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Effect of Body Size and Season on the Concentrations of Cu, Cd, Pb and Zn in *Diastylis rathkei* (Kröyer) (Crustacea: Cumacea) from Kiel Bay, Western Baltic

K. M. SWAILEH* and D. ADELUNG†
**Biology and Biochemistry Department, Birzeit University, P.O. Box 14, Birzeit, West Bank, Israel*
†Abt. Meereszoologie, Institut für Meereskunde an der Universität Kiel, Düsternbrooker Weg 20, 24105 Kiel, Germany

The concentrations of four metals (Cu, Cd, Pb and Zn) in the cumacean *Diastylis rathkei* were estimated from four stations in Kiel Bay, Western Baltic. Metal concentrations in samples from the four stations were not significantly different from each other. Smaller animals were found to contain less Cu and Cd and more Pb and Zn than larger ones. Seasonality in the concentrations of the four metals was observed. Generally, high concentrations of the four metals occurred during the summer months, which corresponds to the main growth period of *D. rathkei*. The ratio between monthly maximum and minimum mean concentrations was greatest for Pb (factor 2.5) and least for Zn (factor 1.4). Cd and Cu had a common ratio of 1.6.

Monitoring metal pollution in the aquatic environment is becoming increasingly important with rapid industrial and technological developments. The use of living organisms as biomonitors for metal pollution has many advantages since they integrate pollution over a period of time, provide a measurement of the bioavailability and biomobility of pollutants, and concentrate pollutants to levels that are far higher than those in the abiotic environment (Phillips, 1977). Unfortunately, interpretation of the data concerning metal concentrations in biomonitors can be complicated by biological as well as environmental processes that can cause variations in these metal concentrations and make comparisons of results between different studies difficult. Examples of these processes are: reproductive cycle, seasonal growth, age, water temperature and salinity, run-off, chelating agents and depth of sampling.

While mussels and oysters are among the best studied organisms for cosmopolitan biomonitoring, other species of local, national or international importance should be considered for monitoring programmes (Rainbow & Phillips, 1993). In addition, most of the monitoring programmes are confined to the nearshore areas which are more susceptible to pollution than offshore areas. This makes the offshore areas poorly studied with regard to pollution. At the present time, dumping waste in the deep sea is a common way of getting rid of material. This necessitates establishing background levels of pollutants in some offshore organisms, an element that might be needed for future monitoring programmes.

The common cumacean crustacean, *Diastylis rathkei* (Kröyer) is of national importance in the Western Baltic as well as in the German Bight. This small crustacean is one of the major benthic producers and the most important food item of Western Baltic demersal fish, such as the dab (*Limanda limanda*), cod (*Gadus morhua*) and flounder (*Platichthys flesus*) (Hertling, 1928; Arntz, 1971, 1974, 1977a,b; Rachor *et al.*, 1982). *Diastylis rathkei* constitutes nearly 9% of the stomach contents of cod (Schulz, 1988), 35% of those of the flounder (Arntz, 1977b) and 13% of those of the dab from the German Bight (Kühl, 1964). *Diastylis rathkei* average ashfree dry weight production in Kiel Bay is nearly 1500 t yr^{-1} and its mean annual abundance is about 500 individuals m^{-2} (Rachor *et al.*, 1982). It is a deposit feeder in muddy sand bottoms (Forsman, 1938) but it can also survive as an epistratum feeder on coarser sediment (Habermehl *et al.*, 1990). The normal life span of this species is 1 year (Krüger, 1940) but < 5% of the population reach a second breeding period (Jarre, 1989). The purpose of this study was to estimate the baseline concentrations of four trace metals (Cu, Cd, Pb and Zn) in this organism from four stations in Kiel Bay, and to investigate the effect of body size and season on its metal concentrations.

