Heavy Metals in Zooplankton and Decapod Crustaceans from the Barents Sea[*]

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Abstract

Trace metals (Cd, Cu, Pb and Zn) were analysed in zooplankton samples and decapod crustaceans collected on cruises of "RV Walther Herwig III" to the Barents Sea (Summer 1991, 1994 and 2000). We found a substantial spatial heterogeneity in the decapod crustacean *Pandalus borealis*, with increasing Cd concentrations from the south (North Cape Bank; 0.7 mg kg^{-1} DW) to the north (north of Svalbard; 4.7 mg kg^{-1}), supporting the hypothesis that the frequently reported Cd-anomaly in polar crustaceans might be extended to the Barents Sea. Regarding various crustaceans and zooplankton collectives (2000) a distinct interspecific heterogeneity of metals was obvious, with lowest Cd concentrations in euphausiids and chaetognaths and highest ones in decapods and hyperiid amphipods; lowest Cu concentrations in chaetognaths and copepods and highest ones in euphausiids and decapods; and lowest Zn concentrations in euphausiids and decapods and highest ones in some copepods. For Pb many values were below or close to the limit of detection, suggesting that Pb concentrations about 0.4 mg kg^{-1} might serve as a regional background value. Results for Cd, Cu and Zn in copepods of this study are largely within the reported range, but high Cd concentrations in copepods from summer in contrast to reported lower ones during winter/spring may be related either to changing accumulation strategies of the copepod species involved or to seasonally changing Cd absorption in copepods from food.

keywords: biomonitoring; trace metals; zooplankton; Cd-anomaly; spatial, intra- and interspecific heterogeneity

1. Introduction

Contamination of the Arctic marine ecosystems with trace metals and other xenobiotics receives continued attention in the scientific literature and international environmental programmes (AMAP, 2004; OSPAR Commission, 2000; Riget et al., 2003). 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 from field samples are often much higher than BCFs estimated from experiments regarding dissolved uptake (Kahle and Zauke, 2002b). 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 are often varying specifically as can be explained and predicted by toxicokinetic modelling (Clason et al., 2004; Luoma and Rainbow, 2005). As a result, we frequently can find different species of zooplankton with greatly varying metal concentrations in the same body of ocean water (Kahle and Zauke, 2003; Petri and Zauke, 1993; Ritterhoff and Zauke, 1997d).

That zooplankton play an important role in the biogeochemical cycling of metals in marine systems in general is well known, especially regarding particle-reactive metals in the water column (Fisher et al., 1991; Lee and Fisher, 1994; Stewart et al., 2005). Thus, in several studies macro- and meso-zooplankton organisms have been specifically used as biomonitors for assessment of the bioavailability of elements in marine systems, covering a variety of spatial and temporal scales (e.g. Kahle and Zauke, 2003; Ritterhoff and Zauke, 1997d; Zauke et al., 1996a). Their potential suitability is largely due to their worldwide presence, their major role in the food webs and their high contribution to the total biomass in marine systems.

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). Those 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 others.

Studies on zooplankton from polar regions have revealed particularly high Cd concentrations referred to as 'Cd-anomaly' (Bargagli et al., 1996; Petri and Zauke, 1993; Ritterhoff and Zauke, 1997d). It was hypothesized that they are related to effective but unselective uptake mechanisms which had evolved due to potential metabolic deficiencies in Cu in the Antarctic Ocean. These findings suggest that probably no global background values can be defined. Instead, regional background values should be established in order to follow possible future trends of metals in biomonitors.

An increasing body of information on metals in marine zooplankton has become available for specific regions such as the North Sea, the Greenland Sea and the Weddell Sea as intensively discussed in Zauke et al. (1996a), Ritterhoff and Zauke (1997d) and Kahle and Zauke

(2003). For other areas such as the Barents Sea recent information on metals in offshore zooplankton and crustaceans is largely lacking, in contrast to other components of the marine food web like inshore benthic invertebrates (Zauke et al., 2003), fish (Zauke et al., 1999), sea birds (Savinov et al., 2003) and marine mammals (Booth and Zeller, 2005).The Barents Sea is an important area for commercial fisheries; with total annual yields reaching 3-4 million tons, it is comparable to Icelandic waters and the Norwegian Sea (Gjosaeter, 1995; Hansen et al., 1996). Because potential contamination of fish with heavy metals due to the marine food web might be crucial for the future exploitation of this resource, information on this issue is urgently needed.

The main goal of this paper is to provide further evidence for a Cd-anomaly in decapod crustaceans from Arctic waters. This hypothesis would be supported by high Cd concentrations compared to literature data from other regions and particularly by an increasing Cd gradient from the south to the north and will be tested using the decapod crustacean *Pandalus borealis*. Because the samples were taken in different years and the size of organisms varied largely, temporal and intraspecific heterogeneities of metals in *P. borealis* had to be evaluated in advance. Furthermore, other zooplankton taxa like copepods were investigated to test their suitability for establishing some regional background values to be used in future biomonitoring studies.

