthic organisms on Antarctic shelves is largely affected by glacier transport of coarse material into the sea, ploughing by floating icebergs, plucking by anchor ice and abrasion of shorelines by fast-, pack- and brash ice or by floes driven ashore and piled up by storms (push ice). These near-shore waters and ice shelves are characterised by phytoplankton showing intense and brief blooms during the austral summer. Due to the low biomass of zooplankton, however, most algal cells sink and become food for very rich benthic communities (sponges, hydroids, tunicates, polychaetes, molluscs, actinarians, echinoderms, amphipods and fish) (BARGAGLI, 2005, p. 270, 251). Not only are the benthic communities disturbed by iceberg scours (the main focus during BENDEX) but it may also be possible for the availability of metals to be increased by this process. Additional information is provided regarding crustaceans collected on a previous expedition (ANT XVI/2) to the Weddell Sea in 1999.

Thus, the main goal of this paper is to provide information on the significance and variability of trace metal concentrations in crustaceans from more or less disturbed areas of the Weddell Sea shelf, considering, among others, a unique collection of amphipod species. In particular, the following questions will be treated. (i) Do we find further evidence for a "Cd anomaly" in crustaceans from Antarctic waters? (ii) Is the heterogeneity of metals within a given taxon of amphipods, living in the same environment, small or large; the result could allow some inference regarding possible metabolic requirements for essential metals like Cu and Zn and regarding other possible inference such as the feeding behavior or growth rate. (iii) Do we find that the metal concentrations in eggs or juveniles in the brood pouch differ from those in female adults of selected decapods and amphipods? (iv) Is there any relationship between metal concentrations and length of organisms as reported in other studies, and (v) can we derive reasonable regional or global background values for some metals in benthic invertebrates from this region (except for Cd where this seems to be impossible owing to physical and chemical characteristics of Southern Ocean waters)?

2. Materials and Methods

2.1. Sampling and Sample Preparation

Samples were collected on two "Polarstern" cruises to the Weddell Sea in Jan/Feb 1999 and Nov/Dec 2003. On cruise ANT XVI/2 (in 1999) samples were taken from two different areas (Figure 1, left panel): at 4 stations from area (A) near the Riiser-Larsen Ice Shelf ($70^{\circ}15.95' - 72^{\circ}52.34' \text{ S}$ and $002^{\circ}47.33' - 019^{\circ}05.57' \text{ W}$; water depth 140–445 m) and at 2 stations from area (B) near the Ronne Ice Shelf ($75^{\circ}22.49' - 75^{\circ}27.93' \text{ S}$ and $054^{\circ}22.50' - 055^{\circ}51.15' \text{ W}$; water depth 415–445 m). On cruise ANT XXI/2 (in 2003, BENDEX) samples were taken from a much smaller area largely coinciding with area (A) in 1999 (Fig. 1, right panel): at 25 stations from area (C) north of Kapp Norvegia (Austasen; $70^{\circ}47.88' - 71^{\circ}07.50' \text{ S}$ and $010^{\circ}30.15' - 011^{\circ}33.92' \text{ W}$; water depth 175–1525 m), at 1 station from area (D) west of Kapp Norvegia ($71^{\circ}19.19' \text{ S}$ and $013^{\circ}57.45' \text{ W}$; water depth 844 m) and at 9 stations from area (E) west of Drescher Inlet at the Riiser-Larsen Ice Shelf ($72^{\circ}47.71' - 72^{\circ}56.81' \text{ S}$ and $019^{\circ}30.25' - 019^{\circ}48.99' \text{ W}$; water depth 446–835 m). While in 1999 each station represents a different waypoint of the ship, in 2003 different station numbers were allocated to each sampling gear employed, although in some cases there was only a slight difference in their positions. Thus we regard the areas A–E as units of the investigation but not the stations.

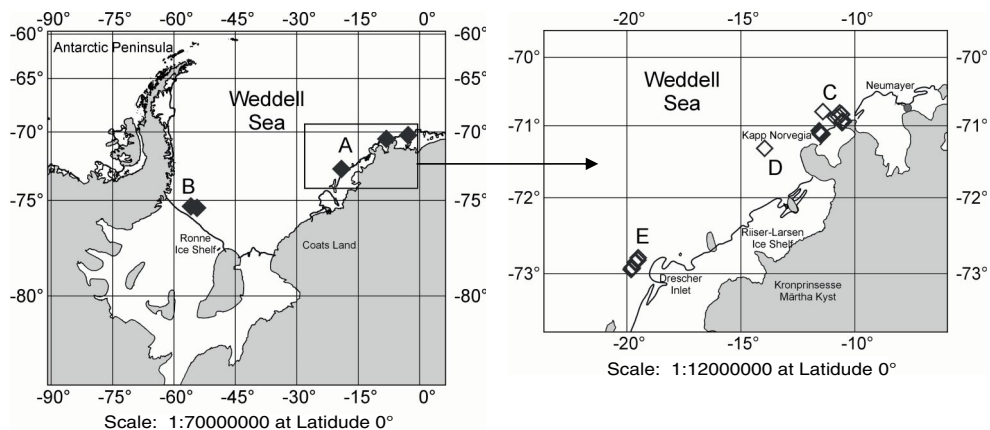


Figure 1. Areas of investigation and sampling locations in the Weddell Sea (A, B, Jan-Feb 1999, left and C, D, E, Nov-Dec 2003, right). See text for more details. Coastlines and ice shelf lines prepared with PanMap 0.9.6 (Pangaea).

In 1999 samples were obtained with an Agassiz trawl and a trap baited with dead fish and exposed to the bottom for 1–3 days. In 2003 samples were obtained again with an Agassiz trawl and additionally with a benthopelagic trawl. This time the traps baited with dead fish were mounted on larger gear like fish traps, amphipod traps or a bottom lander; these were again exposed to the bottom for 2–4 days. Depending on which of these devices was used, our traps were placed 0.2–1.5 m above ground. Fish can be regarded as an appropriate bait, since metal concentrations in muscle tissue – the main body structure consumed by the amphipods and isopods, according to visual inspection upon sampling – are very low (e.g., ZAUKE *et al.*, 1999) with the sole exception of Hg, which was not considered in this study. Thus, a contamination of the samples via food can be completely excluded.

On board ship, the animals were kept alive for 48 h in polyethylene buckets containing seawater of 33.5–34.5 ‰ salinity, at 0 °C without light and food to allow for defecation. This time is a reasonable compromise between achieving gut clearance (ZAUKE *et al.*, 1995) and avoiding excessive stress or even mortality. In fact, no remaining sediment particles could be detected during homogenization of the animals (see below), indicating that a bias in metal concentrations in the crustacean samples is very unlikely to be caused by this process. Upon sampling, the crustaceans were collected and sorted to species level whenever possible under close visual observation, using a binocular microscope to ensure the absence of any foreign particles. Subsequently, the samples were thoroughly rinsed for a few seconds with double-distilled water to remove fine suspended materials, adhering seawater and labile metals, which are not regarded as part of the bioaccumulated metal fraction, from the surface of the animals. Then the animals were dried on good-quality filter paper and stored at –20 °C in Eppendorf caps (2 ml, polypropylene), Petri dishes or good-quality polyethylene bags, depending on their size and number.

In this way we obtained the following samples with more than 6 independent replicates suitable for an interspecific statistical data evaluation: in 1999 the amphipod *Epimeria macrodonta* (WALKER, 1906), the isopod *Ceratoserolis trilobitoides* (EIGHTS, 1833), the decapods *Chorismus antarcticus* (PFEFFER, 1887) and *Notocrangon antarcticus* (PFEFFER, 1887) and the mysiid *Antarctomysis maxima* HOLT and TATTERSALL, 1906; in 2003 the amphipods *Ampelisca richardsoni* KARAMAN, 1975, *Parschisturella carinata* (SCHELLENBERG, 1926), *Tryphosella murrayi* (WALKER, 1903), *Uristes adarei* (WALKER, 1903) and *Waldeckia obesa* (CHEVREUX, 1905), the isopod *Glyptonotus antarcticus* EIGHTS, 1853 and the decapods *Chorismus antarcticus* (PFEFFER, 1887) and *Nematocarcinus lanceopes* BATE, 1888. More samples were collected and analysed with less than 6 replicates, since the taxonomic coverage especially in the 2003 cruise was exceptional and will be useful for future comparisons (see Results section).

In 1999 the following material was obtained to assess possible intraspecific heterogeneities: *E. macrodonta* (juveniles in the brood pouch and females carrying juveniles in the brood pouch), *C. antarcticus* and *N. antarcticus* (eggs and females carrying eggs). In 2003 individual organisms were collected to analyse relationships between metal concentrations and body length for *P. carinata*, *T. murrayi*, *W. obesa* and *C. antarcticus*.

