SCIENTIA MARINA 73(1)
March 2009, 105-115, Barcelona (Spain)
ISSN: 0214-8358
doi: 10.3989/scimar.2009.73n1105

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

KRISTINE JUNG [1], VANESSA STELZENMÜLLER [2] and GERD-PETER ZAUKE [1]

[1] Carl von Ossietzky Universität Oldenburg, ICBM, Postfach 2503, D-26111 Oldenburg, Germany.
E-mail: gerd.p.zauke@uni-oldenburg.de
[2] present address: CEFAS, Pakefield Road, Lowestoft, NR33 0HT, UK.

SUMMARY: Spatial distributions of Cu, Pb, Cd, Ni and Zn concentrations in brown shrimps *Crangon crangon* (Linnaeus, 1758) collected on a cruise of FRV *Walther Herwig III* to the southern North Sea in January 2004, were investigated on a scale of 18 x 18 km to evaluate the range of spatial autocorrelations for the different variables under study. 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 are 6.6 km for Cd, 3.0 km for Ni and 5.2 km for Pb. Contour plots revealed a clear coincidence of high values for Cd, Ni and Pb with low shrimp mean body wet weight. Nevertheless, spatial autocorrelations were rather weak, since classical and geostatistical population estimates for the means and the 95% confidence intervals were in good agreement. The low detected concentrations of Pb in *C. crangon* were in good agreement with reported data for decapod crustaceans from other regions. For Zn reported values were distinctly below our 95% confidence intervals, while for Cu they were slightly above and for Cd distinctly above concentrations in *C. crangon* from this study. For Ni no comparative values exist. We conclude that with this integrated biomonitoring approach metal concentrations could be assessed more precisely and relations between biotic and abiotic variables could be evaluated.

Keywords: Crangon crangon, decapod crustaceans, biomonitoring, metals, geostatistics, spatial analysis.

RESUMEN: ANÁLISIS ESPACIAL DE LA CONCENTRACIÓN DE METALES EN EL CAMARÓN GRIS *CRANGON CRANGON* (LINNAEUS, 1758) EN EL MAR DEL NORTE MERIDIONAL. – Se analiza la distribución espacial de las concentraciones de Cu, Pb, Cd, Ni y Zn en el camarón gris *Crangon crangon* (Linnaeus, 1758) en muestras recolectadas durante una campaña a bordo del BO "Walther Herwig III" en el Mar del Norte meridional en Enero de 2004, en un área de 18 x 18 km. Se investiga el alcance de las funciones de autocorrelación espacial de las distintas variables estudiadas. El análisis de los modelos de semivariograma obtenidos mediante técnicas geostadísticas indica un aumento de la variabilidad para la mayoría de las variables con la distancia. Sólo cuando se toman las muestras a distancias superiores al alcance práctico del semivariograma se puede asumir independencia estocástica entre los datos. Los alcances calculados son 6.6 km para Cd, 3.0 km para Ni y 5.2 km para Pb. Los mapas de isodensidad muestran una clara coincidencia de altos valores de Cd, Ni y Pb con valores bajos de peso medio húmedo de camarón. Sin embargo, las autocorrelaciones espaciales observadas son relativamente débiles, ya que las estimaciones obtenidas para la media y el intervalo de confianza al 95% mediante técnicas geostadísticas y técnicas clásicas coinciden. Los bajos valores de concentración de Pb detectados en *C. crangon* concuerdan con los datos obtenidos para crustáceos decápodos en otras regiones. Para Zn los valores medidos en otras regiones se encuentran de manera consistente por debajo del intervalo de confianza del 95%, mientras que para Cu los valores observados son ligeramente superiores a los nuestros. Para la concentración de Cd en *C. crangon* los valores que se indican en la literatura son claramente superiores a la concentraciones medidas en nuestro estudio, mientras que para Ni no existen valores de comparación. Se concluye que con esta aproximación integrada a la valoración biológica, las concentraciones de metales pueden evaluarse de manera más precisa, así como las relaciones entre variables bióticas y abióticas.