2. Materials and methods

2.1. Sampling and sample preparation

Samples were collected from FRV "Walther Herwig III" (cruise 217, May/June, 2000) in four regions of the Norwegian Sea and the Barents Sea at 25 stations (Fig. 1): area 1, North Cape Bank (71°30.5 'N -71°37.5 'N and 24°50.0 'E -25°13.8 'E, 250-300 m depth); area 2, Central Bank (74°40.6 'N -75°22.2 'N and 31°49.7 'E -32°05.0 'E, 100-320 m depth); area 3, Spitsbergen Bellsund Bank, Hornsund Bank and South Cape Bank (76°30.3 'N -77°26.5 'N and 12°29.9 'E -14°14.5 'E; 220-300m depth) and area 4, Bear Island (74°11.5 'N-74°35.5 'N and 16°09.5 'E-17°26.5 'E, 200-480 m depth). Additional data were evaluated originating from two other cruises of Walther Herwig II and III in the same region; namely cruise 116, July, 1991 at 2 stations (area 5, north of Svalbard: 81°18.0 'N and 18°40.0 'E, 400-500 m depth) and cruise 150, August, 1994 at 3 stations (area 3, Spitsbergen Bellsund Bank: 77°14.4 'N and 12°49.2 'E, 204-211 m depth and area 2, Central Bank: 75°13.6 'N and 32°26.3 'E, 274 m depth).

Zooplankton samples were taken with a vertically towed plankton net (the so-called 'Helgoländer Planktonnetz', a net originally used to catch ichthyoplankton, with a mesh size of 500 µm and a diameter of 1 m), which was hauled at 0.7 m sec^{-1} from depths of 200-460 m, depending on the station. Decapod samples originate from catches with the standard fishing trawl GOV (Chalut à Grande Ouverture Verticale) from depths of 170-330 m, with trawling time of 30 min at a trawling speed of 4 knots.

On board ship zooplankton from the 'Helgoländer Planktonnetz' was transferred to polyethylene buckets and kept alive in sea water collected from depths > 100 m for at least 1-3 h to allow for defecation. We assume complete defecation within this period as gut transit times in

copepods are less than 0.5 h at 18°C (Reinfelder and Fisher, 1991). The animals were then sorted, still alive, under a binocular microscope to the species level, yielding the following samples: *Calanus finmarchicus* (Gunnerus, 1765); *Calanus hyperboreus* Krøyer, 1838; *Euchaeta norvegica* (Boeck, 1872) and *Metridia longa* (Lubbock, 1854) (Copepoda); *Themisto abyssorum* (Boeck, 1870) and *Themisto libellula* (Mandt, 1867) (Amphipoda); *Meganyctiphanes norvegica* (M. Sars, 1875) (Euphausiacea) and *Eukronia hamata* (Möbius, 1875) (Chaetognatha). The decapods from the GOV were treated likewise, yielding the following samples: *Sabinea sarsi* Smith, 1879 and *Pandalus borealis* Krøyer, 1844. The size of *P. borealis* was classified as follows (rostrum to telson): small (5.0-7.5 cm), medium (7.5-10.5 cm), large (10.5-12.0 cm).

Subsequently, the zooplankton samples were briefly, the decapods thoroughly rinsed with double-distilled water, dried on good-quality filter paper and stored at −30°C in Eppendorf caps (2 ml, polypropylene), Petri dishes or good-quality polyethylene bags, depending on their size and number (normally consisting of 20 specimens of *P. borealis* and 7 specimens of *S. sarsi*). To assure the quality of the sampling procedure, we followed the guidelines set up in Zauke et al. (1996a) and Zauke and Petri (1993). The risk of contamination was largely reduced by, firstly, keeping the animals in sea water or on high-quality filter paper whenever possible. Secondly, the absence of contaminating particles such as paint particles was guaranteed by close visual examination of each specimen collected.

2.2. Analytical procedures

Upon arrival in the laboratory in Oldenburg, the frozen crustacean samples were subjected to freeze-drying for 72 h to remove remaining water (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 HNO_3 (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} (µg g^{-1}) dry weight (DW). We obtained the following fresh weight / dry weight ratios: 4.1 for *P. borealis* and *S. sarsi*, 4.7 for *M. norvegica*, 5.1 for *E. norvegica* and *T. abyssorum*, 5.8 for *M. longa*, 6.2 for *C. finmarchicus*, 8.4 for *C. hyperboreus*, and 9.9 for *E. hamata*.

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 (see 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 excellent 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 marine zooplankton and crustaceans. Only for Pb we find many values below or close to the limit of detection. Thus, Pb was not considered in the statistical data evaluation.

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 to be robust against deviations 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 for *P. borealis* by the factors body size, time of sampling and the different study areas within the Barents Sea and adjacent waters as shown in Fig. 1 and for the zooplankton in general by the factor species affiliation. A multiway ANOVA was not applicable due to the resulting low degrees of freedom and the occurrence of empty cells in the different factor levels. Thus, these factors had to be analysed and discussed stepwise.

