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ICBM

Evaluation of spatial and temporal models to assess the bioaccumulation of trace metals in marine invertebrates

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In memoriam

Ekkehard Vareschi

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INTRODUCTION AND OUTLINE OF THIS THESIS

The role of trace metals in marine organisms

Trace metals occur in naturally low background concentrations in the marine environment. These background concentrations are subjected to a complex system of physical, chemical and biological processes and balances between the compartments water body, suspended matter and sediment. Due to their biological role in the nutrient and energy cycle they are addressed as essential metals like copper, nickel and zinc and non-essential metals like cadmium or lead. Both, essential and non-essential trace metals are toxic to aquatic life from a species specific level on (Rainbow, 1993, 1995a). Due to industrialization, heavy metal concentrations in the oceans increased because of high anthropogenic emissions. A high amount of inputs of hazardous substances come from rivers or by direct inputs into the ocean. Mostly coastal areas and estuaries are affected (Haarich and Schmidt, 1993; Essink et al., 2005).

To detect the environmental impact of heavy metals within a given area, analysis of concentrations in water, sediment and biota provide complementary information. Metals occur in different species in water and sediment, but not all of them can be incorporated in animals or plants. The bioavailable fraction is per definition the content of heavy metals incorporated in the organisms (Simkiss and Taylor, 1995; Zauke et al., 1996), providing a time-integrated measure of biologically active metals in the environment (de Kock and Kramer 1994; Rainbow 1995). Conversely, metal concentrations in ambient water are subjected to strong variation due to periodic events, in the time scale from hours to centuries (de Kock and Kramer 1994). Single measurements of heavy metal concentrations in water can therefore only be a snap-shot. To assess the environmental quality regarding heavy metals in the oceans, the bioavailable fraction is of great importance, since possible toxic effects are largely dependent on the bioavailable exposure concentration in seawater (Phillips and Rainbow 1989; Rainbow 1995; Zauke et al. 1996). This fraction is only accessible by determining the amount of metals incorporated in organisms, because this is more dependent on species-specific uptake and detoxification mechanisms and metabolic requirements than on the concentration in the soluble phase (Rainbow, 1988; Depledge, 1989; Rainbow, 1995a).

Investigations on bioaccumulation of heavy metals in organisms provide the evaluation of the bioavailable fraction in ecological systems (biomonitoring). Therefore, information about the accumulation strategies in organisms are required and a calibration is essential, that means a quantification of the potential for bioaccumulation, e.g. by means of toxicokinetic models. A high potential for bioaccumulation allows conclusion for possible future risks in case of an increase of the bioavailable supply. On one hand there might be a development of concentrations of pollutants in organisms, leading to toxic effects, if detoxification strategies are missing or over stressed. On the other hand there might be an accumulation over the food web (Zauke et al., 2001). A general theoretical concept of these coherences is presented in Figure 1.

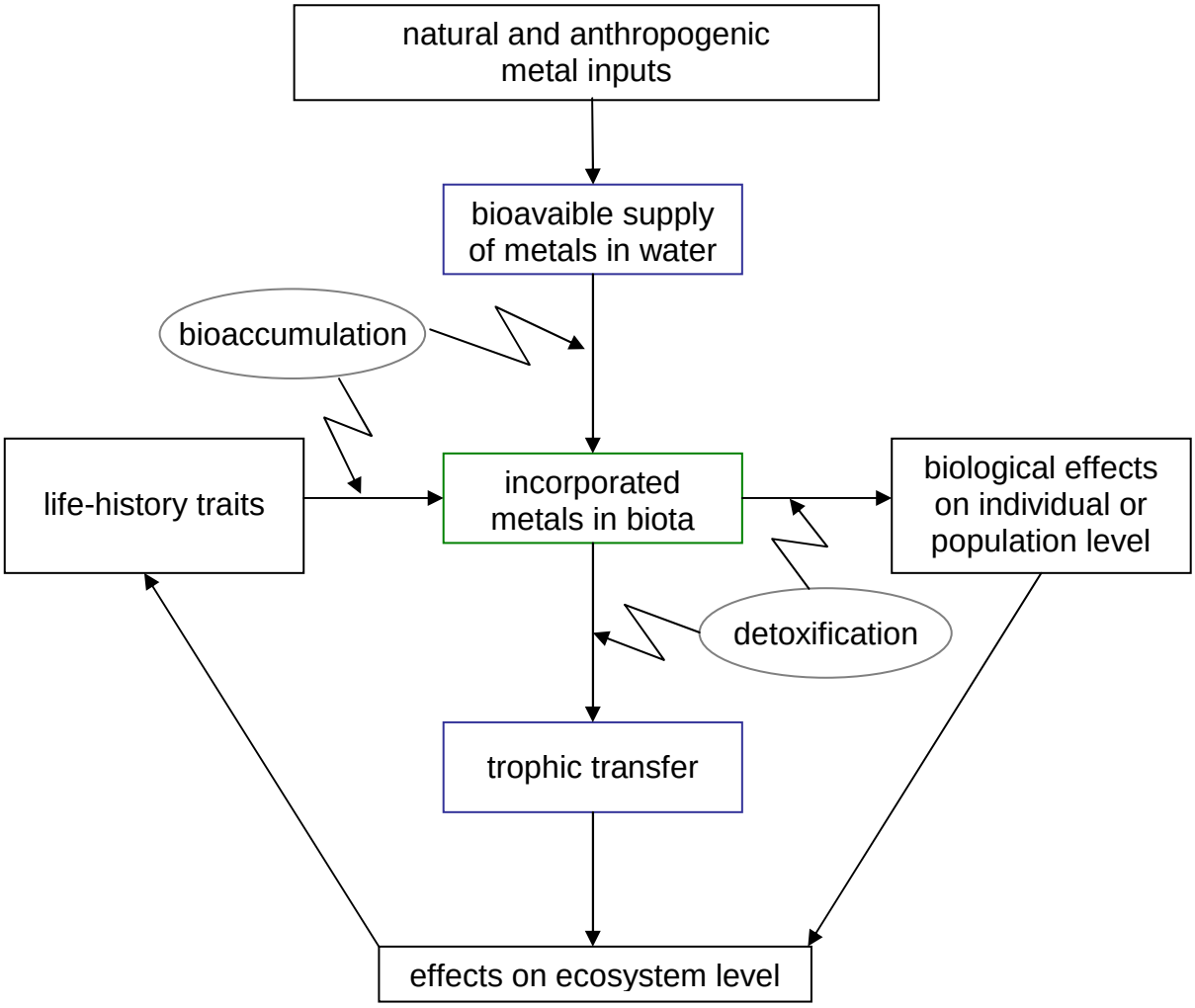

Figure 1: General theoretical concept for the assessment of heavy metals in aquatic systems (after Zauke et al., 2001).

Since marine invertebrates constitute an important path in the trophic transfer of metals or other xenobiotics to higher trophic levels, especially to birds and mammals, crustaceans and bivalves have been chosen, among others, as recommended organisms in baseline studies for the marine environment within the scope of assessment programmes.

For the assessment of bioaccumulation in animals, different parameters and circumstances play an important role. In Figure 2 general aspects and thematic positioning of the present work are shown.

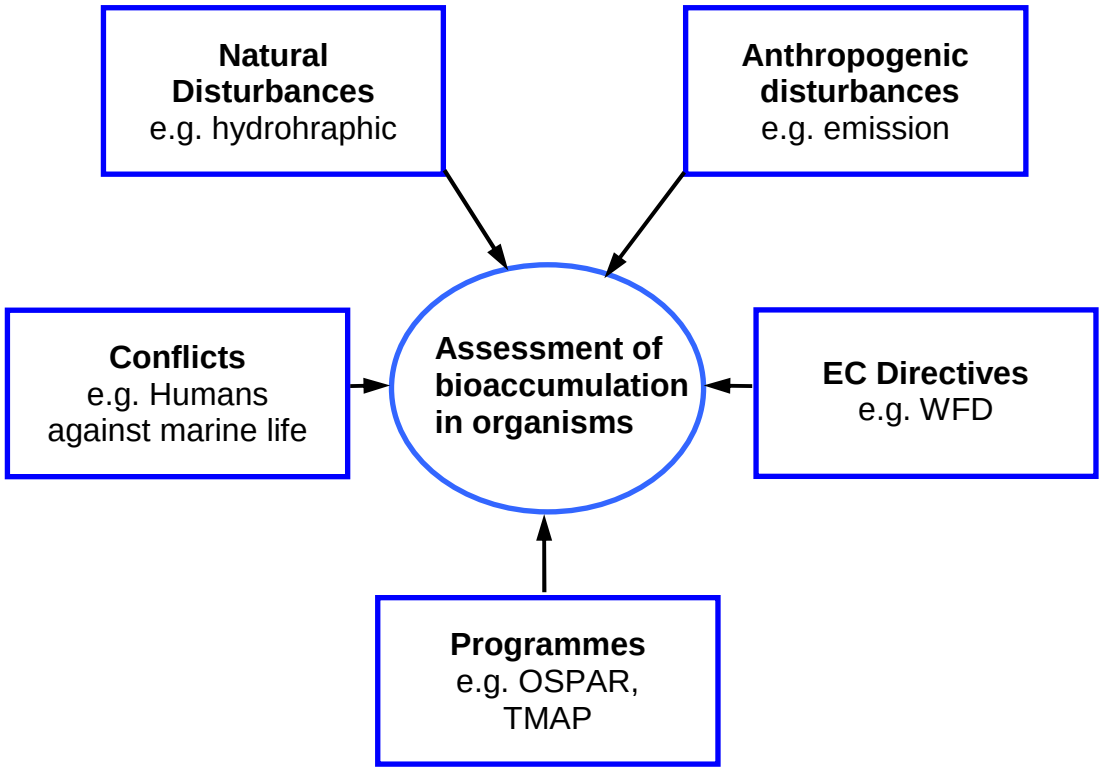

Figure 2: Diagram of the general aspects and thematic positioning of the here presented PhD-thesis. The scopes are outlined in Figure 5.

The North Sea and the Wadden Sea region

The North Sea and with its Wadden Sea region is an area where people live, work and recreate. About 3.7 million people are living along the Wadden Sea coast, with 75,000 of them inside the Wadden Sea Area. The Trilateral Wadden Sea Plan (1997) acknowledges this by stating that economic and social values should also be maintained and enhanced. The Wadden Sea is a coastal area extending over 450 km along the North Sea coast of the Netherlands, Germany and Denmark and is one of the largest wetlands on a global scale (Figure 3). It covers an area of about 14,700 km^2 and the Conservation Area is about 11,200 km^2 (Essink et al., 2005). Anthropogenic influences such as fishing activity, chronic large scale eutrophication, pollution, structural interferences and climate change are thought to influence epifaunal communities on a long-term basis (Hinz et al., 2004). Hence, we have to differentiate between natural and anthropogenic disturbances, and the last ones need to be reduced to a sustainable degree by appropriate management.

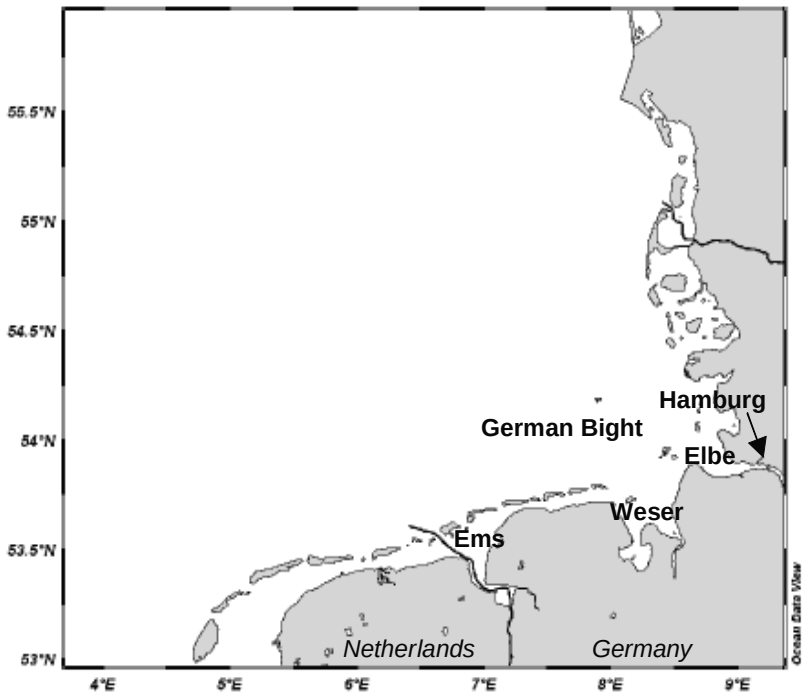

Figure 3: Southern North Sea and the inner German Bight with the Wadden Sea coast from Netherland, Germany and Denmark.

A network of tidal channels, sandbars, mudflats, salt marshes and islands creates a transition zone between land and sea characterized by daily changing flood and ebb tides and high dynamics in salinity, light, oxygen and temperature. This has resulted in a complex system which provides a unique habitat for a rich flora and fauna. The great productivity and size of the Wadden Sea provide a basis for the reproduction of North Sea fish stocks and for its function as a turntable of bird migration. The Wadden Sea is an open system connected with the adjacent North Sea. The quality of water, sediment and marine habitats is, to an important degree, influenced by the North Sea and activities in the catchment area of the discharging rivers.

A decline in the tendency in the pollution of the North Sea was recently reported in the Quality Status Report 2004 by the OSPAR Commission (Essink et al., 2005). Due to the large differences in both annual and river flow, the riverine metal loads into the North Sea and the Wadden Sea vary profoundly. OSPAR reported fluvial metal inputs into the Wadden Sea by the river Elbe with approximately 5.5 t Cd y^{-1}, 200 t Cu y^{-1}, 160 t Pb y^{-1} and 1700 t Zn y^{-1}. For the river Weser 2.5 t Cd y^{-1}, 60 t Cu y^{-1}, 60 t Pb y^{-1} and 700 t Zn y^{-1} were measured. Atmospheric inputs from the Dutch Wadden Sea in 2001 were 158 kg Cd y^{-1}, 3934 kg Cu y^{-1}, 3021 kg Ni y^{-1}, 6984 kg Pb y^{-1} and 16337 kg Zn y^{-1} (Essink et al., 2005). Nickel was excluded in the QSR 2004 due to data not being available for all Wadden Sea subareas, but as a new compound of high priority in both OSPAR and WFD, needs to be included in the monitoring of the Wadden Sea (Essink et al., 2005).

Directives and Protection Programmes

Directives and protection programmes, like the Quality Status Reports (QRS) mentioned above issued by the OSPAR Commission, are implemented in order to provide a framework for the protection, maintenance and improvement of the environment. But they also describe and evaluate recent developments, including the effects the policies implemented, for example by the three Wadden Sea countries in the Trilateral Wadden Sea Cooperation (Essink et al., 2005).

The Water Framework Directive (WFD) (The Council Directive 2000/60/EC) on establishing a framework for Community action in the field of water policy was enacted in December 2000 (European Union, 2000). It aims at a coordination of all water-related measures on a European level. The key elements of the WFD include the protection of all waters, surface and ground waters in a holistic way and achieving a good quality (good ecological status) by 2015. A River Basin Management Plan has to be prepared by 2009 based on the results of an operational monitoring programme (to be established by 2006). River management plans are to be reviewed every 6 years (Essink et al., 2005). The WFD covers all types of surface and ground waters. Coastal waters cover the area up to 1 nautical mile from the baseline and with regard to the chemical status also the territorial waters up to 12 nautical miles.

In the framework of the Habitat Directive a coherent ecological network is being established. The NATURA 2000 will consist of Special Areas of Conservation (SACs) designated according to the Habitat Directive, and the Special Protective Areas of the Bird Directive. All major parts of the Wadden Sea Area have already been included in NATURA 2000.

According to the Bird Directive from 1979 (The Council Directive 79/409/EEC), basically the entire Wadden Sea Area except the main shipping routes and adjacent offshore areas have been designated as Special Protective Area (SPA). The Bird Directive aims at the protection of all species of natural occurring birds in the territories of the EC member states. Additionally, the Flora Fauna Habitat Directive adopted in 1992 (The Council Directive 92/43/EEC), complements the 1979 Bird Directive.

Biomonitoring and Bioaccumulation

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 (Petri and Zauke, 1993; 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 (Rainbow and White, 1989; Zauke and Petri, 1993). Natural and anthropogenic metal inputs influence the bioavailable metal supply in aquatic systems. This bioavailable fraction is usually determined by measuring the metal amount accumulated by organisms, which is the main goal in biomonitoring (e.g. Rainbow, 1993; Ritterhoff et al., 1996). Biomonitoring provides data about the environmental impact and the comparability of measured values, and therefore allows a prognosis. The term biomonitor defines an organism which can be used to establish geographical

and temporal variations in the bioavailability of contaminants by measuring the accumulated concentrations of chemicals in the whole body or in specific tissues (Rainbow, 1995a).

Bioaccumulation is a very complex process, influenced by several environmental factors. For example, the uptake route plays an important role, and it may be that no simple correlations can be established between the concentrations of pollutants in the substrate and in the organism, or that more than one uptake route is involved (Rinderhagen et al., 2000). Metal bioaccumulation (internal exposure) is mechanistically based, but empirically considers geochemical influences, biological differences, and differences among metals (Luoma and Rainbow, 2005).

An important pre-condition for using organisms as biomonitors is a net accumulation strategy (Rainbow, 1995a; Zauke et al., 1996). Investigations on the time course of uptake and clearance of metals in organisms, in relation to external metal exposures, are a first step to assess the significance of metals in aquatic systems. They provide the experimental basis for estimation of kinetic parameters of compartment models and preliminary hypotheses about underlying accumulation strategies. When metal concentrations in organisms do not readily reach a plateau phase, this indicates a net accumulation strategy under the given experimental conditions (Zauke et al., 1995) The concept of toxicokinetic studies was developed for xenobiotics in fish, but has been successfully extended to metals in various aquatic invertebrates (e.g. MacLean et al., 1996; Ritterhoff et al., 1996).

In addition to a net accumulation strategy, further pre-conditions have to be met before organisms can be used as biomonitors. Firstly, animal collectives from different localities must show similar BCFs to allow a comparison between field concentrations of metals in organisms in terms of their bioavailability (Zauke et al., 1995; Zauke et al., 1996). This points to the problem of the calibration of biomonitors. Verification of models involves comparison of model predictions with independent experimental data sets (Rykiel, 1996; Holzbecher, 1997; Bernds et al., 1998).

Classically, studies on metals in biota are related to the species level because this level is implicitly regarded as highly relevant in describing the processes of metal metabolism. This is certainly true in basic physiological studies. Regarding applied investigations, however, we propose to select appropriate units of investigation on the basis of a straightforward calibration procedure, involving assessment of toxicokinetic parameters (BCFs, rate constants), clarification of uptake, elimination and storage mechanisms and validation of invariable BCFs or rate constants under different exposure conditions (see discussions in: Phillips and Rainbow, 1989; Rainbow and White, 1989; Petri and Zauke, 1993; Rainbow, 1993; Viarengo and Nott, 1993; Zauke et al., 1995; Zauke et al., 1996). Such a calibration may eventually lead to consideration of levels below the biological species or to well-defined collectives of organisms above the species level (Zauke et al., 1996).

The involvement of different intracellular metal-handling mechanisms has severe implications for the suitability of bioaccumulation models as predictive tools. First-order kinetics are characteristic for compartment models (where the rate of transfer of a chemical is directly proportional to the concentration in the compartment) unless a nonlinear process is explicitly incor-

porated (Barron et al., 1990). Thus, when passive transport processes like diffusion are the main uptake mechanism (involving uncharged metal species), first-order kinetics will yield reasonable predictions over a wide range of exposure concentrations, independently of the duration of the experiments. This pre-condition is not met when facilitated diffusion and ion channel proteins or carriers are involved (viz. in the case of uptake of charged ionic metal species). Then a limitation to the uptake process might occur at higher exposures, as pointed out by Dallinger (1995) and Rainbow (1997). As a consequence, the relationship between net metal uptake and increasing exposure concentrations will follow a saturation curve (Hudson, 1998; Sunda and Huntsman, 1998). In this case, a verification or extrapolation of (linear) model predictions derived from first-order kinetics will only be successful for the lower range of exposure concentrations, where the uptake process is not limiting the rate of uptake (Ritterhoff and Zauke, 1997; Bernds et al., 1998; Clason and Zauke, 2000). In this situation, application of hyperbolic models might be promising, since they assume a nonlinear process as shown by Kahle and Zauke (2002a, 2002b) for Antarctic copepods and by Clason et al. (2003) for an Antarctic amphipod.

Such kinetic studies provide an experimental basis for the estimation of kinetic model parameters, albeit with a low degree of physiological detail. For an evaluation of the organisms' potential for biomonitoring, this method is adequate (Zauke et al., 1995, and literature cited therein). However, in order to understand the mechanisms of bioaccumulation, more sophisticated investigations would be required, e.g. considering metal distributions in different organs or quantification of subcellular sequestration processes (granules, lysosomes or metallothioneins; Viarengo and Nott, 1993).

Pharmacokinetics describe in detail the uptake and the disposition of chemicals in an organism. The analysis is based on physiological models. Chemicals are measured in different organs of the organism. In toxicokinetic studies the kinetic parameters are calculated by means of compartment models (black box model), where the surrounding medium (water) is the first compartment and the organism is the second. Chemicals are measured in whole organisms. Laboratory experiments are carried out to validate the results from the field study. From differences in sensitivity, eventually occurring between populations from different sampling sites, it can be concluded that animals originating from a polluted site might have developed a greater metal tolerance (Rinderhagen et al., 2000, and literature cited therein).

Knowing which aqueous metal species control the rates of trace metal uptake by aquatic biota is essential for modelling their fate and effects in aquatic systems. Metal speciation has been shown to govern important biotic uptake processes including the acquisition of essential metals and the regulation of growth in phytoplankton (Sunda and Huntsman, 1998), the entry of toxic metals into aquatic food chains (Watras et al., 1998), and the toxic effects of metals on higher organisms, such as fish (Playle, 1998). When 'uptake' is understood more broadly as any direct interaction with an aquatic organism, many significant biologically-mediated transformations of metals, such as redox and methylation reactions, also fall into the category of uptake processes. In the simplest form of uptake, metals are adsorbed onto sites in cell walls and cell membranes without being transported into the cytoplasm. Adsorp-

tive uptake can be substantial for metals that have a high affinity for surfaces (Hudson, 1998).

In reviewing studies of trace metal uptake, evidence is sought for specific mechanism controlling transepithelial metal uptake or loss, and their possible relationship with pumps controlling the regulation of physiologically-important electrolytes. There is evidence of zinc regulation in some crustaceans. However, the widespread occurrence of specific transepithelial regulatory mechanisms for trace metals is unlikely. Metal uptake and toxicity is often negatively associated with salinity. Although this phenomenon is probably related, in part, to trace metal speciation, an associated calcium effect is seen in some species from freshwaters. Several examples exist of interactions between calcium regulation and trace metals, suggesting there may be a number of shared pathways (Wright, 1995).

Monitoring organisms

In the marine coastal ecosystem benthic and demersal organisms play a key role. One basis for an ecological assessment is knowledge of ecological preferences and distribution of benthic living organisms under different environmental conditions. Although information on the abundance of species is essential, the relevance of reproduction and population dynamics should be considered too. As described in Hinz et al. (2004), there is also a seasonal and annual variability in the assemblage of the epifaunal community. Seasonal and annual changes are most likely linked to migratory movements into and out of the area under investigation. Temporal changes in species composition and abundance correlated best with water temperature, while the spatial distribution of the total biomass is correlated with sediment characteristics.

Crustaceans are frequently used as bioindicators and biomonitors in various aquatic systems. One reason is that they are a very successful group of animals, distributed in a number of different habitats including marine, terrestrial and freshwater environments. They are thus interesting candidates for comparative investigations. Some of the special features of crustaceans, particularly of reproduction strategies, may be highly important for the interpretation of data from bioindicator studies using these organisms.

Various bioaccumulation studies have been performed in the Aquatic Ecology group employing different crustaceans from different marine areas: a lot of them considering Antarctic amphipods and copepods (Kahle and Zauke, 2002a, 2002b; Clason et al., 2003; Kahle and Zauke, 2003), and amphipods from the North Sea and UK Estuaries (Clason and Zauke, 2000; Clason et al., 2004a; Clason et al., 2004b). Although other studies have been dealing with bioaccumulation in decapod crustaceans (e.g. Rainbow, 1988; Rainbow and White, 1989; Rainbow, 1995b, 1997; Rainbow et al., 1999; Rainbow et al., 2000; Rainbow and Black, 2005a, 2005b) only few information about bioaccumulation in *Crangon crangon* (Linnaeus, 1758) is available (Dethlefsen, 1978; Amiard et al., 1985; Riisgard and Famme, 1986; Costello and Read, 1994; Mattig et al., 1997; Simas et al., 2001). Because of the economical and ecological importance of the brown shrimp in the Wadden Sea and estuarine areas, bioaccumulation studies on these organisms have to be taken more into consideration. The

brown shrimp *C. crangon* is, like the edible cockle *Cerastoderma edule*, an important element in the marine coastal ecosystem.

In coastal and estuarine waters from the Baltic and the North Sea, the Atlantic coast of North and West Europe and the Mediterranean brown shrimps can be found. The epifaunal decapod, the brown shrimp *C. crangon*, is a typical euryhaline inhabitant (Cieluch et al., 2005). *C. crangon* is considered to be a key species in the coastal waters of the North Sea and in particular in the Wadden Sea, since it occurs in masses and acts both as a highly efficient predator and important prey. The shallow water region of the Wadden Sea is the nursery ground for brown shrimps (Berghahn, 1996). Brown shrimps are important food resources for flatfish, shore crabs, seals, various waders, seagulls and auks (Jensen and Jensen, 1985; Janke, 1999).

Due to the high variable environmental conditions in the North Sea, the spatial structuring of *C. crangon* in the Wadden Sea is controlled by abiotic variables such as sediment quality, sediment dynamics, environmental factors such as sea surface temperature (Hinz et al., 2004) and tidal level and therefore also from sunlight intensity during low water level in the shallow regions of the Wadden Sea (Berghahn, 1996). Biotic factors, for instance predation, competition or interactions between adults and juveniles (Berghahn, 1996), also strongly influence the recruitment and subsequent spatial distribution of *C. crangon*. Furthermore, seasonal migration patterns of brown shrimps, *C. crangon*, are well documented. During autumn shrimps usually migrate from the shallow water areas of the Wadden Sea to offshore areas and then return in spring with the onset of warmer water temperatures. The pattern of migration depends largely on the weather conditions; while during mild winters there may be almost no migration in offshore direction, under moderate to severe winter conditions *C. crangon* is reported to migrate 1 to 90 km offshore (Hinz et al., 2004). Spatial distribution pattern of brown shrimps are largely dependent on relatively warm water conditions.

In many environmental programmes mussels like *Mytilus edulis* (blue mussel watch) or oysters like *Crassostrea gigas* are used as biomonitors. However, due to their importance in the food webs of mud flats, other bivalves such as cockles (e.g. *C. edule*) merit further consideration, as recently suggested by Szefer et al. (1999), Cheggour et al. (2000) and Cheggour et al. (2001). Cockles can be regarded as net accumulators for some metals, as can be inferred from detected linear regression relationships between Cd, Pb and Zn in cockles and the surrounding sediments (Cheggour et al., 2001) or from toxicokinetic bioaccumulation studies showing a distinct uptake and depuration of Cd, Pb and Cu in experimental organisms (G.-P. Zauke and M. Schwager, ICBM, Oldenburg, unpublished data).

The edible cockle *C. edule* is an infaunal suspension feeder, living in the upper few centimetres of the sediment. It burrows just below the sand surface and may reach adult densities of up to several thousands per square meter, mainly in the intertidal zone (de Montaudouin and Bachelet, 1996). This bivalve is one of the dominant and important species in tidal flats of the Dutch, German and Danish Wadden Sea (Flach, 1996), where it represents 8 - 60% of the total macrobenthic biomass (Ramon, 2003). Cockles are important food resources for shrimps, flatfish, shore crabs, oystercatchers, herring gulls and eiders (Jensen and Jensen, 1985). The spatial structuring of *C. edule* in the Wadden Sea is controlled by abiotic vari-

ables such as sediment quality (Ducrotoy and Desprez, 1986), sediment dynamics (Bouma et al., 2001), severe winters or wind-induced effects (Strasser et al., 2003). Biotic factors, for instance predation (Strasser and Gunther, 2001), competition or interactions between adults and juveniles (de Montaudouin and Bachelet, 1996), also strongly influence the recruitment and subsequent spatial distribution of cockles.

Spatial modelling and geostatistics

The patchy nature of the environment coupled with the behaviour of a species determines the spatial arrangement of individuals of that species, and the observed spatial distributions are important for understanding ecological processes. Moreover, identifying spatial patterns is important in order to improve the design and interpretation of surveys and experimental studies, by relating sampling programmes to natural scales of variation (Livingston, 1987; Thrush et al., 1989; Stelzenmüller et al., 2005). Although spatial variability on different ecological scales is an important issue in mudflat ecology (Ysebaert and Herman, 2002; Norén and Lindegarth, 2005; Thrush et al., 2005), it is important to note that the spatial dependence of variables has not explicitly been taken into account in ecological and biomonitoring field studies in this area, despite the well-known importance of spatial dependence in ecology and environmental sciences.

This importance is stressed by the following arguments. Sampling points randomly distributed over an area can yield unbiased estimates of the variable of interest only if the sampling-point observations are independent (Petitgas, 2001). When random sampling is carried out at an appropriate spatial scale, it effectively extinguishes any underlying spatial structure in the distribution of organisms. However, the scale of spatial distribution of the target species is usually unknown, and this factor may result in a bias in the calculation of population estimates (Maynou, 1998). The presence of a spatial structure is indicated by spatial autocorrelation between pairs of samples, *viz.* the realisation of a regionalised variable (e.g. biomass of organisms) at one location influences the realisation at neighbouring locations. Thus, when samples are not taken independently of one another and when the population sampled is spatially structured, the computation of any variance requires a model of the spatial relationships within the population (Matheron, 1971). Spatial autocorrelations are recognised as typical characteristics of ecological units like populations of organisms, but also of other environmental variables (Legendre and Legendre, 1998), and can be analysed and modelled mathematically by geostatistics. Thus, the presence of patches, density gradients and spatially autocorrelated variables may confound designs and affect the validity of inferential statistics. Future studies must integrate the intensity and form of patterns from various spatial and temporal scales if we are to understand the processes responsible for generating pattern (Thrush, 1991; Thrush et al., 2005).

Outline of the thesis

The research described in this thesis is based both on field studies as well as on laboratory experiments. Sampling areas of *C. crangon* and *C. edule* in the German Wadden Sea and the German Bight are presented in Figure 4.

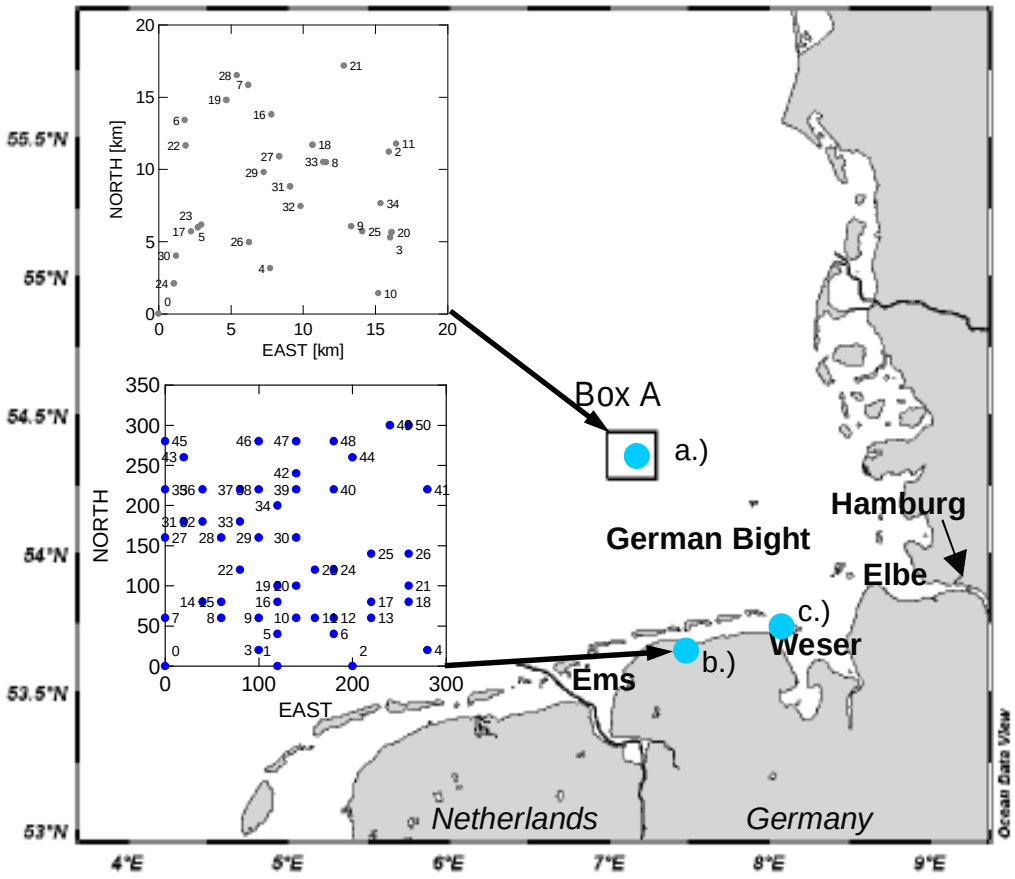

Figure 4: The Wadden Sea coast from Netherland, Germany and Denmark and the inner German Bight, southern North Sea, with the following study areas:

a.) Standard GSBTS sampling area Box A from cruise 259 with FRV "Walther Herwig III" (January 05 – 13, 2004): Chapter 1 and 3.

b.) Spatial location of the investigation area with sampling area of *Cerastoderma edule* at Dornumersiel: Chapter 2.

c.) Sampling Area of the brown shrimp *Crangon crangon* (Linnaeus, 1758) for bioaccumulation experiments in Schillig, German Wadden Sea coast: Chapter 4.

At first, recently established methods for graphite furnace atomic adsorption (Kahle et al., 2003) had to be adjusted to the biological matrix of the brown shrimp and to the different proportions of the elements (Cd, Cu, Ni, Pb and Zn) in the samples (Chapter 1). Samples of

C. crangon collected for the spatial analysis in the German Bight (Box A) were used for this purpose. The modification of the instrumental programmes was mainly relying on the optimization of the measurement range of the calibration curve to enable multielement analysis. Temperature programmes and instrumental parameters were adjusted too.

In a further study, spatial distribution of heavy metal concentrations (Cd, Cu, Ni, Pb and Zn) and biomass indices were investigated in the edible cockle, *C. edule*, from the German Wadden Sea Coast on an ecological scale of 300 x 300 m (Chapter 2). Geostatistical procedures (semivariogram analysis) were applied.

A comparable spatial analysis was done for trace metals (Cd, Cu, Ni, Pb and Zn) in the brown shrimp, *C. crangon*, from the German Bight on a larger ecological scale of 18 x 18 km (Box A) (Chapter 3). On the basis of the variograms (geostatistic), the stochastic independence of the parameters Cd, Cu, Ni, Pb, Zn and mean body wet weight (mbww) was estimated.

Another important aspect of this thesis was to investigate the bioaccumulation behaviour of heavy metals in decapod crustaceans in biological tests due to their high abundances in the benthic community in the Wadden Sea and the North Sea and great importance in the marine food web. For this reason, three independent laboratory experiments on uptake and depuration of Cd, Cu, Pb and Zn in *C. crangon* were carried out (Chapter 4). Therefore juveniles of the brown shrimp, hatched in summer (July) from the North Sea Coast in Schillig, were caught and used in the three toxicokinetic experiments (referred to as *CI*, *CII*, *CIII*). In *CI* significant parameters of a two-compartment model were estimated. Results from *CII* and *CIII* served to verify the two-compartment models from *CI*, involving comparisons of observed and predicted values from the models estimated with independently derived experimental data. A further step of the calibration consisted in a comparative analysis on the sensitivity of *C. crangon*, allowing an assessment of their potential for bioaccumulation by the estimation of the minimal exposure values that would result in a measurable increase of the metal concentration in the animal.

In conclusion, the main objectives in this PhD-thesis were (1) modification of methods for graphite furnace atomic adsorption spectroscopy, (2) detection of spatial distribution and stochastic dependence of heavy metals in two different benthic invertebrates in the Wadden Sea and the North Sea on different ecological scales, and (3) bioaccumulation behaviour of heavy metals in decapod crustaceans in biological tests, to draw conclusions on the field situations and test the suitability for biomonitor organisms (Figure 5).