Palabras clave: Crangon crangon, crustáceos decápodos, valoración biológica, metales, geostadística, análisis espacial.

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), where mussels are frequently used as biomonitors. Although crustaceans may be also regarded as suitable bioindicators and biomonitors in various freshwater systems (Rinderhagen *et al.*, 2000), very little information about such programmes for marine crustaceans can be found, at least not dealing specifically with trace metals. However, due to their great ecological and commercial importance in the intertidal and subtidal zones of 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 northern and western Europe and the Mediterranean. It is a typical euryhaline inhabitant (Cieluch *et al.*, 2005; Viegas *et al.,* 2007) and 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 an important prey. The shallow-water region of the Wadden Sea is the nursery ground for brown shrimps (Berghahn, 1996). Furthermore, they are important food resources for flatfish (*Pleuronectes platessa*), shore crabs (*Carcinus maenas*), seals (*Phoca vitulina*), various waders (Limicolae), seagulls (Laridae) and auks (Alcidae) (Jensen and Jensen, 1985; Janke, 1999), as well as important targets in coastal fisheries (Neudecker and Damm, 2006).

The spatial structuring of *C. crangon* 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 by sunlight intensity during low water level in the shallow regions of the Wadden Sea (Berghahn, 1983). Biotic factors, such as predation (Berghahn, 1996), competition and interactions between adults and juveniles (Berghahn, 1983), also strongly influence the recruitment and subsequent spatial distribution of *C. crangon*.

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 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 should be noted that the spatial dependence of variables has not explicitly been taken into account in ecological and biomonitoring field studies in this area.

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 such as means or confidence intervals (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 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 metal concentrations and mean body wet weight of the brown shrimp *C. crangon* from the German Bight. We employed geostatistical methods recently optimised for the evaluation of fisheries data (Stelzenmüller *et al.*, 2004). 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).

MATERIAL AND METHODS

Sampling area and sample collection

Samples of the decapod crustacean *Crangon crangon* (Linnaeus, 1758) for this analysis were collected from the FRV *Walther Herwig III* (cruise 259, January 5-13, 2004) in an area of the inner German Bight (Box A, Fig. 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) above ground (30-50 m). The height of the gear's vertical opening was around 5 m with a wingspread of around 20 m and the cod end had a fine mesh liner of 20 mm mesh opening. The trawling time was 30 min at a trawling speed of 4 knots (1 knot = 0.514 m s^{-1}). The locations of sampling stations (1-34, Fig. 1) as well as trawl directions were selected randomly within the study area. 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 demersal fish were sorted, counted and weighed. Ten randomly collected shrimp specimens from the by-catch at each station were thoroughly rinsed for a few seconds with double-distilled water to remove fine suspended materials, adhering seawater and labile metals, which are not regarded as part of the bioaccumulated metal fraction, from the surface of the animals. Afterwards they were dried on good-quality filter paper (Type 613, Schleicher and Schuell, Germany) before they were transferred to sterile polystyrene Petri dishes (Greiner bio-one, Frickenhausen, Germany, free from metals), which were numbered, closed tightly with tape and stored deep frozen (at -18°C) for further analysis of metals in the laboratory (see below).

Additionally, in order to determine the dependency between trace metal content and dry weight