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), by non-classical Welch Test (not assuming equality of variances; WS) or by non-parametric Kruskal-Wallis Test (H). In case of two sample comparisons these tests yield the same results as the pooled or separate variance t-Test or the Mann-Whitney Test. 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 non-parametric multiple comparison Z statistic or 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.

3. Results

3.1. Size dependent metal concentrations in Pandalus borealis

Statistical results for size-dependent heterogeneities of metals in *P. borealis* are compiled in Table 2. Parametric tests are used because Lilliefors probabilities > 0.01 indicate that the sample data are distributed normally. The results vary depending on the area and element considered. Regarding area 2 and 3 we find statistically significant differences for Cd and Cu. Larger specimens (see note under Table 2 for definition of sizes) tend to have higher metal concentrations than smaller ones (2.3 – 2.5 vs. 1.3 – 1.4 mg Cd kg^{-1} and 50 - 112 vs. 40 – 50 mg Cu kg^{-1}, respectively). For Zn no significant differences could be detected. Regarding area 5 significant differences are identified for Cu and Zn showing the same size dependency as mentioned above (20 vs. 56 mg Cu kg^{-1} and 48 vs. 58 mg Zn kg^{-1}, respectively). No significant differences are obvious for Cd. The implication of these findings will be discussed more detailed below.

3.2. Temporal and spatial heterogeneities of metals in Pandalus borealis

Temporal heterogeneities of metals in *P. borealis* have to be analysed to decide whether the data can be pooled for the subsequent evaluation of the spatial heterogeneities which is one important goal of this study. For this purpose results are available for areas 2 and 3, unfortunately indicating a temporal heterogeneity for all elements analysed, with the sole exception of Cd regarding area 3, 1994 vs. 2000 ($H = 3.2$; $P = 0.072$; df = 1, 78). Therefore, the data cannot be pooled and are compiled (grouped by area and year) in Table 3 in an increasing sequence of metal concentrations, showing means and 95%-confidence intervals and results of the Lilliefors Test. Since for some groups the null hypothesis (normality of the data) has to be rejected (LIP values < 0.01), comparisons involving those groups is done using the Z statistic, otherwise using the NK procedure.

Despite an increased variability probably introduced by the factor time, a clear increasing spatial trend of Cd in *P. borealis* from south (area 1) to north (area 5) is indicated by the result of the Z statistic, while data from areas 2 and 3 are overlapping (Table 3). Such a trend is missing for Cu and Zn, despite some spatial heterogeneities occurring between areas 2 and 3 in 1994 (Cu: $H = 14.3$; $P = 0.000$; df = 1, 84; Zn: $F = 65.2$; $P = 0.000$; df = 1, 83; notation see Table 2) and between areas 1, 2 and 3 in 2000 (Cu: $H = 30.5$; $P = 0.000$; df = 2, 43; Zn: WS = 9.2; $P = 0.001$; df = 2, 21).

3.3. Interspecific heterogeneities of metals in decapod crustaceans and zooplankton

Results on metal concentrations in various crustaceans and zooplankton collectives (2000) are compiled in Table 4, showing means and 95%-confidence intervals and results of the Lilliefors Test. Since the null hypothesis (normality of the data) has to be rejected only in a few cases (LIP values < 0.01) robust parametric tests are employed. Tests of global null hypotheses (equality of means) indicate significant interspecific heterogeneities for all elements considered (Cd: WS = 78.8; $P = 0.000$; df = 7, 31; Cu: WS = 49.7; $P = 0.000$; df = 8, 24; Zn: WS = 63.3; $P = 0.000$; df = 8, 25). A more detailed analysis of heterogeneities is given by re-

sults of the robust NK procedure (Table 4). For Cd we find lowest concentrations in euphausiids and chaetognaths and highest ones in hyperiid amphipods; for Cu lowest ones in chaetognaths and copepods and highest ones in euphausiids and decapods; and for Zn lowest ones in euphausiids and decapods and highest ones in some copepods. For Pb a statistical comparison is not applicable because many values are below or close to the limit of detection (0.4 mg kg^{-1}); slightly increased values are only obtained for *Themisto abyssorum* (mean ± 95%-confidence interval: 0.8 ± 0.5 mg kg^{-1} d.w., N=6), *Pandalus borealis* (1.0 ± 0.2 mg kg^{-1}, N=47) and *Sabinea sarsi* (1.5 ± 0.3 mg kg^{-1}, N=6).

4. Discussion

4.1. Heterogeneities of metals in Pandalus borealis

The size-dependent increase of Cd and Cu in *P. borealis* from smaller to larger specimens is in good agreement with results reported by Ritterhoff and Zauke (1997c) for the amphipod *Themisto libellula* from the Greenland Sea. Pronounced intraspecific heterogeneities were also reported for some Antarctic crustaceans like 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 contrast to the present paper. Contradictory results were, however, also 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). 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 different samples are composed of differently sized organisms. This is not the case in our study, because samples of *P. borealis* from all areas and times of sampling largely consisted of all size groups. However, since the organisms were analysed individually, we have to encounter an increased variability in all groups, eventually preventing detection of more significant differences in the subsequent statistical analyses.