2.2. Analytical Procedures

Upon arrival in the laboratory in Oldenburg, the frozen crustacean samples were subjected to freeze-drying for 72 h (LYOVAG GT2, Leybold Heraeus). Then the samples were homogenised using a small boron carbide mortar and pestle, to avoid losses of biomass, or a ball mill made of agate. Aliquots of about 10 mg dried material were digested for 3 h at 80 °C with 100 µl HNO₃ (65%, suprapure, Merck) in tightly closed 2 ml Eppendorf reaction tubes (CLASON and ZAUKE, 2000). The digests were made up to 2 ml volume with double-distilled water.

Metal determinations in biological samples were performed using a Varian SpectrAA 880 Zeeman instrument and a GTA 110 graphite tube atomiser with Zeeman background correction according to CLASON and ZAUKE (2000) and KAHLE *et al.* (2003). Ashing and atomisation temperatures were 600 and 1800 °C for Cd, 1000 and 2200 °C for Pb, 800 and 2300 °C for Cu. For Cd and Pb, palladium and magnesium nitrate modifiers were applied. Zn was assayed using an air-acetylene flame (SpectrAA-30, deuterium background correction) and a manual micro-injection method (100 µl sample volume). All metal concentrations in biological tissues are reported in mg kg⁻¹ dry weight (DW).

We obtained the following fresh weight/dry weight ratios for the species included in the statistical evaluation: *N. antarcticus* (4.1), *G. antarcticus* (3.9), *C. antarcticus* (3.8), *N. lanceopes* (3.4), *A. richardsoni* and *E. macrodonta* (3.3), *U. adarei* and *C. trilobitoides* (3.2), *A. maxima* (3.1), *T. murrayi* (2.8), *P. carinata* and *W. obesa* (2.7).

Quality assurance was performed in line with German GLP regulations (ANONYMOUS, 1999), using the following documented criteria: stability of instrumental recalibration, precision of parallel injections (normally showing a coefficient of variation of 1–5%) and analytical blanks (also reflecting the digestion procedure). The precision and validity were evaluated using three certified reference materials which were randomly allocated within the determinations (Table 1). Limits of detection were calculated according to BÜTTNER *et al.* (1980). The analysed values obtained for reference materials are in most cases in good agreement with the certified values, and the limits of detection proved to be adequate for the range of metal concentrations found in this study. For Pb we found many values below or close to the limit of detection, indicating that a contamination of samples can be almost completely excluded (ZAUKE *et al.*, 1996a). Thus, Pb was not considered in the statistical data evaluation (next section). It should be noted, however, that values close to or below the limit of detection are due to the small amount of material digested and analysed, which in turn offers the opportunity to analyse even single and small biological specimen.

2.3. Statistical Procedures

The hypothesis of normal distribution was tested using the Lilliefors Test (referred to as LIP; $\alpha = 0.01$) provided in SYSTAT for Windows (Version 8, WILKINSON, 1998). We selected the 99% significance level because the following parametric tests employed are regarded as robust against devia-

Table 1. Quality assurance using certified reference materials randomly allocated within the determinations. Values are mean \pm 95% CI [mg kg⁻¹ DW].

	TORT 2 (Lobster hepatopancreas)		CRM 278 (Mussel tissue)	
	analysed	certified	analysed	certified
Cd	26.4 \pm 0.9	26.7 \pm 0.6	0.35 \pm 0.02	0.348 \pm 0.007
Pb	0.46 \pm 0.04	0.35 \pm 0.13	2.41 \pm 0.11	2.00 \pm 0.04
Cu	96 \pm 3	106 \pm 10	8.35 \pm 0.25	9.45 \pm 0.13
Zn	170 \pm 4	180 \pm 5	73.4 \pm 2.0	83.1 \pm 1.7

Numbers of independent determinations: 57–59; Limits of detection [mg kg⁻¹ DW] (calculated as 2.6 standard deviations of a “low sample”; BÜTTNER *et al.* (1980): Cd: 0.10; Pb: 0.3; Cu: 1.6; Zn: 12.

tions from the normal distribution. Since they have more power compared to the nonparametric procedures, it is reasonable not to reject these tests too early. The same program was also used to compute means and 95% confidence intervals of metal concentrations in independent subsamples. The experimental units of this study are given by the factors species affiliation, the different study areas within the Weddell Sea as shown in Figure 1 and time of sampling (1999 vs. 2003). Further statistical evaluation was performed with BMDP Dynamics (Release 7.0, DIXON, 1992). Global null hypotheses (*e.g.*, equality of means between the species investigated, *viz.* interspecific heterogeneities) were tested either by classical ANOVA (assuming equality of variances; referred to as F), or by non-classical Welch Test (not assuming equality of variances; WS). In the case of two sample comparisons these tests yield the same results as the pooled or separate variance *t*-Test (referred to as pooled-*t* and separate-*t* value). The adequate procedure was selected after testing for normality by Lilliefors Test (SYSTAT) and equality of variances by Levene Test (BMDP, LS). Null hypotheses were rejected at 95% significance level ($P < 0.05$).

Heterogeneity was analysed in more detail using the Student-Newman-Keuls Multiple Range Test (NK) ($\alpha = 0.05$). The robust NK procedure involves an adjusted significance level for each group of ordered means (DIXON, 1992; p. 585). We did not consider applying a log transformation to the data and employing a classical ANOVA, because in this case the interpretation of the statistical results would be restricted to the transformed data and not to the original ones which are of interest. This disadvantage is avoided by the well-established procedures mentioned above. The statistical evaluation outlined above was employed if at least 6–8 independent replicates were obtained (see above).

2.4. Trophic Characterisation of the Species Analysed

The species collected in this study have different modes of life, habitats and trophic ecology, which might influence the accumulated metal levels. Their trophic type is indicated below, with the main food items found in stomach contents listed if possible (mostly from Weddell Sea studies). Corresponding information is summarised in Tables 2–6 (see Results section) using the abbreviations given below.

2.4.1. Scavengers (SC)

These include the following amphipods: *Abyssorhomene charcoti* (Uristidae); *Abyssorhomene plebs* (Uristidae); *Orchomene* sp. (Lysianassidae); *Orchomenopsis pinguides* (Lysianassidae); *Parschisturella carinata* (Lysianassidae); *Pseudorchomene coatsi*, *Pseudorchomene* sp. (Lysianassidae) and *Waldeckia obesa* (Lysianassidae) (DAUBY *et al.*, 2001; DE BROYER *et al.*, 2004; NYSSSEN *et al.*, 2005; SLATTERY and OLIVER, 1986) and the isopods *Natatolana* spp. (Cirolanidae), for each of which the main food resources have been identified as carrion from fish, invertebrates, birds and mammals (WÄGELE and BRANDT, 1992).

2.4.2. Predators (P)

Epibenthic (macro)predators (EP). These include the following amphipods: *Alexandrella* sp. (Stilipedidae), main food (in *Alexandrella mixta*) identified as ophiuroids (DAUBY *et al.*, 2001); *Epimeriella walkeri* (Epimeriidae), main food ophiuroids and diatoms (DAUBY *et al.*, 2001); *Eusirus perdentatus*, *Eusirus* spp (Eusiridae), main food crustaceans, mainly amphipods, and polychaetes (DAUBY *et al.*, 2001; KLAGES, 1993; KLAGES and GUTT, 1990b; NYSSSEN *et al.*, 2005); *Iphimediella bransfieldi*, *Iphimediella rigida* (Iphimediidae), main food resources largely unknown but main prey of *Iphimediella* sp. are crustaceans (NYSSSEN *et al.*, 2005); the following isopods: *Accalathura gigantissima* (Leptanthuridae), main food amphipods (WÄGELE, 1985); *Anuropus antarcticus* (Anuropidae), trophic type still to be checked; *Paranthura antarctica* (Paranthuridae), main food unknown (WÄGELE and BRANDT, 1990); and finally the following decapods: *Chorismus antarcticus* (Hippolytidae), main food amphipod and isopod crustaceans (GORNÝ *et al.*, 1992).

Epibenthic (macro)predators/opportunistic scavengers (EPOSC). These include the following amphipods: *Eusirus* sp. (*cf. antarcticus*) (Eusiridae), main food crustaceans, primarily amphipods and copepods (DAUBY *et al.*, 2001; DE BROYER *et al.*, 2004); *Hirondellea antarctica* (Lysianassoidea: hiron-delleids), main food hydrozoans and sea anemones (DAUBY *et al.*, 2001); *Tryphosella murrayi* (Lysianassidae), main food crustaceans (amphipods), polychaetes and various carrion (DAUBY *et al.*, 2001) and the isopods: *Ceratoserolis trilobitoides* (Serolidae), main food carrion, benthic crustaceans, polychaetes and krill (WÄGELE, 1986); *Glyptonotus antarcticus* (Chaetiliidae), main food carrion, polychaetes, benthic crustaceans, krill and echinoderms (WÄGELE and BRANDT, 1992).

Epibenthic predators/deposit feeders (EPDF). These include the following amphipods: *Epimeria macrodonta* (Epimeriidae), main food cnidarians, crustaceans (euphausiids), pycnogonids, holothurians and plankton (foraminifers, diatoms, ostracods) (DAUBY *et al.*, 2001); *Liljeborgia georgiana* (Liljeborgiidae), main food crustaceans (copepods, krill, amphipods), polychaetes and cnidarians (DAUBY *et al.*, 2001); *Oediceroides calmani* (Oedicerotidae), main food plankton items (diatoms, radiolarians and copepods), polychaetes and crustaceans (amphipods) (DAUBY *et al.*, 2001).