Materials and Methods

Sampling protocol and analytical procedure

Samples of *D. rathkei* were dredged monthly with the bivalve *Arctica islandica* from four stations in Kiel Bay (Fig. 1 and Table 1) in the period from July 1992 to

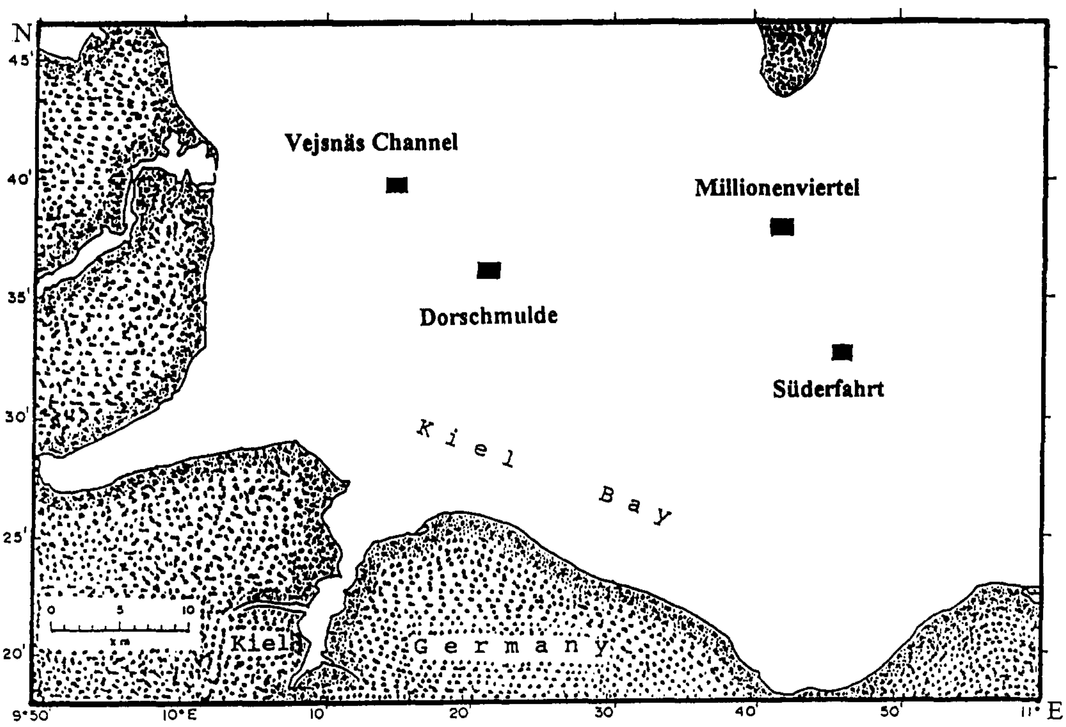

Fig. 1 Kiel Bay showing the location of the stations.

TABLE 1

Descriptions of the four sampling stations.

Station	Depth (m)	Sediment type
Süderfahrt	23	Sandy mud
Millionenviertel	23	Sandy mud
Dorschmulde	21	Mud
Vejsnäs Channel	22	Muddy sand

June 1993. Animals were rinsed from sediment using clean seawater and deep-frozen. In the laboratory, samples were freeze-dried for 1 week, oven-dried at 60°C for 1 day and weighed. Analysis of single individuals for trace metals was not possible since one animal does not provide enough dry weight material for the analysis. Therefore, all samples analysed during this study were composed of pooled animals. Samples of nearly 20 mg dry wt were digested in Teflon tubes with Teflon covers that allow acid reflux to take place. A digestion mixture composed of 1:1 nitric:perchloric super pure acids (Merck) was used (Fischer, 1983). The volume of the mixture was always 10 times the sample weight. Digestion took place in a special block-heater, the temperature of which was gradually increased up to 200°C over the first 5 h. Subsequently, digestion was allowed to continue for a further 10 h. Finally, samples were dried via evaporation at 100°C and volumes were brought to 1 ml using 1 N HCl. Subsequent dilutions using 0.2% HNO_3 were carried out and the concentrations of the four metals determined using a flameless atomic absorption spectrophotometer type Perkin-Elmer 3030 with automatic sampler and Zeeman background corrector. Copepod homogenate (MA-A-1/TM, No. 645) provided by the International Atomic Energy Agency, International Laboratory of Marine Radioactivity, Monaco, was used as reference material and all accepted recoveries were above 90%.

Statistical analysis

All calculations refer to dry weight of tissues. One Way Analysis of Variance (ANOVA) and *t*-test using SYSTAT-programme (Wilkinson, 1992) were applied to investigate differences in metal concentrations.

Results and Discussion

Diastylis rathkei samples collected from the four stations were not significantly different from each other in their metal concentration (Table 2). This organism is highly mobile, making vertical nocturnal migrations in the water column, where it is subjected to drift by water currents. This high mobility led Jarre (1989) to the conclusion that there is only one population of *D. rathkei* in Kiel Bay. In addition, the four sampling stations are located in the offshore area, away from direct effects of possible local coastal pollution, where depth and sediment types are similar. These factors contribute to minimizing possible local differences in metal concentration.