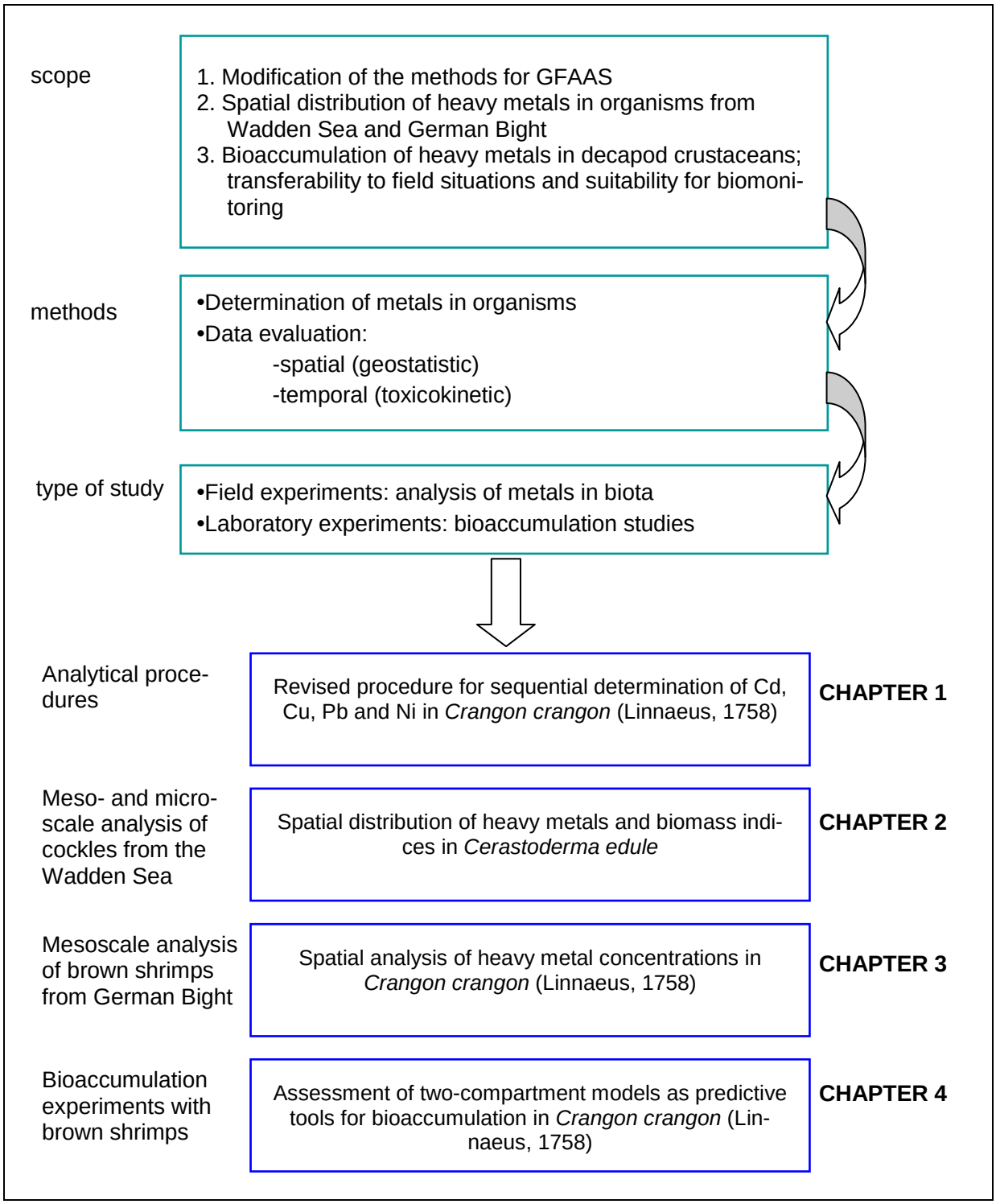

Figure 5: Diagram of particular aspects of the present PhD-thesis and characterization of the methods used and working hypothesis.

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CHAPTER 1

Revised procedure for sequential determination of Cd, Cu, Pb and Ni in the brown shrimp *Crangon crangon* (Linnaeus, 1758) by graphite furnace atomic absorption spectroscopy and Zeeman background correction

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Abstract

In this work a revised method is presented for the analysis of heavy metals in aquatic invertebrates by means of GFAAS (Varian SpectrAA 880 Zeeman instrument and a GTA 110 graphite tube atomiser). Due to different biological matrices it was necessary to modify the sequential analysis to the matrix of the organisms under study. For the optimization of established methods, samples of the decapod crustaceans *Crangon crangon* and two certified standard reference materials were used. Quality assurance was performed in line with German GLP (good laboratory practice) regulations. Precision and validity of the modified methods was evaluated using the two certified reference materials randomly allocated within routine determinations. Stability of instrumental calibration, precision of parallel injections and analytical blanks were in line with requirements from good laboratory practice. Evaluated precision and limits of detection were largely in good agreement with previous studies in this context. Aliquots of 10 mg biological tissues resulted in limits of detection of 0.2 mg Cd kg^{-1}; 3 mg Cu kg^{-1}; 0.4 mg Pb kg^{-1} and 0.1 mg Ni kg^{-1} (DW). The revised methods presented in this chapter are applicable for the analysis of decapod crustaceans, where often only small amounts of biomass are available, especially when bioaccumulation experiments are considered.

Key words: GFAAS, heavy metals, matrices, decapods

1. INTRODUCTION

To assess the environmental quality regarding heavy metals in the oceans, the bioavailable fraction is of great importance, since possible toxic effects are largely dependent on the bioavailable exposure concentration in seawater. This fraction is only accessible by determining the amount of metals incorporated in organisms, because this is more dependent on species-specific uptake and detoxification mechanisms and metabolic requirements than on the concentration in the soluble phase (Rainbow, 1988; Depledge, 1989; Ritterhoff and Zauke, 1998). As a result, we frequently can find different species of marine organism with greatly varying metal concentrations in the same body of ocean water (Petri and Zauke, 1993; Ritterhoff and Zauke, 1997b).

In marine biomonitoring, metal concentrations in different species of aquatic invertebrates are used to assess their role in the biogeochemical cycle within aquatic environments, to evaluate their suitability as monitors of the bioavailable environmental supply or to analyse the internal exposure to potentially toxic substances as a basis for an effect monitoring. Many environmental monitoring programmes record results of past events without understanding the underlying physiological and ecological processes. There is, however, an increasing demand for prospective approaches to detect potential human impact on ecosystems, based on a sound understanding of these processes (Rainbow, 1993, 1995; Zauke et al., 1996). Natural and anthropogenic metal inputs influence the bioavailable metal supply which cannot be detected directly by routine analytical procedures, e.g. by measuring metal concentrations in the soluble phase. The bioavailable fraction can only be assessed by determining incorporated metal levels in organisms which is the main goal in biomonitoring (Phillips and Segar, 1986; Rainbow and Phillips, 1993). This involves field investigations (Prowe et al., 2006; Zauke and Schmalenbach, 2006) as well as bioaccumulation experiments (Clason and Zauke, 2000; Kahle and Zauke, 2002a, 2002b; Clason et al., 2004).

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. These 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 (Rainbow, 1988; Zauke and Petri, 1993).

In recent studies of the Aquatic Ecology Group of the ICBM these aspects have been evaluated in detail, considering various aquatic invertebrates and habitats such as marine zooplankton from the Arctic (Ritterhoff and Zauke, 1997a, 1997b, 1997c, 1997d) freshwater zooplankton and benthos from lakes (Zauke et al., 1998), marine zooplankton from the Iberian Deep Sea (Prowe et al., 2006), benthic invertebrates from German coastal waters and estuaries (Zauke et al., 1995; Ritterhoff et al., 1996; Bernds et al., 1998; Clason and Zauke, 2000) and crustaceans from the Antarctic marine environment (e.g. Weddel Sea, coast line of Wilkes Land; Zauke and Petri, 1993; Keil et al. in press).

Graphite furnace atomic absorption (GFAAS) has gained a reputation in the field of analytical chemistry as a routine technique for the determination of very low levels of trace metals in a

variety of samples types. The Zeeman background correction technique has further enhanced the quality of the results obtained from the graphite tube atomizer. The generation of atoms by means of an electrically heated graphite furnace atomizer is a technique which is complementary to conventional atomic absorption (AA), rather than a technique which replaces it. There are many analytical advantages of flame AA, as giving excellent results, and being simple, convenient and extremely useful, but there are many trace metal analyses which are only possible by means of a graphite furnace atomizer. The advent of 'flameless' atomization, in particular the pyrolytic coated graphite tube furnace atomizer, greatly reduced the physical and chemical limitations imposed by the flame atomization process. Interferences are of different nature to those found in flame atomization but are amenable to control by proper choice of analytical conditions, and chemical pre-treatment. The graphite tube is a confined furnace chamber where pulse vaporization is achieved by raising the temperature with a programmed sequence of electrical power (Rothery, 1988).

Flame atomic absorption spectroscopy (FAAS) systems are subject to sensitivity limitations which restrict the analytical scope of flame methods. Graphite furnace atomizer methods are typically 100 times more sensitive than flame methods for most elements in a wide range of samples. For the determination in GFAAS less sample volume is needed than in FAAS (5-100 µl compared to 300 µl). Furthermore, the sample preparation can be simplified and handling minimized (Rothery, 1988).

Ideally, during the measurement period in furnace AA, the only absorbing species within the optical beam should be the analyte atoms. However, in practice other species may be produced during the sample atomisation process. If these species are capable of absorption at the same wavelength as the analyte sample, and this absorptions overlaps in time the atomic absorption of the analyte, the resulting absorbance measurements will be higher than they should be for the given atom population and the analytical result will therefore not be valid (Rothery, 1988). The perfect background correction system would provide a means for measuring the total absorbance of the analyte in combination with the background, and simultaneously provide a reference measurement due to all of the absorbing species except the analyte. Acceptable correction for background absorption can be achieved when these measurements are made sequentially providing that their separation in time is small compared to the rate at which the absorbance signal is changing. Atomic spectral lines can be shifted by a variety of techniques. The application of magnetic fields (Zeeman Effect) and electric fields (Stark effect) have been widely exploited, although a number of other more sophisticated approaches are also known. The Zeeman Effect has proved to be the most practicable one. By employing the Zeeman Effect to modify the atomic absorption wavelength profile, the residual background absorbance at the analyte wavelength, can then be measured separately. Since only a single source is required, there remain none of the optical alignment problems encountered with their associated accuracy. Furthermore, the Zeeman Effect is generally applicable to all spectroscopic transitions and so encompasses the entire wavelength range.

When analysing samples of marine organisms from the field or from bioaccumulation experiments there is normally only a limited amount of biomass available, thus requiring micro

digestion procedures in combination with multielement determinations. In the past such procedures were employed, involving sequential multielement graphite furnace atomic absorption spectroscopy with deuterium background correction, first using ammonium dihydrogen phosphate as matrix modifier for Cd and Pb (Zauke et al., 1986) and then a palladium nitrate and magnesium nitrate modifier (Zauke et al., 1996; Kahle et al., 2003).

It was figured out that in particular for the analysis of the decapod brown shrimp *Crangon crangon* (Linnaeus, 1758) it was necessary to develop special methods and optimise established methods respectively. As a result of higher concentrations of Cu and Ni in brown shrimps than in other decapod crustaceans but also other crustaceans in general, higher calibration standards and more calibration steps (for Ni) were applied. For the measurement of this decapod species, the boundary conditions of the sequential analysis need to be aligned to the element ratio in the animals, so that all samples can be analysed within the same dilution. The reference samples Tort-2, Lobster hepatopancreas (National Research Council Canada) and CRM No 278R, Mussel Tissue: *Mytilus edulis* (Community Bureau of Reference) were used for this purpose since they are similar in their matrix to the marine organisms mentioned above.

The main objective of this paper was to modify and optimize the methods for graphite furnace atomic absorption (GFAAS) methodology for the Varian SpectrAA 880 Zeeman atomic absorption spectrometer after Kahle et al. (2003), suitable for the analysis of the trace metals cadmium (Cd), copper (Cu), nickel (Ni) and lead (Pb) in the brown shrimp *C. crangon*.

2. MATERIAL AND METHODS

2.1 Samples used in this study

For the analysis samples of the decapod crustacean *Crangon crangon* (Linnaeus, 1758) were collected from FRV "Walther Herwig III" (cruise 259, January 05 – 13, 2004) in an area of the inner German Bight (Box A, see Chapter 3, Figure 3.1), one of the eleven standard sampling areas of the German Small-scale Bottom Trawl Survey (GSBTS) in the North Sea (Ehrich et al., 1998; Ehrich et al., 2007). Fishing was carried out under standard IBTS (International Bottom Trawl Survey) protocol using a standard net GOV (Chalut à Grande Ouverture Verticale), with a trawling time of 30 min at a trawling speed of 4 knots (1 knot = 0.514 m s^{-1}). The locations of 29 sampling stations (station numbers 1-34, see Chapter 3, Figure 3.1) as well as trawl directions were selected randomly within the area under investigation of the survey. The trawl positions were taken as midpoint of the haul converted to an absolute measure in km (easting and northing) relative to 54°27´N and 6°58´E.

On board ship samples of *C. crangon* were taken from the catch while epibenthic fish were sorted, counted and weighed. Ten randomly collected shrimp specimens from the by-catch at each station were thoroughly rinsed with double-distilled water for a couple of seconds to remove possible contaminating particles. Superficial water was carefully removed with good-quality filter paper from the animals before they were transferred to sterile polystyrene Petri dishes, numbered, closed tightly with tape and deep frozen (stored at -18°C) for further

analysis of heavy metals in the laboratory (see below). To assure the quality of the sampling procedure, we followed the guidelines set-up in Zauke and Petri (1993) and Zauke et al. (1996).

Due to the analysis of background values in shrimps from the field, the concentration of the trace metals cadmium (Cd), copper (Cu), nickel (Ni) and lead (Pb) for *C. crangon* were obtained and the methods for the graphite tube atomic absorption were optimized for this decapod species.

2.2 Sample preparation

The samples of marine organisms, and here in particular of the brown shrimp *C. crangon,* are normally freeze-dried (LYOVAG GT2, Leybold Heraeus) and then homogenised using a small boron carbide mortar and pestle or a ball mill made of agate. The reference samples Tort-2, Lobster hepatopancreas (National Research Council Canada) and CRM No 278R, Mussel Tissue: *Mytilus edulis* (Community Bureau of Reference) do not need this treatment since they have already undergone these procedures. In order to have a homogenic powder, the reference samples have to be shaken well before use. Aliquots of about 10 mg dried material were digested for 3 hours at 80°C with 100 µL HNO_3 (65%, suprapure Merck) in tightly closed 2 ml Eppendorf safe-lock reaction tubes (Clason and Zauke, 2000). The digests were allowed to cool down slowly and were subsequently made up to 2 ml volume with bidistilled water. After appropriate dilution, final sample and standard solutions were adjusted to concentrations of 3.25% HNO_3.

2.3 Quality Assurance

Quality assurance was performed in line with German GLP (good laboratory practice) regulations (Anonymous, 2005), 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). Furthermore, precision and validity was evaluated using the certified reference materials Tort-2, Lobster hepatopancreas (National Research Council Canada) and CRM No 278R, Mussel Tissue: *Mytilus edulis* (Community Bureau of Reference) randomly allocated within routine determinations. In the present paper these materials were also used to optimise the methods. Limits of detection were calculated according to Büttner et al. (1980) as mean blank (eventually set to zero) plus 2.6 * standard deviations (SD) of a 'low value'.

2.4 Instrumental Parameters

Metal determinations were performed using a Varian SpectrAA 880 Zeeman instrument and a GTA 110 graphite tube atomiser. All elements were measured in the absorbance and concentration calibration mode using wall atomisation with Zeeman background correction (Rothery, 1988). Calibration graphs where obtained in new rational mode using 5 standards, made of a reslope solution using the auto-mix capabilities of the PSD (Programmable Sam-

ple Dispenser). Approximately every ten samples a reslope, the second or third standard of the calibration curve, was run automatically. Every thirty samples a recalibration was run automatically. The elements where determined in the order of increasing ashing- and atomisation temperatures. After completion of one element (a method in a worksheet of the software) a tube clean was applied to ensure an appropriate calibration zero (cal zero). Under normal conditions, 500-600 firings could be done before the tube had to be changed. The limit of the firing counter was set to 600 firings. Nitrogen gas of grade 5.0 was used.

The rinsing solution contained 0.002% Triton X -100 and 0.065% HNO_3 (suprapure Merck). To reduce the formation of gas bubbles in the syringe and in the capillary, the rinsing solution was prepared from 2-3 days old bidistilled water and the rinse vessel was kept at least half full. Gas bubbles in the dispensing system were removed before each automatic run. The capillary was frequently wiped with a soft tissue soaked with isopropylalcohol to prevent adhesive effects due to the matrix modifier used. Sample cups and reslope containers where sealed with slitted lids to minimise evaporation during the measurements and to prevent the formation of drops on the capillary during the measurements. By using these lids the capillary has to be fitted very accurately in the centre of the graphite tube and the sampling vessels.

Table 1.1: General method parameters and quantities of matrix modifiers used during the analysis of the trace metals cadmium (Cd), copper (Cu), lead (Pb), and nickel (Ni).

	Cd	Cu	Pb	Ni
Measurement mode	Prompt Height	Prompt Area	Prompt Height	Prompt Area
Wavelength [nm]	228.8	327.4	283.3	232.0
Lamp current [mA]	5.0	4.0	5.0	4.0
Slit width [mm]	0.5	0.5	0.5	0.2
Calibration steps	5	5	5	5
Sample volume [µl]	5	20	20	20
Modifier 1* [µl]	5	-	5	8
Modifier 2** [µl]	5	-	5	4
Calibration-range (µg L^{-1})	1-5	40-200	5-25	5-25

* Modifier 1: 0.4 mg ml^{-1} $Pd(NO_3)_2$; ** Modifier 2: 2 g L^{-1} $MgNO_3$

For Cd, Pb, and Ni palladium and magnesium nitrate modifiers adopted from Rettberg and Beach (1989), Welz et al. (1992) and Sneddon and Farah (1994) were applied (see Table 1.1). Both modifiers must be kept in separate vessels on the PSD to prevent any chemical effects which may disturb the injection of samples in the graphite tube. Detailed information regarding the methods employed for the various elements are given in Appendix 1-4 as original printouts from the automatic runs, including signal and calibration graphs. Further general instrument parameters are listed in Table 1.1. They were optimised on basis of information provided in the Varian SpectrAA 880Z software version 3.00 and after Kahle et al. (2003).

2.5 Statistical analysis

Descriptive statistics of the analysed variables cadmium (Cd), copper (Cu), nickel (Ni) and lead (Pb) were calculated with SYSTAT 10.0 for Windows. The parameter analysed were mean, Upper 95% and Lower 95% confidence interval (CI), coefficient of variation (C.V.) and Lilliefors Probability (2-tail) to test whether sample data are distributed normally (Wilkinson, 2000; Wilkinson and Engelman, 2000).

3. RESULTS AND DISCUSSION

The results of the quality assurance using certified reference materials Tort-2, Lobster hepatopancreas (National Research Council Canada) and CRM No 278R, Mussel Tissue: *Mytilus edulis* (Community Bureau of Reference) appear in Table 1.2, indicating in most cases a good agreement between the measured and certified values. At the bottom of the table the limits of detection according to Büttner et al. (1980) are indicated.

Table 1.2: Quality assurance using certified reference materials randomly allocated within the determinations. Values are means ± 95% confidence intervals [mg kg^{-1} DW].

	Tort-2 Lobster hepatopancreas; National Research Council Canada			CRM No 278R Mussel Tissue : *Mytilus edulis*; Community Bureau of Reference		
Ele-	analysed	n	certified	analysed	n	certified
Cd	24.1 ± 1.1	8	26.7 ± 0.6	0.389 ± 0.062	8	0.348 ± 0.007
Cu	109 ± 14	8	106 ± 10	9.17 ± 1.03	7	9.45 ± 0.13
Pb	<0.40	4	0.35 ± 0.13	2.17 ± 0.24	8	2.00 ± 0.04
Ni	2.10 ± 0.42	6	2.5 ± 0.19	0.78 ± 0.04	4	(1.00)

n: Numbers of independent determinations

Limits of detection (according to Büttner et al., 1980): 0.2 mg Cd kg^{-1}; 3 mg Cu kg^{-1}; 0.4 mg Pb kg^{-1} and 0.1 mg Ni kg^{-1} (dry weight).

For lead due to the low limit of detection, the values measured in the shrimps are close to the limit of detection itself. Limits of detection for the other metals proved to be adequate for the range of metal concentrations found in this study for marine crustaceans compared to other publications. The values for limits of detection obtained in this paper are in fairly good agreement with reported values in other studies [mg kg^{-1}]: (a) Cd= 0.1, Cu= 4.7, Ni= 0.74 and Pb= 0.32 in studies on trace metals in calanoid copepods and amphipods from the Weddell Sea (Kahle and Zauke 2002a, 2002b, 2003, 2003); (b) Cd= 0.12, Cu= 2.0, and Pb= 0.4 in a study on zooplankton and decapod crustaceans from the Barents Sea (Zauke and Schmalenbach (2006) and (c) Cd=0.13, Cu=2.6, Ni=0.6-1.0 and Pb=0.15 in a bioaccumulation study from estuaries in UK (Clason et al., 2004).

In Table 1.3 results for the descriptive statistics of the analysed *C. crangon* samples from 29 stations in the North Sea (Box A) are compiled. The values for the coefficient of variation are for all parameters, with the sole exception of lead, below 30%. Lead has a respectively high coefficient of variation of 74%, due to low measured concentrations close to the limit of detection (see note of Table 1.2). Values for the Lilliefors probabilities (LIP) indicate that sample data are distributed normally.

Table 1.3: Descriptive statistics of the variables cadmium (Cd), copper (Cu), nickel (Ni), and lead (Pb) from the analysed samples of *Crangon crangon* from the North Sea. Values are means ± 95% CI [mg kg^{-1} DW].

Variable	N	mean	C.V. [%]	LIP
Cd	29	0.22 ± 0.03	27	0.001
Cu	29	37 ± 2	13	1.000
Ni	29	1.1 ± 0.1	25	0.363
Pb	29	0.75 ± 0.21	74	0.002

Note: N = abundance, C.V. = coefficient of variation, CI = confidence interval, LIP= Lilliefors Probability (2-tail) to test whether sample data are distributed normally, DW = dry weight.

The overall recovery rate is high for all elements analysed in both reference materials (Table 1.2) and the limits of detection are sufficiently low, to make the methodology suitable for the analysis of decapod crustaceans. This was likewise the case in previous studies, employing graphite furnace AAS with deuterium background correction (Ritterhoff and Zauke, 1997b; Kahle et al., 2003). Somewhat surprising is, however, that current limits of detection in this study (as well as in Kahle et al. 2003) are not lower than previously reported ones, despite the fact that the instrument used in recent studies (SpectrAA 880 Zeeman) seems to be more sensitive than the instrument used before (Varian AA-975, GTA-95). However, since the calculation method employed (Büttner et al. 1980) relies on the variability of a 'low value', the micro-digestion procedure will be the limiting factor, eventually due to unavoidable inhomogeneities in the samples.

Since we do not have observed any drift in calibration or underground signals, measurements in the concentration calibration mode is preferable compared to standard addition mode for the analytical performance, regarding time and material effort. Furthermore, standard addition mode requires linear calibration curves within the analytical range. It is noteworthy to mention that peak appearance times largely show good agreements between calibration standards and both reference materials for all elements (see Appendix 1-4), indicating that severe matrix effects have not to be considered. This result is most likely due to the application of chemical modifiers and Zeeman background correction (e.g. Dulude, 1992; Welz et al., 1992; Beisel et al., 1993).

In order to obtain as most information as possible from small sample volumes, the capability to measure multielement sequences within one automatic run is necessary. Furthermore this reduces analytical effort and prevents digests from ageing. However, the concentration ranges of calibration curves, sample volumes and volume reduction factors have to be chosen carefully. Thus, drying and ashing stages must be adapted to the different sample volumes. To prevent high volume reduction or over ranged samples, it is strongly recommended that the concentration range of samples considered is approximately known. The utilisation of slitted lids for the sample cups reduces the evaporation to a minimum.

The Zeeman Effect in furnace atomic absorption provides a background correction measuring the total absorbance in the analyte in combination with the background, and simultaneously provides a reference measurement due to all of the absorbing species except the analyte. Historically, the initial attempt to provide this reference measurement was achieved by incorporating a deuterium continuum source into the spectrometer's optical system. The absorbance measured with the hollow cathode lamp includes the contributions from the analyte, matrix and scattering components while with the deuterium lamp there is essentially no residual atomic absorption (Rothery, 1988).

Several experimental arrangements are possible when exploiting the use of the Zeeman Effect in atomic absorption. Firstly, the field can be applied to either the lamp or to the sample since the Zeeman Effect applies both to absorbing and emitting atom species. Standard hollow cathode lamps do not operate satisfactorily in the strong magnetic field required. Even if acceptable operation were achievable, the split lamp emission profile would result in the background absorption being measured at wavelength slightly removed from the analyte absorption wavelength. Consequently, if the background contained discrete spectral features, this approach could lead to inaccurate background correction. By applying the field directly to the absorbing sample, standard hollow cathode lamps can be used and the background absorbance is measured at the same wavelength as that of the analyte and so the correction for structured background features is optimized (Rothery, 1988).

In conclusion, the methods presented in this paper are applicable for the sequential multielement analysis of decapod crustaceans, where often only small amounts of biomass are available, especially when bioaccumulation experiments are considered. This is supported by almost linear calibration graphs within the analytical range, improving the sensitivity of the analysis, by peak appearance times in excellent conformity comparing standard solutions and other matrices analysed, by a good agreement of analysed and certified values of reference materials, by limits of detection low enough for the measurements of the samples of interrest (with the sole exception of Pb) and by sufficiently narrow 95%-confidence intervals of field samples of brown shrimps. The latter will be explored in more details regarding a spatial analysis in Chapter 3.

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APPENDIX: Instrumental parameters, calibration graphs and selected signal graphics from the determination by Zeeman graphite furnace atomizer

Method: Cadmium (Cd) (Zeeman) - Appendix 1

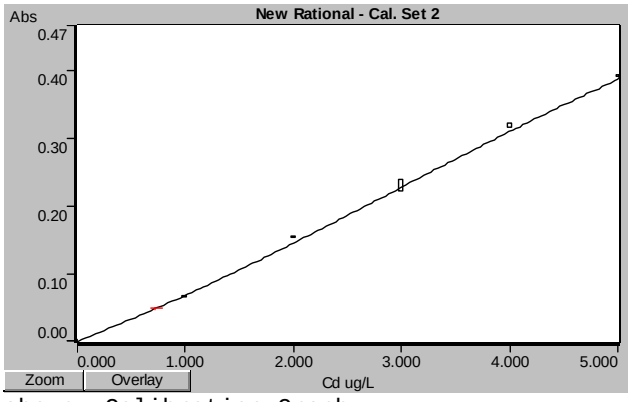
Instrument Mode:	Absorbance
Sampling Mode:	Autonormal
Calibration Mode:	Concentration
Measurement Mode:	PROMT Height
Replicates Standard:	3
Replicates Sample:	3
Total Volume:	15 uL
Sample Volume:	5 uL
Vol. Reduction Factor:	5
Bulk Conc.:	5 ug/L
Modifier 1 Mode:	Co Inject
Modifier 1 Vol.:	5 uL
Modifier 2 Mode:	Co Inject
Modifier 2 Vol.:	5 uL

Furnace Parameters

Step	Temp(C)	Time(s)	Flow (L/min)	Gas Type	Read	Signal Storage
1	80	5.0	3.0	Normal	No	No
2	110	25.0	3.0	Normal	No	No
3	600	5.0	3.0	Normal	No	No
4	600	7.0	3.0	Normal	No	No
5	600	1.0	0.0	Normal	No	Yes
6	1800	0.8	0.0	Normal	Yes	Yes
7	1800	1.0	0.0	Normal	Yes	Yes
8	2400	0.3	3.0	Normal	No	Yes
9	2400	0.8	3.0	Normal	No	Yes

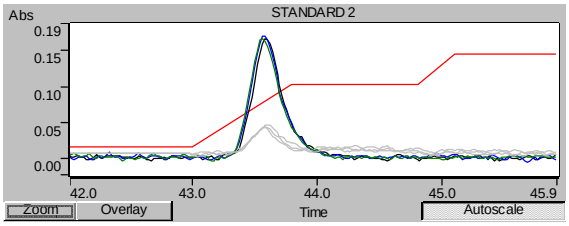
Calibration

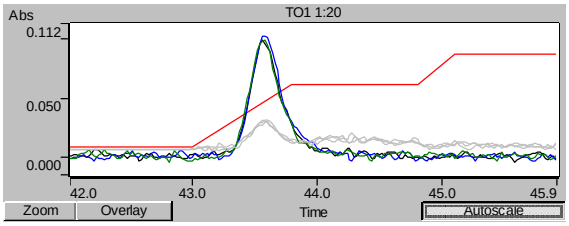
Sample ID	Conc ug/L	%Prec	Mean Abs
CdCAL ZERO	0.0	2.9	0.0056
CdSTANDARD 1	1.0	1.3	0.0648
CdSTANDARD 2	2.0	1.8	0.1525
CdSTANDARD 3	3.0	4.6	0.2293
CdSTANDARD 4	4.0	1.8	0.3161
CdSTANDARD 5	5.0	0.5	0.3901

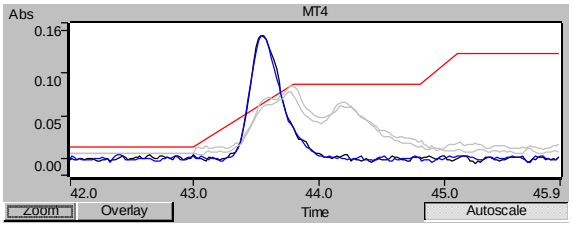


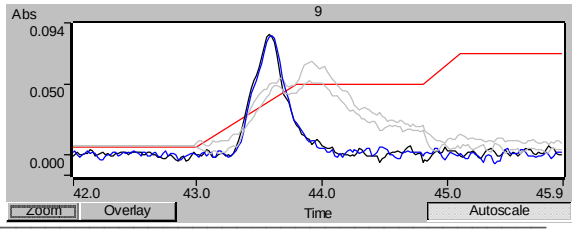
above: Calibration Graph

right from top:
- Signal Graphics for Standard 2
- Reference Material CRM 786R (MT)
- Reference Material Tort-2 (LH)
- Signal Graphics for Sample 9 *C. crangon*









Method: Copper (Cu) (Zeeman) - Appendix 2

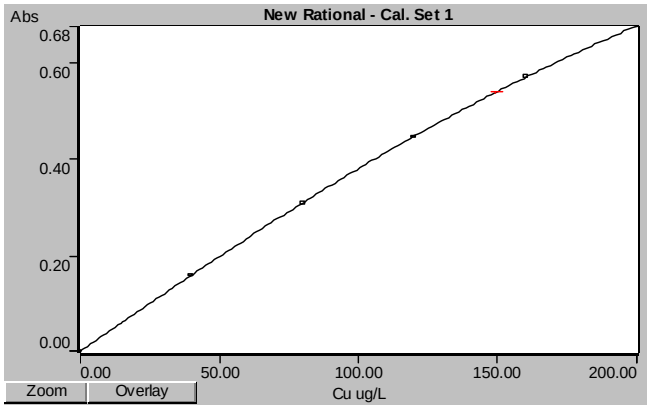
```
Instrument Mode:              Absorbance
Sampling Mode:                Autonormal
Calibration Mode:             Concentration
Measurement Mode:             PROMT Area
Replicates Standard:          3
Replicates Sample:            3
Total Volume:                 20 uL
Sample Volume:                20 uL
Vol. Reduction Factor:        3
Bulk Conc.:                   200 ug/L
```

Furnace Parameters

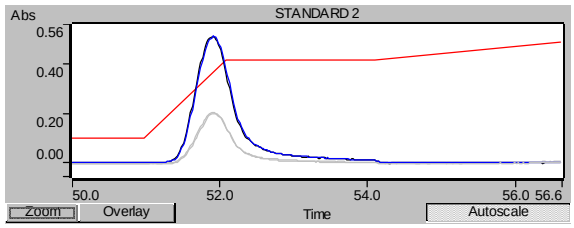
Step	Temp (C)	Time (s)	Flow (L/min)	Gas Type	Read	Signal Storage
1	100	5.0	3.0	Normal	No	No
2	110	25.0	3.0	Normal	No	No
3	120	10.0	3.0	Normal	No	No
4	800	5.0	3.0	Normal	No	No
5	800	5.0	3.0	Normal	No	No
6	800	1.0	0.0	Normal	No	Yes
7	2300	1.1	0.0	Normal	Yes	Yes
8	2300	2.0	0.0	Normal	Yes	Yes
9	2650	2.5	3.0	Normal	No	Yes

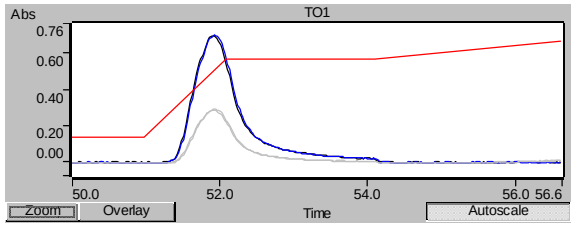
Calibration

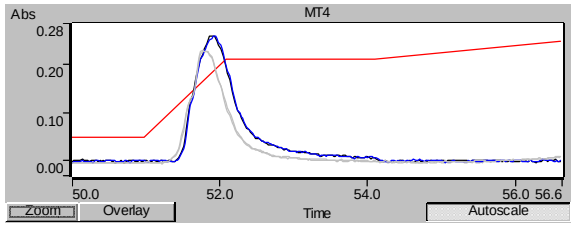
Sample ID	Conc ug/L	%Prec	Mean Abs
CuCAL ZERO	0	>100	0.0000
CuSTANDARD 1	40	1.0	0.1593
CuSTANDARD 2	80	0.5	0.3077
CuSTANDARD 3	120	0.2	0.4451
CuSTANDARD 4	160	0.9	0.5707
CuSTANDARD 5	200	0.6	0.6761

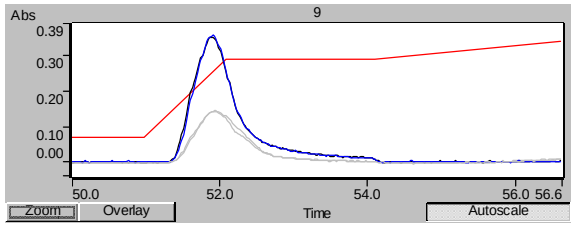


above: Calibration Graph









right from top:
- Signal Graphics for Standard 2
- Reference Material CRM 786R (MT)
- Reference Material Tort-2 (LH)
- Signal Graphics for Sample 9 *C. crangon*

Method: Nickel (Ni) (Zeeman) - Appendix 3

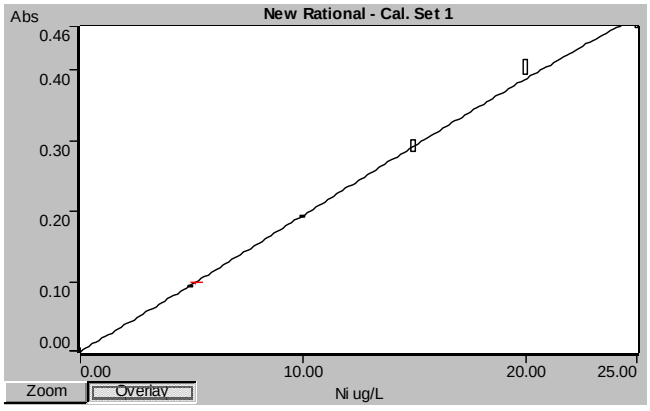
```
Instrument Mode:              Absorbance
Sampling Mode:                Autonormal
Calibration Mode:             Concentration
Measurement Mode:             PROMT Height
Replicates Standard:          3
Replicates Sample:            3
Total Volume:                 32 uL
Sample Volume:                20 uL
Vol. Reduction Factor:        5
Bulk Conc.:                   25 ug/L
Modifier 1 Mode:              Co Inject
Modifier 1 Vol.:              8 uL
Modifier 2 Mode:              Co Inject
Modifier 2 Vol.:              4 uL
```