Benthopelagic/hyperbenthic predators/deposit feeders (BP, to some extent questionable). These include the Mysidacea: *Antarctomysis maxima* (Mysidae), for which no trophic analysis has yet been published; generally Mysidae eat detritus, diatoms and zooplankton (WITTMANN, 1990). Pelagic predators (PP, to some extent questionable). These include the amphipods: *Cyllopus lucasii* (Vibilliidae), however, main food has yet to be checked.

Endobenthic (subsurface) predators (SP). These include the following amphipods: *Heterophoxus videns* (Phoxocephalidae), main food polychaetes, crustaceans (tanais, harpacticoids), diatoms, foraminifers and nematodes (DAUBY *et al.*, 2001; OLIVER *et al.*, 1982).

Epibenthic specialised micropredators (browsers) (EMP). These include the following amphipods: *Echiniphimedia* spp. (Iphimediidae), main food sponges (DAUBY *et al.*, 2001; NYSSSEN *et al.*, 2005); *Gnathiphimedia mandibularis* (Iphimediidae), main food bryozoans (COLEMAN, 1989b; DAUBY *et al.*, 2001; KLAGES and GUTT, 1990a).

2.4.3. Deposit Feeders (DF)

Epibenthic deposit feeders (EDF). These include the following amphipods for which specific trophic analysis have not yet been published: *Hippomedon major* (Lysianassidae), the most common species of which, *H. kergueleni*, is a scavenger and deposit feeder while many other *Hippomedon* species are regarded as deposit feeders; *Orchomenella ultima* and *Orchomenopsis acanthura* (Lysianassidae), *Orchomenella* and *Orchomenopsis* species are usually regarded as deposit feeders and sometimes as opportunistic scavengers; *Paraceradocus gibber* (Melitidae), main food unidentified amorphous organic material, crustaceans, polychaetes, echinoderms, diatoms and sponges (COLEMAN, 1989a; DAUBY *et al.*, 2001) and the following decapods: *Notocrangon antarcticus* (Crangonidae), main food related to sediments but details have to be checked (GORNÝ *et al.*, 1992).

Epibenthic deposit feeders/opportunistic scavengers (EDFOSC). These include the following amphipods: *Uristes gigas* (Uristidae), main food carrion, crustaceans, sponges and diatoms (DAUBY *et al.*, 2001).

2.4.4 Suspension Feeders (SF)

Epibenthic suspension feeders (ESF). These include the following amphipods: *Jassa goniamera* (Ischyroceridae), main food crustacean remains (probably copepods), diatoms, and undefined bodies embedded in mucus (DAUBY *et al.*, 2001).

Epibenthic suspension feeders/deposit feeders (ESFDF). These include the following amphipods: *Ampelisca bouvieri* (Ampeliscidae), main food unknown due to lack of specific trophic analysis, but *Ampelisca* species are generally known as suspension/surface deposit feeders (DAUBY *et al.*, 2001); *Ampelisca richardsoni* (Ampeliscidae), main food various plankton items (mostly diatoms), few sponge spicules and crustacean remains (DAUBY *et al.*, 2001; NYSSSEN *et al.*, 2005).

2.4.5. Associates (Symbionts and Parasites) (ASS)

Symbionts in sponges and ascidians (SYM, in some cases predators?). These include the following amphipods: *Leucothoe spinicarpa* (Leucothoidae) in sponges and ascidians (DE BROYER *et al.*, 2001) but no specific trophic analysis available; *Andaniotes linearis* (Stegocephalidae) in sponges (KUNZMANN, 1996), again no specific trophic analysis available, but Stegocephalidae are mostly micropredators associated with diverse benthic sessile invertebrates and some few species are occasional scavengers (DE BROYER *et al.*, 2004).

Ectoparasites on fish (ECTO). These include the following isopods: *Aega antarctica* (Aegidae), main food fish blood (WÄGELE, 1990).

3. Results

3.1. Intraspecific Heterogeneities of Metals in Crustaceans

Intraspecific heterogeneities of metals found in 1999 for the amphipod *E. macrodonta* comparing juveniles in the brood pouch and females carrying juveniles in the brood pouch are inconsistent depending on the element considered. Statistical tests are not applicable due to low degrees of freedom. Clear differences are obvious for Cd, with concentrations below the limit of detection (< 0.1) in the juveniles vs. $2.5\text{--}7.0\text{ mg kg}^{-1}$ DW in females. For Cu and Zn differences are less distinct and the variability substantial: $6\text{--}14$ vs. $11\text{--}32\text{ mg Cu kg}^{-1}$ and $64\text{--}81$ vs. $40\text{--}336\text{ mg Zn kg}^{-1}$. Pb concentrations are generally low (0.3 vs. 0.8 mg kg^{-1}).

Results obtained in 1999 for the decapods *C. antarcticus* and *N. antarcticus*, comparing eggs and females carrying eggs, are similar to those from *E. macrodonta*. In both decapod species analysed, Cd concentrations in eggs are likewise below the limit of detection while concentrations in females yield 5.5 ± 2.6 (*C. antarcticus*) and $6.2 \pm 0.6\text{ mg kg}^{-1}$ (*N. antarcticus*) (means $\pm 95\%$ CI). Corresponding results for Cu are 10 ± 2 vs. 38 ± 15 (*C. antarcticus*) and 5 ± 2 vs. $51 \pm 6\text{ mg kg}^{-1}$ (*N. antarcticus*), and for Zn 68 ± 5 vs. 54 ± 5 and 70 ± 3 vs. $55 \pm 2\text{ mg kg}^{-1}$, respectively. The detected differences for Cu and Zn are statistically significant ($\alpha = 0.05$): with separate- $t = -4.50$, $df = 7$ and $P = 0.003$ for Cu and pooled- $t = 4.70$, $df = 12$ and $P = 0.001$ for Zn in *C. antarcticus* and with Mann-Whitney statistic = 1.00, $df = 1$ and $P = 0.000$ for Cu and pooled- $t = 9.00$, $df = 66$ and $P = 0.000$ for Zn in *N. antarcticus*. Pb in eggs of both species is below the limit of detection (< 0.3) and in females is also rather low ($0.5\text{--}0.8\text{ mg kg}^{-1}$).

Relationships between body dry weight (DW), Cd, Cu, Zn and body length (BL) of selected crustaceans from the Weddell Sea (Nov–Dec 2003) are displayed in Figure 2. Only for DW and BL could exponential models be estimated, while for the metals analysed no consistent trend is visible.

3.2. Interspecific Heterogeneities of Metals in Crustaceans

Results for interspecific heterogeneities in crustaceans from 1999 and 2003 regarding collectives with a sufficient number of independent replicates to perform statistical analyses are compiled in Tables 2–4. Parametric tests are used throughout, since Lilliefors probabilities indicate that the sample data are distributed normally. Global null hypotheses (equality of means) have to be rejected in all cases (Table 4), giving reason to explore heterogeneities in more detail. These are distinct in the 1999 survey according to the results of the NK procedure (Table 2), ranging from 1.2 (*C. trilobitoides*) to 6.2 mg Cd kg^{-1} DW (*N. antarcticus*), 17 (*E. macrodonta*) to 51 mg Cu kg^{-1} (*N. antarcticus*) and 49 (*C. trilobitoides*) to 89 mg Zn