To study the effect of growth on metal concentration, body weight was considered in this study to be a

TABLE 2

Comparisons between mean metal concentrations ($\mu g\ g^{-1}$ dry wt ± SD) in *D. rathkei* from four stations in Kiel Bay in July 1992.

Station	Cu	Cd	Pb	Zn
Süderfahrt	124.5 ± 5.5	0.45 ± 0.04	9.10 ± 0.93	58.0 ± 4.30
Millionenviertel	130.7 ± 7.5	0.46 ± 0.03	8.20 ± 1.30	64.2 ± 10.2
Dorschmulde	122.6 ± 6.2	0.44 ± 0.02	8.25 ± 0.81	58.7 ± 2.40
Vejsnäs Channel	123.1 ± 3.6	0.43 ± 0.01	7.90 ± 0.81	61.2 ± 4.40
ANOVA	NS	NS	NS	NS

$n=4$ pooled samples per station; each sample contained 3–5 animals of 3–5 mg dry wt.

NS: Not significant at $p < 0.05$.

relevant growth parameter. Jarre (1989) reported that there was no significant sexual growth dimorphism in this species. The two weight classes (≤ 3 and >3 mg dry wt) of *D. rathkei* differed significantly in their metal concentrations (Table 3). Smaller animals contained less Cu and Cd and more Zn and Pb than larger ones. The difference in metal concentration between small and large animals is well known and has been observed by many authors. It could be due to differences in metabolic activity and thus metal metabolism (Williamson, 1980) or due to sexual maturity, since maturation is accompanied by physiological changes and in the affinity of biochemical substances for metals (Oesterberg, 1974). White & Rainbow (1987) however, reported that the decrease in the concentration of some metals with increasing body size of crustaceans may indicate that a significant proportion of these metals may be surface-adsorbed, since smaller specimens have high surface area to volume ratios than larger ones. In this case, metals are not under metabolic influence. The increase in Cd with size of *D. rathkei* may indicate accumulation of this metal from a Cd-rich diet. The increase of Cu concentration with increasing weight of *D. rathkei* could be due to an increase in the concentration of haemocyanin, a copper-containing respiratory pigment, with age. This trend in Cu concentration was observed by White & Rainbow (1987) in the crustacean *Systellaspis debilis*. They reported that young organisms contained little or no haemocyanin.

Metal concentrations in *D. rathkei* during this 1-year study are summarized in Table 4. The mean concentrations of Cu (102.9 μg g^{-1}) and Zn (77.4 μg g^{-1}) are close to the estimated total requirements of these two metals by crustaceans which are 83.7 and 70.8 μg g^{-1}, respectively (White & Rainbow, 1985). These two metals are essential and crustaceans have been reported to be generally good regulators of their total body metal contents (Bryan, 1971; Wright, 1976; White & Rainbow, 1982; Devineau & Amiard-Triquet, 1985; Clark, 1989; Rainbow, 1990).

TABLE 3

Mean ± SD of the concentrations (μg g^{-1} dry wt) of four trace metals in two weight classes of *D. rathkei* from Kiel Bay, in July 1992.

Dry weight (mg)	Cu	Cd	Pb	Zn
≤3	117.6 ± 6.2	0.39 ± 0.029	9.46 ± 0.77	71.9 ± 4.2
>3	125.6 ± 7.0	0.45 ± 0.031	8.37 ± 1.10	61.7 ± 7.4
t-test	$p=0.029$	$p=0.001$	$p=0.038$	$p=0.004$

$n=8$ pooled samples per each dry weight class.

TABLE 4

Metal concentrations (μg g^{-1} dry wt) in *D. rathkei* collected from Kiel Bay in the period from July 1992 to June 1993.

	Cu	Cd	Pb	Zn
Minimum	61.7	0.17	2.9	54.3
Maximum	172.4	0.56	14.8	120.3
Mean ± SD	102.9 ± 19.9	0.3 ± 0.07	7.5 ± 2.5	77.4 ± 10.5

$n=68$ pooled samples.