Distinct spatial heterogeneities of metals in organisms as shown in this study for *P. borealis* (Table 3) may be caused by different metal speciation and hence bioavailabilities between water masses due to variations in the salinity (psu). Information on water masses in our investigation areas (AMAP, 1998; OSPAR Commission, 2000) suggest, however, that the reported salinity differences are too low (North Cape Bank: 34.7 – 34.9 psu; Central Bank: 34.8 – 35.0 psu; Spitsbergen Bellsund Bank: 34.1 – 34.8 psu) to lead to such an effect. Furthermore, there is no evidence of distinctly different metal concentrations in seawater in the Arctic, supporting the hypothesis that the observed spatial differences especially of Cd in *P. borealis* might be related to different accumulation strategies in organisms from our investigation areas (see next sections for further discussion).

4.2. Interspecific heterogeneities of metals in decapod crustaceans and zooplankton

From Table 4 a distinct interspecific heterogeneity of metal concentrations is obvious. Regarding results of the NK procedure it must be taken into account that this test represents a multiple comparison option. If the data vary to a great extent, then possible differences in the lower range might be hidden by higher values, as has been found for Cu. Thus, when only copepods are taken into account then *C. hyperboreus* is significantly different from *C. finmarchicus* and *M. longa*, and these in turn are significantly different from *E. norvegica*. A similar phenomenon was reported by Ritterhoff and Zauke (1997d).

Heavy-metal concentrations in various marine pelagic crustaceans and in zooplankton reported in the literature are compiled in Tables 5 and 6. Generally, Pb concentrations are low (<0.3 – 2.7 mg kg^{-1}) in agreement with results of the present study, probably indicating some regional background values in the organisms under study. Regarding Cd, Cu and Zn the reported patterns are also in good agreement with the interspecific heterogeneities obtained in this study, *viz.* low Cd concentrations in euphausiids (about 0.2 mg kg^{-1}) and high ones in decapods and amphipods (10-50 mg kg^{-1}); low Cu concentrations in copepods (4-10 mg kg^{-1}) and high ones in euphausiids and decapods (30-70 mg kg^{-1}); and finally intermediate Zn concentrations in almost all taxa (30-90 mg kg^{-1}) with the sole exception of copepods, where especially in samples of *Euchaeta* and *Metridia* high concentrations were found (160-500 mg kg^{-1}).

4.3. Hypothesis of a Cd-anomaly and Cu-deficiency in decapods inferred from literature data

The phenomenon of a polar Cd-anomaly which has been intensively discussed in the literature (e.g. Bargagli et al., 1996; Bustamante et al., 1998; Petri and Zauke, 1993; Ritterhoff and Zauke, 1997d) refers to the fact that we find species with low and high Cd concentrations in the same water mass and/or that polar Cd concentrations are higher than values from other regions. Our results compiled in Tables 3 and 4 support this idea, especially regarding the decapod crustacean *Sabinea sarsi*, the copepods *Calanus hyperboreus* and *C. finmarchicus* and the amphipod *Themisto abyssorum*. This view is further supported by the pronounced increase of Cd in *Pandalus borealis* in this study (Table 3), from the North Cape Bank (0.7 mg kg^{-1}; 71°30.5 'N) to waters north of Svalbard (4.7 mg kg^{-1}; 81°18.0 'N).

It is striking that a similar Cd gradient regarding whole-body concentrations (geometric means) was reported for *P. borealis* from western and eastern Greenland waters for the years 1985-1987 (cited from Dietz et al., 1998, p. 485): Nanortalik (1.8 mg kg^{-1}; 60.2°N), Nuuk (2.0 mg kg^{-1}; 64.2°N), Maniitsoq (1.3 mg kg^{-1}; 65.4°N), Ammassalik (2.4 mg kg^{-1}; 65.6°N), Sisimiut (1.8 mg kg^{-1}; 66.9°N), Kangatsiaq (2.6 mg kg^{-1}; 68.3°N), Qeqertarssuaq (4.1- 5.1 mg kg^{-1}; 69.8°N), Uummannaq (2.4 - 4.3 mg kg^{-1}; 71.5°N) and Upernavik (5.2 mg kg^{-1}; 74°N). As emphasized in a recent review (Riget et al., 2004 and the literature cited therein), substantial differences were also reported on more local scales, with two to five times greater Cd concentrations in *Mytlilus edulis* and *P. borealis* from the open sea compared to the inner fjord systems of Greenland.

Reported Cd concentrations in polar decapod crustaceans and hyperiid amphipods are also high (Table 5), but there are some indications that this phenomenon may be extended to

other marine areas like the Iberian Deep Sea Plain (Prowe et al., in press). Most of the regions mentioned so far are considered to be rather isolated, so that high Cd concentrations in polar and deep sea crustaceans cannot be related to anthropogenic influences. Regarding the Antarctic Ocean they are thought to indicate a Cu deficiency (Petri and Zauke, 1993; Zauke and Petri, 1993). It has been hypothesised that a potential copper 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) . Theoretical considerations have suggested metabolic requirements for decapods 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).Thus, some of the *P. borealis* analysed in this study (Table 3) and some decapod crustaceans from other polar regions and from the Iberian Deep Sea Plain (Table 5) might be suffering Cu deficiencies in a sense that the total metabolic requirements have probably not been met.