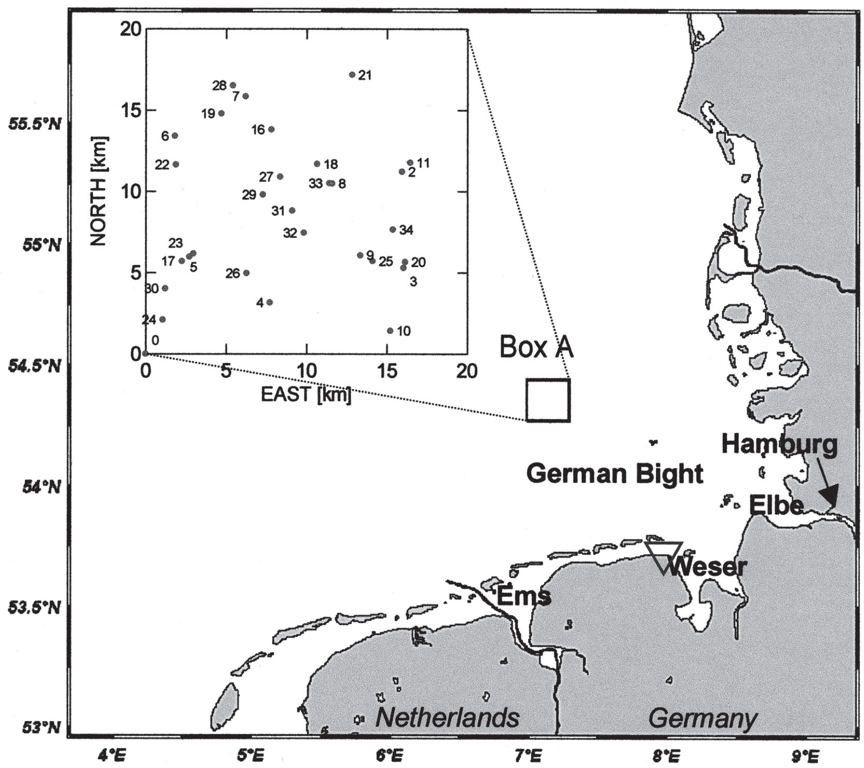

FIG. 1. – Standard GSBTS sampling area Box A and positions of the trawling midpoints from cruise 259 with FRV *Walther Herwig III* (January 05 – 13, 2004) in an area of the inner German Bight, southern North Sea and sampling location for single specimen measurements in Schillig (triangle), German North Sea coast (July 2004 and 2005, respectively).

TABLE 1. – Quality assurance using certified reference materials randomly allocated within the determinations.

Element	Tort-2 Lobster hepatopancreas; National Research Council Canada analysed	n	certified	CRM No 278R Mussel Tissue: *Mytilus edulis*; Community Bureau of Reference 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	2.1 ± 0.4	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

Values are means ± 95% confidence intervals (mg kg^{-1} DW); 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} (DW).

(DW) of brown shrimps, results from individuals of *C. crangon* sampled in July 2004 and 2005 in Schillig at the German Wadden Sea Coast (Fig. 1) were considered.

Sample preparation and analytical procedures

Upon arrival at the laboratory the frozen *C. crangon* samples were defrosted for a short period of time to assess the individual body wet weight. Then the 10 pooled shrimps from each sampling site were transferred back to the polystyrene Petri dishes and stored frozen again at –18°C, before the samples were subjected to freeze-drying for 48 h (Lyovag GT2, Leybold Heraeus). Afterwards, the dry weight was determined and 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 (Jung, 2007, chapter 1). Ashing and atomisation temperatures were 600 and 1800°C for Cd; 1000 and 2200°C for Pb; 800 and 2700°C for Ni and 800 and 2300°C for Cu. 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 and 3.6 ± 0.07 regarding organisms from Box A.

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 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), but not for CRM 278R.

Preliminary data analysis

For each sampling location, the mean body wet weight was standardised (and then termed *mbww*) by the number of 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* in the spatial analysis). For each of these variables descriptive statistics were calculated (Wilkinson and Engelman, 2000) and the normality of the data was tested by the Lilliefors 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, in which point sizes were plotted proportional to the sample value at each sampling site.

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 $\hat{\gamma}$(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 distributions (Cressie, 1991):

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

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 km) 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, in which more weight is given to the points near the origin—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):

$$SD = \frac{C}{C_0+C} \quad \text{and} \quad SD(\%) = \frac{C}{C_0+C} * 100 \tag{2}$$

When this ratio approaches 1 or 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 cross-validation procedure to provide a measurement of the reproduction of the data by the models defined and the perspective kriging procedures. The crossvalidation results 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 the predicted distribution of 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; Webster and Oliver, 2001). 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:

The classical 95% confidence interval

$$95\%CI_{class} = m \pm t * S_m \quad \text{with} \quad S_m = s/\sqrt{n} \tag{3}$$

and the geostatistical 95% confidence interval

$$95\%CI_{geo} = m_k \pm t * S_{m_k} \quad \text{with:} \quad S_{m_k} = ke/\sqrt{n} \tag{4}$$

where S_m is the classical standard error, S_{m_k} is the geostatistical standard error and t-values are 1.96 (otherwise as defined above). Additionally, classical coefficients of variation were calculated as well as the geostatistical estimator modified from Fernandes and Rivoirard (1999) where the spatial structuring of the data is taken into account:

$$CV_{class} = s*100 / m \ (\%) \quad (5a)$$

and

$$CV_{geo} = ke*100 / m_k \ (\%) \quad (5b)$$

For statistical computing we used the R environment (R Development Core Team, 2005) with the package geoR.

RESULTS

Post plots of the measured sample values are presented in Figure 2. For *Zn* and *Cu* the point sizes indicate constant but high values at all sampling stations over the sampling area, whereas the point sizes for *mbww*, *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 sampling stations increased Pb values are obvious. Empirical semivariograms are displayed in Figure 3 and the estimated model parameters nugget, sill and range, the measure of spatial dependency (*SD %*), and the results of the cross-validation are compiled in Table 2. The highest level of spatial dependence is visible for *Ni* (88%), 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 are shown in Figure 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 *Cu* and *Zn*.

A comparison between classical and geostatistical population estimates is shown in Table 3. Regarding the coefficients of variation (CV), the highest variability can be seen for *Pb* which, after