The mean concentrations of the two non-essential metals, Cd and Pb, were 0.3 and 7.5 μg g^{-1}, respectively. Body contents of these metals are generally reported not to be regulated by marine animals (Wright, 1977; Jennings & Rainbow, 1979; White & Rainbow, 1982; Devineau & Amiard-Triquet, 1985). The mean concentration of Cd in *D. rathkei* is, for example, less than that of the 1 μg g^{-1} reported by Bryan (1976) to be typical for decapod crustacean, and much less than that of the 12.8 μg g^{-1} found in the decapod crustacean *S. debilis* (White & Rainbow, 1987). Compared to the mean Cd concentration, mean Pb concentration in *D. rathkei* is much higher. This could be due to the fact that Pb concentration in the sediments of the Baltic, where *D. rathkei* lives, is much higher than the Cd concentration. For example, in the Western Baltic sediment, Cd concentration ranges between 0.17 (Kattegat area) and 0.7 mg kg^{-1} dry wt (The Great Belt area) while that of Pb lies between 20 and 43 mg kg^{-1} dry wt from the same areas, respectively (HELCOM, 1993).

Variation in metal concentration with season has been well-documented in different studies (Bryan, 1973; Cossa *et al.*, 1979; Orren *et al.*, 1980; Boyden & Phillips, 1981; Cain & Luoma, 1990). It is reported to be due to varying seasonal growth rate, reproductive cycle, phytoplankton productivity, water salinity and temperature and run-off. Statistically significant ($p<0.0001$) seasonality in the concentrations of the four metals in *D. rathkei* was observed. High metal concentrations were observed mainly during the summer months (May–August) (Fig. 2). The highest monthly mean concentrations of Zn (95.3), Cu (145.1), Cd (0.39) and Pb (11.1) μg g^{-1} were observed in May, June, July and August, respectively. The lowest monthly mean concentrations of Cd (0.24) and Zn (68.7) μg g^{-1} occurred in August and those of Cu (89.3) and Pb (4.4) μg g^{-1} in December (Fig. 2). The ratio between the seasonal mean maximum and minimum concentrations was greatest for Pb (factor 2.5) and least for Zn (factor 1.4). Cd and Cu had factors of 1.6. The increase in the concentrations of the metals during the summer months corresponds to the main growth period of this species. *Diastylis rathkei* grows most quickly, in both length and weight, during the summer months (June–September) and stops growing in late autumn when maturity is reached (Krüger, 1940; Rachor *et al.*, 1982; Jarre, 1989). Growth could lead to dilution of metals if tissue assimilation exceeds metal accumulation. This does not seem to be the case in *D. rathkei*, perhaps because it feeds on detritus, which is considered to be a metal-rich diet (Rainbow, 1990). Moreover, moulting from the juvenile stage to the mature stage takes place during this growth period. White & Rainbow (1984) found that moulting of crustaceans is associated with a temporary increase in the concentrations of metals inside their bodies, which could be due to an increase in the permeability of the body surface prior to tanning and/or calcification of new cuticle. A small, insignificant increase in the concentration of some metals was observed in winter. During this period the juveniles leave the marsupia (Krüger, 1940) and thus, for the