Zn concentrations in decapods of this study (Table 4) are largely within the range reported for comparable organisms in the literature (Table 5). These results support the conclusions of Rainbow and White (1989), that decapods are able to regulate their Zn body concentrations, probably leading to whole-body concentrations that are comparable worldwide. Estimated enzymatic requirements for Zn in decapods are about 20 mg kg^{-1} (Rainbow, 1993; Rainbow, 1996), which are met for *P. borealis* and *S. sarsi* in this study.

4.4. Further assessment of detected metal concentrations in zooplankton

Regarding copepods, high Cd concentrations found in this study (Table 4) are generally in agreement with data from other polar regions (Table 6, Weddell Sea, Fram Strait, Greenland Sea). Noteworthy are, however, deviations of Cd in copepods reported by Pohl (1992), with high Cd concentrations obtained from organisms collected from surface layers during the Arctic summer (July 1990), in agreement with our data, and by Ritterhoff and Zauke (1997d), who found low Cd concentrations in organisms collected from water depths down to 1500 m by the end of the Arctic winter. Possible explanations for these differences detected in field samples from the two studies may be related either to changing accumulation strategies of the copepods involved or to seasonally changing Cd absorption in copepods from food during summer when they intensively feed on phytoplankton, while the food uptake path is irrelevant in winter (see Ritterhoff and Zauke, 1997d for further discussion). Furthermore, local upwelling might be related to increased Cd bioavailabilities in seawater and hence increased uptake into copepods as hypothesised for the northern North Sea (Zauke et al., 1996a). Thus, we cannot regard our data for copepods as an indication of a Cd-anomaly without further evaluation. This might involve toxicokinetic experiments also in the summer time, since Ritterhoff and Zauke (1997a, b) have not found any relevant uptake of water-borne cadmium in bioaccumulation experiments performed during the winter cruise.

Even if Cu requirements for copepods are considered to be similar to those of decapods (which has to be proven in future studies) they must be lower, because copepods, but also

some hyperiid amphipods like *Themisto*, lack haemocyanin (Gruner et al., 1993). Thus, most of the data compiled in Tables 4 and 6 probably suggest some Cu deficiency for calanoid copepods.

5. Conclusions

Our study provides further evidence for the frequently reported Cd-anomaly in polar crustaceans, especially regarding a pronounced increase of Cd in *Pandalus borealis* from the North Cape Bank to waters north of Svalbard, in good agreement with a similar trend reported for Greenland waters. We suggest that this phenomenon might be related to a corresponding deficiency in essential trace elements like Cu. This could be the consequence of efficient uptake mechanisms for Zn or Cu that cannot discriminate between those elements and Cd. To test this hypothesis, further experimental studies are required, investigating in more detail, e.g., the role of metal-binding proteins in this process. Our data on zooplankton and crustacean samples suggest that Pb concentrations below 1 mg kg^{-1} might serve as a regional background value for comparison in biomonitoring studies. Size dependent, temporal, spatial as well as interspecific heterogeneities of metals detected in this study indicate that the organisms investigated might serve as suitable biomonitors. This has to be further evaluated by toxicokinetic bioaccumulation studies on board ship as previously reported for other polar waters (Kahle and Zauke, 2002a, b; Ritterhoff and Zauke, 1997a, b).

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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		CRM 278		NIST SRM 1566	
	(Lobster hepatopancreas)		(Mussel tissue)		(Oyster tissue)	
	analysed	certified	analysed	certified	analysed	certified
Pb	0.34 ± 0.04	0.35 ± 0.13	1.80 ± 0.08	2.00 ± 0.04	0.40-0.65[b]	0.48 ± 0.04
Cu	106 ± 5	106 ± 10	9.90 ± 0.25	9.45 ± 0.13	58-67[b]	63 ±3.5
Cd	25.8 ± 1.1	26.7 ± 0.6	0.33 ± 0.02	0.348 ± 0.007	3.0-3.6[b]	3.5 ± 0.4
Zn	183 ± 9[a]	180 ± 5	84 ± 4[a]	83.1 ± 1.7	870-1100[b]	852 ± 14

Numbers of independent determinations:[a] 11-13; [b] 4 (range); others: 16-19;
Limits of detection [mg kg^{-1} DW] (calculated as 2.6 standard deviations of a "low sample"; Büttner et al., 1980): Pb: 0.4; Cu 2.0; Cd: 0.12; Zn: 5.

Table 2

Size dependent heterogeneities of trace metal concentrations in *Pandalus borealis* (small, medium, large) from the Central Bank (2000), Spitsbergen Bellsund Bank (2000) and north of Svalbard (1991): Test of global null hypotheses.