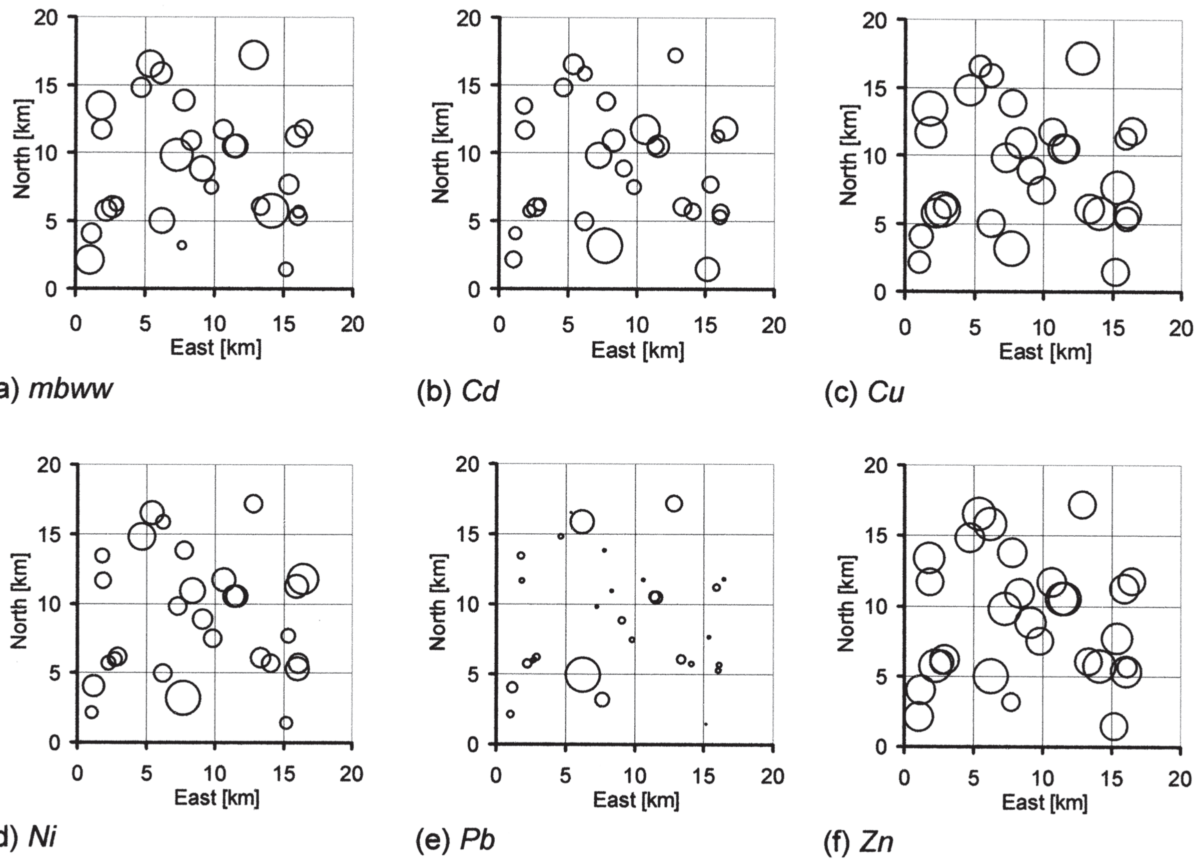

FIG. 2. – Spatial distributions (post plots) of sampling stations within Box A (Fig. 1). Point sizes are relative to the measured values of (a) *mbww* (mean body wet weight of shrimps per sample in g), (b) *Cd*, (c) *Cu*, (d) *Ni*, (e) *Pb* and (f) *Zn* in *Crangon crangon* (mg kg^{-1} DW, respectively).

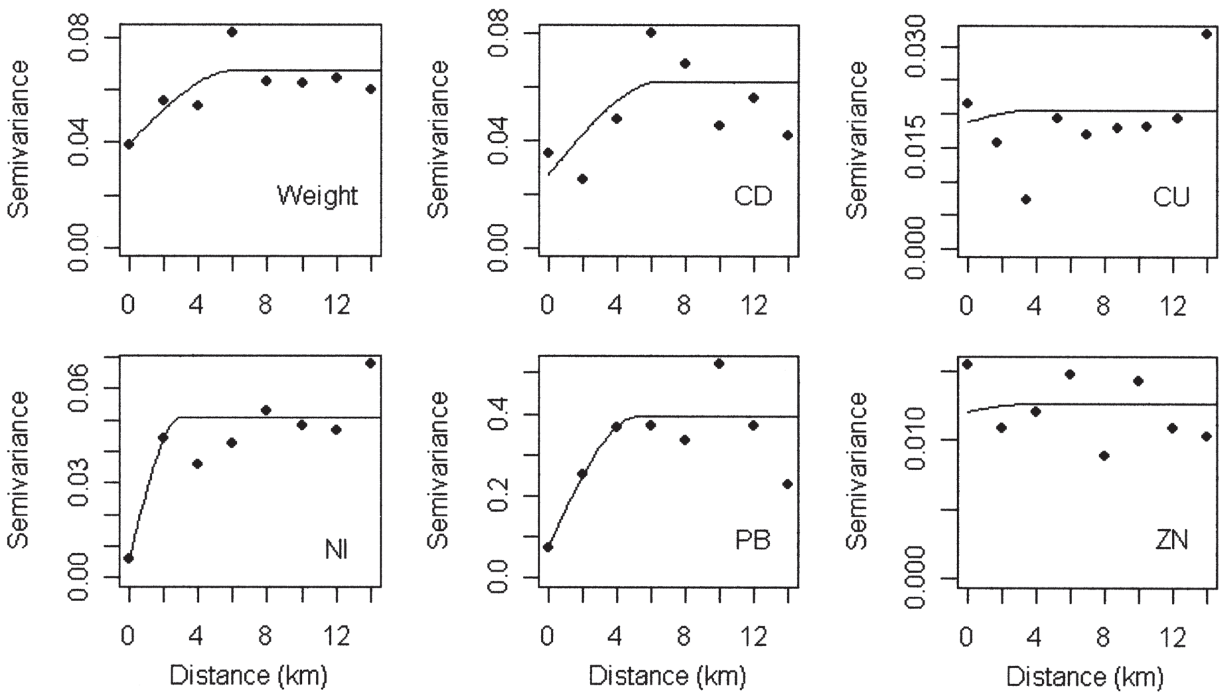

FIG. 3. – Empirical semivariograms with fitted spherical models for weight (*mbww* = mean body wet weight of shrimps per sample in g), *Cd*, *Cu*, *Ni*, *Pb*, and *Zn* in *Crangon crangon* (mg kg^{-1} DW, respectively) within Box A (Fig. 1).