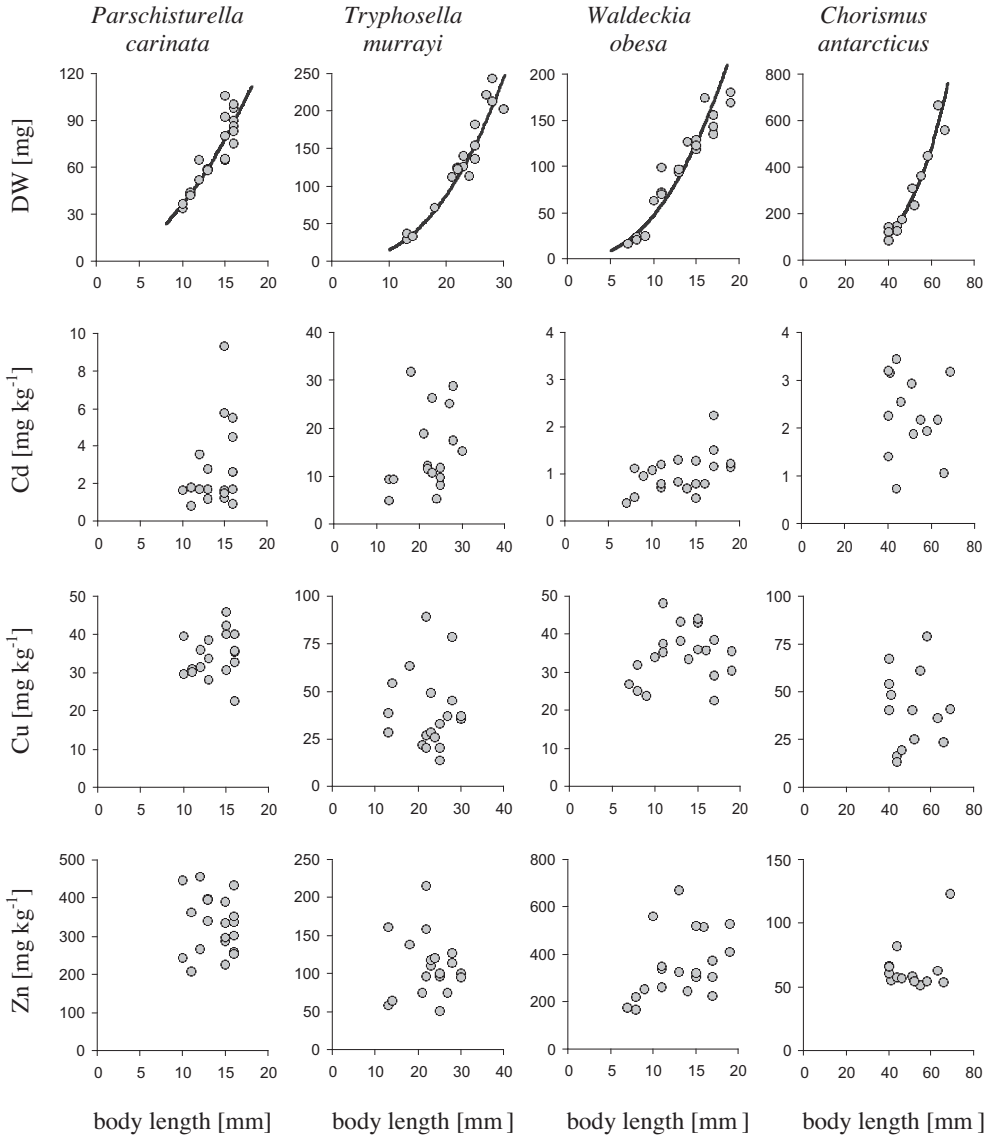


Figure 2. Relationships between body dry weight (DW), Cd, Cu, Zn and body length (BL) of selected crustaceans from the Weddell Sea (Nov–Dec 2003). Only for DW and BL could exponential models be estimated: $DW = 0.46 \cdot BL^{1.9}$ ($R^2 = 0.86$) for *P. carinata*; $DW = 0.05 \cdot BL^{2.5}$ ($R^2 = 0.97$) for *T. murrayi*; $DW = 0.19 \cdot BL^{2.4}$ ($R^2 = 0.91$) for *W. obesa* and $DW = 0.00013 \cdot BL^{3.7}$ ($R^2 = 0.95$) for *C. antarcticus*.

Table 2. Interspecific heterogeneities of cadmium, copper and zinc in crustaceans from the Weddell Sea (Jan–Feb 1999). Sampling areas see Figure 1.

Collective	Taxon	Trophic type	Area	Depth	Mean \pm 95% CI [mg kg ⁻¹ DW]	N	Groups			
							LIP	1	2	3
Cd	<i>Ceratoserolis trilobitoides</i>	Iso	EPOSC	B	445	1.2 \pm 0.5	9	0.118		
	<i>Antarctomyxis maxima</i>	Mys	BP	B	415–445	1.2 \pm 0.2	27	0.025		
	<i>Epimeria macrodonta</i>	Amp	EPDF	B	445	4.6 \pm 1.8	8	1.000		
	<i>Chorismus antarcticus</i> *	Dec	EP	A	140–280	6.1 \pm 2.0	12	0.651		
	<i>Notocrangon antarcticus</i>	Dec	EDF	B	415–445	6.2 \pm 0.6	34	0.208		
Cu	<i>Epimeria macrodonta</i>	Amp	EPDF	B	445	17 \pm 5	12	0.819		
	<i>Chorismus antarcticus</i> *	Dec	EP	A	140–280	29 \pm 8	20	0.132		
	<i>Ceratoserolis trilobitoides</i>	Iso	EPOSC	B	445	31 \pm 8	12	0.668		
	<i>Antarctomyxis maxima</i>	Mys	BP	B	415–445	31 \pm 7	30	0.036		
	<i>Notocrangon antarcticus</i>	Dec	EDF	B	415–445	51 \pm 6	34	0.808		
Zn	<i>Ceratoserolis trilobitoides</i>	Iso	EPOSC	B	445	49 \pm 9	12	0.133		
	<i>Notocrangon antarcticus</i>	Dec	EDF	B	415–445	55 \pm 2	34	1.000		
	<i>Antarctomyxis maxima</i>	Mys	BP	B	415–445	59 \pm 3	30	0.325		
	<i>Chorismus antarcticus</i> *	Dec	EP	A	140–280	63 \pm 8	20	0.111		
	<i>Epimeria macrodonta</i>	Amp	EPDF	B	445	89 \pm 23	9	0.509		

Taxon: Iso = Isopoda, Mys = Mysidacea, Amp = Amphipoda, Dec = Decapoda; Trophic type: EPOSC = epibenthic (macro)predators/opportunistic scavengers, BP = benthopelagic/hyperbenthic predators, EPDF = epibenthic predators/deposit feeders, EP = epibenthic (macro)predators, EDF = epibenthic deposit feeders (see text for more details and references); LIP: Lilliefors probability that sample data are distributed normally ($\alpha = 0.01$); 95% CI: 95% confidence intervals; Bars (|) indicate homogeneous groups according to the Student-Newman-Keuls Multiple Range Test; * not included in the statistical evaluation due to different sampling area; Pb is not displayed because most values are close to the limit of detection (Table 1): 0.4–0.7 mg kg⁻¹ DW for all collectives analysed.

kg⁻¹ (*E. macrodonta*). The same is true for the 2003 survey (Table 3), where we find values ranging from 1.2 (*W. obesa*) to 20.3 mg Cd kg⁻¹ (*T. murrayi*) and from 52 (*N. lanceopes*) to 382 mg Zn kg⁻¹ (*W. obesa*). Only for Cu do we find largely homogeneous concentrations (39–55 mg kg⁻¹) with the sole exception of *N. lanceopes* (169 mg kg⁻¹). Pb concentrations for all collectives analysed are generally low in both surveys, below or close to the limit of detection (< 0.3–0.7 mg kg⁻¹).

Data for crustaceans with insufficient numbers of replicates to perform statistical analyses are compiled in Tables 5 (1999) and 6 (2003), indicating remarkable heterogeneities of metal concentrations within the taxonomic groups investigated. These range, for example, in amphipods (1999) from 1.2 (*W. obesa*) to 38.3 mg Cd kg⁻¹ (*Orchomene* sp.) or from 53 (*Eusirus* sp.) to 409 mg Zn kg⁻¹ (*W. obesa*) and in amphipods (2003) from 0.6 (*G. mandibularis*) to 34.4 mg Cd kg⁻¹ (*O. acanthura*) and from 41 (*E. antarcticus*) to 1244 mg Zn kg⁻¹ (*I. bransfieldi*). The heterogeneity of Cu in amphipods is less pronounced. Within isopods increased Cd, Cu and Zn concentrations are found in *P. antarctica* (1999): 8.7, 261 and 541 mg kg⁻¹, respectively.

4. Discussion

4.1. Intraspecific Heterogeneities of Metals in Crustaceans

The distinct increase of Cd, Cu and Zn from juveniles in the brood pouch to adult females in the amphipod *Epimeria macrodonta* (this study) is in good agreement with results reported by RITTERHOFF and ZAUKE (1997c) for the amphipod *Themisto libellula* from the Green-

Table 3. Interspecific heterogeneities of cadmium, copper and zinc in crustaceans from the Weddell Sea (Nov–Dec 2003). Sampling areas see Figure 1.