	Area	LS	P	Test	Test statistic	P	df
Cd	2	1.4	0.289	F	11.4	0.001	2, 13
Cu	2	3.2	0.073	F	7.9	0.006	2, 13
Zn	2	2.5	0.125	F	2.0	0.183	2, 13
Cd	3	5.1	0.017	WS	23.2	0.000	2, 9
Cu	3	8.8	0.002	WS	39.7	0.000	2, 9
Zn	3	13.7	0.000	WS	2.0	0.195	2, 9
Cd	5	1.7	0.193	F	3.1	0.053	2, 49
Cu	5	18.6	0.000	WS	27.7	0.000	2, 15
Zn	5	1.1	0.347	F	9.3	0.000	2, 48

Size of *P. borealis* (rostrum to telson): small (5.0-7.5 cm), medium (7.5-10.5 cm), large (10.5-12.0 cm). LS: Levene statistic (equality of variances); WS: Welch statistic; F: F-value (ANOVA); P: tail probability (corresponding null hypotheses are rejected when $P < 0.05$); df: degrees of freedom. Sampling areas see Fig. 1. Pb was not evaluated due to many values below or close to the limit of detection (see Table 1).

Table 3

Trace metal concentrations in *Pandalus borealis* from the Norwegian Sea, Barents Sea, Spitsbergen Bellsund Bank and north of Svalbard.

	Area	Sampling region	Year	Mean±95% CI [mg kg^{-1} DW]			N	LIP	Test	Groups 1	2	3	4
Cd	1	North Cape Bank	2000	0.7	±	0.1	8	0.321	Z	|			
	3	Bellsund Bank	2000	1.7	±	0.3	23	0.003			|		
	2	Central Bank	2000	1.8	±	0.2	16	0.677			|		
	3	Bellsund Bank	1994	1.9	±	0.4	57	0.000			|		
	2	Central Bank	1994	2.3	±	0.3	27	0.170			|		
	5	North of Svalbard	1991	4.7	±	0.7	53	0.027				|	
Cu	2	Central Bank	1994	15	±	4	28	0.000	Z	|			
	3	Bellsund Bank	1994	21	±	3	58	0.002		|			
	1	North Cape Bank	2000	36	±	6	8	0.082			|		
	5	North of Svalbard	1991	38	±	7	53	0.000			|		
	2	Central Bank	2000	43	±	3	16	0.659			|	|	
	3	Bellsund Bank	2000	82	±	14	23	0.012				|	
Zn	3	Bellsund Bank	1994	45	±	1	57	0.457	NK	|			
	5	North of Svalbard	1991	53	±	3	52	0.219			|		
	2	Central Bank	1994	56	±	3	28	0.047			|		
	3	Bellsund Bank	2000	67	±	6	20	0.244				|	
	1	North Cape Bank	2000	71	±	6	8	0.643				|	
	2	Central Bank	2000	81	±	4	16	0.974					|

LIP: Lilliefors probability that sample data are distributed normally (α = 0.01); 95% CI: 95% confidence intervals. Z: Z statistic; NK: Student-Newman-Keuls Multiple Range Test; Bars (|) indicate homogeneous groups according to the multiple comparison tests; otherwise as in Tables 2 and 4. Pb was not evaluated due to many values below or close to the limit of detection (see Table 1).

Table 4

Interspecific heterogeneities of trace metal concentrations in zooplankton and decapods from the Norwegian Sea, Barents Sea, Spitsbergen Area and Bear Island (2000): Student-Newman-Keuls Multiple Range Test.

	Collective	Taxon	Mean±95% CI [mg kg^{-1} DW]	N	LIP	Groups 1	2	3	4	5
Cd	*Meganyctiphanes norvegica*	Eup	0.2 ± 0.1	5	0.288	|				
	Eukronia hamata	Cha	0.8 ± 0.2	8	0.867	|				
	Pandalus borealis	Dec	1.6 ± 0.2	47	0.000	|				
	Metridia longa	Cop	2.4 ± 0.6	10	0.265	|				
	Euchaeta norvegica	Cop	1.2 - 4.0*	3	-					
	Calanus hyperboreus	Cop	4.2 ± 1.0	11	0.873		|			
	Sabinea sarsi	Dec	4.3 ± 1.1	20	0.005		|			
	Calanus finmarchicus	Cop	6.3 ± 1.1	12	0.096			|		
	Themisto abyssorum	Amp	10.5 ± 4.0	7	0.257				|	
Cu	*Eukronia hamata*	Cha	4 ± 1	8	0.192	|				
	Calanus hyperboreus	Cop	6 ± 1	11	0.837	|				
	Calanus finmarchicus	Cop	7 ± 1	13	0.597	|				
	Metridia longa	Cop	7 ± 1	10	0.143	|				
	Euchaeta norvegica	Cop	9 ± 4	4	0.913	|				
	Themisto abyssorum	Amp	9 ± 3	7	0.724	|				
	Meganyctiphanes norvegica	Eup	47 ± 11	5	1.000		|			
	Pandalus borealis	Dec	61 ± 9	47	0.000		|			
	Sabinea sarsi	Dec	68 ± 10	17	0.587		|			
Zn	*Sabinea sarsi*	Dec	59 ± 5	20	0.341	|				
	Meganyctiphanes norvegica	Eup	73 ± 11	5	0.886	|	|			
	Pandalus borealis	Dec	79 ± 8	47	0.000	|	|			
	Calanus hyperboreus	Cop	108 ± 22	12	0.006		|			
	Themisto abyssorum	Amp	110 ± 16	8	0.295		|			
	Eukronia hamata	Cha	124 ± 20	8	0.390		|			
	Calanus finmarchicus	Cop	154 ± 32	14	0.510			|		
	Euchaeta norvegica	Cop	251 ± 61	4	1.000				|	
	Metridia longa	Cop	509 ± 53	9	1.000					|