TABLE 2. – Estimated parameters of spherical semi-variogram models (Fig. 3) fitted to sample values of corresponding variables measured for *Crangon crangon* from Box A (Fig. 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 (g), *Cd*, *Cu*, *Ni*, *Pb* and *Zn (mg kg^{-1} DW, respectively)*; *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).

TABLE 3. – Comparison of classical and geostatistical population estimates of the variables mean body wet weight (*mbww*) of shrimps per sample (g), *Cd*, *Cu*, *Ni*, *Pb* and *Zn* (mg kg^{-1} DW) in *Crangon crangon* from the North Sea (Box A).

Variable	m	$95\%CI_{class}$	m_k	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

N = 29; m = arithmetic mean, $95\%CI_{class}$ = classical 95% confidence interval (eq 3), m_k = geostatistical mean (estimated by kriging), ke = mean kriging error, $95\%CI_{geo}$ = geostatistical 95% confidence interval (eq 4), CV_{class} = classical coefficient of variation (eq 5a).and CV_{geo} = geostatistical coefficient of variation (eq 5b), *LIP*= Lilliefors Probability (2-tail) that sample data are distributed normally (α=0.01).

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>α=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 a lesser extent, *Pb.*

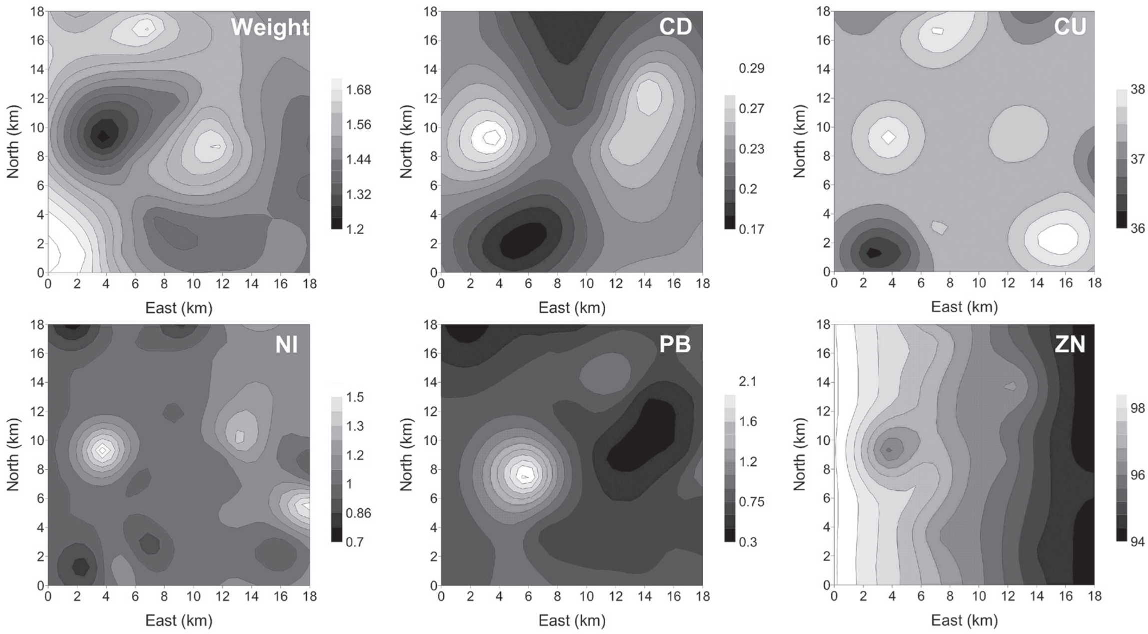

FIG. 4. – Mapped spatial distribution of weight (*mbww* = mean body wet weight of shrimps per sample in g), *Cd*, *Cu*, *Ni*, *Pb* and *Zn* in *Crangon crangon* (mg kg^{-1} DW, respectively) within Box A (Fig. 1) in universal kriging plots showing contour lines.