Furnace parameters

Step	Temp(C)	Time(s)	Flow (L/min)	Gas Type	Read	Signal Storage
1	100	5.0	3.0	Normal	No	No
2	115	40.0	3.0	Normal	No	No
3	130	10.0	3.0	Normal	No	No
4	800	5.0	3.0	Normal	No	No
5	800	1.0	3.0	Normal	No	No
6	800	1.3	0.0	Normal	No	Yes
7	2700	1.4	0.0	Normal	Yes	Yes
8	2700	1.2	0.0	Normal	Yes	Yes
9	2800	1.0	3.0	Normal	No	Yes

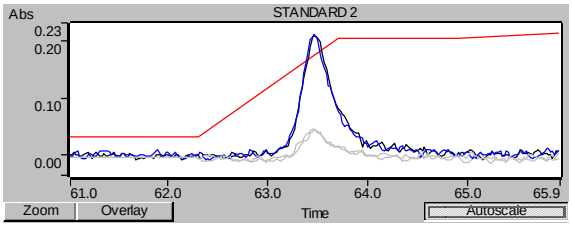
Calibration

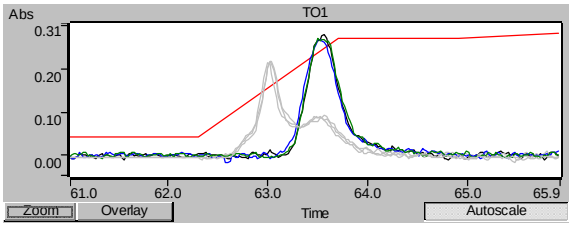
Sample ID	Conc ug/L	%Prec	Mean Abs
NiCAL ZERO	0	31.0	0.0177
NiSTANDARD 1	5	1.3	0.0927
NiSTANDARD 2	10	0.3	0.1916
NiSTANDARD 3	15	3.7	0.2910
NiSTANDARD 4	20	3.2	0.4030
NiSTANDARD 5	25	1.6	0.4627

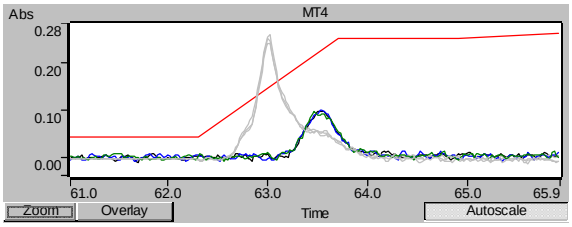


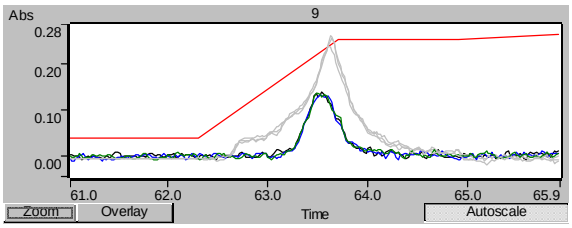
above: Calibration Graph

right from top:
- Signal Graphics for Standard 2
- Reference Material CRM 786R (MT)
- Reference Material Tort-2 (LH)
- Signal Graphics for Sample 9 *C. crangon*









Method: Lead (Pb) (Zeeman) - Appendix 4

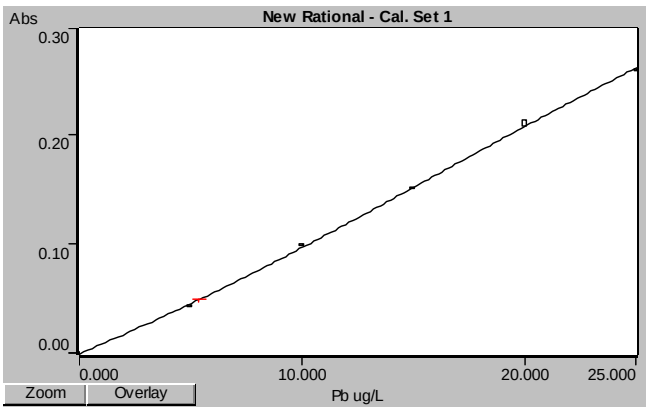
```
Instrument Mode:              Absorbance
Sampling Mode:                Autonormal
Calibration Mode:             Concentration
Measurement Mode:             PROMT Height
Replicates Standard:          3
Replicates Sample:            3
Total Volume:                 30 uL
Sample Volume:                20 uL
Vol. Reduction Factor:        5
Bulk Conc.:                   25 ug/L
Modifier 1 Mode:              Co Inject
Modifier 1 Vol.:              5 uL
Modifier 2 Mode:              Co Inject
Modifier 2 Vol.:              5 uL
```

Furnace Parameters

Step	Temp(C)	Time(s)	Flow (L/min)	Gas Type	Read	Signal Storage
1	90	10.0	3.0	Normal	No	No
2	110	20.0	3.0	Normal	No	No
3	200	5.0	3.0	Normal	No	No
4	1000	5.0	3.0	Normal	No	No
5	1000	5.0	3.0	Normal	No	No
6	1000	1.5	0.0	Normal	No	Yes
7	2200	0.6	0.0	Normal	Yes	Yes
8	2200	1.0	0.0	Normal	Yes	Yes
9	2600	0.2	3.0	Normal	No	Yes
10	2600	0.5	3.0	Normal	No	Yes

Calibration

Sample ID	Conc ug/L	%Prec	Mean Abs
PbCAL ZERO	0	24.5	0.0044
PbSTANDARD 1	5	1.3	0.0430
PbSTANDARD 2	10	2.0	0.0986
PbSTANDARD 3	15	0.5	0.1503
PbSTANDARD 4	20	2.1	0.2092
PbSTANDARD 5	25	0.8	0.2576



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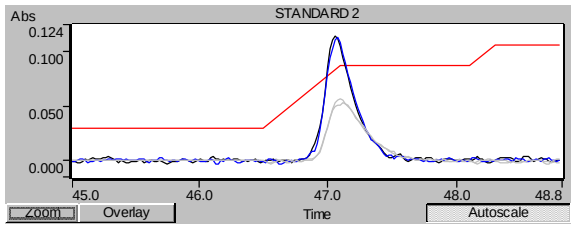

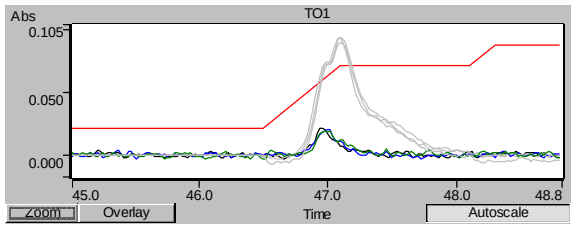

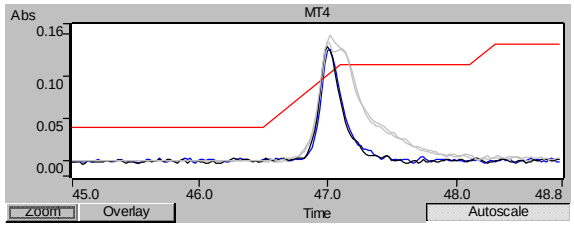

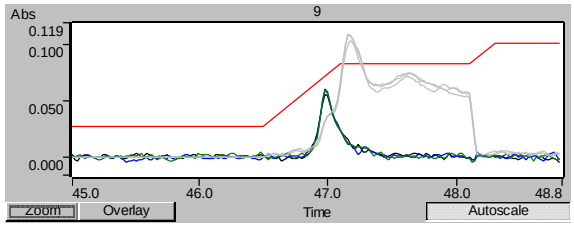

right from top:
- Signal Graphics for Standard 2
- Reference Material CRM 786R (MT)
- Reference Material Tort-2 (LH)
- Signal Graphics for Sample 9 *C. crangon*

CHAPTER 2

Spatial distribution of heavy metal concentrations and biomass indices in *Cerastoderma edule* Linnaeus (1758) from the German Wadden Sea: An integrated biomonitoring approach[*]

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Homepage: http://www.icbm.de/~aqua/index.html

Abstract

The spatial distribution of heavy metal concentrations (*Cu*, *Pb*, *Cd*, *Ni* and *Zn*) and biomass indices (total, soft-body and standardised soft-body weight, *TW*, *SBW* and *SSBW*) in the cockle *Cerastoderma edule* from the German Wadden Sea has been investigated at an intermediate ecological scale of 300 x 300 m to evaluate the range of spatial autocorrelations for the different variables under study and to assess the extent to which biological properties of the organisms may be related to the spatial distribution of metal concentrations in *C. edule* from the sampling area. Semivariogram models obtained by geostatistical procedures indicate a distinct increase in variability for most variables with sampling distance: exceptions are *Cd*, *Pb* and *Ni*, for which a pure nugget-effect model is found. Only if samples are taken at distances above the estimated values for the practical range of the semivariogram, can stochastic independence of the data be assumed. These distances are 30 m for *Cu*, 122 m for *Zn*, 132 m for *SBW*, 156 m for *SSBW* and 170 m for *TW*. The contour plots of the mapped spatial distribution show a clear coincidence of increasing values for *TW*, *SBW* and *SSBW* with increasing relative height above sea level (*RH*) from shoreline to the sea (*viz*. from south to north). The spatial distribution of *SSBW* indicates the cumulation of larger-sized cockles in

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the northern part of the sampling area, while *Cu* concentrations in cockles decreased with increasing distance from shore line. This spatial correlation is supported by results of Mantel's test, taking the relative distance among sample locations into account. In future studies such an approach might be useful when addressing the problem of trophic transfer of energy or pollutants from food (e.g. cockles) to higher trophic levels like fish and seabirds.

Key words: *Cerastoderma edule*, heavy metals, biomonitoring, geostatistic, spatial analysis

1. INTRODUCTION

Biomonitoring of trace metals receives continued attention in the scientific literature and international and national environmental programmes (AMAP, 2005; BMLP, 2005; CCMA, 2005; TMAP, 2005). To assess the quality of the marine environment regarding heavy metals, the bioavailable fraction in 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 on dissolved uptake (Kahle and Zauke, 2002). Thus, the total bioavailable fraction is only accessible by determining the amount of metals incorporated in organisms (Zauke et al., 1996). The accumulation patterns and subsequent accumulated concentrations in marine organisms often vary depending on the species, 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 benthic invertebrates with greatly varying metal concentrations in the same body of seawater (Zauke et al., 1995; Mattig et al., 1997; Zauke et al., 2003).

In many environmental programmes mussels like *Mytilus edulis* or oysters like *Crassostrea gigas* are used as biomonitors. However, due to their importance in the food webs of mud flats, other bivalves such as cockles (e.g. *Cerastoderma edule*) merit further consideration, as recently suggested by Szefer et al. (1999) and Cheggour et al. (2000) and (2001). Cockles can be regarded as net accumulators for some metals, as can be inferred from detected linear regression relationships between Cd, Pb and Zn in cockles and the surrounding sediments (Cheggour et al., 2001) or from toxicokinetic bioaccumulation studies showing a distinct uptake and depuration of Cd, Pb and Cu in experimental organisms (G.-P. Zauke and M. Schwager, ICBM, Oldenburg, unpublished data).

The cockle *C. edule* is an infaunal suspension feeder, living in the upper few centimetres of the sediment. It burrows just below the sand surface and may reach adult densities of up to several thousands per square meter, mainly in the intertidal zone (de Montaudouin and Bachelet, 1996). This bivalve is one of the dominant and important species in tidal flats of the Dutch, German and Danish Wadden Sea (Flach, 1996), where it represents 8 - 60% of the total macrobenthic biomass (Ramon, 2003). Cockles are important food resources for

shrimps (*Crangon crangon*), flatfish (*Pleuronectes platessa*), shore crabs (*Carcinus maenas*), oystercatchers (*Haematopus ostralegus*), herring gulls (*Larus argentatus*) and eiders (*Somateria mollissima*) (Jensen and Jensen, 1985). The spatial structuring of *C. edule* in the Wadden Sea is controlled by abiotic variables such as sediment quality (Ducrotoy and Desprez, 1986), sediment dynamics (Bouma et al., 2001), severe winters or wind-induced effects (Strasser et al., 2003). Biotic factors, for instance predation (Strasser and Gunther, 2001), competition or interactions between adults and juveniles (de Montaudouin and Bachelet, 1996), also strongly influence the recruitment and subsequent spatial distribution of cockles.

The patchy nature of the environment coupled with the behaviour of a species determines the spatial arrangement of individuals of that species, and the observed spatial distributions are important for understanding ecological processes. Moreover, identifying spatial patterns is important in order to improve the design and interpretation of surveys and experimental studies, by relating sampling programmes to natural scales of variation (Livingston, 1987; Thrush et al., 1989; Stelzenmüller et al., 2005). Although spatial variability on different ecological scales is an important issue in mudflat ecology (Ysebaert and Herman, 2002; Norén and Lindegarth, 2005; Thrush et al., 2005), it is important to note that the spatial dependence of variables has not explicitly been taken into account in ecological and biomonitoring field studies in this area, despite the well-known importance of spatial dependence in ecology and environmental sciences.

This importance is stressed by the following arguments. Sampling points randomly distributed over an area can yield unbiased estimates of the variable of interest only if the sampling-point observations are independent (Petitgas, 2001). When random sampling is carried out at an appropriate spatial scale, it effectively extinguishes any underlying spatial structure in the distribution of organisms. However, the scale of spatial distribution of the target species is usually unknown, and this factor may result in a bias in the calculation of population estimates (Maynou, 1998). The presence of a spatial structure is indicated by spatial autocorrelation between pairs of samples, *viz.* the realisation of a regionalised variable (e.g. biomass of organisms) at one location influences the realisation at neighbouring locations. Thus, when samples are not taken independently of one another and when the population sampled is spatially structured, the computation of any variance requires a model of the spatial relationships within the population (Matheron, 1971). Spatial autocorrelations are recognised as typical characteristics of ecological units like populations, but also of other environmental variables (Legendre and Legendre, 1998), and can be analysed and modelled mathematically by geostatistics. Thus, the presence of patches, density gradients and spatially autocorrelated variables may confound designs and affect the validity of inferential statistics. Future studies must integrate the intensity and form of patterns from various spatial and temporal scales, if we are to understand the processes responsible for generating pattern (Thrush, 1991; Thrush et al., 2005).

Because this aspect is largely missing in ecological and biomonitoring studies in the intertidal zone, we present here an integrated approach combining the analysis of the spatial distribution of heavy metal concentrations and biomass indices in the cockle *C. edule* from the German Wadden Sea, sampled on an intermediate ecological scale (Ysebaert and Herman,

2002) of 300 x 300 m. We employed geostatistical methods recently optimised for the evaluation of fisheries data (Stelzenmüller et al., 2004), especially regarding additional small-scale survey data (Stelzenmüller et al., 2005). The goal is to evaluate the range of spatial autocorrelations for the different variables under study and to assess the extent to which biological properties of the organisms are potentially related to the spatial distribution of metal concentrations in *C. edule* from the sampling area.

2. MATERIALS AND METHODS

2.1. Sampling area and collection of cockles

The survey was conducted on the German North Sea coast at Dornumersiel within the German Wadden Sea in May 2001 (Fig. 2.1 A, B). To obtain the sampling sites, a rectangle measuring 300 by 300 metres was defined, referred to below as the sampling area, with vertices given as the Cartesian coordinates (0.0; 0.0: SE) and (300.0; 300.0: NE). Subsequently, a grid was defined with a spacing of 20 m parallel to the x-axis (east) and to the y-axis (north) leading to 256 nodes as potential sampling locations, of which 50 were selected by pseudo-random numbers using SYSTAT for Windows (Version 10, Wilkinson, 2000b). We thus obtained distance classes between 20 and 200 meters. Furthermore, at two randomly chosen meso-scaled sampling locations small-scaled sampling with distance classes between 2 and 16 metres was carried out in order to improve the resolution of small-scale variability (Stelzenmüller et al., 2005). In the field, the SE vertex of the rectangle was placed randomly, approximately 500 metres apart from the high–water level in front of the beach (5951441 N, 2598072 E; Gauß-Krüger coordinates, Potsdam date; TOP50 Karte Niedersachsen; Version 2.0, CD-ROM, Landesvermessung und Geobasisinformation, Niedersachsen). Within this area the 50 pre-defined sampling sites were established (Fig. 2.1 C). Corresponding coordinates and relative heights above sea level were assayed using tachymetry for each sampling location. It was only possible to determine relative heights, since no surveying point or tide gauge was available in this area.

At each sampling location a wooden frame 1 m² in area was positioned at random. This frame was divided by ropes into 16 sub-areas, 3 of which were selected randomly. The cockles, *Cerastoderma edule* Linnaeus (1758), were quantitatively collected from the selected sub-areas (each 1/16 = 0.0625 m²) by sieving the sediment with a mesh size of 1 mm. All cockles per sub-area were counted and the total body mass was weighed. We thus obtained 3 independent replicates per sampling site. The biomass indices at each sampling site are hereinafter expressed as mean values of the three cut-frames selected per site. In addition, approximately 10 living cockles per sampling site were taken from this pool to the laboratory to establish the relation between total and soft-body weight and to quantify the concentrations of heavy metals in the soft-body tissues (see below).

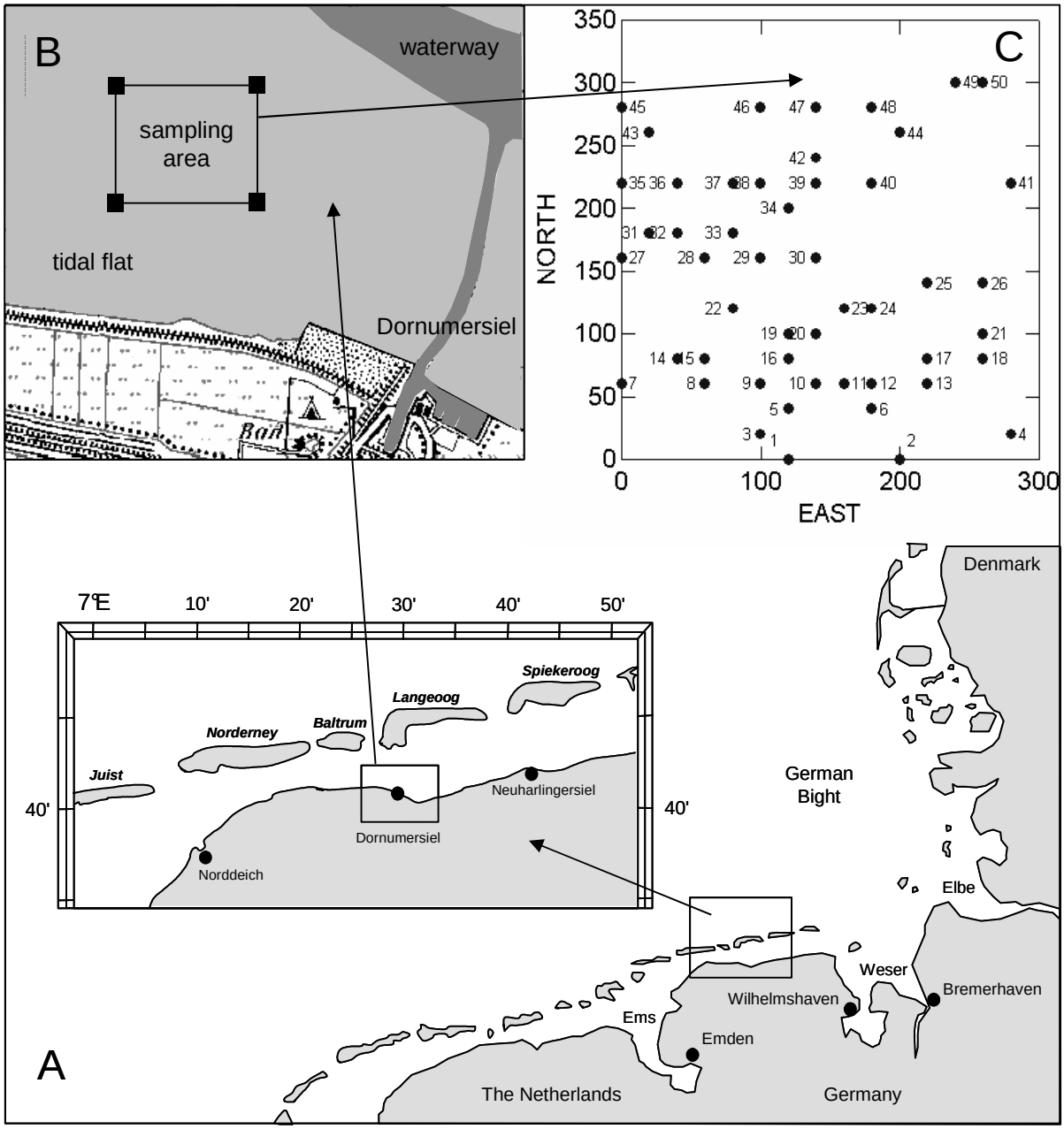

Fig. 2.1: Spatial location of the investigation area with A.) German Bight and East Friesian Islands; B.) Sampling area at Dornumersiel and C.) Sampling localities within the sampling area.

2.2. Sample preparation and analytical procedures

Upon arrival at the laboratory the organisms were kept alive for 48 h in aerated seawater (32 psu; 13°C) to allow defecation. The water was obtai ned from the marine station of the ICBM in Wilhelmshaven. After defecation, the organisms were deep frozen, and after about 2 h each cockle was weighed separately with shell to obtain the individual total body mass. Subsequently, the soft-body tissue was separated from the shell and also weighed to establish total to soft-body weight relationships by applying linear regression using SYSTAT 10

(Wilkinson and Coward, 2000). The pooled soft-body tissues of 10 cockles from each sampling site were briefly rinsed with double-distilled water, dried on good-quality filter paper, transferred to a polystyrene Petri dish and stored at –18°C for subsequent metal analysis.

The frozen *C. edule* samples were subjected to freeze-drying for 48 h (LYOVAG GT2, Leybold Heraeus). Then the samples were homogenised using a small boron carbide mortar and pestle to avoid losses of biomass. 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 safe-lock reaction tubes (Clason and Zauke, 2000). The digests were made up to 2 ml volume with double-distilled water. After appropriate dilution, the final sample and standard solutions were adjusted to concentrations of 3.25% HNO_3.

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 with methods modified from 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; 800 and 2700 °C for Ni. For Cd, Pb and Ni, palladium and magnesium nitrate modifiers were applied. Zn was assayed using an air-acetylene flame (Varian 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).

Table 2.1: Quality assurance using certified reference materials randomly allocated within the determinations. Values are means ± 95% confidence intervals [mg kg^{-1} DW].

	TORT-2 (Lobster hepatopancreas)		CRM 278R (Mussel tissue)	
Element	analysed	certified	analysed	certified
Cd	out of range	26.7 ± 0.6	0.509 ± 0.045	0.348 ± 0.007
Cu	117 ± 17	106 ± 10	9.74 ± 0.22 [c]	9.45 ± 0.13
Ni	1.57 ±1.05 [a]	2.50 ± 0.19	(0.69 ± 0.25)	(1.00 ±)
Pb	0.78 ± 0.56 [b]	0.35 ± 0.13	2.59 ± 0.20	2.00 ± 0.04
Zn	201 ± 26 [a]	180 ± 5	95.5 ± 13.5	83.1 ± 1.7

*Notes***:** Numbers of independent determinations: [a] 6; [b] 7; [c] 16; others: 10.

Limits of detection [mg kg^{-1} DW] (based on determination of CRM 278R) calculated as 2.6 standard deviations of a "low sample", (Büttner et al., 1980): Cd: 0.16; Cu 1.1; Pb: 0.7; Ni: 0.9. For Zn no limits of detection could be calculated due to high readings.

Quality assurance was performed in line with German GLP regulations (Anonymous, 2005), 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 two certified reference materials which were randomly allocated within the determinations (Table

2.1). The analysed values for the reference materials were largely in good agreement with the certified values. Limits of detection calculated according to Büttner et al. (1980) proved to be adequate for the range of metal concentrations found in this study.

2.3. Preliminary analysis

For each sampling location, the relative heights above sea level obtained from the tachymetry, referred to as *RH*, are given in centimetres (cm); the total weights and the soft-body weights of the cockles (referred to as *TW* and *SBW*) are given in grams per 1/16 m². At each station, an individual regression model (see above) was used to compute the soft-body weight from the total weight and abundance of cockles. Furthermore, the soft-body weight was standardised (and then termed *SSBW*) by the number of cockles at each station, to obtain an indicator of the mean size of individual cockles at each station. Concentrations of copper, lead, cadmium, nickel and zinc in soft-body tissues of the cockles are given in mg kg^{-1} DW (referred to as *Cu*, *Pb*, *Cd*, *Ni* and *Zn*). For each of these variables descriptive statistics were calculated (Wilkinson and Engelman, 2000) and the normality of the data was tested by the Lilliefors Probability Test (Wilkinson, 2000a), again using SYSTAT 10.

In order to investigate possible trends within the data linear and non-parametric regressions with one covariate (Bowman and Azzalini, 1997) (relative height) were carried out (Kaluzny et al., 1998). Significant linear trends within the data with the relative height were detected for *TW*, *SBW*, *SSBW* and *Cu* (Table 2.2). These trends were taken into account for subsequent structural analysis. Furthermore, to visualise the spatial distribution of the sample values, post plots were created, where sample values were plotted proportional to the sample value at each sampling site.

Table 2.2: Parameters of linear parametric and non-parametric regressions of variables measured for *Cerastoderma edule* (dependent) on relative height above sea level (*RH*, independent). Significant p-values (<0.05) indicate spatial trends to be considered in the subsequent geostatistical analysis.

Variable	a	b	R^2 (%)	F-statistic	p-value
TW	-176	1.75	20.6	13.5	0.001
SBW	-75.5	0.74	17.8	11.2	0.002
SSBW	-1.38	0.02	21.7	14.0	0.001
Cu	8.98	-0.02	30.2	21.3	< 0.001

a = constant and b=slope of the linear regression; degrees of freedom: 46.

TW: total weight of *C. edule* (g 1/16 m^{-2}); *SBW*: soft-body weight (g 1/16 m^{-2}); *SSBW*: standardised soft-body weight (g per individual cockle); *Cu*: copper concentrations in *C. edule* (mg kg^{-1} DW).

2.4. Structural analysis and surface mapping

For this study the structure of spatial variability of *Z*(*x*) (*TW*, *SBW*, *SSBW*, *Cu*, *Pb*, *Cd*, *Ni* and *Zn*) was assessed by an empirical covariance function. Empirical semivariograms $\hat{\gamma}(\mathrm{h})$ were used to describe the spatial structure of the sample data. The semivariogram outlines the spatial correlation of data, measuring the half variability between data points as a function of their distance. In the absence of spatial autocorrelation among samples the semivariance is equal to the variance of *Z*(*x*). When a significant linear trend was encountered, data were detrended (Kaluzny et al., 1998). Omnidirectional and directional semivariograms were computed using the robust "modulus" estimator, which is supposed to be resistant against extreme values and skewed data distribution (Cressie, 1991):

$$\hat{\gamma}(h) = \left\{ \frac{1}{N(h)} \sum_{x_i-(x_i+i)\sim h} \left| Z(x_i+h) - Z(x_i) \right|^{\frac{1}{2}} \right\}^4 \Big/ (0.914 + (0.988/N(h))) \tag{1}$$

where $Z(x_i)$ is the realisation of the variable under study at station x_i, $Z(x_i+h)$ is another realisation separated from x by a discrete distance h (measured in m) and $N(h)$ is the number of pairs of observations separated by h. Directional semivariograms (for *RH*, *TW*, *SBW* and *SSBW*) were computed along the east-west direction and were corrected for the detected presence of a geometric anisotropy (Kaluzny et al., 1998). The parameters nugget (C_0), sill (C) and range (a) of spherical and Gaussian models were fitted automatically to the empirical semivariograms (Cressie, 1991). To reduce subjectivity and to ensure reproducibility of the fit, a weighted least squares procedure was employed, where more weight is given to the points near the origin, which is the crucial part in determining the variogram parameters.

Furthermore, to measure the strength of spatial dependence within sample data a ratio of structural variance (C) to sample variance (C_0+C) was computed (Robertson and Freckmann, 1995):

$$SD = \frac{C}{C_0 + C} \tag{2}$$

When this ratio approaches 1, the spatial dependence is high over the range of modelled separation distances. Conversely, when the ratio approaches 0, spatial dependence is low. Low spatial dependence indicates a high sampling and/or analytical error, or a spatial variability occurring at scales smaller than the minimum distance separating small sampling pairs. Sokal (1978) related the diameter of an aggregation of a species to the modelled range. The effective range (*ra*) for spherical models is equal to the estimated range and in case of a Gaussian model the effective range is $\sqrt{3}a$.

In order to assess the goodness-of-fit (*gof*) of the different models, for each fitting procedure an index recommended by Fernandes and Rivoirard (1999) was computed:

$$gof = \frac{\sum_h N(h)\left[\hat{\gamma}(h) - \gamma(h)\right]^2}{\sum_h N(h)\left[\hat{\gamma}(h)\right]^2} \tag{3}$$

where $N(h)$ is the number of pairs used to compute the semivariogram, $\hat{\gamma}(h)$ is the empirical semivariogram and $\gamma(h)$ is the fitted model. The closer the *gof* to 0, the better the fit. In addition, the observed data were cross-validated by ordinary kriging, which provides a measurement of the reproduction of the data by the model defined and the kriging procedure. The results of this jack-knifing method are given by standardised errors. If the mean of this standardised error (*Zscore*) is zero and the standard deviation (*SD-Zscore*) approximately 1, then the model and the method employed provide an adequate reproduction of the data (Isaaks and Srivastava, 1989).

Mapping of density surfaces of the predicted sample values of *TW*, *SBW*, *SSBW*, *Cu*, and *Zn* was carried out with ordinary kriging and universal kriging (kriging in the presence of a drift when a linear trend was detected). For punctuate estimates of variable values at unsampled locations, the estimator given by Webster (2001) was employed:

$$\hat{Z}(X_o) = \sum_{k=0}^{K} \sum_{i=0}^{N} \alpha_k \lambda_i f_k(X_i) \tag{4}$$

where λ_i are charging weights attributed to each $Z(X_i)$, f_k represents the drift by a set of functions, $f_k(x)$, k = 0,1,...K, of our choice (here simple polynomials of the order 1) and a_k are the unknown coefficients. To apply this interpolation method, a grid was drawn over the area investigated with a mesh size of 3 m. The uncertainty of the estimation of ordinary and universal kriging was expressed by computing the square root of the kriging variance (kriging error).

Additionally, partial Mantel's tests were computed (Mantel, 1967; Smouse et al., 1986) taking the relative distance among sample locations into account (Fortin and Dale, 2005; Legendre et al., 2005) to provide information on the spatial relationships between the kriging estimates for the biological variables and the measured heavy-metal concentrations, while accounting for the spatial structuring and the intercorrelation with relative height (*RH*). Also significant spatial overlapping was tested among the biological variables to explore whether the total weight and soft-body weight are related to cockle size (*SSBW*). Tests were calculated with 1000 permutations based on the Spearman rank correlation (Best and Roberts, 1975). Thus the hypothesis “No spatial correlation between the two matrices, while controlling the space effect” was tested for all possible pairings of biotic and abiotic variables. Rufino (2004) used Mantel's test to test for significant spatial overlapping of kriging estimates of crustacean biomass and sediment parameters.

When it is possible to fit an appropriate spatial model, the mean of the corresponding variable estimated by kriging is expected to be similar to the sample mean (Isaaks and Srivastava, 1989). Furthermore, in order to compare the classical and geostatistical estimates, the arithmetic mean (m), the mean sample value estimated by kriging (m_K), the classical standard deviation (s) and the square root of the mean kriging error (*ke*) were calculated. Subsequently, the following parameters were estimated:

$$S_m = s / \sqrt{n} \quad \text{(the classical standard error)} \tag{5}$$

$$S_{m_k} = ke/\sqrt{n} \text{ (the geostatistical standard error)} \quad (6)$$

$$95\%CL_{class} = m \pm t \cdot S_m \text{ (the classical 95\% confidence interval)} \quad (7)$$

$$95\%CL_{geo} = m_k \pm t \cdot S_{mk} \text{ (the geostatistical 95\% confidence interval)} \quad (8)$$

with t-values as 1.96. Additionally, classical and geostatistical coefficients of variation were calculated using the classical estimator:

$$CV_{class} = s / m \ (\%) \quad (9)$$

where *s* is the corresponding standard deviation, and the geostatistical estimator modified from Fernandes and Rivoirard (1999):

$$CV_{geo} = ke / m_K \ (\%) \quad (10)$$

where the spatial structuring of the data is taken into account.

Spatial statistical analyses were conducted using the software package R (Ihaka and Gentleman, 1996) and the library geoR (Ribeiro and Diggle, 2001).

3. RESULTS

The post plots of the measured sample values are presented in Fig. 2.2. The empirical semivariograms show that almost all variables are spatially autocorrelated with the exceptions of *Cd*, *Pb* and *Ni*, where only pure nugget effect models could be fitted (Fig. 2.3). Gaussian models best described the spatial structures of the biological variables, while spherical models are fitted to the abiotic variables *RH*, *Cu* and *Zn*. The parameters nugget, sill and range of the models fitted, the values of goodness-of-fit statistic, the measure of spatial dependency, the practical range as well as the results of the crossvalidation are compiled in Table 2.3, indicating that in all cases valid models are obtained. The greatest practical range is calculated for *TW* (170 m) and the smallest one for *Cu* (30 m). The highest level of spatial dependency is measured for *RH*, while the lowest level is detected for *Cu*.

The mapped distribution of relative height above sea level (Fig. 2.4) shows an increase with increasing distance from the shore line from south to north. The spatial distributions of the total biomass (*TW*) and soft-body weights (*SBW*) of cockles show similar south to north gradients of increasing biomass index with increasing distance from the shore line. The spatial distribution of the standardized soft-body weight (*SSBW*) demonstrates the cumulation of larger sized cockles in the northern part of the sampling area. The mapped *Cu* distribution shows a consistent decrease of *Cu* concentration within the cockles with increasing distance from shore line. Conversely, Zn in cockles is rather patchily distributed.

A comparison of classical and geostatistical estimations of means and 95% confidence intervals of the variables studied is provided in Table 2.4, indicating a substantial reduction of the variability for *TW*, *SBW*, *SSBW* and to lesser extent for Cu. Results of the partial Mantel's

test (Table 2.5) show a significant spatial correlation between *SSBW* and the other biological variables, indicating that the biomass and soft-body weight of cockles is linked to cockle size. Furthermore, there is significant spatial overlapping of copper concentration with all biotic variables. Thus, at stations where cockle size (total biomass or soft-body weight) is similar, copper concentration tend also to be similar. A significant spatial correlation with *Zn* is only obvious for *TW*.