Explanations see Tables 2 and 3; Taxon: Eup = Euphausiacea, Dec = Decapoda, Cop = Copepoda, Amp = Amphipoda, Cha = Chaetognatha; * range (not included in the statistical evaluation); Pb was not evaluated due to many values below or close to the limit of detection (see Table 1).

Table 5

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

Species	Taxon	Region	Cd	Cu	Pb	Zn	Ref
Systellaspis debilis	Dec	Atlantic, African Coast	22	55	-	70	1
Systellaspis debilis	Dec	Atlantic, Azores Islands	13	-	-	50	2
Systellaspis debilis	Dec	N.E. Atlantic Ocean	12	67	-	53	3
Systellaspis debilis	Dec	N.E. Atlantic Ocean	11	56	-	41	4
Systellaspis debilis	Dec	Iberian Deep Sea Plain	16	49	0.6	62	5
Acanthephyra exima	Dec	Atlantic, Azores Islands	3.0	-	-	-	2
Acanthephyra purpurea	Dec	N.E. Atlantic Ocean	3.0	36	-	46	4
Acanthephyra spec.	Dec	Iberian Deep Sea Plain	6.1	56	0.6	52	5
Bentheogennema intermedia	Dec	Iberian Deep Sea Plain	11	36	0.4	74	5
Benthesicymus iridescens	Dec	Iberian Deep Sea Plain	15	55	0.4	79	5
Sergia spec.	Dec	Iberian Deep Sea Plain	1.9	17	0.5	67	5
Hymenodora glacialis	Dec	Greenland Sea	6.7	16	< 0.3	37	6
Hymenodora glacialis	Dec	Fram Strait	9.2	12	< 0.3	52	6
Chorismus antarcticus	Dec	Weddell Sea	13	93	1.6	44	7
Notocrangon antarcticus	Dec	Weddell Sea	13	67	0.8	46	7
euphausiids	Eup	Iberian Deep Sea Plain	0.8	25	0.3	72	5
euphausiids	Eup	Central North Sea	0.2	28	0.7	70	8
Euphausia superba	Eup	Weddell Sea	3.5	66	0.3	33	7
Thysanoessa inermis	Eup	Greenland Sea	< 0.1	39	< 0.3	78	6
Meganyctiphanes norvegica	Eup	N. North Sea/Atlantic	0.5	26	1.0	45	8
Meganyctiphanes norvegica	Eup	Greenland Sea	0.4	35	< 0.3	42	6
Meganyctiphanes norvegica	Eup	Mediterranean	0.1	-	0.5	-	10
Hyperia spec	Amp	Northern North Sea	51	26	1.8	72	8
Themisto abyssorum	Amp	Greenland Sea	28	24	1.0	92	6
Themisto abyssorum	Amp	Fram Strait	28	22	1.2	86	6
Themisto libellula	Amp	Greenland Sea	34	26	0.4	61	6
Themisto libellula	Amp	Fram Strait	24	23	0.5	61	6
Themisto compressa	Amp	NE Atlantic	70	-	-	-	9
Eukronia hamata	Cha	Greenland Sea	1.1	3	< 0.3	69	6

taxa: Dec = Decapoda; Eup = Euphausiacea; Amp = Amphipoda; Cha = Chaetognatha

references: 1: Ridout et al., 1985; 2: Leatherland et al., 1973; 3: White and Rainbow, 1987; 4: Ridout et al., 1989; 5: Prowe et al., in press; 6: Ritterhoff and Zauke, 1997d; 7: Petri and Zauke, 1993; 8: Zauke et al., 1996a; 9: Rainbow and White, 1989; 10: Fossi et al., 2002; 11: Kahle and Zauke, 2003; 12: Pohl, 1992.

Table 6

Mean trace metal concentrations in copepods from different regions of the world [mg kg^{-1} DW].