Collective	Taxon	Trophic type	Area	Depth	Mean \pm 95% CI [mg kg ⁻¹ DW]	N	LIP	Groups						
								1	2	3	4			
Cd	<i>Waldeckia obesa</i>	Amp	SC	C/E	252–835	1.2 \pm 0.5	11	0.014						
	<i>Chorismus antarcticus</i>	Dec	EP	C	228–337	2.5 \pm 0.4	24	0.173						
	<i>Parschisturella carinata</i>	Amp	SC	C/E	279–835	2.9 \pm 1.7	6	1.000						
	<i>Ampelisca richardsoni</i>	Amp	ESFDF	C	119–288	4.8 \pm 1.5	7	0.440						
	<i>Glyptonotus antarcticus</i>	Iso	EPOSC	C	175–309	6.0 \pm 2.0	6	0.383						
	<i>Uristes adarei</i>	Amp	EDFOSC	C	119–309	6.7 \pm 2.3	8	0.254						
	<i>Nematocarcinus lanceopes</i>	Dec	EP	C/D	844–1525	9.0 \pm 1.5	11	1.000						
	<i>Tryphosella murrayi</i>	Amp	EPOSC	C	244–406	20.3 \pm 7.4	9	1.000						
	Cu	<i>Waldeckia obesa</i>	Amp	SC	C/E	252–835	39 \pm 5	10	0.664					
		<i>Parschisturella carinata</i>	Amp	SC	C/E	279–835	40 \pm 11	7	0.419					
<i>Glyptonotus antarcticus</i>		Iso	EPOSC	C	175–309	42 \pm 18	6	1.000						
<i>Chorismus antarcticus</i>		Dec	EP	C	228–337	43 \pm 7	25	1.000						
<i>Uristes adarei</i>		Amp	EDFOSC	C	119–309	43 \pm 21	8	0.575						
<i>Tryphosella murrayi</i>		Amp	EPOSC	C	244–406	47 \pm 9	9	1.000						
<i>Ampelisca richardsoni</i>		Amp	ESFDF	C	119–288	55 \pm 19	8	0.999						
<i>Nematocarcinus lanceopes</i>		Dec	EP	C/D	844–1525	169 \pm 56	11	0.531						
Zn		<i>Nematocarcinus lanceopes</i>	Dec	EP	C/D	844–1525	52 \pm 2	11	0.670					
		<i>Chorismus antarcticus</i>	Dec	EP	C	228–337	59 \pm 3	24	0.167					
	<i>Glyptonotus antarcticus</i>	Iso	EPOSC	C	175–309	85 \pm 9	6	0.117						
	<i>Uristes adarei</i>	Amp	EDFOSC	C	119–309	102 \pm 18	7	0.817						
	<i>Tryphosella murrayi</i>	Amp	EPOSC	C	244–406	157 \pm 54	9	0.538						
	<i>Ampelisca richardsoni</i>	Amp	ESFDF	C	119–288	259 \pm 61	8	0.745						
	<i>Parschisturella carinata</i>	Amp	SC	C/E	279–835	296 \pm 63	7	0.504						
	<i>Waldeckia obesa</i>	Amp	SC	C/E	252–835	382 \pm 99	11	1.000						

Notation as in Table 2; Trophic type: SC = scavengers, ESFDF = Epibenthic suspension feeders/deposit feeders, EDFOSC = Epibenthic deposit feeders/opportunistic scavengers (see text for more details and references); Bars (|) indicate homogeneous groups according to the Student-Newman-Keuls Multiple Range Test; Pb is not displayed because most values are close to the limit of detection (see Table 1); < 0.3–0.4 mg kg⁻¹ DW for all collectives analysed.

Table 4. Interspecific heterogeneities of cadmium, copper and zinc in crustaceans from the Weddell Sea (Jan–Feb 1999, see Table 3 and Nov–Dec 2003, see Table 4); test of global null hypotheses.

	Year	LS	P	Test	Test statistic	P	df
Cd	1999	8.3	0.000	W	98.1	0.000	3, 19
Cu	1999	2.6	0.055	F	18.1	0.000	3, 84
Zn	1999	20.4	0.000	W	6.8	0.002	3, 22
Cd	2003	10.8	0.000	W	25.1	0.000	7, 23
Cu	2003	12.5	0.000	W	4.0	0.005	7, 25
Zn	2003	12.4	0.000	W	42.9	0.000	7, 23

LS: Levene statistic (equality of variances); W: Welch-test (not assuming equality of variances); F: F-test (ANOVA); P: tail probability (corresponding null hypotheses are rejected when $P < 0.05$); df: degrees of freedom. *Chorismus antarcticus* not included in the statistical evaluation due to different sampling area; Pb is not displayed because most values are close to the limit of detection (Table 1).

Table 5. Interspecific heterogeneities of cadmium, copper and zinc in crustaceans from the Weddell Sea (Jan–Feb 1999). Results of singular samples where statistical evaluations (see Table 3) are not applicable ($N < 6$). Values are ranges; sampling areas see Figure 1.

Collective	Taxon	Trophic type	Area	Depth	Cd	Cu	Zn	N
<i>Ampelisca bowieri</i>	Amp	ESFDF	A	140–415	4.0–8.6	31–75	140–263	4
<i>Echiniphimedia scotti</i>	Amp	EMP	A	280	2.4	9	78	1
<i>Epimeria macrodonta</i>	Amp	EPDF	A	140–425	4.7–6.1	5–23	96–129	2
<i>Eusirus</i> sp.	Amp	EPOSC	A	140–280	2.3–7.8	7–22	53–58	5
<i>Gnathiphimedia</i> sp.	Amp	EMP	A	140–280	1.1–1.5	13–22	47–163	3
<i>Heterophoxus videns</i>	Amp	SP	A	140	12.8	70	284	1
<i>Oediceroides calmani</i>	Amp	EPDF	A	280	23.6–30.1	29–64	54–79	4
<i>Orchomene</i> sp.	Amp	SC	A	280–445	8.9–38.3	19–81	165–273	5
<i>Paraceradocus gibber</i>	Amp	EDF	A	140	3.6–4.2	5–16	39–50	3
<i>Waldeckia obesa</i>	Amp	SC	A	280	1.2	40	409	1
<i>Notocrangon antarcticus</i>	Dec	EDF	A	280	6.5	36	59	1
<i>Aega antarctica</i>	Iso	ECTO	A	280–430	1.0–6.8	6–21	85–157	3
<i>Ceratoserolis trilobitoides</i>	Iso	EPOSC	A	140	2.9	20–26	51–68	2
<i>Paranthura antarctica</i>	Iso	EP	A	280	8.7	261	541	1
<i>Antarctomysis maxima</i>	Mys	BP	A	280	1.8–3.4	7–36	53–90	3
<i>Ampelisca bowieri</i>	Amp	ESFDF	B	415	7.3	53	131	1
<i>Echiniphimedia scotti</i>	Amp	EMP	B	445	0.7	25	84	1
<i>Epimeria robusta</i>	Amp	EPDF	B	415	5.4	34	122	1
<i>Eusirus</i> sp.	Amp	EPOSC	B	415–445	2.0–13.3	17–51	61–69	4
<i>Leucothoe spinicarpa</i>	Amp	ASS	B	445	23.5	38	166	1
<i>Orchomene</i> sp.	Amp	SC	B	445	5.1	160	677	1
<i>Waldeckia obesa</i>	Amp	SC	B	445	0.6	38	211	1
<i>Glyptonotus antarcticus</i>	Iso	EPOSC	B	445	5.3	36	72	1
<i>Natatolana</i> sp.	Iso	SC	B	445	4.6	37	105	1

Notation see Table 2; Trophic type: EMP = epibenthic specialised micropredators (browsers), SP = endobenthic (subsurface) predators, ECTO = ectoparasites on fish, ASS = associates (symbionts and parasites) (see text for more details and references); Pb is not displayed because most values are close to the limit of detection (Table 1): 0.4–1.0 mg kg⁻¹ DW for most collectives analysed.

land Sea (0.1 vs. 50 mg Cd kg⁻¹ DW and 3 vs. 37 mg Cu kg⁻¹) with the sole exception of Zn, where differences were less pronounced or absent. This applies also to the decapods *Chorismus antarcticus* and *Notocrangon antarcticus* when Cd and Cu concentrations in eggs are compared with those in adult females (this study).

Theoretical considerations have suggested metabolic requirements for decapod crustaceans from temperate waters such that about 7–15 mg kg⁻¹ DW of total body Cu would be sufficient for enzymatic requirements and about 25 mg kg⁻¹ would satisfy the haemocyanin component demand, giving a total of about 30–40 mg Cu kg⁻¹ (RAINBOW, 1993; RAINBOW, 1996). The relatively low Cu concentrations in egg samples of decapods found in the present study, but also in juvenile amphipods, support the hypothesis that the enzymatic requirements and haemocyanin component demand for Cu in early life-history stages are probably not met without a distinct bioaccumulation of this essential element after hatching.

This view is supported by the fact that for many amphipods from temperate or polar waters a high potential for bioaccumulation of copper was reported on the basis of toxicokinetic studies, yielding the following bioconcentration factors (BCFs): 1400 (*Themisto abyssorum*; Greenland Sea; RITTERHOFF and ZAUKE, 1997a; RITTERHOFF and ZAUKE, 1997b);

Table 6. Interspecific heterogeneities of cadmium, copper and zinc in crustaceans from the Weddell Sea (Nov–Dec 2003). Results of singular samples where statistical evaluations (see Table 4) are not applicable ($N < 6$). Values are ranges; sampling areas see Figure 1.