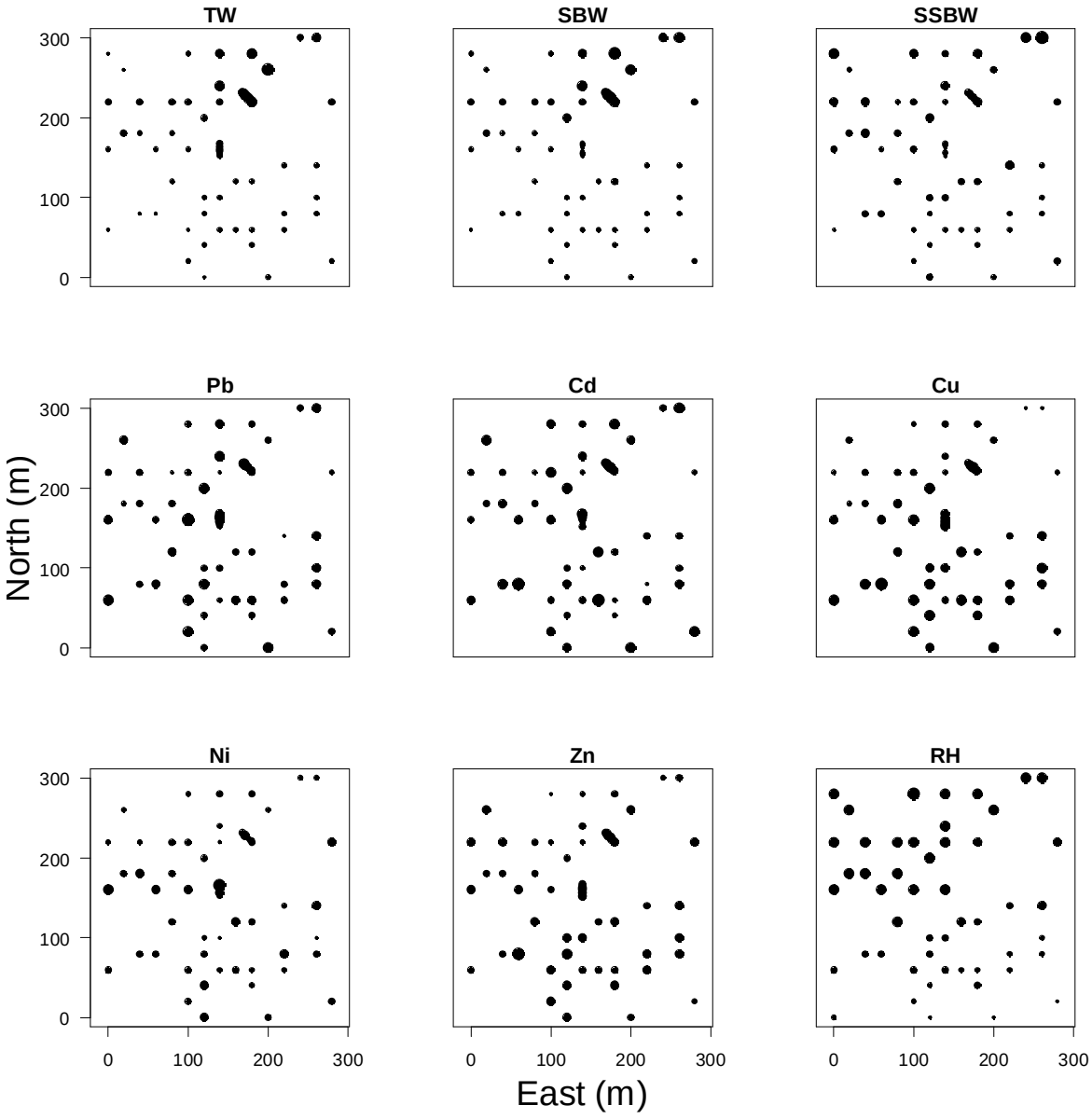

Fig. 2.2: Spatial distributions (post plots) of sampling stations. Point sizes are relative to the measured values of *TW* (total weight), *SBW* (soft-body weight), *SSBW* (standardised soft-body weight), *Pb*, *Cd*, *Cu*, *Ni*, and *Zn* in *C. edule* and *RH* (relative height above sea level).

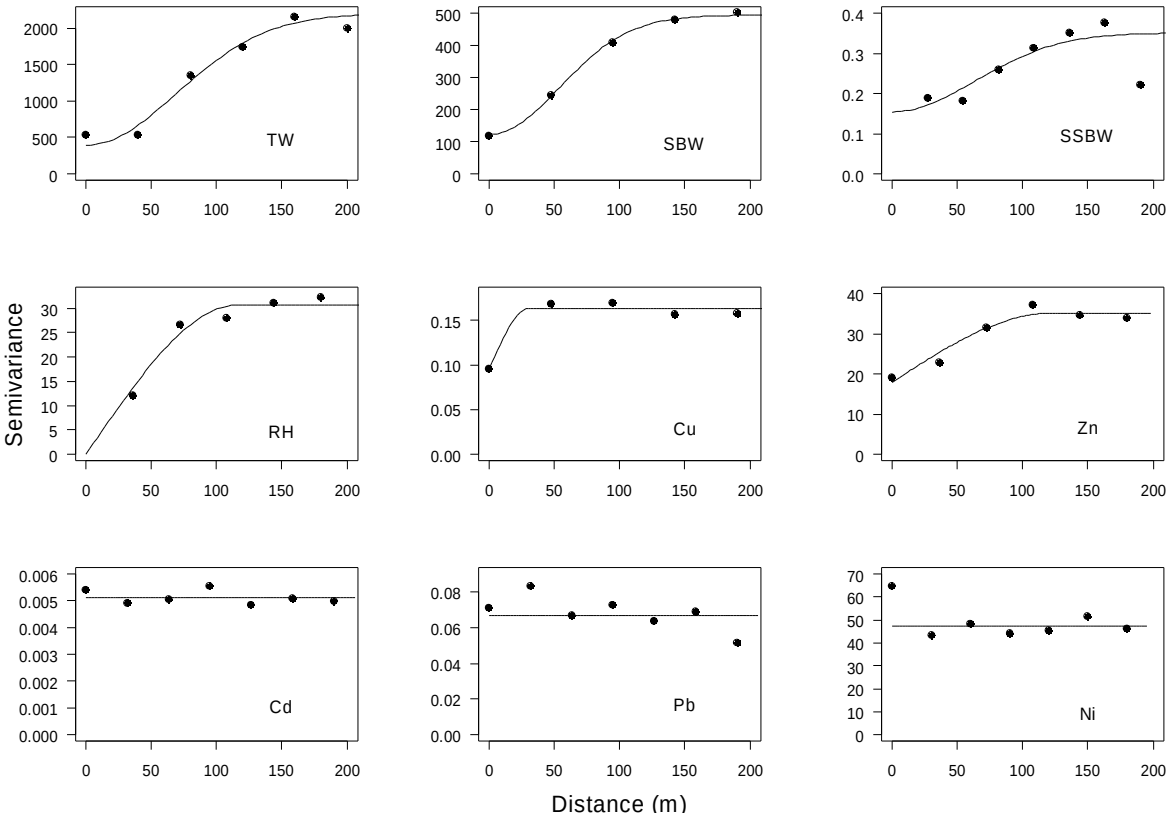

Fig. 2.3: Empirical semivariograms with fitted Gaussian, spherical and nugget effect models of *TW* (total weight), *SBW* (soft-body weight), *SSBW* (standardised soft-body weight), *Pb*, *Cd*, *Cu*, *Ni*, and *Zn* in *C. edule* and *RH* (relative height above sea level).

Table 2.3: Estimated parameters (C_0, C, a) of semivariogram models fitted to the sample values of corresponding variables measured for *Cerastoderma edule* (see Fig. 2.3), practical ranges (*ra*) (to assess the extent of the spatial autocorrelations) and indicators to assess the quality of the estimates (*gof, Zscore, SD-Zscore*).

variable	model	C_0	C	a	*ra* (m)	$C/(C_0+C)$	*gof*	*Zscore*	*SD-Zscore*
TW	gau	391	1802	98	170	0.82	0.00	-0.01	1.71
SBW	gau	122	372	76	132	0.75	0.00	0.00	1.13
SSBW	gau	0.15	0.20	90	156	0.56	0.02	0.01	1.31
RH	sph	0.00	30.8	117	117	1.00	0.00	-0.01	1.86
Cu	sph	0.10	0.07	30	30	0.42	0.00	-0.01	1.10
Zn	sph	17.9	17.2	122	122	0.49	0.00	0.00	1.19
Cd	nug	0.01	-	-	-	0	0.00	-	-
Pb	nug	0.07	-	-	-	0	0.01	-	-
Ni	nug	47.3	-	-	-	0	0.01	-	-

variables: see Table 2.2; models: spherical (sph), Gaussian (gau) and pure nugget (nug); model *parameters*: nugget (C_0), partial sill (C) and range (a); practical range (*ra*); quality indicators: measure of spatial dependency $C/(C_0+C)$, values of the "goodness - of - fit" statistic (*gof*), mean standardised error of the crossvalidation (*Zscore*) and standard deviation of the standardised error (*SD-Zscore*).

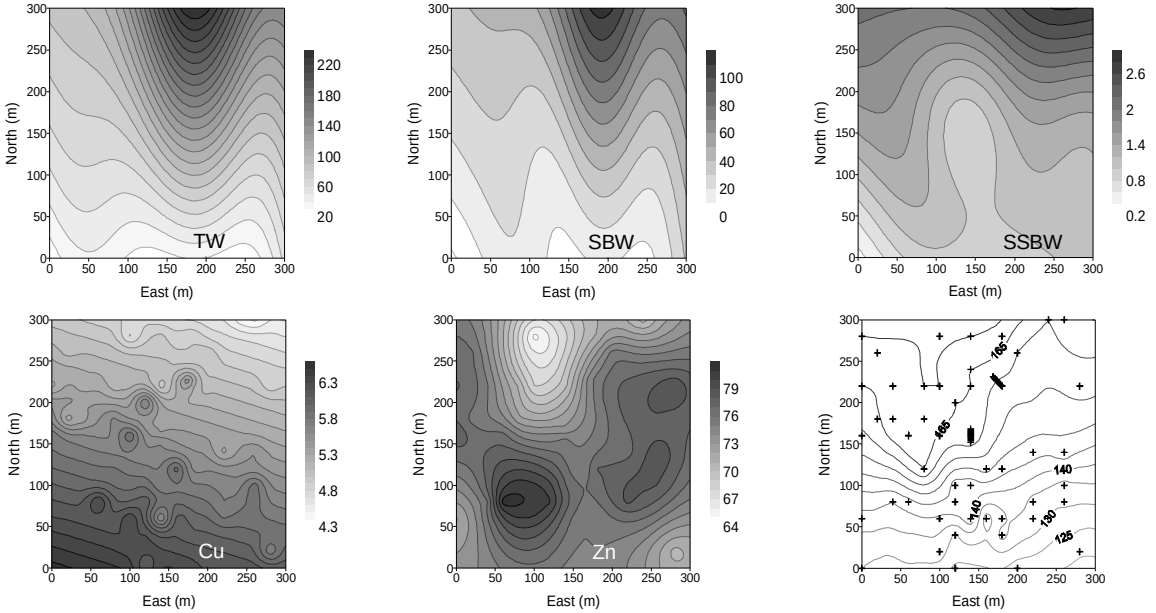

Fig. 2.4: Mapped spatial distribution of *TW* (total weight), *SBW* (soft-body weight), *SSBW* (standardised soft-body weight), *Pb*, *Cd*, *Cu*, *Ni*, and *Zn* in *C. edule* and *RH* (relative height above sea level). Contour lines of *RH* are presented together with the sampling locations.

Table 2.4: Population estimates for biological variables and metal concentrations in *Cerastoderma edule* from the sampling area at Dornumersiel (2001) based on classical and geostatistical procedures (see Materials and Methods section for more details).

		classical estimation				geostatistical estimation			
Variable	LIP	mean	±	95%CI$_{class}$	CV_{class}	m_K	±	95%CI$_{geo}$	CV_{geo}
TW (g 1/16 m^{-2})	0.000	101	±	15	62	90	±	5	24
SBW (g 1/16 m^{-2})	0.000	39	±	8	79	37	±	5	33
SSBW (g Ind^{-1})	0.701	1.33	±	0.16	48	1.38	±	0.10	32
Pb (mg kg^{-1} DW)	0.602	0.85	±	0.06	30	n.a.	-	-	-
Cd (mg kg^{-1} DW)	0.164	0.31	±	0.14	22	n.a.	-	-	-
Cu (mg kg^{-1} DW)	0.327	5.5	±	0.1	10	5.5	±	0.1	7
Ni (mg kg^{-1} DW)	0.379	64	±	13	11	n.a.	-	-	-
Zn (mg kg^{-1} DW)	0.022	75	±	2	8	75	±	1	7

variables: see Table 2.2; LIP: Lilliefors probability (α=0.01); 95%CI$_{class}$: classical 95% confidence interval; CV_{class}: classical coefficient of variation; m_K: mean sample value estimated by kriging; 95%CI$_{geo}$: geostatistical 95% confidence interval; N=63-65; n.a.: not applicable.

Table 2.5: Partial Mantel correlations (*r*) of kriging values, estimated at a grid size of 20 by 20 meters, between total weight (*TW*), soft-body weight (*SBW*), standardised soft-body weight (*SSBW*) and the metal concentrations (*Cu, Zn*) in soft-body tissues of *Ceratoderma edule*. P-values <0.05 (in brackets) indicate significant spatial correlations between variables compared.

	SBW	*SSBW*	*Cu*	*Zn*
TW	0.804 (p<0.001)	0.326 (p<0.001)	0.614 (p<0.001)	0.071 (p=0.001)
SBW	-	0.504 (p<0.001)	0.642 (p<0.001)	0.014 (p=0.278)
SSBW	-	-	0.640 (p<0.001)	0.016 (p=0.243)
Cu	-	-	-	0.069 (p<0.001)

variables: see Tables 2 and 4.

4. DISCUSSION

4.1. Spatial analysis and mapping

The Lilliefors probabilities compiled in Table 2.4 indicate that we can assume a normal distribution of all variables, with the sole exception of *TW* and *SBW*. These exceptions stress the necessity to employ the robust "modulus" estimator which is supposed to be resistant against skewed data distributions (Cressie, 1991). The quality criteria compiled in Table 2.3 suggest valid spatial model estimations in any case, and justify the consideration of linear trends in the spatial analysis due to results shown in Table 2.2. The semivariogram models shown in Fig. 2.3 indicate a distinct increase of the variability for most variables with sampling distance. Only for *Cd*, *Pb* and *Ni* do we find a pure nugget effect model. The fact that increasing semivariogram models reach their sill within the maximum distance considered suggest that the pre-selected scale of this investigation (300 x 300 m) is adequate. Furthermore, it is obvious that the additional small-scaled sampling points at two locations have improved the estimation of the semivariogram models regarding small distance classes. This is especially true in the case of *Cu*, for which without these sampling points we would only have been able to estimate a pure nugget effect model comparable to *Cd*, *Pb* and *Ni*.

The contour plots of the mapped spatial distribution of the variables under study (Fig. 2.4) show a clear coincidence of increasing values for *TW* and *SBW* with increasing relative height above sea level (*RH*). In the intertidal zone, tidal elevation is of prime importance for filter feeders because it sets the submersion time and hence determines their feeding period and their supply of food (Peterson and Black, 1987; de Montaudouin and Bachelet, 1996; Ysebaert et al., 2003). This is just the opposite of what we have found. In our investigation

area *RH* increases seawards due to a sandy bar in the north, indicating that probably the sediment composition is an important factor influencing the spatial distribution of cockles, due to sediment quality (Ducrotoy and Desprez, 1986) or sediment dynamics (Bouma et al., 2001; Ysebaert and Herman, 2002).

Spatial patterns in benthic bivalve populations are the integrative effect of recruitment by settling larvae, interactions between established adults and settling larvae or early juveniles (de Montaudouin and Bachelet, 1996), migration, passive resuspension and mortality (Armonies, 1992, 1996), competition for food and space (de Montaudouin and Bachelet, 1996), and finally predation by shrimps, flatfish, shore crabs, oystercatchers, herring gulls and eiders (Jensen and Jensen, 1985). Passive transport may result in long-distance dispersal and may enable the specimens to settle, at a later time and with a larger size, away from the area occupied by the recently metamorphosed larvae (Armonies, 1992). If specimens are able to react to increasing environmental deterioration, they may choose to leave. As an adaptive strategy, they need to find a balance between risks of migration in the water column, the risks of staying in the sediment, and the chance to find a better habitat. Potential factors regulating migratory activity may be food, disturbance, predators, the density of conspecifics, hydrography, or the sediment composition.

There is some conflicting evidence regarding the circumstances in which competition for food might become important for cockles. In our study the mean abundance of cockles in the investigation area (± 95% confidence interval) is 455 ± 60 individuals m^{-2}. Kamermans et al. (1992) found that even densities of 2000 cockles m^{-2} had no effect on growth of laboratory-reared bivalves, while in other studies on benthic suspension feeders the occurrence of either direct interference by competition for space (Peterson and Andre, 1980), or sediment-mediated interactions (Jensen and Jensen, 1985) has been demonstrated on rare occasions.

In our investigation area the fauna was devasted in early summer 1996 by the occurrence of large-scale "black spots". This phenomenon has frequently been encountered in studies regarding mudflats of the southern North Sea (Böttcher et al., 1998; Rusch et al., 1998; Freitag et al., 2003). Recruitment of cockles, but also of other invertebrates took place in autumn 1996, indicating that large adults in our study originate from this singular event. However, spatial patterns in *SSBW* (Fig. 2.4), an indicator of the mean size of individual cockles, suggest further recruitment events between 1996 and 2001. Furthermore, this situation may influence the future development of the spatial distribution of cockles, since the metabolism increases with body size at a faster rate than does the pumping rate in cockles (as in other bivalve species). Hence, there is a negative relationship between the clearance/respiration ratio and body size, which has been considered a factor reducing the growth efficiency of larger individuals (Iglesias and Navarro, 1991).

4.2. Population estimates for Cerastoderma edule and implications for biomonitoring

Application of classical statistical procedures assumes stochastic independence of the data (Petitgas, 2001). Our results clearly indicate spatial autocorrelations for the variables *TW*, *SBW*, *SSBW*, *Cu* and *Zn* regarding the chosen scale of investigation, as can be seen from increasing semivariograms (Fig. 2.3). Thus it is only when samples are taken at distances above the estimated values for the practical range (Table 2.3) that stochastic independence of the data can be assumed. These are 30 m for *Cu*, 122 m for *Zn*, 132 m for *SBW*, 156 m for *SSBW* and 170 m for *TW*. If samples are taken at smaller distances, classical population estimates like means and confidence intervals might be biased (Maynou, 1998) and the survey precision might be overestimated, as can be seen from the comparison of classical and geostatistical coefficients of variation compiled in Table 2.4. Only for *Cd*, *Pb* and *Ni* do the pure nugget effect models obtained (Fig. 2.3, Table 2.3) indicate that stochastically independent data might be obtained even at distances below 20 m.

The spatial distribution of the standardized soft-body weight (*SSBW*) indicates the cumulation of larger-sized cockles in the northern part of the sampling area, while the mapped *Cu* distribution shows a consistent decrease of *Cu* concentration in cockles with increasing distance from shore line (Fig. 2.4). The inverse relationship found for *Cu* with *RH* but also with *SSBW* is supported by the results of Mantel's test (Table 2.5), clearly showing a correlation between copper concentrations in and the individual body weight of *C. edule* while considering the relative spatial distance among sampling locations. These results confirm frequently reported size-dependent bioaccumulation of Cu in cockles inferred from field studies (Fig. 2.5), showing a higher potential for Cu accumulation in smaller specimens.

Results in Table 2.4 indicate good agreements between classical and geostatistical means for the variables *TW*, *SBW*, *SSBW*, *Cu* and *Zn*, while for *Pb*, *Cd* and *Ni* such a comparison is not possible. Thus, in our case study a bias of the classical mean is not obvious for the given spatial sampling design on a grid of 20 x 20 m distance. Conversely, the geostatistical confidence intervals and coefficients of variation are much lower than the classical ones, clearly demonstrating the validity of the spatial approach. Thus, for a literature comparison geostatistical estimates should be used if applicable.

Trace metal concentrations in soft-body tissues of cockles, *C. edule* (if not indicated otherwise), from different regions of the world are compiled in Table 2.6. Data from other locations of the German North Sea coast are within the confidence intervals of this study (Table 2.4) or only slightly exceeding these limits, while data from various estuaries in SW England provide some information on metal concentrations in cockles from sites which are supposed to be contaminated (Bryan et al., 1985), reaching 120 mg Pb kg^{-1}, 6 mg Cd kg^{-1}, 174 mg Cu kg^{-1} and 303 mg Zn kg^{-1}. Comparable Pb and Cd concentrations are also reported for the Isle of Man (Southgate et al., 1983), but, most surprisingly, also from locations in Morocco (Cheggour et al., 2000; 2001). The latter have been related to the application of agrochemicals and industrial effluents from a neighbouring phosphate processing plant.

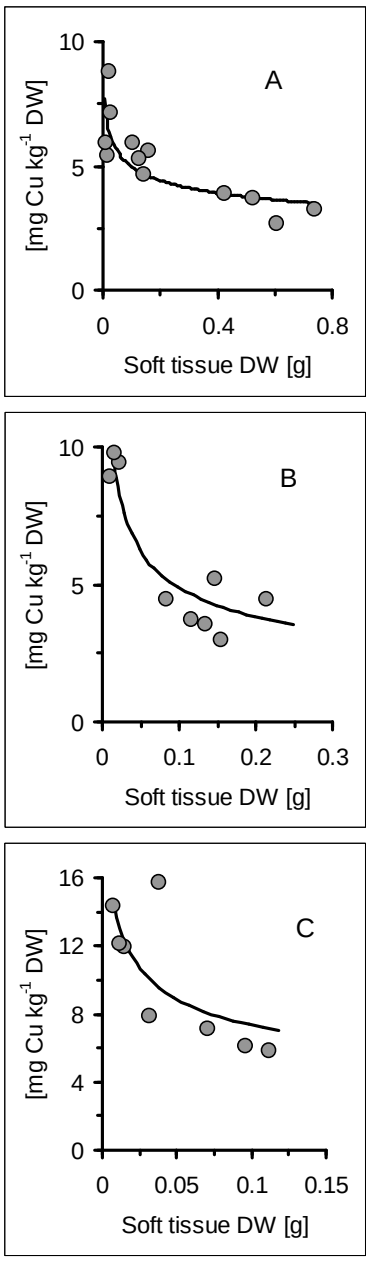

Fig. 2.5: Relationship between Cu and soft tissue dry weight in *C. edule* from different locations of the German Bight, North Sea: (A) Norderney (model: $y = 3.33\ x^{-0.17}$; $R^2 = 0.65$), (B) Stollhamm ($y = 2.18\ x^{-0.35}$; $R^2 = 0.83$) and (C) Wurster Watt ($y = 3.94\ x^{-0.27}$; $R^2 = 0.50$) (G.-P. Zauke and H.-J. Janßen, ICBM, Oldenburg, unpublished data, November 1991).

Table 2.6: Trace metal concentrations in soft tissues of cockles, *Cerastoderma edule* (if not indicated otherwise), from different regions of the world (mg kg^{-1} DW).

Region	Pb	Cd	Cu	Zn	Ref
Dornumer Siel, Germany (North Sea)	0.9	0.31	6	75	1
Norderney, Germany (North Sea)	0.5-0.8	0.16-0.31	3-6	59-82	2
Stollham, Jade, Germany (North Sea)	1.0-1.6	0.70-0.98	6-13	64-111	3
Wursten, Cuxhaven, Germany (North Sea)	0.6-1.3	0.19-0.32	5-10	82-153	3
Jade, Germany (North Sea)	0.6-1.1	0.50-0.90	6-19	97	4
Poole, UK (North Sea)	0.3	1.0	3	57	5
various estuaries, SW England	0.4-**120**	0.4-**6.0**	4-**174**	46-**303**	6
Spiekeroog, Germany (North Sea)	0.9-3.9	0.16-0.25	-	-	7
Isle of Man, UK (Irish Sea)	23-370	1.6-6.3	10	44-139	8
Shannon, Ireland (Atlantic Ocean)	-	-	15	60	9
Etang de Thau, France (Mediterranean)	0.2-5.0	0.4-1.3	9-17	60-84	10
Oum er Rbia, Morocco (Atlantic Ocean)	8.2-16.0	0-4.9	2-26	42-380	11
Sidi Moussa, Morocco (Atlantic Ocean)	8.1-30.0	1.0-4.0	6-38	75-151	12

bold: possibly contaminated locations;

references; 1: this paper; 2: (Zauke et al., 1995); 3: G.-P. Zauke and H.-J. Janßen, ICBM, Oldenburg, unpublished data (1991/92); 4: (Dittmer, 1982) (recalculated from WW); 5: (Bryan, 1984); 6: (Bryan et al., 1985); 7: (Bietz et al., 1997); 8: (Southgate et al., 1983); 9: (O'Leary and Breen, 1997);10: (Szefer et al., 1999)(*C. glaucum*; data taken from figures); 11: (Cheggour et al., 2000); 12: (Cheggour et al., 2001).

5. CONCLUSION

Spatial autocorrelations found for total and soft-body weights of cockles at the chosen scale of investigation suggest that classical statistical procedures should be applied with caution in ecological field studies on this scale. Only at distances above 130 m will it potentially be possible to obtain independent replicates, while below this distance explicit spatial procedures like geostatistics should be employed. If samples are taken at the shorter distances, classical population estimates like means and confidence intervals might be biased and the survey precision overestimated. Our results for *Cu* and *Zn* suggest that these arguments may be also extended to biomonitoring studies performed at this scale of investigation, but not for *Cd*, *Pb* and *Ni*. Finally, the spatial correlation between *Cu* concentrations in and the individual body weight of *C. edule* that is found by taking the relative distance among sampling locations into account provides an example of an integrated approach combining aspects of biomonitoring with ecological field work. In future studies such an approach might be useful when addressing the problem of trophic transfer of energy or pollutants from food (e.g. cockles) to higher trophic levels such as fish and seabirds.

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CHAPTER 3

Spatial analysis of heavy metal concentrations in the brown shrimp *Crangon crangon* (Linnaeus, 1758) from the southern North Sea

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Abstract

The spatial distribution of heavy metal concentrations (*Cu*, *Pb*, *Cd*, *Ni* and *Zn*) in the brown shrimp *Crangon crangon* (Linnaeus, 1758) collected on a cruise of FRV “Walther Herwig III" to the southern North Sea in January 2004, has been investigated at an ecological scale of 18 x 18 km (10 x 10 nautical miles) to evaluate the range of spatial autocorrelations for the different variables under study and to assess the extent to which biological properties of the organisms may be related to the spatial distribution of metal concentrations in *C*. *crangon* from the sampling area. Semivariogram models obtained by geostatistical procedures indicated a distinct increase in variability for most variables with sampling distance. Only if samples are taken at distances above the estimated values for the practical range of the semivariogram, can stochastic independence of the data be assumed. These distances are 6.6 km for *Cd*, 3.6 km for *Cu*, 3.0 km for *Ni*, 5.2 km for *Pb*, and 3.5 km for *Zn*. Classical and geostatistical population estimates for the means are in good agreement. Conversely, we observed a reduction of variability for *Cd, Ni, Pd* and *Zn* by the application of the geostatistical approach. The contour plots of the mapped spatial distribution revealed a cumulation of heavier brown shrimps in the samples in the south-western corner of the sampling area and a clear coincidence of high values for *Cd*, *Ni* and *Pb* with low shrimp mean body wet weight. Beyond, measured *Cu*, *Pb* and *Zn* concentrations were largely within the reported range, while *Cd* concentrations in *C*. *crangon* from the North Sea were far below the values in de-

capod crustaceans reported from other regions. We conclude that with this integrated biomonitoring approach heavy metal concentrations could be assessed more precisely and relations between biotic and abiotic variables could be evaluated. Hence, we suggest such an approach also for future studies addressing the problem of trophic transfer of energy or pollutants from food (e.g. brown shrimp) to higher trophic levels like fish and seabirds.

Key words: *Crangon crangon*, decapoda, biomonitoring, heavy metals, geostatistic, spatial analysis

1. INTRODUCTION

Biomonitoring of trace metals receives continued attention in the scientific literature and international and national environmental programmes (AMAP, 2005; BLMP, 2005; CCMA, 2005; TMAP, 2005). To assess the quality of the marine environment regarding heavy metals, the bioavailable fraction in 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 on dissolved uptake (Kahle and Zauke, 2002). Thus, the total bioavailable fraction is only accessible by determining the amount of metals incorporated in organisms (Zauke et al., 1996). The accumulation patterns and subsequent accumulated concentrations in marine organisms often vary depending on the species, 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 benthic invertebrates with greatly varying metal concentrations in the same body of seawater (Zauke et al., 1995; Mattig et al., 1997; Prowe et al., 2006; Zauke and Schmalenbach, 2006).

The bioavailable fraction is of great importance to assess the environmental quality regarding heavy metals in the oceans, since possible toxic effects are largely dependent on the bioavailable exposure concentration in seawater. This fraction is only accessible by determining the amount of metals incorporated in organisms, because this is more dependent on species-specific uptake and detoxification mechanisms and metabolic requirements than on the concentration in the soluble phase (Rainbow, 1988; Depledge, 1989; Ritterhoff and Zauke, 1998).

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. These 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 (Rainbow and White, 1989; Zauke and Petri, 1993). Classically, studies on metals

in biota are related to the species level because this level is implicitly regarded as highly relevant in describing the processes of metal metabolism.

In the marine ecosystem benthic and demersal organisms play a key role. One basis for an ecological assessment is knowledge of ecological preferences and distribution of benthic living organisms under different environmental conditions. Although information on the abundance of species is essential, the relevance of reproduction and population dynamics should be considered too. They contribute to the biochemical cycling of nutrients, provide habitat structures for other organisms and serve as an important food source for fish (Ehrich et al., 2007). Anthropogenic influences such as fishing activity and chronic large-scale eutrophication are thought to influence epifaunal communities on a long-term basis, but have been considered not to be responsible for the short-term variability in the assemblage of epifaunal community structures (Hinz et al., 2004). Water temperature is considered to be the major factor influencing the epifaunal community structure and species abundance (Hinz et al., 2004). When the 3℃ isothermal borders are shifted further offshore during cold winters, benthos organisms are likely to migrate into deeper and therefore warmer water.

There are many environmental programmes established where, for example, mussels are used as biomonitors. Although crustaceans are frequently used as bioindicators and biomonitors in various aquatic systems (Rinderhagen et al., 2000), hardly any information about such programmes for marine crustaceans could be found, with an exception from the Canadian Science Advisory Secretariat, Department of Fisheries and Oceans, establishing a framework for the assessment of lobsters (DFO, 2006), assessments of shrimps stocks (DFO, 2006), and an Integrated Fisheries Management plan (DFO, 2003). However, due to their great importance in the food webs of the intertidal zone in the Wadden Sea and subtidal zone in the North Sea, crustaceans such as the brown shrimp *Crangon crangon* (Linnaeus, 1758) merit further consideration.

The epifaunal decapod, the brown shrimp *Crangon crangon* (Linnaeus, 1758), can be found in coastal and estuarine waters in the Baltic and the North Sea, the Atlantic coast of North and West Europe and the Mediterranean. It is a typical euryhaline inhabitant (Cieluch et al., 2005). *C. crangon* is considered to be a key species in the coastal waters of the North Sea and in particular in the Wadden Sea, since it occurs in masses and acts both as a highly efficient predator and important prey. The shallow water region of the Wadden Sea is the nursery ground for brown shrimps (Berghahn, 1996). The organisms burrow just below the sand surface during the day, so that only the eyes and antennae can be seen. Brown shrimps are important food resources for flatfish (*Pleuronectes platessa*), shore crabs (*Carcinus maenas*), seals (*Phoca vitulina*), various waders (Limicolae), seagulls (Laridae) and auk birds (Alcidae) (Jensen and Jensen, 1985; Janke, 1999).

Due to the high variable environmental conditions in the North Sea, the spatial structuring of *C. crangon* in the Wadden Sea is controlled by abiotic variables such as sediment quality, sediment dynamics, environmental factors such as sea surface temperature (Hinz et al., 2004) and tidal level and therefore also from sunlight intensity during low water level in the shallow regions of the Wadden Sea (Berghahn, 1983). Biotic factors, for instance predation (Berghahn, 1996), competition or interactions between adults and juveniles (Berghahn,

1983), also strongly influence the recruitment and subsequent spatial distribution of *C. crangon*. Furthermore the seasonal migration patterns of the brown shrimp *C. crangon* are well known. During autumn the shrimps migrate from the shallow water areas of the Wadden Sea to offshore areas and then return in spring with the onset of increasing water temperatures. The pattern of migration depends largely on the weather conditions, while during mild winters there may be nearly no migration offshore, under moderate to severe winter conditions *C. crangon* is reported to migrate 1 to 90 km offshore (Hinz et al., 2004). The distribution of *C. crangon* is largely dependent on relatively warm water conditions.

The patchy nature of the environment coupled with the behaviour of a species determines the spatial arrangement of individuals of that species, and the observed spatial distributions are important for understanding ecological processes. Moreover, identifying spatial patterns is important in order to improve the design and interpretation of surveys and experimental studies, by relating sampling programmes to natural scales of variation (Livingston, 1987; Thrush et al., 1989; Stelzenmüller et al., 2005; Jung et al., 2006; Stelzenmüller et al., 2006). Although spatial variability on different ecological scales is an important issue in marine ecology (Ysebaert and Herman, 2002; Norén and Lindegarth, 2005; Thrush et al., 2005), it is important to note that the spatial dependence of variables has not explicitly been taken into account in ecological and biomonitoring field studies in this area, despite the well-known importance of spatial dependence in ecology and environmental sciences.

This importance is stressed by the following arguments. Sampling points randomly distributed over an area can yield unbiased estimates of the variable of interest only if the sampling-point observations are independent (Petitgas, 2001). When random sampling is carried out at an appropriate spatial scale, it effectively extinguishes any underlying spatial structure in the distribution of organisms. However, the scale of spatial distribution of the species under study is usually unknown, and this factor may result in a bias in the calculation of population estimates (Maynou, 1998). The presence of a spatial structure is indicated by spatial autocorrelation between pairs of samples, *viz.* the realisation of a regionalised variable (e.g. biomass of organisms) at one location influences the realisation at neighbouring locations. Thus, when samples are not taken independently of one another and when the population sampled is spatially structured, the computation of any variance requires a model of the spatial relationships within the population (Matheron, 1971). Spatial autocorrelations are recognised as typical characteristics of ecological units like populations, but also of other environmental variables (Legendre and Legendre, 1998), and can be analysed and modelled mathematically by geostatistics. Thus, the presence of patches, density gradients and spatially autocorrelated variables may confound designs and affect the validity of inferential statistics. Future studies must integrate the intensity and form of patterns from various spatial and temporal scales if we are to understand the processes responsible for generating pattern (Thrush, 1991; Thrush et al., 2005).

Because this aspect is largely missing in ecological and biomonitoring studies in the coastal zone, we present here an integrated approach combining the analysis of the spatial distribution of heavy metal concentrations and mean body wet weight (*mbww*) in the brown shrimp *C. crangon* from the German Bight, sampled on an ecological scale of 18 x 18 km (10 x 10

nautical miles) in one of the eleven standard sampling areas of the German Small-scale Bottom Trawl Survey (GSBTS) in the North Sea (Box A, Ehrich et al., 1998; Ehrich et al., 2007). We employed geostatistical methods recently optimised for the evaluation of fisheries data (Stelzenmüller et al., 2004), especially regarding additional small-scale (Stelzenmüller et al., 2005) and meso-scale survey data (Stelzenmüller et al., 2006). This integrated approach has also been successfully applied in a recent study on the cockle *Cerastoderma edule* from the German Wadden Sea (Jung et al., 2006).

The goal of this paper is to evaluate the range of spatial autocorrelations for the different variables under study and to assess the extent to which biological properties of the organisms are potentially related to the spatial distribution of metal concentrations in *C. crangon* from the sampling area.

2. MATERIAL AND METHODS

2.1 Sampling area and sample collection

Samples of the decapod crustacean *Crangon crangon* (Linnaeus, 1758) for this analysis were collected from FRV "Walther Herwig III" (cruise 259, January 05 – 13, 2004) in an area of the inner German Bight (Box A, see Figure 1), one of the eleven standard sampling areas of the German Small-scale Bottom Trawl Survey (GSBTS) in the North Sea (Ehrich et al., 1998; Ehrich et al., 2007). Fishing was carried out under standard IBTS (International Bottom Trawl Survey) protocol using a standard net GOV (Chalut à Grande Ouverture Verticale), with a trawling time of 30 min at a trawling speed of 4 knots (1 knot = 0.514 m s^{-1}). The locations of sampling stations (1-34, see Figure 3.1) as well as trawl directions were selected randomly within the area of investigation of the survey. The trawl positions were taken as midpoint of the haul converted to an absolute measure in km (easting and northing) relative to 54°27´N and 6°58´E.

On board ship the samples of *C. crangon* were taken from the catch while epibenthic fish were sorted, counted and weighed. Ten randomly collected shrimps specimen from the by-catch at each station were thoroughly rinsed for a few seconds with double-distilled water to remove possible contaminating particles. Superficial water was carefully removed with good-quality filter paper from the animals before they were transferred to sterile polystyrene Petri dishes, numbered, closed tightly with tape and deep frozen (stored at -18°C) for further analysis of heavy metals in the laboratory (see below). To assure the quality of the sampling procedure, we followed the guidelines set-up in Zauke and Petri (1993) and Zauke et al. (1996).

Additionally, in order to determine the dependency between trace metal content and weight (DW) in brown shrimps, results from single measured individuals of *C. crangon* sampled in July 2004 and 2005 in Schillig at the German Wadden Sea Coast were consulted.