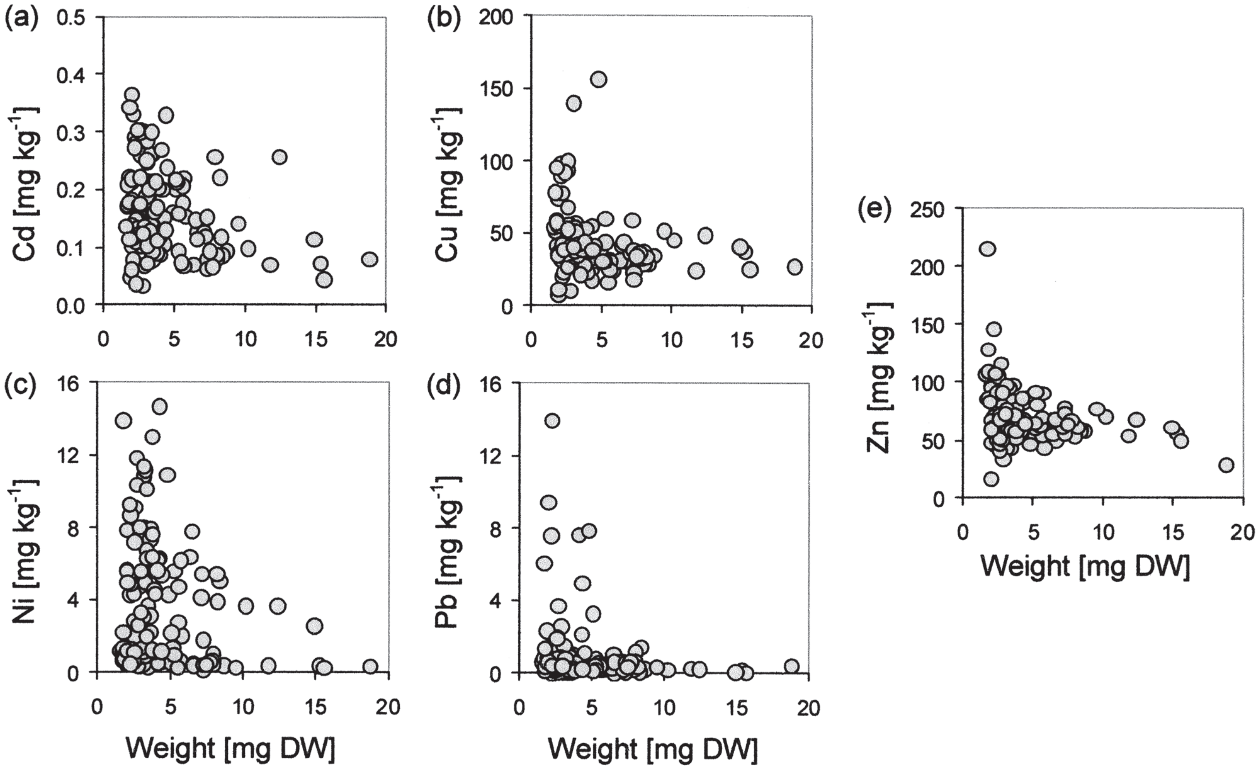

FIG. 5. – Relationships between concentrations of (a) *Cd*, (b) *Cu*, (c) *Ni*, (d) *Pb* and (e) *Zn* (mg kg^{-1} DW, respectively) and body dry weight (mg) for individually measured *Crangon crangon* sampled in July 2004 and 2005 in Schillig, German Wadden Sea Coast (Fig. 1).

Metal determinations in single specimens of *C. crangon* collected at a nearshore locality (Schillig) are shown in Figure 5, indicating, despite a substantial variability, decreasing metal concentrations with increasing weight of organisms, although it was not possible to obtain reasonable linear or non-linear regression models.