Collective	Taxon	Trophic type	Area	Depth	Cd	Cu	Zn	N
<i>Abyssorhomene charcoti</i>	Amp	SC	C	406	3.1	21	242	1
<i>Abyssorhomene plebs</i>	Amp	SC	C	406	1.6–3.7	50–57	74–108	3
<i>Alexandrella n. sp.</i>	Amp	EP	C	337	2.7	8	118	1
<i>Andaniotes linearis</i>	Amp	ASS	C	274	2.2	15	73	1
<i>Cylopus lucasi</i>	Amp	PP	E	452–506	4.2–5.7	36–48	74–108	3
<i>Echiniphimedia echinata</i>	Amp	EMP	C	295	6.6	49	66	1
<i>Echiniphimedia hodgsoni</i>	Amp	EMP	C	295	2.1	28	99	1
<i>Epimeriella cf walkeri</i>	Amp	EP	C	244–268	2.2–3.0	5–20	48–77	3
<i>Eusirus antarcticus</i>	Amp	EPOSC	C	309–394	6.8–17.7	9–48	41–74	4
<i>Eusirus antarcticus, leo</i>	Amp	EPOSC	C	268	10.3	17	64	1
<i>Eusirus perdentatus</i>	Amp	EP	C	268	15.5–32.2	47–75	72–94	3
<i>Gnathiphimedia mandibularis</i>	Amp	EMP	C	228–268	0.6–4.9	11–18	212–395	2
<i>Hippomedon major</i>	Amp	EDF	C	268–279	4.7–8.6	26–207	386–913	2
<i>Hippomedon sp.</i>	Amp	EDF	C	252–406	8.1–20.4	95–112	98–134	3
<i>Hironellea antarctica</i>	Amp	EPOSC	C	279–406	5.3–7.7	7–13	107–440	2
<i>Iphimediella bransfieldi</i>	Amp	EP	C	268–333	1.9–23.9	18–23	255–1244	2
<i>Iphimediella rigida</i>	Amp	EP	C	244–337	0.3–0.8	18–27	97–120	2
<i>Iphimediella sp.</i>	Amp	EP	C	288	24.6	17	329	1
<i>Jassa goniamera</i>	Amp	ESF	C	268–309	2.4–3.0	8–11	60–73	3
<i>Liljeborgia georgiana</i>	Amp	EPDF	C	268–337	1.1–2.1	15–19	72–80	2
<i>Orchomenella cf ultima</i>	Amp	EDF	E	524–835	2.0–4.2	31–37	178–274	3
<i>Orchomenopsis acanthura</i>	Amp	EDF	C	228–406	23.6–34.4	45–223	110–155	3
<i>Orchomenopsis pinguides</i>	Amp	SC	C	253–394	3.0–5.7	29–49	88–158	4
<i>Paraceradocus gibber</i>	Amp	EDF	C	268–274	5.4–6.0	9–18	54–62	2
<i>Pseudorhomene coatsi</i>	Amp	SC	E	526	3.0	31	192	1
<i>Pseudorhomene n. sp.</i>	Amp	?	E	526	2.8	35	155	1
<i>Tryphosella cf intermedia</i>	Amp	EPOSC	C	394–406	8.7–13.4	50–120	105–205	2
<i>Tryphosella sp.</i>	Amp	EPOSC	C	394	7.8–15.6	29–42	86–192	3
<i>Uristes gigas</i>	Amp	EDFOSC	C	288	0.9	48	62	1
<i>Accalathura gigantissima</i>	Iso	EP	C	337	5.1	100	280	1
<i>Anuropus (antarcticus)</i>	Iso	EP	E	456	8.4–10.7	70–80	47–50	3
<i>Ceratoserolis trilobitoides</i>	Iso	EPOSC	C	274–288	0.9–1.8	34–37	47–49	2
<i>Natatalana sp.</i>	Iso	SC	C/E	288–524	1.6–5.5	9–73	76–151	2

Notation see Table 2; Trophic type: PP = pelagic predator, ESF = epibenthic suspension feeder (see text for more details and references), Pb is not displayed because most values are close to the limit of detection (Table 1): 0.4–0.8 mg kg⁻¹ DW for most collectives analysed.

400–780 (*Chaetogammarus marinus*; estuaries Devon UK; CLASON *et al.*, 2004b); 1700–2900 (*Paramoera walkeri*; Casey station; Antarctic; CLASON *et al.*, 2003) and 4032 (*Abyssorhomene plebs*; synonymous to *Orchomene plebs*; Weddell Sea; KAHLE and ZAUKE, 2003a). Estimated enzymatic requirements for Zn in decapods are about 20 mg kg⁻¹ (RAINBOW, 1993; RAINBOW, 1996), which is far below the concentrations in juveniles and eggs reported in this study. Most interestingly, the potential for bioaccumulation of Zn is less pronounced compared to Cu or even absent in the studies cited above, yielding the following BCFs: 190 in *T. abyssorum*; absent in *C. marinus*; 670–2400 in *P. walkeri*. Only for *A. plebs* was the BCF reported for Zn (5200) high enough to be comparable to Cu.

We can infer from these data that body surfaces of juveniles in the brood pouch and eggs are probably not permeable to Cu (and Cd), in contrast to older life-history stages where bioavailable element species are taken up across external or internal permeable body surfaces from surrounding water or from food (VIARENGO and NOTT, 1993; WANG, 2002).

Results compiled in Figure 2 do not indicate a size-dependent bioaccumulation of Cd, Cu and Zn in the amphipods and decapods analysed. Information from the literature on this issue is contradictory and inconsistent. For *Pandalus borealis* from the Barents Sea a size-dependent increase of Cd and Cu was reported from smaller to larger specimens (ZAUKE and SCHMALENBACH, 2006), in good agreement with the hypothesis discussed above. Pronounced intraspecific heterogeneities were also reported for some Antarctic crustaceans such as the isopod *Ceratoserolis trilobitoides*, the decapod *Notocrangon antarcticus* and the amphipod *Eusirus propeperdentatus* (ZAUKE and PETRI, 1993), showing an increase of whole-body Cu concentrations with increasing body length for the decapod and the amphipod. The detected heterogeneity in the isopod was related to the sex status. In both studies such increase was absent for Zn, in agreement with the present paper. Contradictory results were obtained for estuarine gammarids in toxicokinetic studies, where kinetic model parameters (k_1 : rate constants for uptake and BCF: bioconcentration factors) for Cd, Cu, Pb and Zn decreased with increasing body length of the amphipod *Gammarus zaddachi* (WANG and ZAUKE, 2004). A similar tendency was reported for the amphipod *A. plebs* from the Weddell Sea regarding field as well as experimental conditions (KAHLE and ZAUKE, 2003a).

Not only sex and body length may be important in determining accumulated metal concentrations in amphipods, but also the fecundity status of the females, as has been shown for gammarids from a Weser estuary modelling study (BÄUMER *et al.*, 1991). Not taking into account such effects may lead to a bias in the study if, for example, spatially or temporally different samples are composed of differently sized organisms. However, this is not likely in our study due to the absence of metal-size relationships at least in some of the species investigated.

The dry weight (DW) to body length (BL) relationships in three amphipod and one decapod species can be appropriately expressed by a power function (Figure 2). Due to linear relationships between wet weight (WW) and DW (with constants as 0 and slopes (R^2 -values) as 2.65 (0.89) for *P. carinata*, 2.83 (0.97) for *T. murrayi*, 2.73 (0.97) for *W. obesa* and 3.80 (0.99) for *C. antarcticus*) the exponents of the power model give the same results for WW-BL and DW-BL, respectively. Exponents near to the value 3 indicate that the organisms grow symmetrically (or isometrically) while deviations from the value 3 indicate an allometric growth; for values > 3 the organism becomes "heavier for its length" as it grows larger. Our data varying between 1.9 (*P. carinata*) and 3.7 (*C. antarcticus*) thus indicate different body shapes of the organisms investigated. Exponents of the power model near 3 were reported for the estuarine amphipod *Gammarus zaddachi* (WANG and ZAUKE, 2002, there misleadingly related to allometric growth) and the Antarctic amphipod *Paramoera walkeri* (CLASON *et al.*, 2003). For the hyperiid Arctic amphipod *Themisto libellula* a slightly smaller value (2.6) was reported, indicating an allometric growth (RITTERHOFF and ZAUKE, 1997c), as was found here for *T. murrayi* and *W. obesa* of this study.

There is limited information available to explore possible spatial or temporal heterogeneities of metals in crustaceans in this study. In the 1999 survey two separated areas were considered (Figure 1, A and B, north to south distance about 5°). Due to the low number of replicates (at times single measurements) only a preliminary assessment is possible. However, information compiled in Table 5 does not suggest a consistent tendency for 5 species of amphipods. Conversely, a clear trend of increasing Cd concentrations from south to north (distance about 10°) was reported for the decapod *P. borealis* from the Barents Sea (0.7 vs. 4.7 mg kg⁻¹ DW; ZAUKE and SCHMALENBACH, 2006) and from Greenland waters (1.3 vs. 5.2 mg kg⁻¹; RIGET *et al.*, 2004 and the literature cited therein).