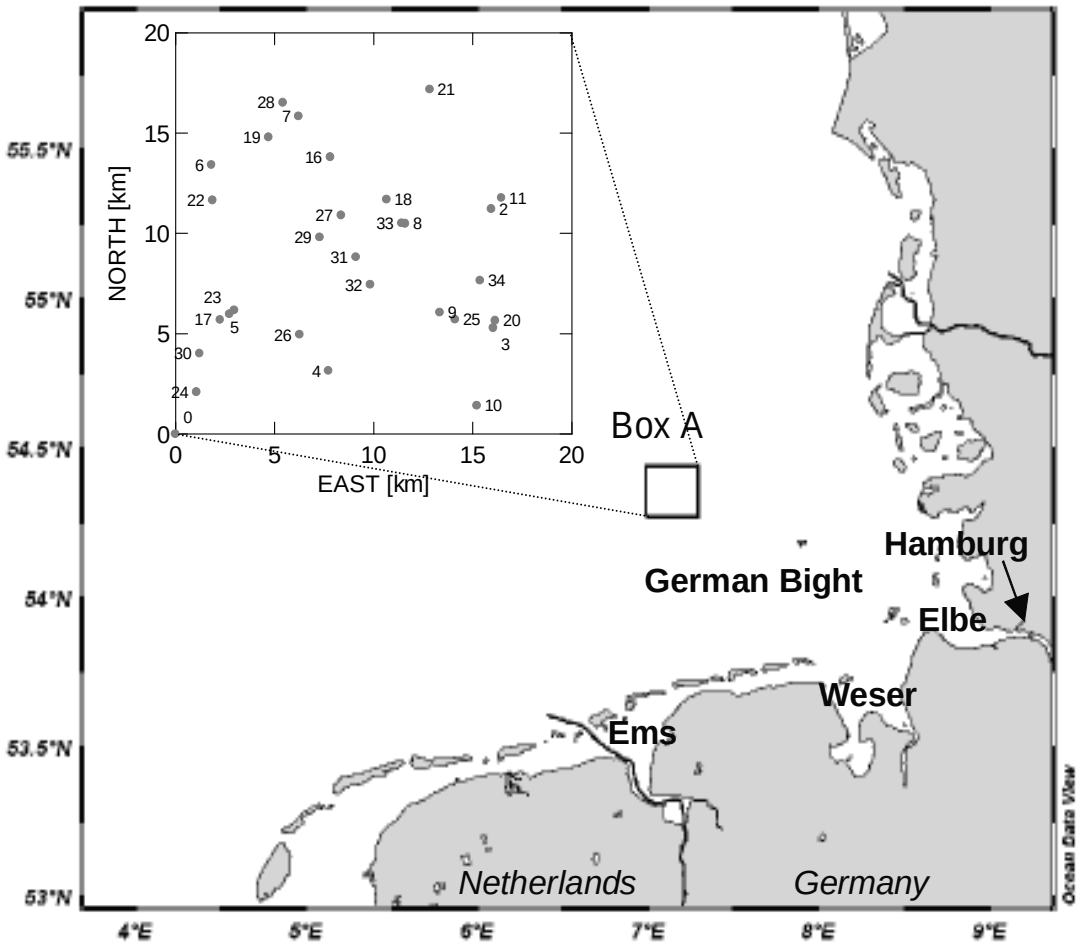

Figure 3.1: Standard GSBTS sampling area Box A and positions of the trawling midpoints from cruise 259 with FRV "Walther Herwig III" (January 05 – 13, 2004) in an area of the inner German Bight, southern North Sea.

2.2 Sample preparation and analytical procedures

Upon arrival at the laboratory the frozen *C. crangon* samples were defrosted and per station each brown shrimp was weighed separately to obtain the individual body wet weight and to calculate to total mean body wet weight of the whole sample per station. The 10 pooled *C. crangon* from each sampling site were transferred back to the polystyrene Petri dish and stored at –18℃ for subsequent metal analysis. The frozen samples were subjected to freeze-drying for 48 h (Lyovag GT2, Leybold Heraeus) and the dry weight was determined. Then the samples were homogenised using a small boron carbide mortar and pestle to avoid losses of biomass. Aliquots of about 10 mg dried material were digested for 3 h at 80℃ with 100 µl HNO_3 (65%, suprapure, Merck) in tightly closed 2 ml Eppendorf safe-lock reaction tubes (Clason and Zauke, 2000). The digests were made up to 2 ml volume with double-distilled water. After appropriate dilution, the final sample and standard solutions were adjusted to concentrations of 3.25% HNO_3.

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 modified from Clason and Zauke (2000) and Kahle et al. (2003). Ashing and atomisation temperatures were 600 and 1800°C for Cd; 800 and 2300°C for Cu; 800 and 2700°C for Ni; 1000 and 2200°C for Pb. For Cd, Ni and Pb, palladium and magnesium nitrate modifiers were applied. Zn was assayed using an air-acetylene flame Varian SpectrAA 30 with 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). Wet weight to dry weight conversion factors for *C. crangon* were 5.3 ± 0.1 (mean ± 95% CI) regarding organisms from Schillig (mean body weight 2-20 mg DW) and 3.6 ± 0.07 (Box A, mean body weight 1.5 g DW), indicating a higher water content in the smaller animals.

Quality assurance was performed in line with German GLP regulations (Anonymous, 2005), 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 two certified reference materials which were randomly allocated within the determinations (Table 3.1). The analysed values for the reference materials were largely in good agreement with the certified values. Limits of detection calculated according to Büttner et al. (1980) proved to be adequate for the range of metal concentrations found in this study, with the sole exception of Pb, where values were below the limit of detection for the certified reference material TORT-2 (Lobster hepatopancreas).

Table 3.1: Quality assurance using certified reference materials randomly allocated within the determinations. Values are means ± 95% confidence intervals [mg kg^{-1} DW].

	Tort-2 Lobster hepatopancreas; National Research Council Canada			CRM No 278R Mussel Tissue : *Mytilus edulis*; Community Bureau of Reference		
Element	analysed	n	certified	analysed	n	certified
Cd	24.1 ± 1.1	8	26.7 ± 0.6	0.389 ± 0.062	8	0.348 ± 0.007
Cu	109 ± 14	8	106 ± 10	9.17 ± 1.03	7	9.45 ± 0.13
Pb	<0.40	4	0.35 ± 0.13	2.17 ± 0.24	8	2.00 ± 0.04
Ni	2.10 ± 0.42	6	2.5 ± 0.19	0.78 ± 0.04	4	(1.00)
Zn	184 ± 15	4	180 ± 6	77.1 ± 9.9	4	83.1 ± 1.7

n: Numbers of independent determinations

Limits of detection (according to Büttner et al., 1980): 0.2 mg Cd kg^{-1}; 3 mg Cu kg^{-1}; 0.4 mg Pb kg^{-1}; 0.1 mg Ni kg^{-1} and 16 mg Zn kg^{-1} (dry weight).

2.3 Preliminary analysis

For each sampling location, the mean body wet weight was standardised (and then termed *mbww*) by the number of brown shrimps at each station, to obtain an indicator of the mean weight of individual brown shrimps collected at each station. Due to the fact that brown shrimps appeared as by-catch of the GOV, the mean weight of the brown shrimp samples can only be estimated for the 10 randomly collected organisms in one sample and not for the mean weight of *C. crangon* from the whole catch. Concentrations of cadmium, copper, nickel, lead and zinc in the brown shrimps are given in mg kg^{-1} DW (referred to as *Cd*, *Cu*, *Ni*, *Pb* and *Zn*). For each of these variables descriptive statistics were calculated (Wilkinson and Engelman, 2000) and the normality of the data was tested by the Lilliefors Probability Test (Wilkinson and Coward, 2000) using SYSTAT 10.

In order to investigate possible trends within the data linear and non-parametric regressions with one covariate (Bowman and Azzalini, 1997) were carried out (Kaluzny et al., 1998). These trends were taken into account for subsequent structural analysis. Furthermore, to visualise the spatial distribution of the sample values, post plots were created, where sample values were plotted proportional to the sample value at each sampling site.

2.4 Structural analysis and surface mapping

For this study the structure of spatial variability of $Z(x)$ (*mbww*, *Cd*, *Cu*, *Ni*, *Pb* and *Zn*) was assessed by an empirical covariance function. Empirical semivariograms $\hat{\gamma}(\mathrm{h})$ were used to describe the spatial structure of the sample data. The semivariogram outlines the spatial correlation of data, measuring the half variability between data points as a function of their distance. In the absence of spatial autocorrelation among samples the semivariance is equal to the variance of $Z(x)$. When a significant linear trend was encountered, data were detrended (Kaluzny et al., 1998). Omnidirectional and directional semivariograms were computed using the robust "modulus" estimator, which is supposed to be resistant against extreme values and skewed data distribution (Cressie, 1991):

$$\hat{\gamma}(h) = \left\{ \frac{1}{N(h)} \sum_{x_i-(x_i+i)\sim h} \left| Z(x_i+h) - Z(x_i) \right|^{\frac{1}{2}} \right\}^4 \Big/ (0.914 + (0.988/N(h))) \tag{1}$$

where $Z(x_i)$ is the realisation of the variable under study at station x_i, $Z(x_i+h)$ is another realisation separated from x by a discrete distance h (measured in m) and $N(h)$ is the number of pairs of observations separated by h. The parameters nugget (C_0), sill (C) and range (a) of spherical models were fitted automatically to the empirical semivariograms. To reduce subjectivity and to ensure reproducibility of the fit, a weighted least squares procedure was employed, where more weight is given to the points near the origin, which is the crucial part in determining the variogram parameters (Cressie, 1991).

Furthermore, to measure the strength of spatial dependence (*SD*) within sample data a ratio of structural variance (C) to sample variance (C_0+C) was computed (Robertson and Freckmann, 1995):

$$SD = \frac{C}{C_0 + C} \tag{2}$$

When this ratio approaches 1 or 100% (for *SD (%)* = *SD* x 100), the spatial dependence is high for the range of modelled separation distances. Conversely, when the ratio approaches 0, spatial dependence is low. Low spatial dependence indicates a high sampling and/or analytical error, or a spatial variability occurring at scales smaller than the minimum distance separating small sampling pairs. Sokal and Oden (1978) related the diameter of an aggregation of a species to the modelled range. The effective range for spherical models is equal to the estimated range.

We used the crossvalidation procedure to provide a measurement of the reproduction of the data by the models defined and the perspective kriging procedures. The results of this jackknifing method are given by standardised errors (or residuals, *viz*. the difference between the observed and predicted values). If the mean of this standardised error (*Zscore,), viz*. the expectation value of the residuals, is zero and the standard deviation (*SD-Zscore*) approximately 1, then the model and the method employed provide an adequate reproduction of the data (Isaaks and Srivastava, 1989).

Mapping of density surfaces of the predicted sample values of *mbww*, *Cd*, *Cu*, *Ni*, *Pb* and *Zn* was carried out with ordinary kriging and universal kriging with external drift (in the presence of a trend). For punctuate estimates of variable values at unsampled locations, the estimator given by Webster and Oliver (2001) was employed:

$$\hat{Z}(X_o) = \sum_{k=0}^{K} \sum_{i=0}^{N} \alpha_k \lambda_i f_k(X_i) \tag{3}$$

where λ_i are charging weights attributed to each $Z(X_i)$, f_k represents the drift by a set of functions, $f_k(x)$, k = 0,1,...K, of our choice (here simple polynomials of the order 1) and a_k are the unknown coefficients. To apply this interpolation method, a grid was drawn over the area investigated with a mesh size of 0.5 km.

When it is possible to fit an appropriate spatial model, the mean of the corresponding variable estimated by kriging is expected to be similar to the sample mean (Isaaks and Srivastava, 1989). Furthermore, in order to compare the classical and geostatistical estimates, the arithmetic mean (*m*), the mean sample value estimated by kriging (m_K), the classical standard deviation (*s*) and the square root of the mean kriging error (*ke*) were calculated. Subsequently, the following parameters were estimated:

$$S_m = s / \sqrt{n} \quad \text{(classical standard error)} \tag{4}$$

$$S_{m_k} = ke / \sqrt{n} \quad \text{(geostatistical standard error)} \tag{5}$$

$$95\%CI_{class} = m \pm t \cdot S_m \quad \text{(classical 95\% confidence interval)} \tag{6}$$

$$95\%CI_{geo} = m_k \pm t \cdot S_{mk} \quad \text{(geostatistical 95\% confidence interval)} \tag{7}$$

with t-values as 1.96. Additionally, classical and geostatistical coefficients of variation were calculated using the classical estimator:

$$CV_{class} = s / m \ (\%) \tag{8}$$

where *s* is the corresponding standard deviation, and the geostatistical estimator modified from (Fernandes and Rivoirard, 1999):

$$CV_{geo} = ke / m_K \ (\%) \tag{9}$$

where the spatial structuring of the data is taken into account. For statistical computing we used the R environment (R Development Core Team, 2005) with the package geoR.

3. RESULTS

Post plots of the measured sample values are presented in Figure 3.2. For *Zn* the point sizes indicate constant but high values (similar to *Cu* and *mbww*) at all sampling stations over the sampling area, whereas the point sizes for *Cd*, *Ni* and *Pb* seem to reflect different values at the sampled stations. Furthermore, we observe higher *Cd* values occurring in the central area, while only at 3-5 points increased Pb values are obvious. Empirical semi-variograms are displayed in Figure 3.3 and the estimated model parameters nugget, sill and range, the measure of spatial dependency (*SD %*), as well as the results of the crossvalidation are compiled in Table 3.2. The highest level of spatial dependence is visible for *Ni* (89%), while the lowest level is detected for *Zn* (8%). Furthermore, the largest range is calculated for *Cd* (6.6 km) and the smallest one for *Ni* (3.0 km).

The mapped spatial distributions for the variables under study appear in Figure 3.4, indicating some clearly marked patches of increased values for mean body weight of shrimps per sample as well as for *Cd*, *Ni* and *Pb*, but, less distinctive patches for the metals *Cu* and *Zn*.

A comparison between classical and geostatistical population estimates is shown in Table 3.3. Regarding the coefficients of variation (CV), highest variability can be seen for *Pb* which,

after *Cd*, reflects the second lowest metal concentration. Highest concentrations of metals in the animals can be found for *Zn*. The values of *mbww*, *Cu* and *Ni* are normally distributed as indicated by the Lilliefors probabilities (LIP > 0.01). Generally, mean values calculated classically and geostatistically are in good agreement, as well as the variability of *Cd*, *Ni* and *Zn* and, to lesser extent, of *Pb.*

Metal determinations in single specimen of *C. crangon* collected at a nearshore locality (Schillig) are shown in Figure 3.5, indicating, despite a substantial variability, decreasing metal concentrations with increasing weight of organisms (*mbww),* although it was not possible to obtain reasonable linear or non-linear regression models.

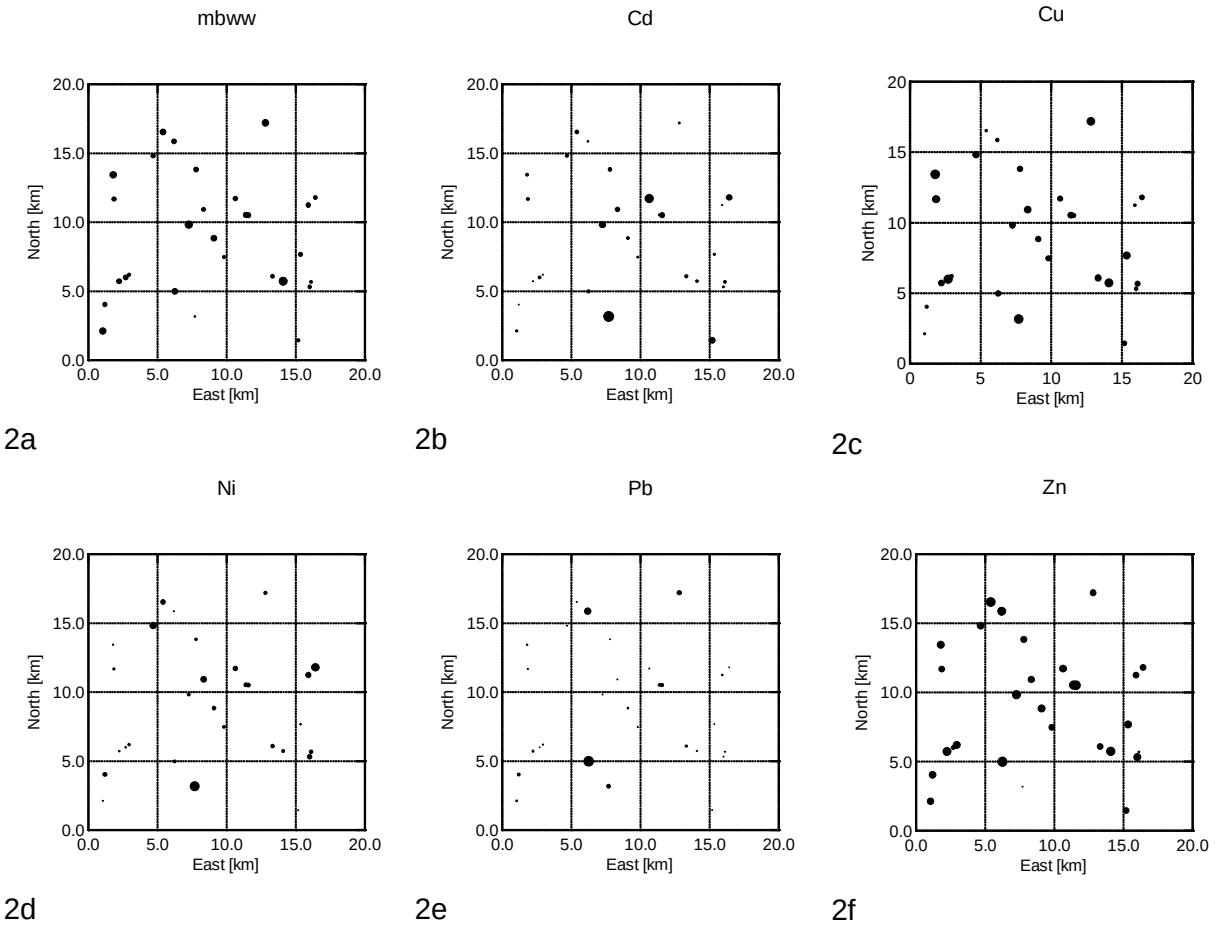

Figure 3.2: Spatial distributions (post plots) of sampling stations within Box A (Figure 3.1). Point sizes are relative to the measured values of *mbww* (mean body wet weight of shrimps per sample) (a), *Cd* (b), *Cu* (c), *Ni* (d), *Pb* (e) and *Zn* (f) in *Crangon crangon.*

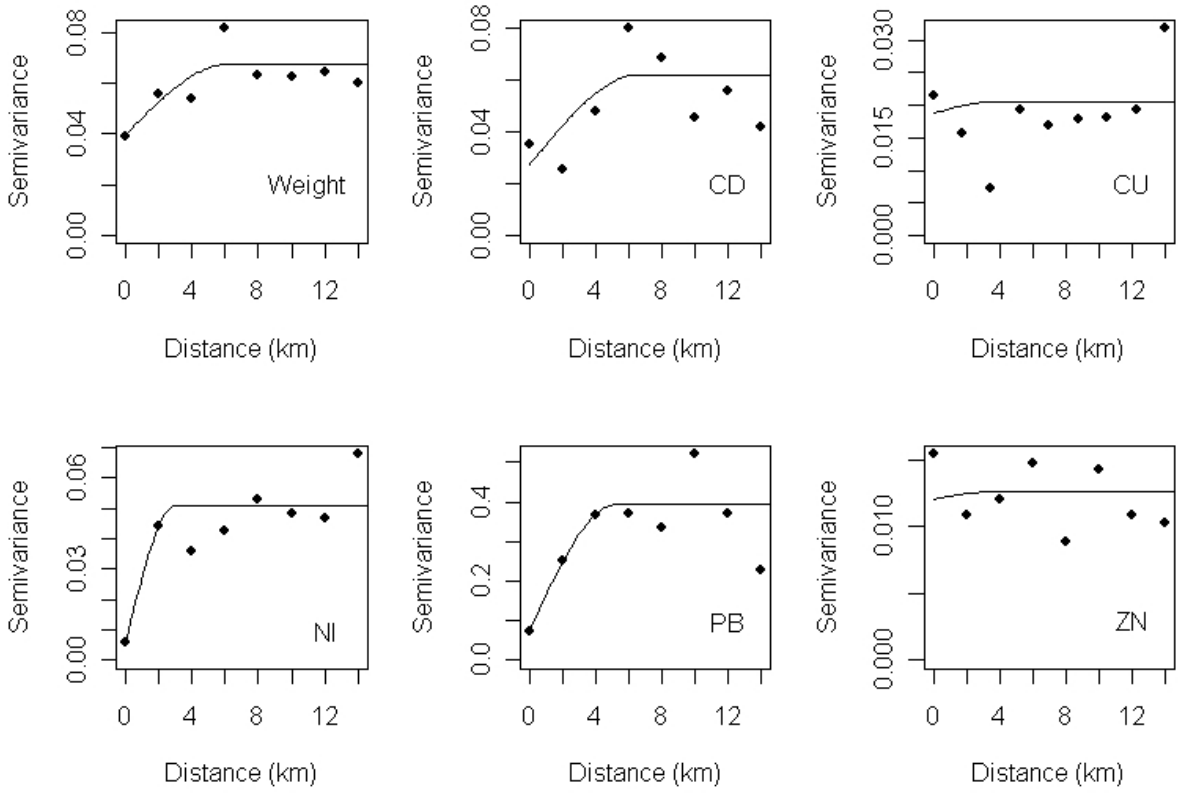

Figure 3.3: Empirical semi-variograms with fitted spherical models for weight (*mbww* = mean body wet weight of shrimps per sample), *Cd*, *Cu*, *Ni*, *Pb*, and *Zn* in *Crangon crangon*.

Table 3.2: Estimated parameters of spherical semi-variogram models (Figure 3.3) fitted to sample values of corresponding variables measured for *Crangon crangon* from Box A (Figure 3.1).

Variable	Trend	C_0	C	a	*SD (%)*	*Zscore*	*SD Zscore*
mbww	east	0.040	0.028	6.3	41	0.05	1.5
Cd	north	0.027	0.035	6.6	56	0.04	1.4
Cu	none	0.019	0.002	3.6	10	-0.01	0.8
Ni	east	0.006	0.045	3.0	88	0.02	1.3
Pb	north	0.075	0.319	5.2	81	0.22	3.5
Zn	east	0.012	0.001	3.5	8	0.02	1.3

variables: *mbww* = mean body wet weight of shrimps per sample, *Cd* = cadmium, *Cu* = copper, *Ni* = nickel, *Pb* = lead and *Zn* = zinc; *model parameters*: C_0 = nugget, C = sill and a = range (km); *goodness-of-fit*: *SD (%)* = relative measure of spatial dependence (eq 2); *Zscore*= mean of standardised errors (residuals) and corresponding standard deviations (*SD Zscore*) derived from crossvalidation procedure; expectation values 0 and 1, respectively.

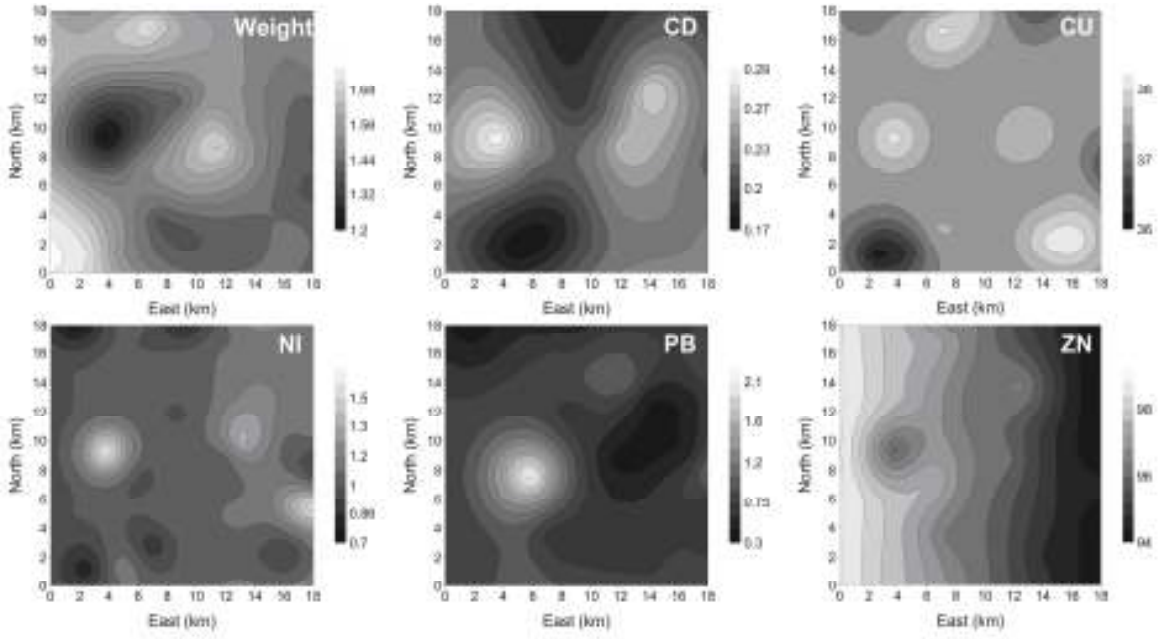

Figure 3.4: Mapped spatial distribution of weight (*mbww* = mean body wet weight of shrimps per sample), *Cd*, *Cu*, *Ni*, *Pb* and *Zn* in *Crangon crangon* in universal kriging plots showing contour lines. Metals were measured in mg kg^{-1} DW. Mean body wet weight was measured in g.

Table 3.3: Comparison of the classical and geostatistical population estimates of the variables mean body wet weight (*mbww*) of shrimps per sample, cadmium (*Cd*), copper (*Cu*), nickel (*Ni*), lead (*Pb*) and zinc (*Zn*) in *Crangon crangon* from the North Sea. Metals were measured in mg kg^{-1} DW; mean body wet weight (*mbww*) was measured in g.

Variable	m_{class}	95%CI_{class}	m_{geo}	ke	95%CI_{geo}	CV_{class}(%)	CV_{geo}(%)	LIP
mbww	1.5	0.14	1.5	0.39	0.14	26	26	0.309
Cd	0.22	0.02	0.23	0.05	0.02	26	24	0.001
Cu	37	2	37	5.4	2	13	15	1.000
Ni	1.1	0.1	1.1	0.2	0.1	24	22	0.363
Pb	0.8	0.2	0.8	0.5	0.2	74	61	0.002
Zn	97	4	97	11	4	12	12	0.002

Notes: N = 29; arithmetic mean (m_{class}), classic 95% confidence interval (95%CI_{class}), geostatistical mean (m_{geo}), mean kriging error (ke), geostatistical 95% confidence interval (95%CI_{geo}), classical coefficient of variation (CV_{class}).and geostatistical coefficient of variation (CV_{geo}), LIP= Lilliefors Probability (2-tail) that sample data are distributed normally, DW = dry weight.

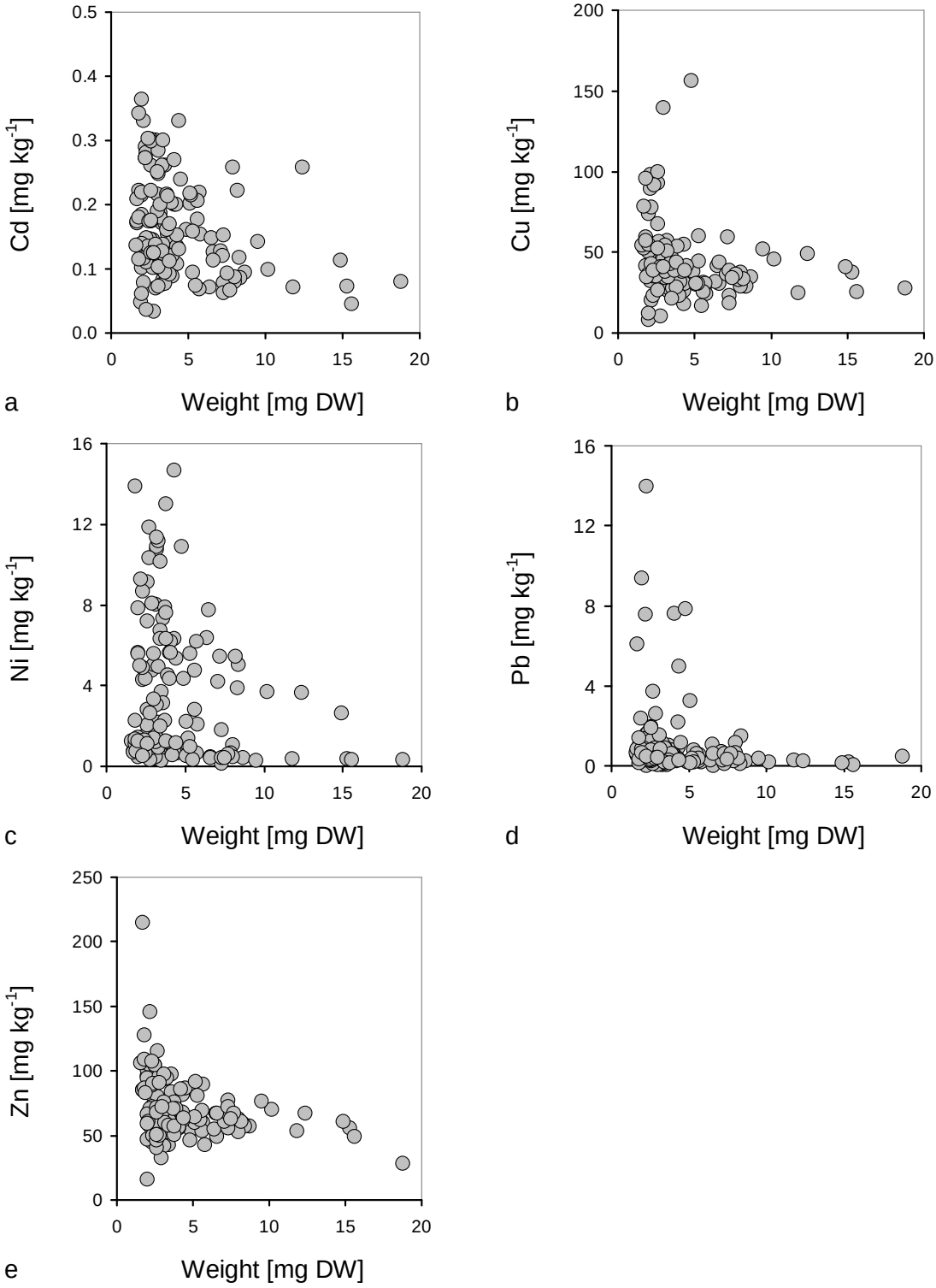

Figure 3.5: Relationships between wet weight and metal concentrations of Cd (a), Cu (b), Ni (c), Pb (d) and Zn (e) from single measured individuals of *Crangon crangon* sampled in July 2004 and 2005 in Schillig, German Wadden Sea Coast. Metals are given in mg kg^{-1} DW.

4. DISCUSSION

Spatial analysis and mapping

The Lilliefors probabilities compiled in Table 3.3 indicate that we can only assume a normal distribution of the variables *mbww*, *Cu* and *Ni* (α = 0.01), stressing the necessity to employ the robust "modulus" estimator which is supposed to be resistant against skewed data distributions (Cressie, 1991). The *goodness-of-fit* criteria compiled in Table 3.2 suggest valid spatial model estimations for *Ni* and *Pb* with the relative measure of spatial dependence (88 and 81%, respectively) approaching the maximum value of 100%, irrespective of the fact that the *Zscore* and the *SD Zscore* of the crossvalidation procedure shows some deviation from a perfect agreement for *Pb* (0 and 1, respectively). Moderately acceptable values *for SD* (%) are obvious for *mbww* and *Cd,* while the spatial dependence is low for *Cu* and *Zn*. Overall we can conclude that reasonable model estimates are obtained for all variables under study, with the sole exception of *Cu* and *Zn*, in agreement with the visual inspection of the semi-variogram models depicted in Figure 3.3. These indicate a distinct increase of the semi-variance (the dissimilarity) for all variables but Cu and Zn with sampling distance.

However, regarding the classical and geostatistical 95% confidence intervals (Table 3.3) no relevant reduction of the variability of all variables under study is visible, taking a spatial relationship explicitly into account. Thus, although most of the model estimates are reasonable in terms of the *goodness-of-fit* criteria mentioned above, the absence of a relevant reduction of the variability by the geostatistical procedure suggest that the spatial autocorrelation is rather week and the results of the spatial modelling must be regarded with some caution. Consequently, the CV values for metals in shrimps are within a range to be expected as the imprecision of the analytical procedure (see results for reference materials; Table 1.2, Chapter 1).

The fact that increasing semi-variogram models reach their sill within the maximum distance considered suggests that the given scale of this investigation (18 x 18 km) is adequate. However, additional small-scaled sampling points at randomly chosen locations could furthermore improve the estimation of the semi-variogram models regarding small distance classes as shown for *C. edule* (Jung et al., 2006). Regarding *C. edule*, this was especially true in the case of copper, for which without these additional small-scaled sampling points only a pure nugget effect model could have been estimated. Unfortunately, this was not possible in this study and thus eventually prevented better model estimates.

The contour plots of the mapped spatial distribution of the variables under study are depicted in Figure 3.4. Despite the constraints discussed above, they show a clear coincidence of increasing values for *Cd*, *Ni* and *Pb* and also similar spatial patterns in comparison with weight. In Figure 3.5 the relationships between metal concentration in individually measured organisms and their corresponding body wet weight are depicted (shrimps collected in July 2004 and 2005 from the intertidal zone). Due to the large variability within the analysed data from the single measurements, no significant linear or non-linear regression models ($R^2 > 0.5$) could be fitted for the metals. Nevertheless, a distinct decrease of metal concentrations with increasing mean body wet weight is apparent. These findings are in agreement with coinciding patches of increased metal concentrations shown in Figure 3.4. *Cd* and *Ni*, and to a

lesser extent *Pb*, have patches with high metal concentrations in areas where the mean body wet weights are lowest. Furthermore, the detected aggregation of higher values for mean body wet weight in the south-west corner of the sampling area is consistent with findings of distributions of epifaunal total biomasses in the same sampling area (Box A) (Hinz et al. 2004), showing high biomass levels in the south-west corner decreasing towards the north-east corner in accordance with the mud percentages.

Only for the relationship between mean body wet weight and length of organisms was it possible to obtain a significant non-linear regression model (Figure 3.6). The exponent of the model —the corpulence factor — slightly exceeds the value of 3, indicating that the shape of the organisms' body is longer in relation to its weight.

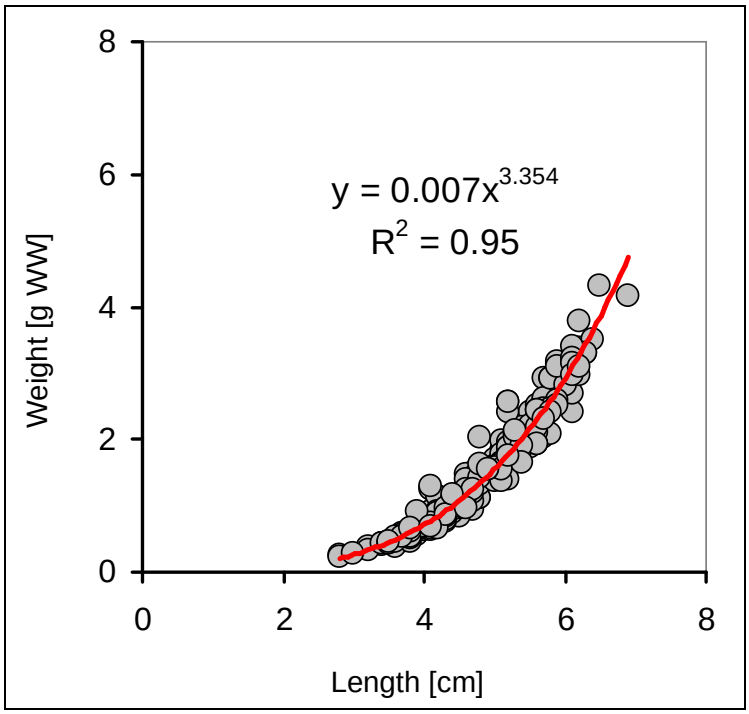

Figure 3.6: Relationships between mean body wet weight and length in all sampled organisms of *Crangon crangon* (cruise 259 with FRV "Walther Herwig III", January 2004).

Generally we might expect metal concentrations in organisms from nearshore areas to be higher compared to offshore areas, due to higher possible contaminations in inshore regions. With increasing distance from the shoreline, a dilution of the fluvial discharge from estuaries with seawater takes places or otherwise metals are removed from the water column by sedimentation. OSPAR reported fluvial metal inputs into the Wadden Sea by the river Elbe with approximately 5.5 t Cd y^{-1}, 200 t Cu y^{-1}, 160 t Pb y^{-1} and 1700 t Zn y^{-1}. For the river Weser 2.5 t Cd y^{-1}, 60 t Cu y^{-1}, 60 t Pb y^{-1} and 700 t Zn y^{-1} were measured. Nickel was excluded in the QSR 2004 due to data not being available for all Wadden Sea subareas, but as a new compound of high priority in both OSPAR and WFD, needs to be included in the monitoring of the Wadden Sea (Essink et al., 2005).