Species	Region	Cd	Cu	Pb	Zn	Ref
Calanus finmarchicus	Greenland Sea	0.3	4	< 0.3	86	6
Calanus finmarchicus	Fram Strait	0.3	5	< 0.3	93	6
Calanus finmarchicus	Greenland Sea	7.7	8	1.6	164	12
Calanus finmarchicus	Fram Strait	6.8	5	0.7	192	12
*Calanus finmar/helgol.**	German Bight	0.7	4	1.0	110	8
*Calanus finmar/helgol.**	Southern North Sea	1.8	7	1.0	129	8
*Calanus finmar/helgol.**	Central North Sea	3.2	7	1.0	123	8
*Calanus finmar/helgol.**	Northern North Sea	5.0	8	1.0	98	8
*Calanus finmar/helgol.**	N. North Sea/Atlantic	10.9	7	1.0	70	8
Calanus hyperboreus	Greenland Sea	0.7	4	< 0.3	88	6
Calanus hyperboreus	Fram Strait	0.8	6	0.3	104	6
Calanus hyperboreus	Greenland Sea	3.0	4	0.5	85	12
Calanus hyperboreus	Fram Strait	-	5	0.6	81	12
Rhincalanus gigas	Weddell Sea	3.7	11	0.2	432	11
Calanus propinquus	Weddell Sea	5.6	26	0.5	191	11
Calanoides acutus	Weddell Sea	4.6	10	0.3	183	11
Euchaeta norvegica	Greenland Sea	0.1	5	< 0.3	172	6
Euchaetidae	Iberian Deep Sea Plain	3.8	10	0.2	160	5
Metridia longa	Greenland Sea	0.6	6	0.5	389	6
Metridia longa	Fram Strait	0.7	8	0.6	351	6
Metridia curticauda	Weddell Sea	9.6	21	0.5	278	11
Metridia gerlachei	Weddell Sea	10.2	26	0.7	518	11
Centropages spec	Central North Sea	2.5	17	2.5	391	8
Acartia spec	Central North Sea	1.7	15	2.0	491	8
Temora spec	Central North Sea	1.7	34	2.7	524	8

* mixed collectives of *C. finmarchicus* and *C. helgolandicus*; references: see Table 5.
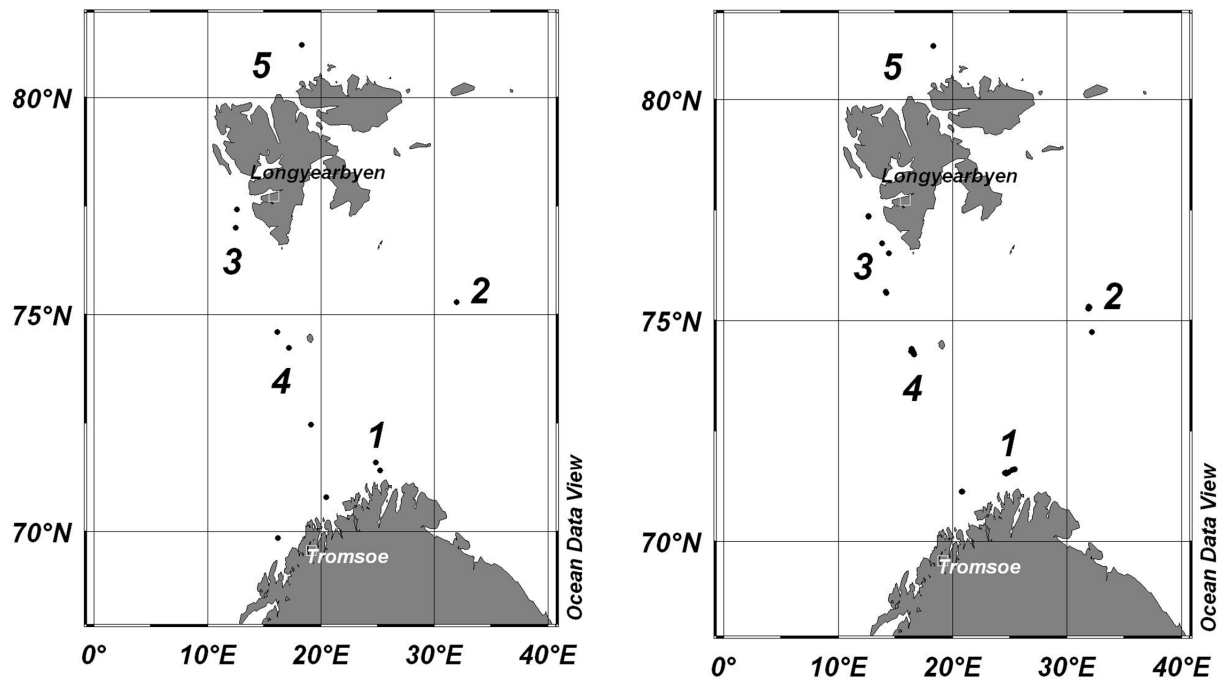
Figure 1: Areas of investigation and sampling locations in the Norwegian Sea and the Barents Sea for the 'Helgoländer Planktonnetz' (left) and the GOV (Chalut à Grande Ouverture Verticale) (right) with (1) North Cape Bank; (2) Central Bank; (3) Spitsbergen Bellsund Bank, Hornsund Bank and South Cape Bank, (4) Bear Island and (5) north of Svalbard (see section 2.1 for further details).