DISCUSSION

Spatial analysis and mapping

The calculated Lilliefors probabilities (Table 3) indicate that we can only assume a normal distribution of the variables *mbww*, *Cu* and *Ni* ($\alpha = 0.01$), 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 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 (0 and 1, respectively) for *Pb*. 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. These indicate a distinct increase of the semivariance (the dissimilarity) for all variables but *Cu* and *Zn* with sampling distance.

However, regarding the classical and geostatistical 95% confidence intervals (Table 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 weak and that the results of the spatial modelling must be regarded with some caution. Consequently, most of the *CV* values for metals in shrimps are within a range to be expected because of the variability of the imprecision of the analytical procedures employed.

The fact that increasing semivariogram 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 semivariogram models regarding small distance classes, as shown for *Cerastoderma edule* in the German Wadden Sea (Jung *et al.*, 2006). 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, it was not possible to employ such a design in this study, a fact which might have prevented better model estimates.

The contour plots of the mapped spatial distribution of the variables under study are depicted in Figure 4 and the relationships between metal concentrations in individually measured organisms and their corresponding body dry weight are presented in Figure 5. Due to the large variability within analysed data from 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 dry weight is apparent. These findings are in agreement with coinciding patches of increased concentrations of *Cd* and *Ni*, and to a lesser extent *Pb* (Fig. 4.), at locations where the mean body wet weights of shrimps (*mbww*) are lowest. Furthermore, the detected aggregation of higher values for *mbww* in the southwest 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 southwest corner decreasing towards the northeast corner, in fairly good agreement with the grain size of the sediments (percentages of the silt fraction <63 μm).

Implications for biomonitoring

In the German Bight environmental conditions are highly variable. Only limited information on ecology and biomonitoring of marine invertebrates explicitly taking spatial autocorrelations into account is available (Jung *et al.*, 2006). Application of classical statistical procedures assumes stochastic independence of the data (Petitgas, 2001). Our results indicate at least weak spatial autocorrelations at the scale of investigation for *Ni* and *Pb* and, to a lesser extent, for *mbww* and *Cd*, as can be inferred from increasing semivariograms (Fig. 3) and from the fitted semivariogram models (Table 2). Thus, only when samples are taken at distances above the estimated values for the corresponding ranges can stochastic independence of the data be assumed. These are 6.6 km for *Cd*, 3.0 km for *Ni* and 5.2 km for *Pb*. 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 is not

TABLE 4. – Mean concentrations of the trace metals decapods from different regions of the world (mg kg^{-1} DW).

Species	Region	*Cd*	*Cu*	*Pb*	*Zn*	Ref
Acanthephyra purpurea	NE Atlantic	3.0	36	-	46	1
Acanthephyra sp.	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 sp.	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	NE 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 case in our study, as can be seen from the comparison of classical and geostatistical coefficients of variation and confidence intervals compiled in Table 3. However, it does not invalidate the geostatistical approach employed here, since it will always be an a posteriori conclusion.

Trace metal concentrations in decapods from different regions of the world are compiled in Table 4, suggesting 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. In particular, low reported Pb concentrations are in good agreement with our data for *C. crangon* (Table 3). For Zn reported values are distinctly below our 95% confidence intervals, while for Cu they are slightly above and for Cd distinctly above concentrations in *C. crangon* from this study. For Ni no comparative values exist.

In conclusion, spatial autocorrelations (although rather weak) found for *Ni*, *Pb*, *Cd* and *mbww* in 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 distances 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 shorter distances, classical population estimates like means and confidence intervals could 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 for 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

We thank the captain and crew of the FRV *Walther Herwig III* on cruise 259, as well as S. Ehrich (Bundesforschungsanstalt für Fischerei, Hamburg) for their kind cooperation. Metal determinations and data analysis are part of the PhD thesis of K. Jung at the C.v.O. University of Oldenburg.

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Scient. ed.: F. Maynou.
Received March 14, 2008. Accepted July 8, 2008.
Published online December 22, 2008.