However, such a decrease towards offshore areas must not always be the case as has been shown for Cd in calanoid copepods on a larger spatial scale (Zauke et al. 1996), with lower values in the southern North Sea to a sharp increase of high values in the central North Sea and the Scottish Isles. A comparison between results obtained for the nearshore locality of Schillig (Figure 3.5) and Box A (Table 3.3) yields contradictory conclusions. While for Cd and Zn we find an increase from nearshore to offshore locations (taking into account the heavier weight of the organisms from Box A), results for Cu, Ni and Pb tend to be similar. Thus, the findings reported here for Cd in Crangon are in fairly good agreement with the trend cited above for copepods.

Time series on *C. crangon* densities in the German Wadden Sea show a considerable degree of interannual variability (Siegel et al., 2005). The brown shrimp *C*. *crangon* is a less dominant species in the epifaunal assemblage, but still it varies annually and seasonally in its importance of structuring these compositions. Spatial patterns in benthic populations are positively correlated with sediment characteristics, influenced to a major extent by water temperature and therefore also by seasonal variability (Hinz et al., 2004; Siegel et al., 2005). If specimens are able to react to increasing environmental deterioration, they may choose to leave. As an adaptive strategy, they need to find a balance between risks of migration and staying hidden in the upper level of the sediments, and the chance to find a better habitat. Potential factors regulating migratory activity are similar to other benthic organisms, for example to *C. edule* (Jung et al., 2006). This may be availability and search for food, disturbance, hydrography, predators, the density of concurrent species, or the sediment composition.

The spatial structure, that can be revealed by geostatistical analysis, for example a spatial pattern of high density patches or local dominance by one or more species, make it difficult to predict relative species composition at a given sampling site, thus challenging the concept of a community of ecologically interacting decapod crustaceans. Local factors, such as hydrographic processes, sediment resuspension and mud content, might help to explain heterogeneities in the spatial distribution of decapod crustaceans (Maynou et al., 1996, Hinz et al., 2004). Furthermore, this might also help to explain spatial distributions in metal concentrations in areas under investigation. Spatial structures depend on the species and they can also vary with time of day and sampling period (Stelzenmüller et al., 2006).

The mobility of most epifaunal species suggests that the sampling scale have to be much larger than for that of relatively sedentary infauna. If too small scales are chosen, it might be possible to detect changes, but it will be difficult to distinguish if the observed changes are fundamental long-term changes of if they are caused by the spatial discontinuity derived from redistribution of organisms, e.g. through drifting or migration (Hinz et al., 2004).

Size estimates for Crangon crangon and implications for biomonitoring

In the German Bight environmental conditions are highly variable. Only limited information on ecology and biomonitoring of marine invertebrates is available explicitly taking spatial autocorrelations into account (Jung et al., 2006). Application of classical statistical procedures assumes stochastic independence of the data (Petitgas, 2001). Our results indicate at least week spatial autocorrelations at the scale of investigation for *Ni* and *Pb* and, to lesser extent, for *mbww* and *Cd*, as can be inferred from increasing semi-variograms (Figure 3.3) and from the estimated semi-variogram models (Table 3.2). Thus it is only when samples are taken at distances above the estimated values for the corresponding ranges that stochastic independence of the data can be assumed. These are 6.6 km for *Cd*, 3.0 km for *Ni* and 5.2 km for *Pb*. In order to obtain independent samples for monitoring of all variables considered, the distance between sampling points has to be above 6.6 km. If samples are taken at smaller distances, classical population estimates like means and confidence intervals might be biased (Maynou et al., 1996; Maynou, 1998) and the survey precision might be overestimated. This would be not the case in our study as can be seen from the comparison of classical and geostatistical coefficients of variation compiled in Table 3.3. However, this does not invalidate the geostatistical approach employed here, since it will always be an *a posteriori* conclusion. The mapped *Zn* distribution shows a decrease of *Zn* concentration in brown shrimps from west to east (Figure 3.4). The inverse relationship found for *Cd*, *Ni* and *Pb*, clearly showing a spatial correlation between cadmium, nickel and lead concentrations in the individual body weight of *C. crangon* when the spatial autocorrelation is explicitly taken into account.

The biological actions of Zn and Cd, for example, are very different. Zinc is indispensable to life, but can be toxic at increased concentrations. Cadmium is generally be regarded as non-essential having no known physiological function, but having an injurious effect on living organisms. Literature data reveal that bioaccumulation patterns of these two metals differ to a great extent in different aquatic organisms. It is known that another decapod shrimp, *Palaemon elegans*, is able to regulate Zn (White and Rainbow, 1985; Nugegoda and Rainbow, 1988, 1989, 1989). In situ studies confirmed that zinc concentrations in the organisms are largely independent of metal levels in the environment (White and Rainbow, 1982). Findings of Devineau and Amiard-Triquet, (1985) demonstrate that *Palaemon serratus* is able to regulate its total-body levels of zinc during the early stages of its life cycle. Unlike zinc, cadmium is not regulated in marine crustaceans, e.g. *C. crangon* (Dethlefsen, 1978).

Trace metal concentrations in decapods from different regions of the world are compiled in Table 3.4. It is very striking, that information on the genus *Crangon* is completely missing. However, Pb concentrations in decapods from other regions are within the 95%-confidence intervals of this study regarding Box A (Table 3.3), while for Zn reported values are slightly above and for Cu slightly below these intervals. Conversely, Cd concentrations in *C. crangon* from this study are much lower than those in decapods from other regions. For *Ni* no comparative values exist. Comparing different regions such as the Iberian Deep Sea Plain (Prowe et al., 2006), the Greenland Sea (Ritterhoff and Zauke, 1997) and the Barents Sea (Zauke and Schmalenbach 2006) the results compiled in Table 3.4 suggest overall low Pb

concentrations < 1 mg kg^{-1} and almost constant Zn concentrations around 50-80 mg kg^{-1}, but, greatly varying Cd and Cu concentrations.

Table 3.4: Mean concentrations of the trace metals cadmium (Cd), copper (Cu), lead (Pb) and zinc (Zn) in decapods from different regions of the world [mg kg^{-1} DW].

Species	Region	Cd	Cu	Pb	Zn	Ref
Acanthephyra purpurea	NO Atlantic	3.0	36	-	46	1
Acanthephyra spec.	Iberian Deep Sea Plain	6.1	56	0.6	52	2
Bentheogennema intermedia	Iberian Deep Sea Plain	10.7	36	0.4	74	2
Benthesicymus iridescens	Iberian Deep Sea Plain	14.9	55	0.4	79	2
Chorismus antarcticus	Weddell Sea, Antarctic	13.0	93	1.6	44	3
Hymenodora glacialis	Greenland Sea, Arctic	6.7	16	< 0.3	37	4
Hymenodora glacialis	Fram Strait, Arctic	9.2	12	< 0.3	52	4
Notocrangon antarcticus	Weddell Sea, Antarctic	13.0	67	0.8	46	3
Pandalus borealis	Barents Sea, Arctic	1.6	61	< 0.4	79	5
Sabinea sarsi	Barents Sea, Arctic	4.3	68	< 0.4	59	5
Sergia spec.	Iberian Deep Sea Plain	1.9	17	0.5	67	2
Systellaspis debilis	Atlantic, African Coast	22.0	55	-	70	1
Systellaspis debilis	Atlantic, Azores	13.0	-	-	50	6
Systellaspis debilis	NO Atlantic	12.0	67	-	53	7
Systellaspis debilis	Iberian Deep Sea Plain	16.3	49	0.6	62	2

Ref = references: 1: Ridout et al. (1989); 2: Prowe et al. (2006); 3: Petri and Zauke (1993); 4: Ritterhoff and Zauke (1997); 5: Zauke and Schmalenbach (2006); 6: Leatherland et al. (1973); 7: White and Rainbow (1987). For nickel (Ni) no comparative values exist.

The higher Zinc concentrations found in *C. crangon* of this study compared to other decapod crustaceans could be due to a higher metabolic requirement. Nugegoda and Rainbow (1988) reported that the decapod *Palaemons elegans* is clearly able to regulate total body zinc at higher external concentration in the presence of the chelating complex EDTA. Due to the chelator this increased ability to regulate Zn is correlated with a reduction in the uptake of Zn by the prawn. Assuming that zinc concentrations in seawater of the German Bight are lower than those of nearshore regions, higher Zn concentrations in shrimps from Box A indicate a higher potential for bioaccumulation compared to organisms from nearshore stations (e.g. Schillig).

5. CONCLUSION

At least week spatial autocorrelation found for *Ni*, *Pb*, *Cd* and *mbww* (mean body wet weight) of brown shrimps at the chosen scale of investigation suggest that classical statistical procedures should be applied with caution in ecological field studies on this scale. Only at dis-

tances above 6.6 km will it be possible to obtain independent replicates for all variables under study (with the exception of Cu and Zn), while below these distance explicit spatial procedures like geostatistics should be employed. If samples are taken at the shorter distances, classical population estimates like means and confidence intervals might be biased and the survey precision might be overestimated. This recommendation is valid although no clear reduction of the variability of trace metals in shrimps within Box A has been encountered, taking a spatial autocorrelation explicitly into account. One reason might be that no additional small scale sampling could be done during the present survey. In future studies such an approach might be useful when addressing the problem of trophic transfer of energy or pollutants from food (e.g. brown shrimps) to higher trophic levels such as fish and seabirds.

Acknowledgements

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CHAPTER 4

Assessment of two-compartment models as predictive tools for the bioaccumulation of trace metals in the brown shrimp *Crangon crangon* (Linnaeus, 1758) from the German Wadden Sea

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Abstract

The objective of the present study is to evaluate the suitability of the brown shrimp *Crangon crangon* (Linnaeus, 1758) from the German Wadden Sea as a biomonitor for the trace metals Cd, Cu, Pb and Zn and to analyse whether the two-compartment model could be used as a predictive tool to assess environmental quality. The focus here is on the verification of model parameters with independent experimental observations and on the statistical analysis of the results. The decapods tested accumulated metals upon exposure and it was possible to estimate significant model parameters of two-compartment models for Cd and Pb. The elements Cu and Zn need further evaluation, since linear predictions from the two-compartment models were not applicable, possibly due to high background concentrations in the animals along with high variances in the samples. Kinetic BCFs at theoretical equilibrium were: 1135 for Cd and 772 for Pb. A tentative estimation showed the following sequence of sensitivity of *C. crangon* to an increase of soluble metal exposure: 0.4 µg Cd L^{-1} and 1.5 µg Pb L^{-1}. Available information can be used to quantify a measure of agreement or disagreement between bioaccumulation in the investigated decapod. This can be regarded as an important step in the calibration of biomonitors which is necessary to assess the potential for bioaccumulation on a large geographical scale.

Key words: German Wadden Sea, trace metals, toxicokinetic models, bioaccumulation, biomonitoring, decapods, model verification, sensitivity of biomonitors.

1. INTRODUCTION

Many environmental monitoring programmes record results of past events without understanding the underlying ecological processes. However, there is an increasing demand for prospective approaches to detect potential human impact on ecosystems, based on a sound understanding of these ecological processes. This is in line with the precautional principle. Natural and anthropogenic metal inputs influence the bioavailable metal supply which cannot be detected by routine analytical procedures measuring metal concentrations in the soluble phase. This bioavailable fraction is usually determined by measuring the metal accumulated in organisms, which is the main goal in biomonitoring (e.g. Rainbow, 1993, 1995).

The bioavailable fraction from food and water is of great importance to assess the environmental quality regarding heavy metals in aquatic systems. 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), for example, in benthic amphipods derived from field samples 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., 1996). 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., 2004b; Luoma and Rainbow, 2005). As a result, we frequently can find different species of marine invertebrates with greatly varying metal concentrations in the same body of ocean water (Petri and Zauke, 1993; Zauke and Schmalenbach, 2006).

To detect potential human impact on ecosystems, natural background concentrations of chemicals in organisms and their fluctuations have to be well established (Petri and Zauke, 1993; Zauke and Petri, 1993). Another pre-condition is the knowledge of accumulation strategies. This depends on the biological species and the element considered (Rainbow et al., 1990), the exposure regime applied (Borgmann and Norwood, 1995), cation homeostasis mechanisms (e.g. (Viarengo and Nott, 1993) as well as life-history influences on metal accumulation and the development of an adequate experimental design on different spatial and temporal scales (Zauke et al., 1996; and literature cited therein).

In many environmental programmes mussels are used as biomonitors. Although crustaceans may be also regarded as suitable bioindicators and biomonitors in various freshwater systems (Rinderhagen et al., 2000), only little information about such programmes for marine crustaceans could be found, with the sole exception of the Canadian Science Advisory Secretariat, Department of Fisheries and Oceans, establishing a framework for the assessment of lobsters (DFO, 2006), assessments of shrimps stocks (DFO, 2006), and an Integrated Fisheries Management plan (DFO, 2003). However, due to their great importance in the food webs of the intertidal zone in the Wadden Sea and subtidal zone in the North Sea, crustaceans such as the brown shrimp *Crangon crangon* (Linnaeus, 1758) merit further consideration.

The epifaunal decapod *C. crangon* can be found in coastal and estuarine waters in the Baltic and the North Sea, the Atlantic coast of North and West Europe and the Mediterranean. It is a typical euryhaline inhabitant (Cieluch et al., 2005). *C. crangon* is considered to be a key species in the coastal waters of the North Sea and in particular in the Wadden Sea, since it occurs in masses and acts both as a highly efficient predator and important prey. The shallow water region of the Wadden Sea is the nursery ground for brown shrimps (Berghahn, 1996). The organisms burrow just below the sand surface during the day, so that only the eyes and antennae can be seen. Brown shrimps are important food resources for flatfish (*Pleuronectes platessa*), shore crabs (*Carcinus maenas*), seals (*Phoca vitulina*), various waders (Limicolae), seagulls (Laridae) and auk birds (Alcidae) (Jensen and Jensen, 1985; Janke, 1999).

Mathematical and statistical techniques provide a means of summarising and assessing the experimental observations available in the research area of the ecotoxicology of metals and xenobiotic substances. Pharmacological and biochemical research groups dealing with enzyme kinetics and drug metabolism have developed a suite of ideas and models which have been adopted to explain metal effects in aquatic biota in a mechanistic fashion. Examples are given (a) in an extension of the compartment model taking into account a maximum number of metal binding sites in the animals with the uptake rate constant either independent or dependent on the external metal exposure (Borgmann and Norwood, 1995) or (b) in the biotic ligand approach, BLM (Paquin et al., 2002; Paquin et al., 2002). In order to understand the mechanisms of bioaccumulation, sophisticated investigations are required, e.g. considering metal distributions in different organs or quantification of subcellular sequestration processes (granules, lysosomes or metallothioneins; Viarengo and Nott, 1993).

In contrast to such mechanistic or physiological models, the dynamic compartment model does not have a high degree of physiological detail. A normal requirement for an assessment model is that it should be as simple as possible, which can be achieved with a low number of parameters (parsimonious modelling) and a "black-box" approach to dynamic processes. In environmental studies the estimation of bioconcentration factors (BCFs) at theoretical equilibrium is of great importance. For an evaluation of the organisms potential for biomonitoring, this method is adequate and has often been used in studies with aquatic invertebrates (e.g. Zauke et al., 1996; Ritterhoff and Zauke, 1997b; Clason and Zauke, 2000; Kahle and Zauke, 2003; Clason et al., 2004b; and literature cited therein).

However, if a limitation of the uptake rate should occur at higher exposures, the relationship between net metal uptake and increasing exposure concentrations will follow a saturation curve. In this case, a verification or extrapolation of (linear) model predictions derived from first-order kinetics will only be successful for the lower range of exposure concentrations, where the uptake process is not limiting the rate of uptake (Ritterhoff and Zauke, 1997b; Bernds et al., 1998; Clason and Zauke, 2000). In this case, a hyperbolic toxicokinetic model allowing non-linear extrapolations could be employed Kahle and Zauke (2002a, 2002b, 2003). Alternatively, predictions derived from compartment models could be adjusted, taking into account a relationship between kinetic parameters such as k_1, k_2 or BCF to the external

metal exposure (C_{wi}) as has been previously described for amphipods (Wang and Zauke, 2004; Clason et al., 2004a; Clason et al., 2004b).

The objective of the present study is to evaluate the suitability of *C. crangon* as a biomonitor for the trace metals Cd, Cu, Pb and Zn, and to analyse whether the two-compartment model could be used as a predictive tool to assess environmental quality. The focus here is on the verification of model parameters with independent experimental observations and on the statistical analysis of the results. Finally the concept of the sensitivity of decapods for biomonitoring will be elaborated in detail. All these aspects are component of a process which is referred to as the calibration of biomonitors. Detailed experimental studies on bioaccumulation of metals in decapods from the German Bight, southern North Sea, and the development and verification of toxicokinetic models as a predictive tool are completely missing as yet.

2. MATERIAL AND METHODS

2.1 Definition of terms

The term *collective* used throughout this paper denotes a decapod sample taken at a given locality at a given time which is regarded as the basic unit of observation. By using this word we emphasise that neither a strict random sampling of these organisms nor a definite (*viz*. destructive) species determination of *all specimens* collected is possible in any case (Zauke et al., 1996). In toxicokinetic experiments the time course of uptake and depuration of metals in organisms is evaluated. Here each treatment represents one single *experimental unit* (Hurlbert, 1984), whereas the independent subsamples taken at different times (see below) represent *evaluation units* (Hurlbert and White, 1993). The term *uptake* denotes the incorporation of substances into the body of organisms, and *clearance* (excretion, depuration) denotes the loss of substances from the body (e.g. Rainbow et al., 1990). *Net accumulation* means that organisms are unable to match rates of excretion to rates of uptake, while *regulation* means that organisms are able to maintain constant body concentrations over a certain range of exposures. Strictly speaking, these definitions are valid only for a given set of experimental conditions, e.g. the range of external metal exposures applied and the time chosen for uptake phases, so that hypotheses based on them should be regarded only as tentative. According to OECD (1981) a *two-compartment model* involves test solutions or other exposure media as the first, and organisms as the second compartment. Note that in other studies this may be termed a *one-compartment model*.

2.2 Sampling and test organisms

Juveniles of *Crangon crangon* (Linnaeus, 1758) from the summer hatch were sampled at the German North Sea Coast in Schillig (Figure 4.1) in July 2004 and 2005. They were caught during falling water with small fishing nets and kept in polypropylene buckets for transportation supplied with in-situ seawater. Upon arrival at the laboratory the organisms were sorted from sand and plant debris. The test organisms were acclimatised for 48 hours in a constant-temperature climate room at 13°C, with a 12 hours day and 12 hours night photoperiod. They

were kept in polypropylene buckets with fresh aerated seawater before being randomly allocated to experimental vessels of different treatments. Seawater (30.4 psu) for keeping the animals and the experiments was obtained from the marine station of the ICBM in Wilhelmshaven. Salinity was measured using a Microprocessor Conductivity Meter LF 196 with the conductivity cell TetraCon 96 A-4 (WTW GmbH, Wissenschaftlich Technische Werkstätten, Weilheim). The identification of *C. crangon* was performed according to Smaldon (1979) and Hayward and Ryland (1990, 1990).

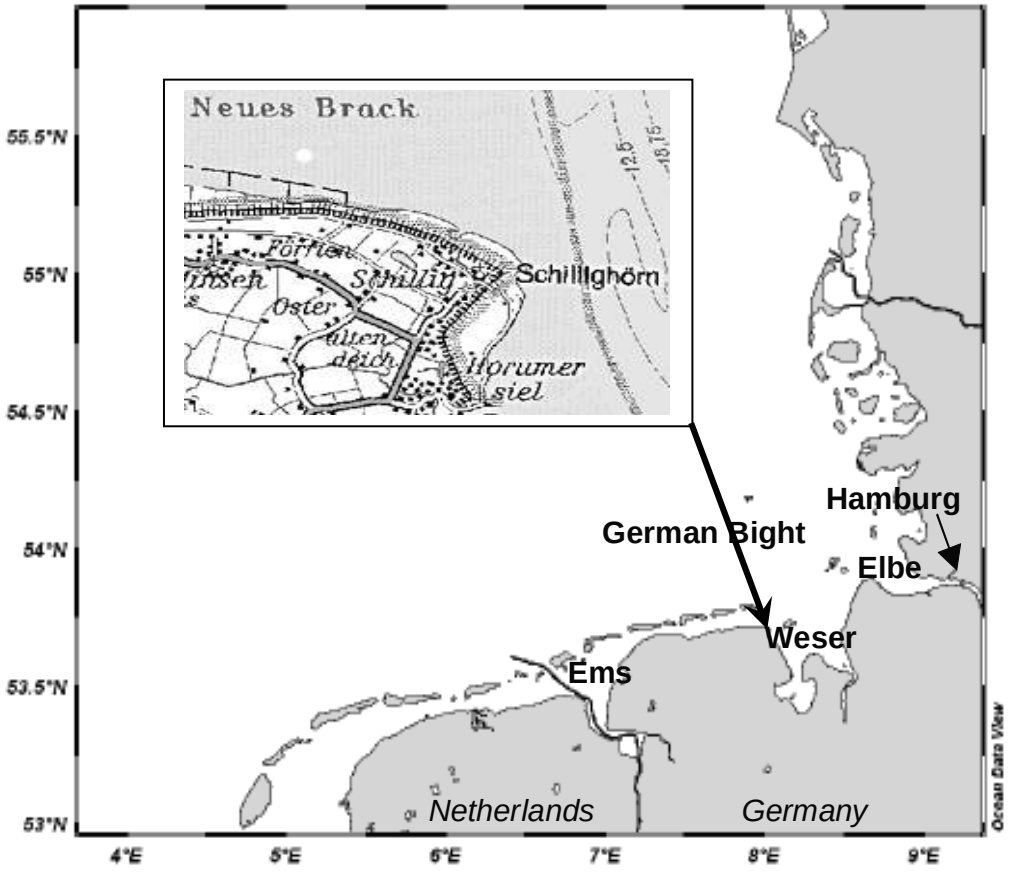

Figure 4.1: Sampling area of the decapod crustacean *Crangon crangon* in Schillig, North Sea Coast, Germany. Sampling took place in July 2004 and 2005.

2.3 Set-up of toxicokinetic studies

2.3.1 General considerations

For the set up of the of all three toxicokinetic studies sterile polyethylene petridishes were used as experimental vessels. The petridishes had the measurements of 94 x 16 mm, with a possible water volume of 10 ml. During the experiments one single organism of *C. crangon* in each experimental vessel was kept respectively in order to prevent loss of organisms through cannibalism among the brown shrimps. Consequently, each petridish was considered as one sample.

The adsorptive capacity of the experimental vessels was saturated by pre-soaking them with test solutions for three days prior to the start of the experiments. During all toxicokinetic studies no sediment was provided in the experimental vessel to avoid loss of metals through adsorption on sediment particles. The semi-static approach (which is in line with official test protocols, e.g. OECD (1981) was selected in this study because of its simplicity and convenience. Water in the semi-static experiments was renewed every day and spiked with a new stock test solution with metals.

The selection of elements in the mixture, non-essential elements Cd and Pb as well as potentially essential elements Zn and Cu, was the same as considered in German governmental monitoring programmes (Anonymous, 2005). It has been shown previously for amphipods that application of simultaneous exposure will most probably not create artefacts in comparison to single element dosing (Clason et al., 2003).

In this investigation three time-dependent toxicokinetic studies were performed, the exposures and controls being run in five independent replicates *sensu* Hurlbert (1984). Soluble metal levels in ambient seawater of the North Sea in general and the German Bight in particular reported from various sources are relatively low: 0.009-0.013 µg Cd L^{-1}; 0.17-0.30 µg Cu L^{-1}; 0.036-0.051 µg Pb L^{-1} and 1.0-1.8 µg Zn L^{-1} (Haarich and Schmidt, 1993, 1993; Scholten et al., 1998). The chosen nominal metal concentrations are presented in Table 4.1. Thus, the nominal metal concentrations in this study were about 40 to 500 times higher than reported concentrations in seawater (see above), which is a compromise between realistic environmental exposures and much higher experimental exposures known to result in a detectable increase of metal accumulation in organisms (Ritterhoff et al., 1996; Clason and Zauke, 2000).

For the set up of the experiments and prior to the daily water change, 1 ml of a metal solution, including Cd, Cu, Pb and Zn, was transferred by pipette to a 10 l polypropylene bucket and made up to a volume of 5 l with seawater. The resulting concentration of metals equals after the dilution approximately the nominal values. Buckets were also pre-soaked with the same mixture three days prior to the start of the toxicokinetic studies to saturate the adsorptive capacity of the bucket. For the set up of the control only a volume of 5 l of seawater was transferred to a 10 l polypropylene bucket.

Nominal and measured total recoverable metal concentrations in the controls (seawater) and exposures (C_{Wi}) throughout the uptake phase of the toxicokinetic studies *CI*, *CII* and *CIII* [µg L^{-1}] are presented Table 4.1. The measured values for C_{W1} are largely in good agreement for Cd and Pb with the nominal values (Table 4.1). For Zn and Cu concentrations are over the nominal values due to high background concentrations in the controls.

Table 4.1: Nominal (n) and measured (m) total recoverable metal concentrations (mean ± 95% CI for measured values) in the controls (seawater) and exposures (C_{Wi}) throughout the uptake phase of the toxicokinetic studies *CI*, *CII* and *CIII* [µg L^{-1}].

			metal concentrations in seawater [µg l^{-1}]			
Study	**C_{Wi}**		**Cd**	**Cu**	**Pb**	**Zn**
Control	-	m	< 0.05	12 ± 2.2	0.1 ± 0.4	17 ± 10.2
nominal up-take	C_{Wn}	n	5	30	20	60
Uptake CI	C_{W1}	m	5.0 ± 0.0	51 ± 17	18 ± 4	74 ± 51
Uptake CII	C_{W2}	m	4.2 ± 0.6	47 ± 12	19 ± 9	66 ± 10
Uptake CIII	C_{W3}	m	4.5 ± 0.6	50 ± 8	20 ± 7	70 ± 11

Limits of detection for the control values (according to Büttner et al. (1980): 0.05 µg Cd L^{-1}; 6.1 µg Cu L^{-1}; 1.0 µg Pb L^{-1} and 29 µg Zn L^{-1}. n.a.: no data available due to technical reasons.

The experimental design for the toxicokinetic studies is described below and in Table 4.2. At the days of sampling five samples were taken from the experimental metal treatments and five from the control treatments. Each sample consisted of one individual of *C. crangon*, superficial seawater was rinsed thoroughly for a few seconds with double-distilled water and dried by placing them on good quality filter paper (Type 613, Schleicher and Schuell, Germany; (Zauke et al., 1996). Each animal was subsequently stored in one Eppendorf reaction tube (2.0 ml, polypropylene). Five independent replicates (*sensu* Hurlbert (1984)) per treatment were thus obtained. All samples were stored frozen (-20℃) for metal determination.

As water samples for the polarography, a volume of 50 ml was taken with a pipette a day prior to sampling of organisms out of each 5 l bucket from the experimental and control treatments before renewal of test solutions in the semi-static approach. The water samples were transferred to pre-cleaned 50 ml polyethylene containers for subsequent measurement of metal concentrations by polarography (see results in Table 4.1) and deep frozen at -20℃.

The experiments were run with each animal in a separate experimental vessel (see above). In toxicokinetic study *CI* one set-up consisted of 60 petridishes for the metal exposure, respectively, and another set-up of 60 petridishes for the control. In the studies *CII* and *CIII* one set-up consisted of 100 petridishes for the metal exposure, respectively, and another set-up of 100 petridishes for the control. Additional ten animals were randomly sampled as experimental start values for metal exposure and control. For analysing metals in the organisms five specimen of *C. crangon* were randomly sampled from each experimental set-up on days 0, 3, 6, 8, 10, 12 and in case of *CI* additionally on day 14. The organisms sampled were stored separately in 2 ml Eppendorf safe-lock reaction tubes, weighed (soft-body wet weight) and deep frozen. During the daily water changes dead and moulted animals with their corresponding exuvia were removed from the experiments and treated as the samples described above for further processing, thus transferred to separate 2 ml Eppendorf safe-lock reaction

tubes (marked to allow a reference to the corresponding animal) and stored deep frozen for further analysis.

The brown shrimps were fed after water change with in seawater dissolved pulverised fish food ("Staubfutter" by TeraMin) to keep them healthy and in good conditions over the duration of the experiments. Due to the fast feeding of the solution through the organisms, is the adsorption of metals on food particles and therefore incorporation over the food path only of minor relevance.

Table 4.2: Experimental design of the toxicokinetic studies *CI*, *CII* and *CIII* with the brown shrimp *Crangon crangon*.

Toxicokinetic study	Experimental design	Goal
CI	uptake phase: 8 d; clearance phase: 6 d; soluble multiple exposure to Cd, Cu, Pb and Zn (C_{W1} in Table 4.1)	Model evaluation
CII	uptake phase: 12 d; soluble multiple exposure to Cd, Cu, Pb and Zn (C_{W2} in Table 4.1)	Model verification
CIII	uptake phase: 12 d; soluble multiple exposure to Cd, Cu, Pb and Zn (C_{W3} in Table 4.1)	Model verification

2.3.2 Set-up of toxicokinetic study *CI*

To study the time course of metal uptake, usually test organisms are exposed for a 10-day uptake phase to a metal mixture; this is usually followed by a 10-day clearance phase (see Clason and Zauke, 2000; Clason et al., 2003; Clason et al., 2004b). However, it was not applicable in this case due to high experienced mortality rates in the beginning of the investigations. In toxicokinetic study *CI* the individuals of *C. crangon* were exposed for an 8-day uptake phase to a metal mixture; which was followed by a 6-day clearance phase. Nevertheless, it was possible to estimate kinetic parameters for the uptake and clearance (see Data evaluation section).

2.3.3 Set-up of toxicokinetic study *CII*

Toxicokinetic study *CII* was set-up in order to verify the two-compartment models obtained in *CI*. Results from study *CI* gave hints on how the experimental set-up had to be optimized. Study *CII* was started with an extended uptake phase of 12 days. For the verification of the toxicokinetic models from *CI* only the uptake phase is considered. Furthermore, it was con-

cluded from *CI*, that the animals should be fed from the beginning of the experiment on, to prevent loss through starvation. In *CIII*, feeding was done every second day right after renewal of the test solution, on day 0, 2, 4, 6, 8, and 10. Upon daily water changes dead and moulted animals together with their corresponding exuvia (this time resulting in one sample, respectively) were removed from the experiments and transferred to 2 ml Eppendorf safe-rock reaction tubes and stored deep frozen (-20℃).

2.3.4 Set-up of toxicokinetic study *CIII*

Further adjustments of the experimental design led to toxicokinetic study *CIII*. This was set-up, such as *CII*, in order to verify the two-compartment models obtained in *CI,* again only considering the uptake phase. The decapods were exposed for a 12-day to the metal mixture specified in Table 4.1. The experiments were run as described for toxicokinetic study *CI* and *CII* with each animal in a separate experimental vessel. During daily water changes dead animals and exuvia of moulted animals were removed from the experiment and treated as samples and transferred to 2 ml Eppendorf safe-rock reaction tubes and stored deep frozen (-20℃).

2.4 Analytical procedures

The frozen decapod samples were subjected to freeze-drying for 48 h (LYOVAG GT2, Leybold Heraeus). Due to the small sample volume of the single animals and the single exuvia sampled on each occasion, it was not necessary to homogenise the samples to avoid losses of biomass. Instead the total dry material ranging from 0.2 to 9.5 mg was digested directly in the Eppendorf reaction tubes in which they had been stored and freeze-dried for 3 h at 80℃ with 100 µl HNO_3 (65%, suprapure, Merck) (Clason and Zauke, 2000). The digests were made up to 2 ml volume with double distilled water. After appropriate dilution, the final sample and standard solutions were adjusted to concentrations of 3.25% HNO_3.

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 Kahle et al., 2003). Ashing and atomisation temperatures were 700 and 2100℃ for Cd; 800 and 2500℃ for Pb; and 800 a nd 2300℃ for Cu. For Cd and Pb, palladium and magnesium nitrate modifiers were applied. Zn was assayed using an air-acetylene flame (Varian SpectrAA 30, with 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).

Quality assurance was performed in line with German GLP regulations (Anonymous, 2005), 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). For biological samples, the precision and validity was evaluated using two certified reference materials which were randomly allocated within the determinations (see Table 4.3). The analysed values for the reference materials were

largely in good agreement with the certified values with the sole exception of Pb. However, in this case these values are below the limit of detection which was calculated according to Büttner et al. (1980).

Table 4.3: Quality assurance using certified reference materials randomly allocated within the determinations. Values are means ± 95% confidence intervals [mg kg^{-1} DW].

	Tort-2 Lobster hepatopancreas; National Research Council Canada			CRM No 278R Mussel Tissue : *Mytilus edulis*; Community Bureau of Reference		
Element	analysed	n	certified	analysed	n	certified
Cd	25.4 ± 0.7	36	26.7 ± 0.6	0.392 ± 0.02	36	0.348 ± 0.007
Cu	100 ± 5	36	106 ± 10	8.84 ± 0.61	36	9.45 ± 0.13
Pb	0.7 ± 0.18	35	0.35 ± 0.13	2.53 ± 0.22	36	2.00 ± 0.04
Zn	183 ± 7	36	180 ± 6	78.4 ± 3.3	36	83.1 ± 1.7

n: Numbers of independent determinations

Limits of detection (according to Büttner et al., 1980): 0.2 mg Cd kg^{-1}; 4.7 mg Cu kg^{-1}; 1.4 mg Pb kg^{-1}; and 25 mg Zn kg^{-1} (dry weight).

Water samples from the exposure treatments were analysed in standard addition mode by polarography (Metrohm 757 VA Trace Analyser, 757 Stand, CH-9101 Herisau, Programm 5.746.0100, following DIN 38406 Teil 16) using a hanging mercury drop electrode (HMDE) and a reference electrode filled with 3M KCl. The linearity of the additions was checked by linear regression analysis leading to adjusted R^2-values ≥ 0.984. Prior to analyses, samples were degassed with nitrogen (grade 5.0). Peaks were detected employing differential pulse anodic stripping voltammetry (DPASV) at -0.63 V (Cd), -0.20 V (Cu), -0.47 V (Pb), and -1.05 V (Zn). Limits of detection for the seawater samples were calculated using the control samples displayed in Table 4.1 according to Büttner et al. (1980) and were < 0.05 µg Cd L^{-1}; 6.1 µg Cu L^{-1}; 1.0 µg Pb L^{-1} and 29 µg Zn L^{-1}.

2.6 Data evaluation

2.6.1. Two-compartment models

Two-compartment models, with test solutions as first and organisms as second compartment, and first order kinetics are used to evaluate and compare toxicokinetics of metals in marine organisms (OECD, 1981). Changes in metal concentrations with time are:

for the first compartment: $$\frac{dC_W}{dt} = 0 \quad (1a)$$

and for the second compartment: $$\frac{dC_A}{dt} = k_1 * C_W - k_2 * C_A \quad (1b)$$

where C_W is the metal concentration in seawater during uptake phase [mg kg^{-1}, approx. equivalent to mg L^{-1}]; t is time; C_A is metal concentration in the animals [mg kg^{-1} DW]; k_1 is the rate constant for uptake phase [d^{-1}] and k_2 is the rate constant for clearance phase [d^{-1}]. From Equation (1a) it is obvious that C_W is assumed to be constant in any given experiment, or in practice, that only minor variations are acceptable. According to Equation (1b), metal concentrations in test organisms will increase in proportion to exposure ($k_1 * C_W$) but, in parallel, will decrease in proportion to the metal concentrations already accumulated in the organisms due to depuration ($-k_2 * C_A$). Thus, the model employed is totally defined by the rate constants without considering any physiological or morphological details of organisms (black-box approach). This approach has been successfully employed for various aquatic crustaceans (Zauke et al., 1995; Ritterhoff et al., 1996; Clason and Zauke, 2000; Clason et al., 2004b).

The integrated forms of these equations are employed to estimate model parameters regarding toxicokinetic study *CI* simultaneously for the uptake and clearance phases from Equations (2a) and (2b), respectively, using non-linear regression methods. The uptake phase (time: $0 < t \leq t_e$, with t_e = end of uptake phase) is described by:

$$C_A = C_{Con} + C_W * \frac{k_1}{k_2} * \left(1 - e^{-k_2 * t}\right) \tag{2a}$$

and the clearance phase ($t > t_e$) is described by:

$$C_A = C_{Con} + C_W * \frac{k_1}{k_2} * \left(e^{-k_2 * (t - t_e)} - e^{-k_2 * t}\right) \tag{2b}$$

where C_{Con} represents the background metal concentration in brown shrimps from the controls [mg kg^{-1} DW] (values for C_{con} from the toxicokinetic studies *CI* in Table 4.4); otherwise as defined above. The parameter k_1 is involved in the clearance phase to define the starting point for the metal depuration (Butte, 1991).