Of the large number of species analysed in this study, only seven occurred in both surveys: *Ceratoserolis trilobitoides*, *Chorismus antarcticus*, *Eusirus perdentatus*, *Glyptonotus antarcticus*, *Paraceradocus gibber* and *Waldeckia obesa*. Results can be compared by linear regression, which will result in a slope equal to one and a constant equal to zero in case of a complete agreement between the two surveys. For Cu we find $Cu_{1999} = 0.73 \cdot Cu_{2003} + 5$ ($R^2 = 0.922$), indicating slightly higher Cu concentrations in the 2003 survey. For Cd and Zn this analysis is hampered by the fact that the regression is largely determined by one pair of high observed values, respectively, involving *E. perdentatus* with 15.2 (1999) vs. 23.0 (2003) mg Cd kg⁻¹ and *W. obesa* with 310 vs. 382 mg Zn kg⁻¹. Pb is low in both surveys. Only for *C. antarcticus* (Table 2 and 3) do we have sufficient numbers of replicates for a statistical analysis, indicating significantly higher Cd concentrations in 1999 vs. 2003 (separate- $t = 4.02$; $df = 12$; $P = 0.002$), lower Cu concentrations in 1999 vs. 2003 (pooled- $t = -2.72$; $df = 43$; $P = 0.001$) and equal Zn concentrations (separate- $t = 1.30$; $df = 25$; $P = 0.205$). Although samples from both surveys were obtained from the same geographical region, it is impossible to make any inference about temporal trends, since only two temporal observations are available as yet.

4.2. Interspecific Heterogeneities of Metals in Crustaceans

Heavy-metal concentrations in various marine crustaceans reported in the literature are compiled in Table 7. Most often Pb concentrations are low (< 1 mg kg⁻¹) in agreement with results of the present study, probably indicating regional or even global background values in the organisms under study. Slightly enhanced concentrations (> 2 mg kg⁻¹) were reported for some gammaridean amphipods from temperate waters, which might be related to anthropogenic influences, but also for the isopod *G. antarcticus* from Elephant Island, where such influence is very unlikely.

Low Pb concentrations in organisms indicate a low bioavailability of this element in seawater. This conclusion is supported by low reported Pb concentrations in the Weddell Sea (3.3–9.3 ng l⁻¹; FLEGAL *et al.*, 1993; SANUDO-WILHELMY *et al.*, 2002) compared to other ocean waters and is consistent with findings that Pb is particle-reactive and characterised by scavenging (HENDERSON and MAIER-REIMER, 2002; LIN *et al.*, 2000). Furthermore, zooplankton may be effective in removing this nonessential element by uptake from seawater and by grazing, packaging it into rapidly sinking faecal pellets (WANG, 2002), depending on the biomass. Thus, Pb is deposited in sediments and is not available in the water column. On the other hand, many marine crustaceans show a high potential for bioaccumulation of available lead in toxicokinetic experiments, resulting in high BCFs especially in polar waters: *e.g.*, 1800 in *T. abyssorum* (RITTERHOFF and ZAUKE, 1997b); 1600 – 6300 in *P. walkeri* (CLASON *et al.*, 2003) and 2900 in *A. plebs* (KAHLE and ZAUKE, 2003a). The time scale for uptake and depuration of Pb in these studies is large enough to suggest incorporation into biological tissues as the relevant mechanism, due to biological half lives ranging from 5 to 23 days. Conversely, physical adsorption onto the exoskeleton would occur within much shorter time intervals (minutes to hours).

For decapod crustaceans reported Cd concentrations (Table 7) are largely high, in agreement with the present study (Tables 2–3). This phenomenon had frequently been referred to as the hypothesis of a polar “Cd anomaly” (BARGAGLI *et al.*, 1996; PETRI and ZAUKE, 1993; ZAUKE and SCHMALENBACH, 2006), but it can be extended to other deep sea waters like the Iberian Deep Sea Plain (PROWE *et al.*, 2006). It is interesting to note that, in contrast to Pb, Cd concentrations in Weddell Sea water are relatively high (60–94 ng l⁻¹; NOLTING and DEBAAR, 1994; WESTERLUND and ÖHMAN, 1991) compared to other ocean waters. This also applies to some Antarctic sediments (*e.g.*, Ross Sea) compared to Arctic sediments (Greenland, Alaska) as compiled by BARGAGLI (2005, p. 230). The enhanced bioavailability of Cd

Table 7. Mean trace-metal concentrations in amphipods, decapods and isopods from different regions of the world [mg kg^{-1} DW].

Species	Taxon	Region	Cd	Cu	Pb	Zn	Ref
<i>Chaetogammarus marinus</i> *	Amp	estuaries, Devon, UK	0.2	79	1.5	80	1
<i>Gammarus locusta</i> *	Amp	North Sea, Helgoland	0.1	64	2.0	51	2
<i>Gammarus zaddachi</i> *	Amp	Ems-estuary, Germany	0.1	83	2.8	79	2
<i>Gammarus oceanicus</i> *	Amp	Grunnfjord, northern Norway	0.4	20	2.0	90	3
<i>Hyperia</i> sp.	Amp	northern North Sea	51.0	26	1.8	72	4
<i>Abyssochomene plebs</i> *	Amp	Weddell Sea, Antarctic	10.5	21	0.7	98	5
<i>Paraceradocus gibber</i>	Amp	Elephant-I., Antarctic	3.8	53	1.4	63	6
<i>Paramoera walkeri</i> *	Amp	Casey Station, Antarctic	2.8	19	0.7	67	7
<i>Paramoera walkeri</i> *	Amp	Ross Sea, Antarctic	4.8	14	0.1	78	15
<i>Themisto abyssorum</i>	Amp	Greenland Sea, Arctic	28.0	24	1.0	92	8
<i>Themisto abyssorum</i>	Amp	Fram Strait, Arctic	28.0	22	1.2	86	8
<i>Themisto abyssorum</i>	Amp	Barents Sea, Arctic	10.5	9	< 0.4	110	9
<i>Themisto libellula</i>	Amp	Greenland Sea, Arctic	34.0	26	0.4	61	8
<i>Themisto libellula</i>	Amp	Fram Strait, Arctic	24.0	23	0.5	61	8
<i>Waldeckia obesa</i>	Amp	Robert-I., Antarctic	4.3	15	–	16	10
<i>Waldeckia obesa</i>	Amp	King-George-I., Antarctic	1.1	81	0.3	301	6
<i>Acantheephyra purpurea</i>	Dec	NO Atlantic	3.0	36	–	46	12
<i>Acantheephyra</i> spec.	Dec	Iberian Deep Sea Plain	6.1	56	0.6	52	13
<i>Bentheogennema intermedia</i>	Dec	Iberian Deep Sea Plain	10.7	36	0.4	74	13
<i>Benthescymus iridescens</i>	Dec	Iberian Deep Sea Plain	14.9	55	0.4	79	13
<i>Chorismus antarcticus</i>	Dec	Weddell Sea, Antarctic	13.0	93	1.6	44	6
<i>Hymenodora glacialis</i>	Dec	Greenland Sea, Arctic	6.7	16	< 0.3	37	8
<i>Hymenodora glacialis</i>	Dec	Fram Strait, Arctic	9.2	12	< 0.3	52	8
<i>Notocrangon antarcticus</i>	Dec	Weddell Sea, Antarctic	13.0	67	0.8	46	6
<i>Pandalus borealis</i>	Dec	Barents Sea, Arctic	1.6	61	< 0.4	79	9
<i>Sabinea sarsi</i>	Dec	Barents Sea, Arctic	4.3	68	< 0.4	59	9
<i>Sergia</i> spec.	Dec	Iberian Deep Sea Plain	1.9	17	0.5	67	13
<i>Systellaspis debilis</i>	Dec	Atlantic, African Coast	22.0	55	–	70	12
<i>Systellaspis debilis</i>	Dec	Atlantic, Azores	13.0	–	–	50	11
<i>Systellaspis debilis</i>	Dec	NO Atlantic	12.0	67	–	53	14
<i>Systellaspis debilis</i>	Dec	Iberian Deep Sea Plain	16.3	49	0.6	62	13
<i>Aega antarctica</i>	Iso	Weddell Sea, Antarctic	0.9	8	0.3	121	6
<i>Ceratoserolis trilobitoides</i>	Iso	Weddell Sea, Antarctic	2.0	42	0.4	40	6
<i>Glyptonotus antarcticus</i>	Iso	Robert-I., Antarctic	4.6	21	–	31	10
<i>Glyptonotus antarcticus</i>	Iso	Elephant-I., Antarctic	1.5	149	2.5	64	6
<i>Natatolana</i> spec.	Iso	Elephant-I., Antarctic	6.7	68	0.9	86	6

Notation see Table 2; * data of control organisms in toxicokinetic experiments; references: 1: CLASON *et al.* (2004b); 2: CLASON and ZAUKE (2000); 3: CLASON *et al.* (2004a); 4: ZAUKE *et al.* (1996a); 5: KAHLE and ZAUKE (2003a); 6: PETRI and ZAUKE (1993); 7: CLASON *et al.* (2003); 8: RITTERHOFF and ZAUKE (1997d); 9: ZAUKE and SCHMALENBACH (2006); 10: DEMORENO *et al.* (1997); 11: LEATHERLAND *et al.* (1973); 12: RIDOUT *et al.* (1989); 13: PROWE *et al.* (2006); 14: WHITE and RAINBOW (1987); 15: BARGAGLI (2001).

in Antarctic coastal marine environments is probably due to the rapid regeneration of the metal in the water column and/or to rapid mineralization in surface sediments during early diagenesis. During the austral summer the upwelling of waters favours the ad/absorption of Cd on phytoplankton cells, and primary consumers' uptake of metals directly from water and food, probably leading to high Cd concentrations in zooplankton as reported for some copepods from the Weddell Sea (3.7–10.2 mg kg^{-1} DW; KAHLE and ZAUKE, 2003b). Marine diatoms respond very rapidly to Cd by producing cysteine-rich polypeptides (phytochelatins)

– playing a major role in the storage of metals and their detoxification. Diatoms and benthic macro-algae, for example, at Terra Nova Bay, have mean Cd concentrations of about 2.5 mg kg^{-1} DW. Diatoms are taken up by sponges through their inhalant water systems or directly by other invertebrates such as the amphipod *P. walkerii*. Huge accumulation of Cd also occurs in the digestive gland and kidney of Antarctic molluscs like *Yoldia eightsii*, *Adamussium colbecki* and *Laternula elliptica* (BARGAGLI, 2005; p. 252, 270ff.).