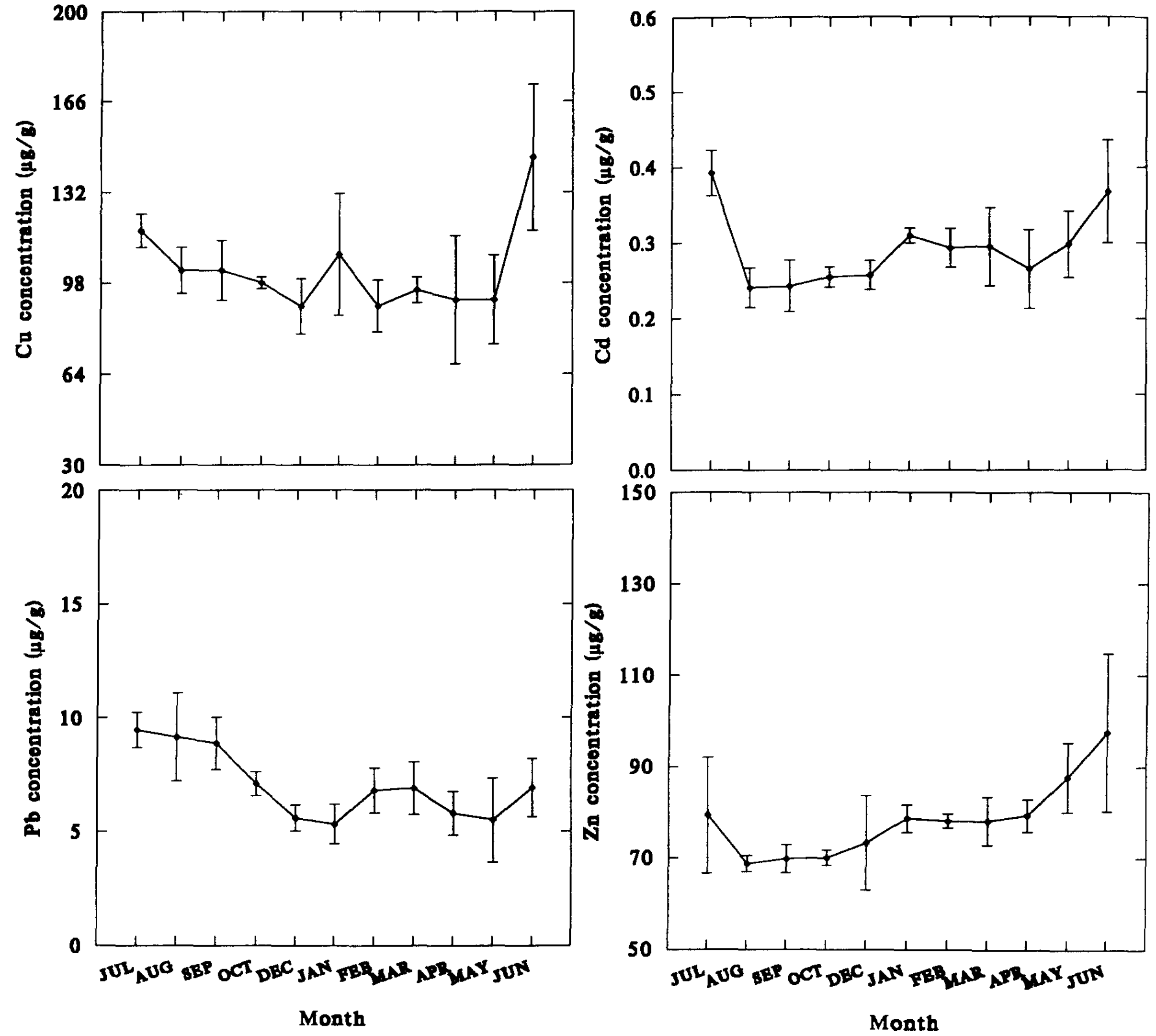

Fig. 2 Seasonal profiles for the concentrations (µg g^{-1} dry wt ± SE) of Cu, Cd, Pb and Zn in *D. rathkei* from Kiel Bay in the period from July 1992 to June 1993. $n=4$–8 pooled samples per month ($p < 0.0001$).

first time, may come into direct contact with the sediment. This could lead to influx of some metals inside their bodies or it could be only an adsorption effect.

There is no biomagnification of Pb in the three species of fish from the Western Baltic that are reported to feed intensively on *D. rathkei*. The Pb concentration in the liver of the three species, which is always much higher than that in the muscle, is still much less than that measured in *D. rathkei* (Table 5). Cd biomagnification is not clear, at least in cod and flounder, but Cd concentration in the liver of dab is 10 times higher than that in *D. rathkei*. Nott & Nicolaidou (1990) found that metals detoxified in phosphate granules by the detritus feeding gastropod, *Cerithium vulgatum*, are not biologically available to the carnivorous gastropod *Murex trunculus*. They suggested that the transfer of detoxification along a food chain can result in reduction of the bioavailability of metals.

TABLE 5

Comparisons between metal concentrations (µg g^{-1} dry wt) in the liver of three demersal fish species from the Western Baltic and their important food item *D. rathkei*.

Species and area	Cu	Cd	Pb	Zn
Gadus morhua (cod)				
Kattegat (1988)*	45	0.5	0.17	90
Platichthys flesus (flounder)				
Great Belt (1987)*	52	0.5	0.17	170
The Sound (1987)*	80	0.5	0.17	116
Limanda limanda (dab)				
Kattegat (1988)*	27	3.0	2.3	70
Diastylis rathkei				
Kiel Bay, present study	102.9	0.3	7.4	77.4

*HELCOM (1990).

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