Bioconcentration Factors (BCFs) as well as uptake rates ($U_p = k_1 * C_W$) derived from toxicokinetic studies have frequently been used to evaluate the potential of organisms for bioaccumulation of soluble or particle-bound metals (see Clason and Zauke, 2000; Clason et al., 2004b; and literature cited therein). To avoid confusion, the experimentally derived BCFs are referred to as kinetic BCFs (in contrast to field BCFs obtained from field data of metals in organisms and in the surrounding water). Thus, for the two-compartment model, kinetic BCFs at theoretical equilibrium are calculated as:

$$BCF = \frac{k_1}{k_2} = \lim \frac{C_A}{C_W} \quad (for \ t \to \infty) \tag{3}$$

The advantage of this approach is that the uptake phase of experiments may be much shorter than would be necessary to reach, let us say, 95% of the measured metal concentration in organisms at equilibrium within the experimental time. Estimated values for BCF_{field}

were obtained from background metal concentrations in *C. crangon*, C_{con} (see Table 4.4), and reported data for metals in seawater (Haarich and Schmidt, 1993, 1993; Scholten et al., 1998; values see above).

2.6.2. Verification of model parameters

Verification of models involves comparison of model predictions (here from toxicokinetic study *CI*) with data from independent experiments (here from *CII* and *CIII*).

In particular, to verify a two-compartment model for a given element from toxicokinetic study *CI* with data from a time-dependent study *CII* or *CIII*, values for C_{con} and C_{Wi} from *CII* or *CIII* and the model parameters k_1 and k_2 from *CI* are taken. Then a prediction can be made with these parameters using Equation (2a), where C_A is a non-linear function of time t with C_W being constant, all other parameters being constant within a given experiment (e.g. Ritterhoff and Zauke, 1997b; Clason and Zauke, 2000; Clason et al., 2004b).

The goodness-of-fit (*gof*) between observed data (dependent) and model predictions (independent) can be assessed by linear regression analysis, assuming that the slope yields the value "1" in the case of perfect agreement. Thus, when the estimated 95% confidence intervals of the slope include the value "1", this can be regarded as a fairly good agreement. Otherwise, values of the slope not equal to one can be used to quantify the disagreement between the two experiments and the degree to which predictions under- or over-estimate the observed data.

2.6.3. Sensitivity of brown shrimps as biomonitors

The assessment of the sensitivity of organisms is a crucial part of the concept of biomonitoring: how large must an increase of external soluble metal exposure be to produce a detectable increase of metal concentrations in the organisms? This problem is very similar to concepts regarding limits of detection in analytical chemistry (e.g. blank + 2.6 SD of a "low value"; Büttner et al., 1980). A first approximate solution to this problem in biomonitoring has been suggested by Clason and Zauke (2000). Basically, the minimum increment in body concentration that can be detected ($\min \Delta C_A$) is obtained from the following equations, based on the assessment of the number of samples necessary to get an estimate within a selected distance from the mean with a given probability (Southwood and Henderson, 2000):

$$N = \left(\frac{t - value * error}{\min \Delta C_A} \right)^2 \tag{4a}$$

with some re-arrangement and replacing error by SD_{con} (standard deviation of control organisms) leading to:

$$\min \Delta C_A = \frac{t - value * SD_{con}}{\sqrt{N}} \tag{4b}$$

Thus, an accumulated metal concentration in the animal significantly larger than the initial value (the concentration of field samples or control organisms, C_{con}) must be:

$$\min C_A > C_{con} + \min \Delta C_A \quad (4c)$$

Regarding Equation (4b) a one sided *t-value* is appropriate. Furthermore, the result will be strongly dependent on the selected probability of the *t-value* (normally 95%), the number of independent experiments *N* (equal to 5 in study *CI*). In the next step, simulations of an uptake experiment of a given duration are performed for compartment models using Equation 2a. Metal exposures (C_{Wi}) are varied systematically until metal concentrations in the animals (C_A) exceed the value of C_{con} + $min\Delta C_A$. The resulting exposure is referred to as the minimal increment in exposure concentrations ($min\Delta C_W$) and can be regarded as sensitivity of organisms as biomonitors.

2.6.4 Statistical computations

All statistical calculations in this study were done with SYSTAT for Windows (Version 10). Two-compartment models were evaluated using the NONLIN subroutine and piecewise regression option (Engelman, 2000). The goodness-of-fit was assessed with the aid of corrected R^2-values (approximate coefficient of determination) and approximate t-ratios of the estimated parameters (Bates and Watts, 1988, p. 90). Note that model estimates are obtained with metal exposures expressed in mg L^{-1}, while in the following figures those are expressed in µg L^{-1} for convenience. Linear regression was performed to compare observed and predicted data from independent experiments using the regression subroutine (Wilkinson, 2000). All simulations were done in Microsoft Office Excel 2003 with the model parameters estimated in SYSTAT.

3. RESULTS

The cumulative moulting of *C. crangon* is displayed in Figure 4.2(a). All toxicokinetic studies differ distinctly: in *CII* moulting is much more intensive compared to *CI* and *CII*. On the other hand, we only find minor differences between controls metal treatments, respectively. The cumulative mortality of brown shrimps is displayed in Figure 4.2(b). In *CI* a distinct increase is obvious from day one on. The loss of animals in the experiments could be reduced due to changes in the set-ups of the following toxicokinetic studies. Although in *CII* mortality is lowest in all studies, moulting was highest in this experiment. At the end of the toxicokinetic studies, mortality in *CI* and *CIII* is distinctly higher in the control than in those with multiple metal exposures in the uptake phase. A slight impact of metal exposure on mortality is only visible in *CII*.

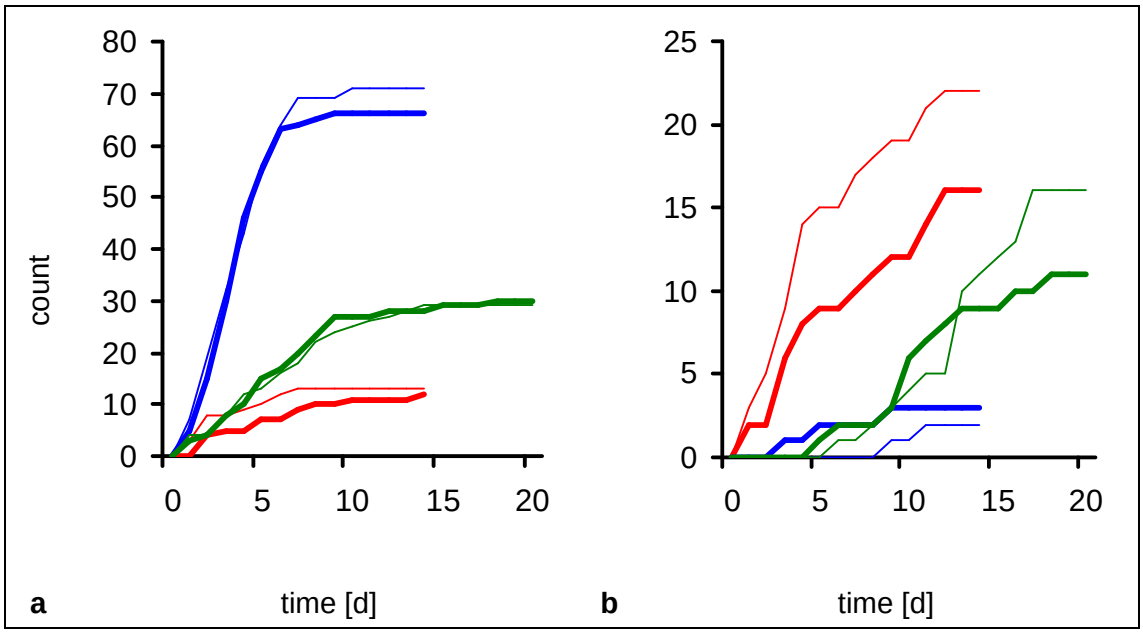

Figure 4.2: Cumulative moulting (a) and cumulative mortality (b) of the brown shrimp *Crangon crangon* in the three toxicokinetic experiments. *CI* control = red fine line; *CI* metal = red thick line; *CII* control = blue fine line; *CII* metal = blue thick line; *CIII* control = green fine line; *CIII* metal = green thick line.

The control values for *C. crangon* from all three toxicokinetic studies are reported in Table 4.4.

Table 4.4: Control values (C_{con}) for *Crangon crangon* from toxicokinetic studies *CI*, *CII* and *CIII*. Values are means ± 95% confidence intervals [mg kg^{-1} DW].

	Cd			Cu			Pb			Zn		
	mean ± 95% CI	SD	N	mean ± 95% CI	SD	N	mean ± 95% CI	SD	N	mean ± 95% CI	SD	N
CI	0.16 ± 0.02	0.08	45	50 ± 6	21	45	0.77 ± 0.29	0.95	44	75 ± 7	21	44
CII	0.18 ± 0.03	0.08	36	32 ± 3	9	35	0.97 ± 0.35	1.01	34	63 ± 5	15	35
CIII	0.16 ± 0.04	0.13	53	37 ± 2	8	52	0.71 ± 0.35	1.21	48	66 ± 5	18	53

Notes: SD = standard deviation, N = number of measurements

The time-dependent uptake of metals in *C. crangon* is shown in Figure 4.3 and the estimated parameters of the two-compartment models appear in Table 4.5. For Cd and Pb it was possible to estimate model parameters of the time-dependent uptake study for each exposure concentration. Generally, the organisms accumulate metals upon external metal exposure with the exception of Cu and Zn (Figure 4.3(c) and 4.3(d)) where no model could be estimated.

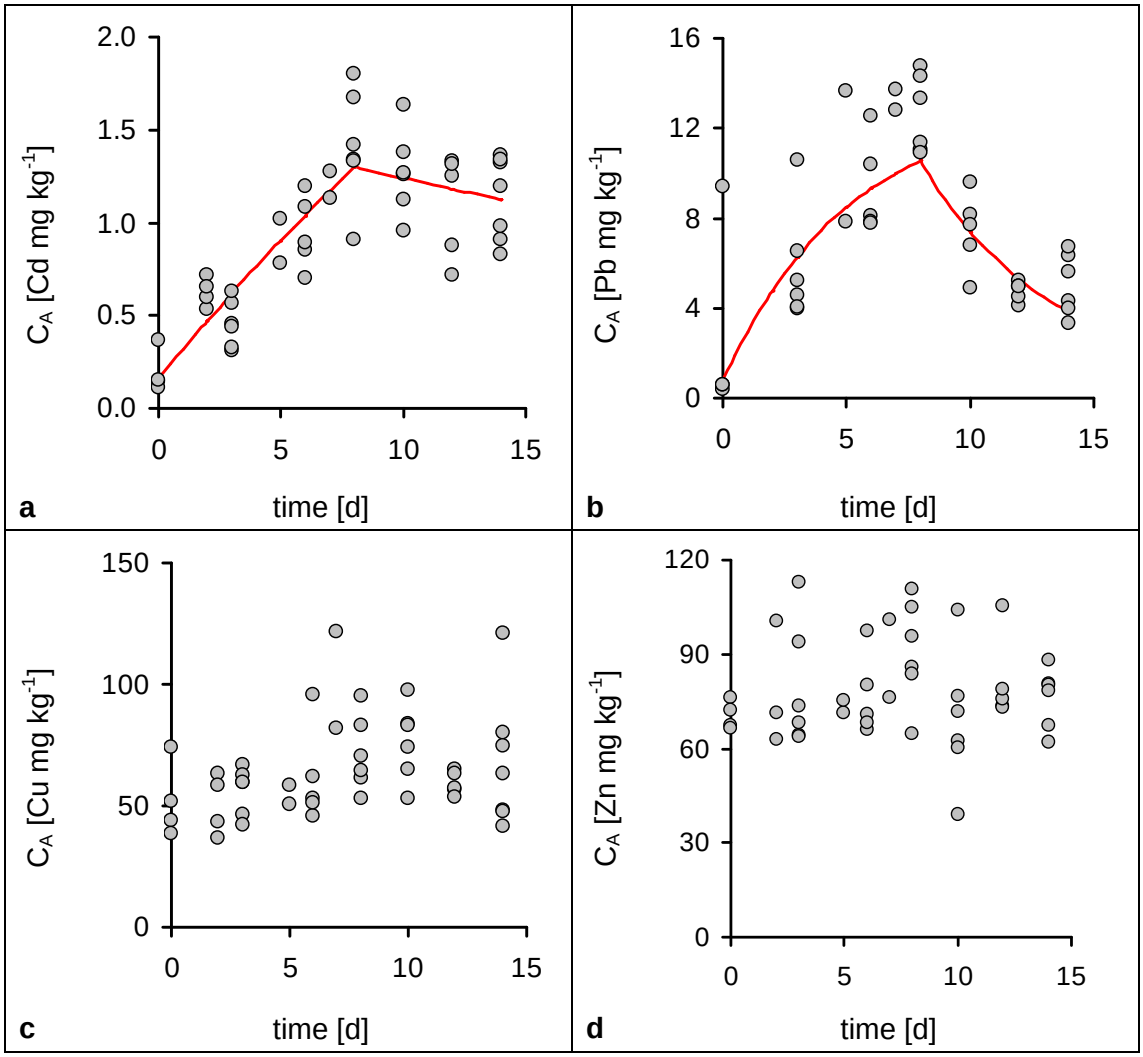

Figure 4.3: Time course of uptake and clearance [mg kg^{-1} DW] of Cd, Pb (a, b),Cu (c), and Zn (d) in brown shrimps (notation in Table 4.2) from toxicokinetic study *CI*. Models could be calculated only for Cd (a) and Pb (b); see red line. Metal exposures see table 4.1. Comparison of observed values (filled circles) with predictions of two-compartment models (Cd and Pb; red line; using equation (1) and (2); kinetic data in Table 4.5). Day 8: end of uptake phases; see text for more details.

The BCFs at theoretical equilibrium decrease from Cd to Pb. Regarding approximate t-ratios, all model parameters for metal uptake and clearance are significantly different from zero (*viz.* the confidence intervals do not include the value zero) and the corrected R^2 values indicate that a sufficiently high proportion of the variance is represented by the compartment models (see details in Table 4.5).

Table 4.5: Toxicokinetics of metals in *Crangon crangon* from toxicokinetic study *CI*; results of two-compartment models using equations (2a) and (2b) (see text for more details).

metal	$k_1 \pm 95\%$ CI	t-value k_1	$k_2 \pm 95\%$ CI	t-value k_2	BCF_{kin}	df	R^2
Cd	31.8 ± 5.5	11.6	0.028 ± 0.028	2.0	1135	46	0.744
Pb	150 ± 33	9.1	0.194 ± 0.061	6.5	772	40	0.639

Notes:
metal exposure see Table 4.1; C_{con} = mean metal concentrations in brown shrimps from control experiments, see Table 4.4 [mg kg^{-1} DW]; k_1 = rate constant for uptake [d^{-1}]; k_2 = rate constant for clearance [d^{-1}]; critical t-value (two sided): $t_{40;\ 0.05} = 2.02$; BCF_{kin} = bioconcentration factor = k_1 / k_2 (DW basis); df = degrees of freedom; R^2 = approximate coefficient of determination. No significant model could be estimated for Cu and Zn.

Results of the time-dependent uptake studies CII and CIII for Cd and Pb are compiled in Figures 4.4 and 4.5, those for Cu and Zn in Figure 4.6.

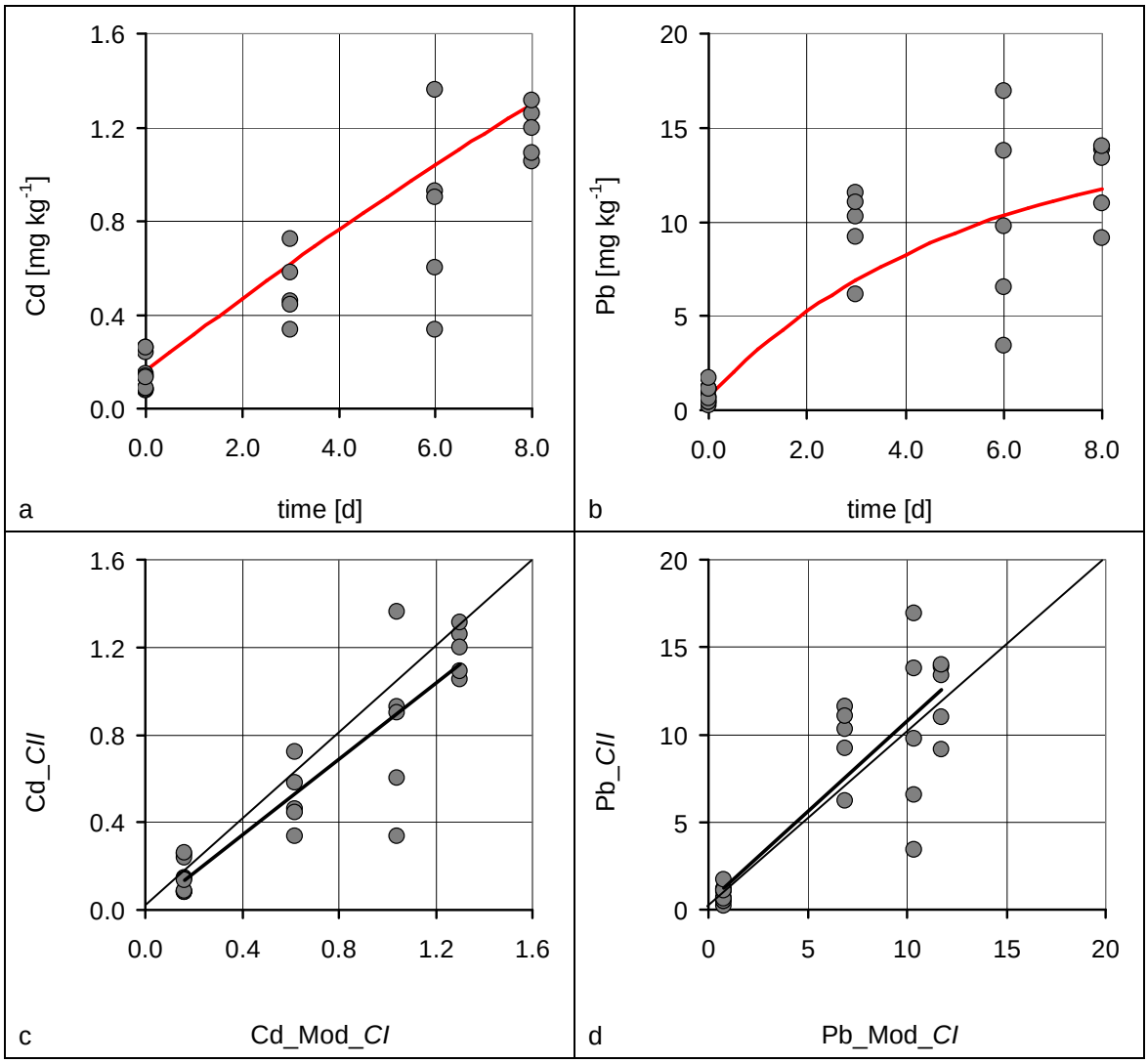

Figure 4.4: Time-dependent bioaccumulation of Cd and Pb in the decapod *Crangon crangon* from the North Sea Coast from toxicokinetic study *CII* (uptake phase 12 d), compared to model predictions (two-compartment model) from toxicokinetic study *CI* (uptake phase 8 d).

(a) Net-uptake of observed Cd from *CII* (filled circles), compared to model predictions from *CI* (red line).

(b) Net-uptake of observed Pb from *CII* (filled circles), compared to model predictions from *CI* (red line).

(c) Cd, linear regression of model predictions (x-axis) and observed data (y-axis) (parameters as given above, dotted line: slope=1).
C_A-*CII* = (0.9 ± 0.2)**model-CI* + 0.1, R^2 = 0.826 (slope ± 95% CI).

(d) Pb, linear regression of model predictions (x-axis) and observed data (y-axis) (parameters as given above, dotted line: slope=1).
C_A-*CII* = (1.0 ± 0.3)**model-CI* – 0.8, R^2 = 0.755 (slope ± 95% CI).

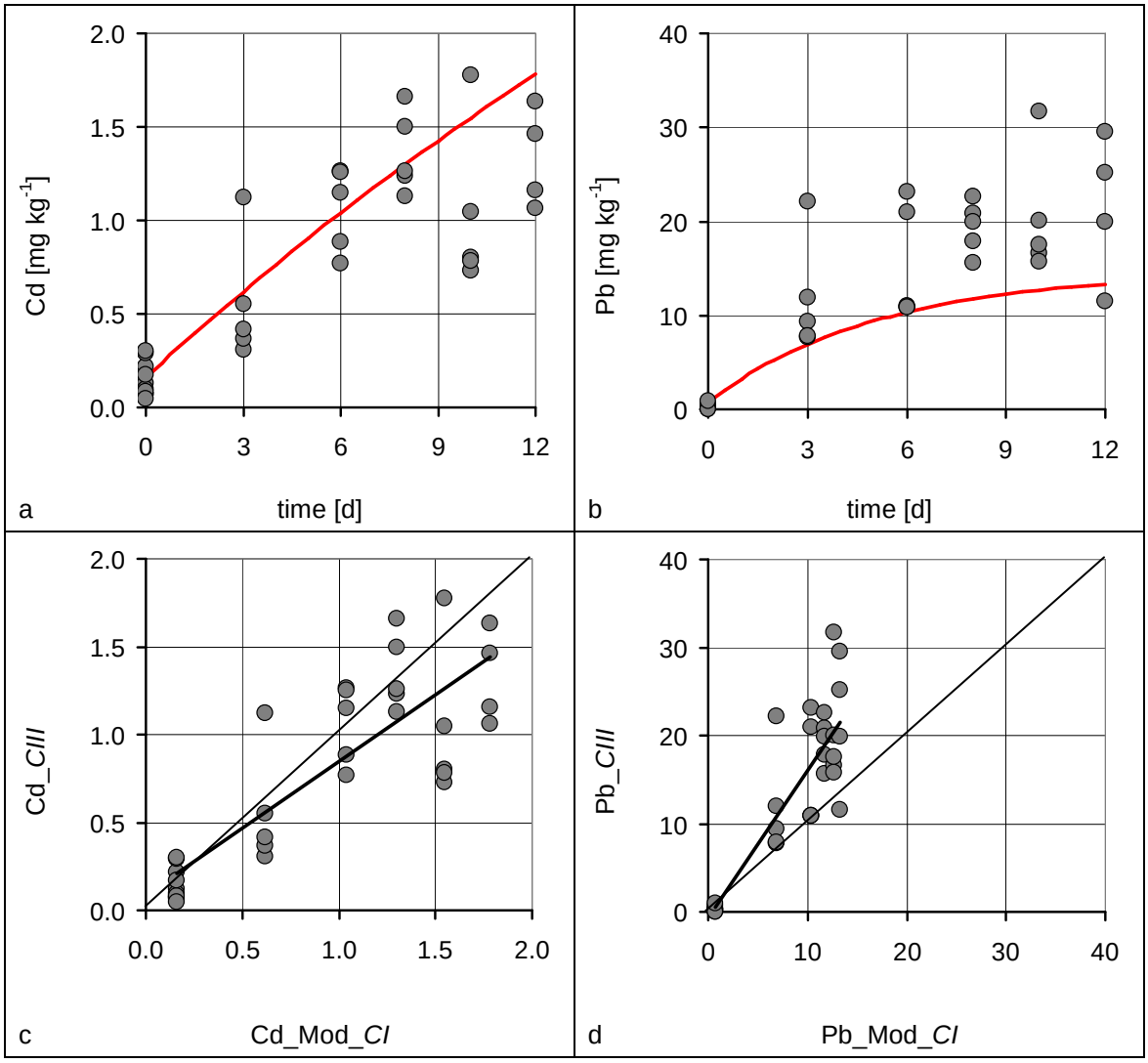

Figure 4.5: Time-dependent bioaccumulation of Cd and Pb in the decapod *Crangon crangon* from the North Sea Coast from toxicokinetic study *CIII* (uptake phase 12 d), compared to model predictions (two-compartment model) from toxicokinetic study *CI* (uptake phase 8 d).

(a) Net-uptake of observed Cd from *CIII* (filled circles), compared to model predictions from *CI* (red line).

(b) Net-uptake of observed Pb from *CIII* (filled circles), compared to model predictions from *CI* (red line).

(c) Cd, linear regression of model predictions (x-axis) and observed data (y-axis) (parameters as given above, dotted line: slope=1).
C_A-*CIII* = (0.8 ± 0.2)**model-CI* - 0.003, R^2 = 0.710 (slope ± 95% CI).

(d) Pb, linear regression of model predictions (x-axis) and observed data (y-axis) (parameters as given above, dotted line: slope=1).
C_A-*CIII* = (1.7 ± 0.3)**model-CI* + 0.5, R^2 = 0.763 (slope ± 95% CI).

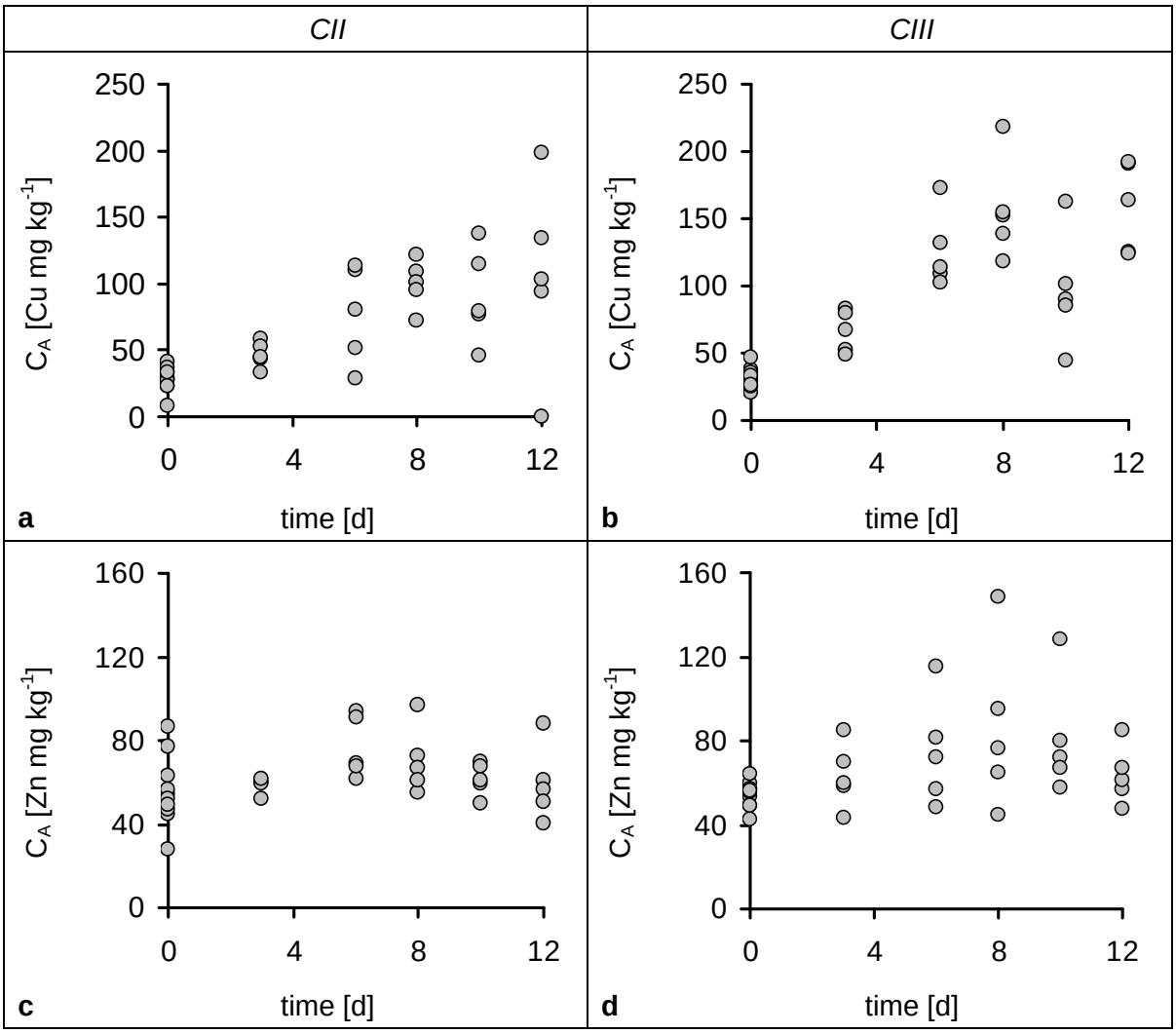

Figure 4.6: Time course of uptake and clearance [mg kg^{-1} DW] of Cu and Zn in brown shrimps (notation in Table 4.2) from toxicokinetic study *CII* (a, c) and *CIII* (b, d). No models could be obtained. Metal exposures: 51 Cu µg L^{-1}, 74 Zn µg L^{-1}. Day 12: end of uptake phases; see text for more details.

Again the organisms responded with a distinct uptake of Cd and Pb in both toxicokinetic studies (Figures 4.4. and 4.5.), but not so for Cu and Zn (Figure 4.6). Furthermore, measured data for Cd and Pb from time-dependent bioaccumulation experiments (toxicokinetic study *CI;* filled circles) are compared to model predictions for *CII* and *CIII* given by the red lines (see Material and Methods section and notes to Figures 4.4. and 4.5. for details). The assessment of this comparison involving linear regression analysis will be discussed in detail in the section Verification of toxicokinetic model parameters (see below).

4. DISCUSSION

Modelling of uptake and clearance kinetics

Statistical information compiled in Table 4.5 for *C. crangon* generally suggest good model estimates for Cd and Pb: the parameters for *CI* are statistically different from zero and the R^2 values indicate that a sufficiently high proportion of the variance is represented by the models. These R^2 values are in good agreement with other bioaccumulation models (regarding uptake and clearance) described in the literature for marine invertebrates such as amphipods, copepods or polychaetes (Zauke et al., 1995; Ritterhoff et al., 1996; Ritterhoff and Zauke, 1997b; Bernds et al., 1998; Clason and Zauke, 2000; Clason et al., 2003; Clason et al., 2004b). Conversely, for Cu and Zn (C_{W1} = 51 µg L^{-1}) no models could be obtained. One reason might be that Cu and Zn concentrations were already high in the control organisms (Table 4.4), indicating that the experimental metal exposure was eventually not high enough to cause a detectable uptake during the experiments. Furthermore, high background values of Cu and Zn in seawater of the control experiments also occurring in the clearance phase, most probably prevented any depuration.

Kinetic BCFs obtained in our experiments (1135 for Cd and 772 for Pb, Table 4.5) are in most cases distinctly higher for Cd and lower for Pb than the ranges observed in amphipods reported for other marine systems. Those include 150 - 630 for Cd and 1600 - 7000 for Pb in *Paramoera walkeri* (Clason et al., 2003), 400 - 500 for Cd and 970 - 1200 for Pb in *Gammarus zaddachi* (Clason and Zauke, 2000), 400 – 1200 for Cd in *G. zaddachi* and *Gammarus salinus* (Zauke et al., 1995; Ritterhoff et al., 1996) and 1800 for Pb in *Themisto abyssorum* (Ritterhoff and Zauke, 1997b). Only regarding *Chaetogammarus marinus* (Clason et al., 2004a) are the BCFs within the same range for Pb (150 – 630 for Pb). Estimated kinetic BCF published by Dethlefsen (1978) for *C. crangon* ranged between 2070 (exposure to 0.01µg Cd L^{-1}) and 153 (exposure to 0.1 µg Cd L^{-1}).

Estimated values for BCF_{field} were obtained from background metal concentrations in *C. crangon*, (C_{con}; Table 4.4), and reported data for metals in seawater (Haarich and Schmidt, 1993, 1993; Scholten et al., 1998; see above) yielding in 17000 for Cd regarding the German Bight and the North Sea Coast and 15000 for Pb regarding the German Bight, 8000 regarding the German Coast and 20000 regarding the North Sea. This variability highlights the problem of the comparative sensitivity of organisms used for biomonitoring, and the necessity for calibration, which will be discussed below in more detail.

The results discussed so far allow some inferences regarding the accumulation strategy of the organisms involved. This depends on the biological species and the element considered (Rainbow et al., 1990), the exposure regime applied (Borgmann and Norwood, 1995), cation homeostasis mechanisms (e.g. Viarengo and Nott, 1993) as well as life-history influences on metal accumulation and the development of an adequate experimental design on different spatial and temporal scales (Zauke et al., 1996; and literature cited therein). Regarding exposures for which linear predictions with a two-compartment model are possible, it is reasonable to assume a net accumulation strategy. Thus, a net accumulation strategy can be inferred for Pb and Cd in *C. crangon*.

Verification of toxicokinetic model parameters

To demonstrate the potential of toxicokinetic models as a predictive tool, model data have to be verified with independent experimental data (Ritterhoff and Zauke, 1997b; Clason and Zauke, 2000; Kahle and Zauke, 2002b; Clason et al., 2004a). This is an important step in the calibration of biomonitors. However, in this paper only for Cd and Pb is it possible to predict modelling results from the classical two-compartment model estimated in *CI* for toxicokinetic studies CII and CIII, since no modelling results could be obtained for Cu and Zn (Figure 3.3c, d). We find a fairly good agreement between predicted and observed data for independent time-dependent uptake studies *CII* and *CIII* (Figure 4.4a, band 4.5, demonstrated by the red lines in the panels on top and by the results of the linear regression analysis shown in the panels on the bottom of the corresponding Figures.

Consequently, the 95%-confidence intervals of the slopes of the linear regression between observed and predicted values include the value "1" in case of *CII* (Figure 4.4). For *CIII* a good agreement with the model predictions is obvious for Cd, but for Pb the predictions underestimate the observed data, and hence the 95%-confidence intervals of the slope does not include the value “1”. This must be taken into account when attempting to interpret metal concentrations in field samples of these decapods as an indicator of the bioavailable supply in the environment. It supports furthermore the idea that consideration of the same biological species might not be always reasonable within the concept of biomonitoring without appropriate calibration procedures.

Whether linear predictions with compartment models are applicable to higher exposures to brown shrimps cannot be elucidated, since no concentration dependent studies have been performed. It is interesting to note that linear predictions with compartment models were successfully verified in other studies for more elements, such as Pb (up to 100 µg L^{-1}), Cu (up to 120 µg L^{-1}) and Ni (up to 60 µg L^{-1}) in the amphipod *C. marinus* (Clason et al., 2004a); for Pb (up to 24 – 36 µg L^{-1}) and Cu (up to 240 µg L^{-1}) in the amphipod *T. abyssorum* from the Greenland Sea (Ritterhoff and Zauke, 1997b), for Pb (up to 60 µg L^{-1}) and for Cu (up to 240 µg L^{-1}) in *G. zaddachi* from German estuaries (Clason and Zauke, 2000) as well as for Pb (up to 70 µg L^{-1}) in the copepod *Calanoides acutus* from the Weddell Sea (Kahle and Zauke, 2002b).

However, if compartment models are used as a prognostic tool, in the case of saturation, the valid range of external metal exposures must eventually be limited to a smaller range as has been pointed out in other studies on bioaccumulation in marine invertebrates (Ritterhoff and Zauke, 1997b; Bernds et al., 1998; Clason and Zauke, 2000; Clason et al., 2004b).

Inferences about accumulation strategy

An important pre-condition for using organisms as biomonitors is a net accumulation strategy (Zauke et al., 1996). Accumulation strategies depend on the biological species and the element considered (Rainbow et al., 1990), the exposure regime applied (Borgmann and Norwood, 1995), cation homeostasis mechanisms (e.g. Viarengo and Nott, 1993) as well as life-history influences on metal accumulation and the development of an adequate experimental

design on different spatial and temporal scales (Zauke et al., 1996; and literature cited therein).

The results discussed so far allow some inferences regarding the accumulation strategy of the organisms involved. A net accumulation strategy can be inferred for Pb and Cd in *C. crangon* from Figure 4.3 and Table 4.5, since the organisms do not readily reach an eqillibrium within the uptake phase. Apart from the above mentioned argument that an experimental uptake of Cu and Zn was probably not detectable due to relatively high background values in the organisms, this might also be an indication of a regulation of these elements within the constraints of the experimental set-up.