With only a few exceptions, the Cu and Zn concentrations in decapods reported worldwide are within the same range of $40\text{--}90 \text{ mg Cu kg}^{-1}$ and $40\text{--}80 \text{ mg Zn kg}^{-1}$, in agreement with results of this study, indicating that these elements are regulated. Some lower reported Cu concentrations (*C. antarcticus*, *H. glacialis*, *Sergia* sp.; $12\text{--}29 \text{ mg kg}^{-1}$) suggest that these organisms might be suffering Cu deficiencies in a sense that the estimated total metabolic demand for this element ($30\text{--}40 \text{ mg kg}^{-1}$; see Section 4.1) has probably not been met. This is not the case for the reported range of Zn concentrations compared to estimated enzymatic requirements of 20 mg kg^{-1} (RAINBOW, 1996; RAINBOW and PHILLIPS, 1993). It has been hypothesised that a potential Cu deficiency might be related to an increased uptake of Cd due to insufficient selectivity of the uptake process for the essential element Cu, e.g., when metal-binding proteins are involved (RITTERHOFF and ZAUKE, 1998; ZAUKE and PETRI, 1993).

The hypothesis of a possible Cu deficiency for some crustaceans is, however, not supported by reported concentrations of dissolved essential elements in surface waters of the Weddell Sea shelf, which were higher than in surface waters of other marine environments (e.g., $30\text{--}300 \text{ ng Cu l}^{-1}$; $260\text{--}760 \text{ ng Zn l}^{-1}$; NOLTING and DEBAAR, 1994; WESTERLUND and ÖHMAN, 1991). In the Southern Ocean, bioutilised trace metals such as Zn, Cu and Ni do not usually show co-limitations with Fe and, under Fe-depleted conditions, their concentrations in waters are not decreased by algal uptake as expected (BARGAGLI, 2005; p. 212).

There is one exception not consistent with this view, viz. the high Cu concentrations we found for *Nematocarcinus lanceopes* (Table 3), reaching $169 \pm 56 \text{ mg kg}^{-1}$. Since this crustacean was caught at depths of $844\text{--}1525 \text{ m}$, high body Cu concentrations may result from higher amounts of dissolved copper in deeper waters; this would indicate, during the end of the summer and in absence of a well-defined water column stratification, a “scavenging-type” distribution which overlaps its “nutrient-type” behaviour (FRACHE *et al.*, 2001). However, this does not apply generally to other deep-sea decapod crustaceans, as can be seen from Table 7. Furthermore, available data on the vertical distribution of Cu in the water column of the Weddell Sea do not indicate a distinct increase of Cu with depth during the austral summer (NOLTING and DEBAAR, 1994; WESTERLUND and ÖHMAN, 1991). Thus, the different behavior between amphipods and decapods in the same marine environment could also be due to the different diet or detoxification mechanisms.

The most striking feature of this study is the enormous interspecific heterogeneity of Cd and Zn in the amphipod species investigated during both surveys (Tables 2–3 and 5–6), in good agreement with reported data compiled in Table 7. Generally, Cd concentrations in gammaridean amphipods from temperate waters are much lower than those in polar amphipods, despite the fact that potential anthropogenic influences are more likely in the waters mentioned at the top of Table 7 (consistent with higher reported Pb concentrations).

Moreover, the enormous heterogeneity of Cd and Zn in the amphipods investigated supports the hypothesis of the “Cd anomaly” and suggests that there is probably no consistent metabolic demand for the essential element Zn in this taxon, in contrast to decapod crustaceans as mentioned above. Different environmental conditions cannot account for our findings for amphipods, since the organisms were largely caught within the same area and water depth.

A relationship between the trophic types of the organisms investigated as described above (see Materials and Methods section) and accumulated metal concentrations is difficult to elucidate. From Table 3 it can be inferred that scavengers tend to show relatively low Cd but relatively high Zn concentrations. On the other hand, Cd concentrations in organisms which

are opportunistic scavengers (EPOSC), and in some other types, tend to be relatively high. Relatively high Zn concentrations in scavengers, but to some extent also in opportunistic scavengers, can be also found in Tables 5 and 6, but there are examples contradicting this view, for example the isopod *Natanolana* sp. (Table 5) or the amphipod *A. plebs* (Table 6). Furthermore, we can also find relatively high Cd concentrations in predators and opportunistic scavengers (EPOSC). These results support the hypothesis that the high diversity of trophic types among the Antarctic crustaceans investigated may account to some degree for the high variability of metal concentrations encountered in the different samples. However, even if the trophic type has a substantial influence on accumulated metal levels in organisms, it will interfere with other biological or physiological characteristics of the animals under study, which are largely unknown as yet. In this case variation of metal accumulation patterns between different (often related) taxa may occur irrespective of their trophic position.

5. Conclusion

In conclusion, our study provides further evidence for the frequently reported “Cd anomaly” in polar crustaceans including decapods, isopods and amphipods, and suggests that Pb concentrations well below 1 mg kg^{-1} might serve as a regional or even global background value for comparison in biomonitoring studies. Increasing Cu concentrations from eggs of decapods or juveniles in the brood pouch of an amphipods species to adult females indicate that the enzymatic requirements and haemocyanin component demand for Cu in early life-history stages is probably not met without a distinct bioaccumulation of this essential element after hatching. Most interestingly, Cd also increases. This could be the consequence of efficient uptake mechanisms for Cu that cannot discriminate between this element and Cd. In contrast to the early life-history stages, we did not find a size-dependent bioaccumulation of Cd, Cu and Zn in adult decapods and amphipods. Cu and Zn concentrations in decapods are largely within the range reported worldwide, indicating that these elements are regulated. The enormous heterogeneity of Cd and Zn in many amphipod species suggests largely varying accumulation strategies and that there is probably no consistent metabolic demand for essential elements in this taxonomic group; this possibility should be investigated in more detail in future studies involving, for example, investigations on the intracellular sequestration of these elements. Finally, there are some indications that the trophic type of the organisms investigated might be, at least partially, related to the accumulated metal concentrations.

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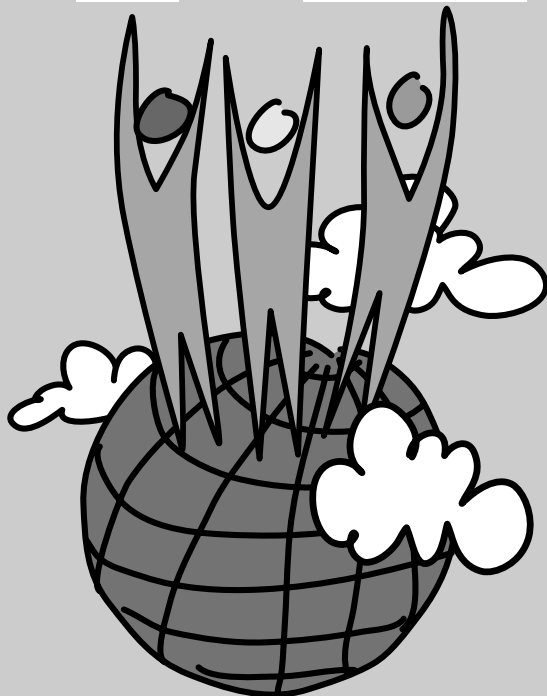
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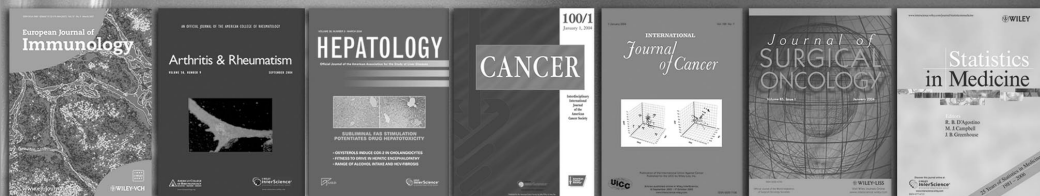
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