The shape of uptake curves found in experiments by Dethlefsen (1978) indicate two different mechanisms of uptake in *C. crangon* for various Cd concentrations. The first part of the uptake curves in their study, seemed to be superimposed by more than one compartment. Following a rapid uptake of Cd reaching an early equilibrium after 8 to 24 hours, a loss of cadmium from the brown shrimps occurred. This might be due to the fact that an incipient oversaturation of available binding sites is possible reduced in the further course of the experiment. Fluctuations in the cadmium concentrations might reflect the parallel changes in the calcium metabolism, because after the moult Ca is transported from storage sites to the integuments of the exoskeleton.

White and Rainbow (1982) and Nugegoda and Rainbow (1987) reported that the decapod *Palaemon elegans* regulates the body concentration in zinc when exposed to a wide range of dissolved Zn concentrations. With increasing Zn exposure the rate of Zn uptake by the prawn increases until a point of regulation breakdown, hence it exceeds the maximum rate of Zn excretion under ambient physicochemical conditions (Nugegoda and Rainbow, 1987, 1988, 1989). The authors also demonstrate that a rise in temperature increases the rate of Zn uptake by *P. elegans* and correspondingly causes a decrease in the threshold seawater zinc concentration at which Zn regulation breaks down and net accumulation of body zinc begins (Nugegoda and Rainbow, 1987, 1988). But also the reduction in salinity causes an increase in Zn uptake (Nugegoda and Rainbow, 1989).

Unlike Zn, Cd is not regulated in many marine crustaceans, e.g. *Carcinus maenas* (Wright, 1977; Jennings and Rainbow, 1979), *Pandalus montagui* (Ray et al., 1980) and *Crangon crangon* (Dethlefsen, 1978). Larvae of *Paelmon serratus* did not regulate Cd over a wide range of overloads studied by (Devineau and Amiard-Triquet, 1985).

Net accumulation was reported for example in amphipods for Pb, Cr, Co, Cu, Ni and (to lesser extent) for Cd in *C. marinus* (Clason et al., 2004a), for Pb, Cu, Zn and (to lesser extent) for Cd in *P. walkeri* (Clason et al., 2003), for Cd, Pb, Cu and Zn in *G. zaddachi* (Clason and Zauke, 2000), for Cu in *Echinogammarus pirloti* (Rainbow and White, 1989), for Pb in *Gammarus pseudolimnaeus* (MacLean et al., 1996) and for Cu and Pb in *T. abyssorum* (Ritterhoff and Zauke, 1997a, 1997b). (Borgmann and Norwood, 1995) found a net uptake of Cu and Zn in *Hyalella azteca* in short term experiments, but in long term experiments the animals were able to regulate Cu, whilst a net accumulation strategy was reported for Cd and Pb (Borgmann et al., 1993; Borgmann and Norwood, 1999). A tendency for Zn regulation

was also described for *H. azteca* (Borgmann et al., 1993) and some decapod crustaceans (Rainbow, 1993) as well as for *C. marinus* (Clason et al., 2004a).

Implications for biomonitoring — the problem of calibration and the sensitivity of the brown shrimp as a biomonitor

Kinetic BCFs provide a first attempt to assess the potential of organisms for bioaccumulation. A more informative approach and an important step in the calibration of biomonitors is the assessment of their sensitivity to changing concentrations (Clason and Zauke, 2000; Clason et al., 2004a; Clason et al., 2004b), since it incorporates information on the reproducibility of analytical results, background values of metals in control organisms and a simulation based on the results of estimated model parameters.

When using the models applied here as a predictive tool for waterborne metal uptake, the emphasis is on modeling a short-term response of organisms to a short-term increase of bioavailable soluble metal species. Experimentally derived kinetic data such as BCFs explicitly reflect the fact that data on uptake of waterborne metals are valid only for the given set of experimental conditions, e.g. the range of external metal exposures applied and the time chosen for uptake phases; therefore, hypotheses based on them should be regarded only as tentative. In the field other metal species might be predominant, and the metals taken up might be subject to subcellular sequestration (see discussions e.g. in Viarengo and Nott, 1993; Dallinger, 1995; Ritterhoff and Zauke, 1998). Furthermore, other uptake routes such as food, particulate matter or sediments have to be taken into account (e.g. Wang and Fisher, 1999; Wang, 2002). Therefore, field BCFs are normally much higher than experimental BCFs.

Information on the sensitivity of the decapod *C. crangon* from the German Wadden Sea Coast as biomonitor estimated by the minimal increment in exposure concentrations ($\min\Delta C_W$) necessary to exceed the value of C_{con} + $\min\Delta C_A$ (see Equations 4a-c) in a simulation with a two-compartment model (Equation 2a) is summarized in Table 4.6. The error term is given by SD_{con}, largely representing the experimental variability.

The estimated $\min\Delta C_W$-values (*viz.* the sensitivity of *C. crangon* as a biomonitor, see Material and method section for details) are summarised in Table 4.6 for exposure C_{W1}. Sensitivity data for decapods, especially *C. crangon*, comparable to these results, could not be found in the literature. Reported ranges of $\min\Delta C_W$-values for other crustaceans from previously reported investigations are 0.25 - 0.5 µg Cd L^{-1}; 2 - 3 µg Pb L^{-1}; 30 - 40 µg Cu L^{-1} and 70 - 150 µg Zn L^{-1} for *G. zaddachi* from German coastal waters (Clason and Zauke, 2000), 0.6 µg Cd L^{-1}, 16 µg Cu L^{-1} and 0.8 µg Pb L^{-1} for *C. marinus* from south-west England (Clason et al., 2004a) and 0.8 - 3.0 µg Cd L^{-1}, 0.12 - 0.25 µg Pb L^{-1}, 0.9 - 3.0 µg Cu L^{-1} and 9 - 26 µg Zn L^{-1} for *P. walkeri* from Antarctic waters (Clason et al., 2003).

Table 4.6: Sensitivity of *Crangon crangon* estimated from modelling results of toxicokinetic study *CI* as biomonitor.

C_{W1} [µg L^{-1}]	metal	BCF	C_{Con} [mg kg^{-1}]	$min\Delta C_A$ [mg kg^{-1}]	$min\Delta C_W$ [µg L^{-1}]
5.0	Cd	1135	0.16	0.24	0.4
18	Pb	772	0.77	1.67	1.5

Notes:
C_{con}: metal concentration in decapods of the control from toxicokinetic study; $min\Delta C_A$ = minimum increment in body concentration which can be detected; $min\Delta C_W$ = minimal increment in exposure concentrations necessary to exceed the value of C_{con} + $min\Delta C_A$ in the decapods (animals) during a simulation of a 8-days uptake phase; see Material and method section for more details; otherwise as in Table 4.4 and 4.5.

The assessment of the sensitivity of organisms is a crucial part of the concept of biomonitoring: how large must an increase of external soluble metal exposure be to produce a detectable increase of metal concentrations in the organisms? The minimal increment in exposure concentrations ($min\Delta C_W$) that is necessary to exceed the sum of the concentration in control organisms in *C. crangon* and the minimum increment of the body concentration is 0.4 mg Cd kg^{-1} and 1.5 mg Pb kg^{-1} from this work. These $min\Delta C_A$ values in the brown shrimp are for Cd in good agreement with values detected *G. zaddachi* from German coastal waters (Clason and Zauke, 2000) and for *C. marinus* from south-west England (Clason et al., 2004a). Values from Antarctic decapods were increased up to seven-times. Reported sensitivity values for lead are higher in gammarids from German coastal waters and up to six-times lower in decapods from Antarctic waters. Consequently, *C. crangon* seems to be more sensitive for Cd than Antarctic decapods, but less sensitive for Pb. Furthermore, there is no consistent trend in the sensitivity of the brown shrimp *C. crangon* compared to other species in the literature, but available information can be used to quantify a measure of agreement or disagreement between bioaccumulation in different decapods.

5. CONCLUSIONS

Our results on bioaccumulation of Pb and Cd in the brown shrimp *C. crangon* clearly demonstrate the potential of toxicokinetic compartment models as a predictive tool and a suitable instrument for the calibration of organisms for biomonitoring. Two-compartment models were successfully fitted for these elements in a first toxicokinetic study leading to model constants statistically different from zero. These could be used to predict accumulation of Cd and Pb in two independent uptake studies. This view is further supported by a net accumulation strategy found for these elements, within the constraints of our experimental design. The elements Cu and Zn need further evaluation, since linear predictions from the two-compartment models obtained for C_{W1} were not applicable. Thus, we obtain the following BCFs for the theoretical equilibrium: 1135 for Cd and 772 for Pb.

The estimated sensitivities of *C. crangon* to an increase of experimental dissolved metal concentrations were 0.4 µg Cd L^{-1} and 1.5 µg Pb L^{-1}. There is no consistent trend in the sensitivity of the brown shrimp *C. crangon* compared to other species in the literature, but available information can be used to quantify a measure of agreement or disagreement between bioaccumulation in different decapods. This can be regarded as an important step in the calibration of biomonitors which is necessary to assess the potential for bioaccumulation on a large geographical scale. The estimates of sensitivity for biomonitoring derived from toxicokinetic experiments are, however, suggested to be substantially lower under field conditions. To test this hypothesis further studies are required, e.g. considering field BCFs as model constants in BCF-exposure relationships.

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SYNOPSIS AND PERSPECTIVES FOR THE APPLICATION OF SPATIAL AND TEMPORAL (TOXICOKINTIC) MODELS IN MARINE BIOMONITORING

The goal of biomonitoring-programmes, like the Trilateral Monitoring and Assessment Program (TMAP, Common Wadden Sea Secretariat), the German “Bund-/Länder-Messprogramm (BLMP)” or activities of the OSPAR Commission (Quality Status Report) is to get information about the bioavailable fraction of heavy metals and other water pollutants in the marine environment by chemical analysis of substances in organisms and hence to assess the risks for humans and nature. The interpretation of data obtained in this way is problematic due to following reasons.

Although it is intended to make statements of the spatial distribution of heavy metals in organisms from different marine regions, eventually showing different degrees of contamination, procedures of spatial statistics (geostatistic) are not taken explicitly into consideration. Instead it is most often assumed that data obtained are spatially independent and therefore can be evaluated by means of classical statistics. However, this assumption has to be critically evaluated and can possibly lead to a bias in the estimates of means and variances of the variables investigated, when spatial autocorrelations occur. This can be detected by the application of geostatistical procedures (e.g. variogram analysis).

A further weak point in the established monitoring programmes is, that no explicit information of the accumulation strategy of the collectives investigated (not only the species investigated) is considered in the assessment of measured metal concentrations in organisms. On the other hand, investigations on toxicokinetics, *viz*. on uptake and depuration of metals in organisms, and the subsequent estimation of kinetic compartment models have been proven to be a useful tool in several studies. The modelling parameters obtained in this way can be used to assess and quantify the agreement or disagreement of the accumulation strategies in the collectives of interest. Only this information allows the comparability of measured metal concentrations in samples of the same species from different areas or of samples from different species. Furthermore, it is possible to quantify the sensitivity of biomonitors by means of model simulations. These approaches can be regarded as a calibration of organisms for biomonitoring, a necessary precondition for the interpretation of monitoring data.

Due to the fact that even closely related species can show different accumulation strategies for certain metals, a too narrow taxonomic scale of monitoring programmes is problematic (for example focusing on the blue mussel *Mytilus edulis* as representative of marine invertebrates).

This thesis aims at providing concepts and information necessary for the assessment of data from monitoring programmes, e.g. proposed by the European Water Framework Directive and which could be used as well in programmes of the OSPAR Commission and TMAP. Therefore, the applicability of further species for biomonitoring was investigated, e.g. of the cockle *Cerastoderma edule* as an important representative of inbenthic communities in the

Wadden Sea as well as of the brown shrimp *Crangon crangon* as an important representative of epibentic and demersal communities in the Wadden Sea and the southern North Sea with substantial regional economic impact. For both species the problem of spatial dependence was addressed on two different scales. In either case the goal of the studies was to quantify the range of spatial dependence and to obtain information from which distance on data of samples will be stochastically independent. In addition, relationships between metal concentrations in organisms and other variables (for example weight of the investigated animals) were analysed. Additionally, bioaccumulation of the dissolved heavy metals Cd, Cu, Pb and Zn was investigated for brown shrimps in laboratory experiments and analysed by means of two-compartment models. The model parameters obtained were verified with independent experimental data and interpreted in terms of the accumulation strategies. In a final step the sensitivity of *C. crangon* for biomonitoring purposes was evaluated by means of model simulations.

At first, recently established methods for graphite furnace atomic adsorption had to be adjusted to the biological matrix of the brown shrimp and to the different proportions of the elements (Cd, Cu, Ni, Pb and Zn) in the samples (Chapter 1). Samples of *C. crangon* collected for the spatial analysis in the German Bight (Box A) were used for this purpose. The modification of the instrumental programmes was mainly relying on the optimization of the measurement range of the calibration curve to enable multielement analyses. Temperature programmes and instrumental parameters were adjusted too. Largely linear calibration curves and coinciding peak appearance times for different samples (calibration standards, samples of brown shrimps and reference samples) were detected, indicating that matrix effects could be largely excluded. Furthermore, a quality assurance was achieved by a good agreement between measured and certified values of standard reference materials.

In a further study, the spatial distribution of heavy metal concentrations (Cd, Cu, Ni, Pb and Zn) and biomass indices were investigated in the edible cockle *C. edule* from the German Wadden Sea Coast on an ecological scale of 300 x 300 m (Chapter 2). Geostatistical procedures (semivariogram analysis) revealed a distinct increase of the variability (or dissimilarity) for most variables with increasing distance of the sampling points. For the metals Cd, Ni and Pb the variograms did not increase. Only if samples are taken at distances above the estimated values for the practical range of the semivariogram, can stochastic independence of the samples be assumed. In this work the range was 30 m for Cu, 122 m for Zn and 132 m for soft-body weight. Isolines of the spatial distribution of biomass variables showed a distinct coincidence between increasing values for the soft-body weight with increasing relative height above sea level from the coast in direction to the open sea. While the spatial distribution of the standardised soft-body weight indicated a cumulation of larger-sized cockles in direction to the open water, copper concentration in cockles decreased with increasing distance from the shoreline.

A comparable spatial analysis was done for trace metals (Cd, Cu, Ni, Pb and Zn) in *C. crangon* from the German Bight on a larger ecological scale of 18 x 18 km (Box A) (Chapter 3). On the basis of the variograms, a stochastic independence of the estimated values for distances above 6.6 km for Cd, 3.0 km for Ni and 5.2 km for Pb could be assumed, while for Cu

and Zn the spatial dependence was only low. Isolines of the spatial distribution for the mean wet weights of the analysed animals showed a cumulation of heavier brown shrimps in samples from the south-western corner of the investigation area and a clear coincidence of high concentrations of Cd, Ni and Pb with simultaneous low mean wet weights in the animals. Measured metal concentrations for Cu, Pb and Zn were largely within the range of values reported for other decapod crustaceans from other regions, only Cd concentrations were distinctly lower.

Furthermore, three independent laboratory experiments on uptake and depuration of Cd, Cu, Pb and Zn in *C. crangon* were carried out (Chapter 4). Therefore, juveniles of brown shrimps hatched in summer (July) were caught from the North Sea Coast in Schillig and deployed in the three toxicokinetic experiments (*CI*, *CII*, *CIII*). In *CI* significant parameters of a two-compartment model could be estimated for Cd and Pb, but not for Cu and Zn, due to missing trends of the data in association with a high variability. Results from *CII* and *CIII* served to verify the two-compartment models of Cd and Pb from *CI*. Comparison of observed and predicted values from the models estimated would in case of a complete agreement result in a straight line with the slope 1. By means of the parameters of a linear regression analysis the degree of disagreement of specific toxicokinetic models could be quantified, an important precondition for the assessment of measured metal concentrations in field samples of brown shrimps. A further step of the calibration consists in a comparative analysis on the sensitivity of *C. crangon*, indicating their potential for bioaccumulation (by the estimation of the minimal exposure values that still would give a measurable increase of the metal concentration in the animal).

The results obtained and additional information from the literature clearly demonstrate the suitability of the cockle, *C. edule*, and the brown shrimp, *C. crangon*, as biomonitors for selected heavy metals. For both species first information was obtained, from which distance of sampling points on a stochastic independence of the data can be assumed and when explicit spatial methods have to be employed. Toxicokinetic analysis of *C. crangon* indicated the suitability as biomonitor for Cd and Pb, but not for the essential elements Cu and Zn, eventually due to the high background values in the field. As is shown in this study taking cockles and brown shrimps as an example, concepts on spatial analysis and toxicokinetics of heavy metals should in future be an integral component of monitoring programmes.

Zusammenfassung und Ausblick zur Anwendung räumlicher und zeitlicher (toxicokinetischer) Modelle im marinen Biomonitoring

Das Ziel von Biomonitoring-Programmen wie dem Trilateral Monitoring and Assessment Program (TMAP, Common Wadden Sea Secretariat), dem Bund-Länder-Messprogramm (BLMP) oder den Aktivitäten der OSPAR Commission (Quality Status Report) besteht darin, über eine chemische Analytik von Schwermetallen und Umweltchemikalien Hinweise in Organismen auf deren bioverfügbaren Anteil in der Meeresumwelt zu erlangen und daraus mögliche Risiken für Mensch und Umwelt abzuschätzen. Die Interpretation der so gewonnenen Daten ist jedoch aus folgenden Gründen problematisch.

Obwohl Aussagen zur räumlichen Verteilung beispielsweise von Schwermetallen in Organismen aus unterschiedlich belasteten Meeresgebieten intendiert sind, werden Verfahren der räumlichen Statistik nicht explizit in Betracht gezogen. Stattdessen wird davon ausgegangen, dass die gewonnenen Daten räumlich unabhängig sind und mit Hilfe der klassischen Statistik ausgewertet werden können. Diese Voraussetzung muss jedoch nicht erfüllt sein und kann bei Vorliegen einer räumlichen Autokorrelation, wie sie durch Anwendung der Geostatistik (Variogrammanalyse) erkannt werden könnte, zu einer Verzerrung der Schätzwerte für Mittelwerte und Varianzen der Untersuchungsvariablen führen.

Ein weiterer Schwachpunkt der etablierten Monitoring-Programme besteht darin, dass für die Bewertung der gemessenen Metallkonzentrationen in Organismen keine expliziten Informationen zur Akkumulationsstrategie der untersuchten Kollektive (d.h. nicht nur der betrachteten Arten) herangezogen werden. Hierzu haben sich Untersuchungen zur Toxicokinetik, d.h. zur Aufnahme und Ausscheidung von Metallen in Organismen, und ihre Modellierung mit kinetischen Kompartment-Modellen in vielen Studien als nützliches Werkzeug erwiesen. Mit Hilfe der so gewonnenen Modellparameter können Übereinstimmungen oder Abweichungen der Akkumulationsstrategien in den einzelnen Untersuchungskollektiven erkannt und quantifiziert werden und ermöglichen so überhaupt erst die Vergleichbarkeit der gemessenen Metallkonzentrationen in Proben der gleichen Art aus unterschiedlichen Gebieten oder aus Proben unterschiedlicher Arten. Darüber hinaus ist es durch Modellsimulationen möglich, die Sensitivität von Biomonitoren zu quantifizieren. Die genannten Ansätze lassen sich als Kalibrierung von Organismen für ein Biomonitoring zusammenfassen – eine notwendige Bedingung für die Interpretation von Monitoringdaten.

Aufgrund der Tatsache, dass schon nahe verwandte Arten unterschiedliche Akkumulationsstrategien für Metalle zeigen können, ist auch eine zu enge taxonomische Ausrichtung der etablierten Monitoring-Programme (z. B. die Miesmuschel *Mytilus edulis* als Vertreter der marinen Evertebraten) problematisch.

Das Ziel der vorliegenden Arbeit bestand darin, in exemplarischen Studien einen Beitrag zur Bewertbarkeit von Daten aus Monitoringprogrammen zu leisten, wie sie beispielsweise in der EU-Wasserrahmenrichtlinie für Küstengewässer gefordert wird und in Programmen der OSPAR Commission und des TMAP ihre Verwendung finden könnten. Hierzu wurde die Eig-

nung weiterer Arten untersucht. Die Eignung der Herzmuschel *Cerastoderma edule* als ein wichtiger Vertreter der inbentischen Lebensgemeinschaften des Wattenmeeres, sowie die der Nordseegarnele *Crangon crangon* als ein wichtiger Vertreter der epibenthischen und demersalen Lebensgemeinschaften des Wattenmeeres und der südlichen Nordsee mit erheblicher regionaler wirtschaftlicher Bedeutung wurden untersucht. An beiden Arten wurde die Problematik räumlicher Abhängigkeiten exemplarisch auf zwei unterschiedlichen Maßstabsebenen herausgearbeitet. In beiden Fällen bestand das Ziel darin, die Reichweite räumlicher Abhängigkeiten zu quantifizieren und Aussagen darüber zu gewinnen, ab welcher Entfernung mit stochastisch unabhängigen Daten gerechnet werden kann. Darüber hinaus sollten Zusammenhänge zwischen Metallkonzentrationen in den Organismen und anderen Variablen (z. B. Gewicht der Versuchstiere) aufgezeigt werden. Am Beispiel der Nordseegarnele wurde ferner die Bioakkumulation der gelösten Schwermetalle Cd, Cu, Pb und Zn aus dem Meerwasser in Laborexperimenten erfasst und mit Hilfe von Zwei-Kompartment-Modellen analysiert. Die so gewonnenen Modellparameter wurden mit unabhängigen experimentellen Daten verifiziert und im Hinblick auf die Akkumulationsstrategie interpretiert. Zuletzt wurde mit Hilfe von Modellsimulationen die Sensitivität von *C. crangon* zum Biomonitoring abgeschätzt.

Zunächst mussten bereits etablierte Methoden der Graphitrohr Atomabsorptionsspektroskopie an die biologische Matrix und die Elementverhältnisse (Cd, Cu, Ni, Pb und Zn) der Nordseegarnelen, angepasst werden (Kapitel 1). Hierzu wurden die für die räumliche Analyse in der Deutschen Bucht (Box A) genommenen Proben herangezogen. Die Modifikation der Methodenprogramme bezog sich im wesentlichen auf die Optimierung des Messbereichs der Bezugsgrößenreihe, um eine sequentielle Multielementanalyse möglich zu machen. Ferner wurden die Temperaturprogramme und instrumentellen Parameter angepasst. Es wurden weitgehend lineare Bezugsgrößenreihen und übereinstimmende „Peak-Appearance-Zeiten“ für unterschiedliche Probenarten (Kalibrierstandards, *Crangon*-Proben, Referenzproben) gefunden, so dass Matrixeffekte weitgehend ausgeschlossen werden konnten. Die Qualität der Analytik wurde darüber hinaus durch die gute Übereinstimmung zwischen gemessenen und zertifizierten Werten von Standardreferenzproben nachgewiesen.

In einer weiteren Studie wurde die räumliche Verteilung von Schwermetallkonzentrationen (Cd, Cu, Ni, Pb und Zn) und Biomasseindizes in der essbaren Herzmuschel *C. edule* aus dem deutschen Wattenmeer auf einer mittleren ökologischen Skala von 300 x 300 m untersucht (Kapitel 2). Geostatistische Verfahren (Semivariogrammanalyse) ergaben einen deutlichen Anstieg der Variabilität (d.h. der Unähnlichkeit) der meisten Variablen mit der zunehmenden Entfernung der Probenorte. Lediglich für die Metalle Cd, Ni und Pb stieg das Variogramm nicht an. Nur wenn die Proben in einem größeren Abstand als die errechneten Schätzwerte für den Einflussbereich (range) des Variogramms genommen werden, kann eine stochastische Unabhängigkeit der Proben angenommen werden: in dieser Arbeit 30 m für Cu, 122 m für Zn und 132 m für die Weichkörperbiomasse. Isolinien der räumlichen Verteilung der Biomassevariablen zeigten einen eindeutigen Zusammenhang zwischen ansteigenden Werten für die Weichkörperbiomasse mit steigender relativer Höhe über dem Meeresspiegel von der Küste in Richtung auf die offene See. Während die räumliche Verteilung der standardisierten Weichkörperbiomasse auf eine Anreicherung größerer Herzmuscheln zum

offenen Wasser hin hinwies, nahm die Kupferkonzentration in den Herzmuscheln gleichzeitig mit der Entfernung von der Küstenlinie ab.

Eine vergleichbare räumliche Analyse wurde für Metalle (Cd, Cu, Ni, Pb und Zn) in *C. crangon* aus der Deutschen Bucht auf einer größeren ökologischen Skala von 18 x 18 km (Box A) durchgeführt (Kapitel 3). Anhand der Variogramme konnte eine stochastische Unabhängigkeit der Daten für Entfernungen größer 6.6 km für Cd, 3.0 km für Ni und 5.2 km für Pb angenommen werden. Für Cu and Zn war die räumliche Abhängigkeit nur gering ausgeprägt. Isolinien der räumlichen Verteilung der mittleren Nassgewichte der Versuchstiere zeigten eine Anhäufung schwererer Nordseegarnelen in den Proben in der süd-westlichen Ecke des Untersuchungsgebietes und eine deutliche Übereinstimmung hoher Gehalte an Cd, Ni und Pb mit gleichzeitigem niedrigen mittlerem Nassgewicht der Tiere auf. Die gemessenen Metallkonzentrationen für Cu, Pb und Zn waren größtenteils innerhalb des Bereichs für Werte anderer gemessener dekapoder Krebstiere aus anderen Regionen, lediglich Cd-Konzentrationen waren deutlich geringer.

Des weiteren wurden insgesamt drei unabhängige Laborexperimente zur Aufnahme und Ausscheidung von Cd, Cu, Pb und Zn in *C. crangon* durchführt (Kapitel 4). Dafür wurden jeweils juvenile Nordseegarnelen des Sommerlarvenfalls (Juli) an der Nordseeküste bei Schillig gefangen und in die drei Versuche eingesetzt (*CI*, *CII* und *CIII*). In *CI* konnten signifikante Parameter eines Zwei-Kompartment-Modells für Cd und Pb ermittelt werden, nicht jedoch für Cu und Zn aufgrund eines fehlenden Trends in den Daten in Verbindung mit hoher Variabilität. Die Ergebnisse aus der Studie *CII* und *CIII* dienten dazu, die Modelle von Cd und Pb aus *CI* zu verifizieren. Bei einem Vergleich von beobachteten und vom Modell vorhergesagten Werten, würde im Falle einer vollständigen Übereinstimmung eine Gerade mit der Steigung 1 resultieren. Mit Hilfe der Parameter einer linearen Regressionsanalyse lies sich das Ausmaß der Übertragbarkeit der spezifizierten toxicokinetischen Modelle quantifizieren und für eine Bewertung von gemessenen Metallkonzentrationen in Freilandkollektiven heranziehen. Ein weiterer Schritt zur Kalibrierung ergab sich aus einer vergleichenden Analyse der Sensitivität von *C. crangon* hinsichtlich ihres Potentials zur Bioakkumulation (über Schätzung der minimalen Expositionswerte, die noch zu einem messbaren Anstieg der Metallkonzentrationen in den Tieren führen würden).

Die durchgeführten Untersuchungen in Verbindung mit der Auswertung von Literaturdaten sprechen eindeutig für die Eignung der Herzmuschel, *C. edule*, und der Nordseegarnele, *C. crangon*, für ein Biomonitoring ausgewählter Schwermetalle. Für beide Arten ließen sich exemplarisch Angaben darüber ermitteln, ab welcher Entfernung von Probenorten stochastische Unabhängigkeit der Daten angenommen werden kann und wann explizite räumliche Verfahren eingesetzt werden müssen. Toxicokinetische Analysen ergaben für *C. crangon* eine Eignung zum Biomonitoring für Cd und Pb, nicht jedoch für die essentiellen Elemente Cu und Zn, nicht zuletzt wegen der relativ hohen Hintergrundwerte in Freilandproben. Die hier am Beispiel von Herzmuscheln und Nordseegarnelen ausgeführten Untersuchungskonzepte zur räumlichen Analyse und zur Toxicokinetik von Schwermetallen sollte zukünftig ein wesentlicher Bestandteil von Monitoring-Programmen sein.

Curriculum Vitae

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School education

08/1981-07/1985	Elementary school Weingarten
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07/1991	Graduation Level 1 certificate (Mittlere Reife)
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Academic education

10/1995-09/1998	**Study on** Diploma Biolo**gy, University Fridericiana to Karlsruhe (TU), Degree Intermediate Examinations (Vordiplom)**
10/1998-12/2002	Study on **Diploma Biology**, Carl von Ossietzky University Oldenburg, Degree Graduate Biologist, main studies in ecology, zoology and biochemistry
Diploma thesis	„Strategies for the ecological assessment of running waters with respect to the European Water Framework Directive (WFD)“

Professional positions

07/2003-12/2006	**Scientific employee**, working group Aquatic Ecology, ICBM, Carl von Ossietzky University Oldenburg, Germany; updating position for the promotion of young scientists; **PhD-position**; responsibility for teaching; preparation of manuals; supervision and guidance in practical courses
01/-30/04/2003	**Scientific employee**, working group Aquatic Ecology, ICBM, Carl von Ossietzky University Oldenburg, Germany; Updating a research document about **analysis of toxicity in Wadden Sea sediments** on behalf of the Lower Saxony Wadden Sea Foundation, Germany
02/2003	**Scientific employee**, working group Aquatic Ecology, ICBM, Carl von Ossietzky University Oldenburg, Germany; employee for the intensive course “**faunistical assessment of running waters**”
05/2002-01/2003	**Scientific student assistant**, working group Aquatic Ecology, ICBM, Carl von Ossietzky University Oldenburg, Germany; **Consultant** for a project on the **oxygen demand in the river Ems** due to the construction of a river barrier in Gandersum, promoted by NLWK-Aurich Projektteam Emssperrwerk

Dissertation

07/2003 - 07/2007	Evaluation of spatial and temporal models to assess the bioaccumulation of trace metals in marine invertebrates

Practical experiences

05/2001-03/2002	Altogether three limited contracts as scientific student assistant, working group Aquatic Ecology, ICBM, Carl von Ossietzky University Oldenburg: **tutor** for advanced course "**aquatic ecology**"; tutor for intensive course "**faunistical assessment of running waters**"; laboratory work for "measurement of **heavy metals in biota** and sediments from the Weser Estuary"
10/2000-02/2001	**Revision and layout** of contributions for conference proceeding for VAAM-conference 2001, Carl von Ossietzky University Oldenburg, Germany
12/1998-02/2000	**Sorting of ground water samples**, working group Zoosystematic and Morphology, Carl von Ossietzky University Oldenburg
05/1997-09/1998	**Data research and presentation**, Department Biotechnology, Fraunhofer Institut für Systemtechnik und Innovationsforschung (Fhg-ISI), Karlsruhe

Further education

11/2005-10/2006	Participation at **Mentoring-Projekt** women@tec pro.doc, center for equalization of women, Carl von Ossietzky University Oldenburg
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06/2006	**Workshop** „Natura 2000 – developments in the Elbe Estuary", BSU Hamburg, Amt für Naturschutz
02/2006	**Symposium** „Alarming signals from the polar regions", Hamburg
10/2002	**Workshop** „EC-Water Framework Directive", Wasserbauingenieure, University Essen
09/2002	**Workshop** „Nature conversation and the EC-Water Framework Directive", Alfred-Töpfer-Naturschutzakademie, Schneverdingen

Conference

09/2004	Deutsche Limnologische Gesellschaft, Jahrestagung, Potsdam

Research cruises

01/2004	Cruise with fishery research vessel „Walther Herwig III“ into the German Bight; BFA, Institut für Fischerei, Hamburg, Fahrtleitung Dr. Siegfried Ehrich
03/2000	Cruise with „Uthörn“ into the German Bight for investigations on plankton in the North Sea; Alfred-Wegener-Institut für Polar- und Meeresforschung, Fahrtleitung PD Dr. Sigrid Schiel

Field trips

07-08/1999	Marine biology, Millport (Isle of Cumbrae), Scotland; Great Britain
06/1999	Ecology, Sardinian, Italy

International experience

09/1994-04/1995	AuPair stay in Los Angeles, USA

Articles in reviewed journals, reports and posters

Jung, K., Stelzenmüller, V. & Zauke, G.-P. 2006: Spatial distribution of heavy metal concentrations and biomass indices in *Cerastoderma edule* Linnaeus (1758) from the German Wadden Sea: An integrated biomonitoring approach. Journal of Experimental Marine Biology and Ecology 338: 81–95.

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10/1995-09/1998	Diplom Biologie**, Universität Fridericiana zu Karlsruhe (TU),** Abschluss Vordiplom
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Diplomarbeit	„Strategien zur ökologischen Bewertung von Fließgewässern im Hinblick auf die Europäische Wasserrahmenrichtlinie (EU-WRRL)“

Berufliche Erfahrungen

07/2003 - 12/2006	**wissenschaftliche Angestellte**, AG Aquatische Ökologie, ICBM, Carl von Ossietzky Universität Oldenburg; Stelle zur **Förderung wissenschaftlichen Nachwuchs**; **Promotionsstelle**; Lehrverpflichtung; Erstellung von Praktikumsmaterialien; Betreuung und Leitung von Praktika
04/2003	**wissenschaftliche Mitarbeiterin**, AG Aquatische Ökologie, ICBM, Carl von Ossietzky Universität Oldenburg; **Aktualisierung eines Forschungsberichts** zur Analyse der **Toxizität von Wattenmeersedimenten** im Auftrag der Niedersächsischen Wattenmeerstiftung
02/2003	**wissenschaftliche Angestellte**, AG Aquatische Ökologie, ICBM, Carl von Ossietzky Universität Oldenburg (0,5 BAT IIa); Mitarbeiterin für das **Vertiefungspraktikum Faunistische Bewertung von Fließgewässern**
05/2002 - 01/2003	**wissenschaftliche Hilfskraft**, Werkvertrag AG Aquatische Ökologie, ICBM, Carl von Ossietzky Universität Oldenburg; **Gutachtertätigkeit zur Sauerstoffzehrung in der Ems** aufgrund des Baues eines Sperrwerks bei Gandersum gefördert durch NLWK-Aurich Projektteam Emssperrwerk

Dissertation

06/2003 - 07/2007	Bewertung von räumlichen und zeitlichen Modellen zur Abschätzung der Bioakkumulation von Spurenmetallen in marinen Evertebraten

Praktische Erfahrungen

05/2001 - 03/2002	insgesamt drei befristete Verträge als **wissenschaftliche Hilfskraft** in der AG Aquatische Ökologie, ICBM, Carl von Ossietzky Universität Oldenburg: **Beteiligung an universitärer Lehre einschließlich der Erstellung von Unterrichtsmaterialien** für Fortgeschrittenen Praktikum **Aquatische Ökologie**; Vertiefungspraktikum **Faunistische Bewertung von Fließgewässern**; **Messung von Metallen in Biota und Sedimenten aus der Wesermündung**
10/2000 - 02/2001	**Revision und Setzung von Vortragsbeiträgen für Tagungsband** zur VAAM-Tagung 2001, Ausrichter Carl von Ossietzky Universität Oldenburg
12/1998 - 02/2000	**Sortieren von Grundwasserproben**, AG Zoosystematik und Morphologie, Carl von Ossietzky Universität Oldenburg
05/1997 - 09/1998	**Datenrecherche und Präsentation** im Fraunhofer Institut für Systemtechnik und Innovationsforschung (Fhg-ISI) in Karlsruhe, Abteilung Biotechnologie

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02/2006	**Symposium** „Warnsignale aus den Polargebieten“, Hamburg
10/2002	**Workshop** „EU-Wasserrahmenrichtlinie“, Wasserbauingenieure, Universität Essen
09/2002	**Workshop** „Naturschutz und EU-Wasserrahmenrichtlinie“, Alfred-Töpfer-Naturschutzakademie, Schneverdingen

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Forschungsausfahrten

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Exkursionen

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Artikel in Reviewed Journals, Poster und Berichte

Jung, K., Stelzenmüller, V. & Zauke, G.-P. 2006: Spatial distribution of heavy metal concentrations and biomass indices in *Cerastoderma edule* Linnaeus (1758) from the German Wadden Sea: An integrated biomonitoring approach. Journal of Experimental Marine Biology and Ecology 338: 81–95.

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Erklärung

Hiermit versichere ich, dass ich diese Arbeit selbstständig verfasst und keine anderen, als die angegebenen Quellen und Hilfsmittel benutzt habe. Die Dissertation wurde in Teilen bereits veröffentlicht.

Oldenburg, den

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(Kristine Jung)

Erklärung

Hiermit versichere ich, dass die Dissertation weder in ihrer Gesamtheit noch in Teilen einer anderen wissenschaftlichen Hochschule zur Begutachtung in einem Promotionsverfahren vorliegt oder vorgelegen hat.

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(Kristine Jung)