Bull. Environ. Contam. Toxicol. 31, 535–542 (1983)

Influence of an Elevated Cadmium Level in the Food on Growth and Food Conversion of *Nereis succinea*

H.-G. Neuhoff

Institut für Meereskunde an der Universität Kiel, Düsternbrooker Weg 20, 2300 Kiel 1, Federal Republic of Germany

In recent times increasing amounts of cadmium have been introduced into the aquatic environment as could be traced in sediments (ERLENKEUSER et al. 1974). Cadmium, however, has various negative physiological effects (THEEDE 1980) among which sublethal Cd pollution is reported to affect reproduction, development and growth (REISH and CARR 1978; LEHNBERG and THEEDE 1979; WATLING 1982). Growth and reproduction, however, are the premise for the existence of a population. In order to analyse effects of Cd on growing organisms, in this study the polychaete Nereis succinea was exposed to sublethal Cd concentrations in the food. As growth related parameters may be influenced by the season as well, experiments were carried out in spring (April to June) and summer/autumn (August to October).

MATERIAL AND METHODS

N. succinea was collected from Kiel Fjord, Western Baltic Sea. The polychaete feeds on detritus (GOERKE 1971) and reproduces at temperatures >14 °C from May to October (KINNE 1954). Maturing individuals metamorphose to the swarming Heteronereis which dies after spawning.

Experiments were performed at a natural, dimmed daylight cycle, 15 °/oo S and 15 °C. The high temperature was used to assure a proper feeding habit of the animals which were observed to reduce or cease feeding below 10 °C (MANGUM 1969). Each test individual (30 per aquarium and experiment) was provided with a Petri-dish (ø = 5 cm) the cover of which was equipped with two opposing chimney-like glass tubes (7.5 cm long) in which the worm lived (NEUHOFF 1979 a). Water exchange in these housings, which were immersed in aquaria, was maintained by the inhabitants' undulating movements. The covered and filter-equipped polypropylen aquaria contained 30 l (pre-filtered) water which was pumped through the charcoal-granula filter bed at a rate of 180 l/h. While the filter bed was exchanged preceding each experiment, the total water content was renewed weekly. Test water was aerated by re-injecting the charcoal-filtered water into the aquarium.

For food preparation 4.2 g Agar Agar were added to 150 ml boiling deionized-water. The solution was stirred while it cooled to 60 °C. Then 25 g lyophilized and ground Mytilus tissue and the Cd quantity desired (Cd-chloride diluted in 10 ml water) were added. The pulp was stirred while it cooled to 30 °C and was then poured into Petri-dishes. Two food types were prepared: Type I (for control experiments) with 3 µg and type II with 97 µg Cd/g dry weight. Cd concentration in type I originated from the natural Cd background of the Mytilus tissue used. Food was stored deep frozen (-28 °C) and thawed in portions for daily use.

A 10-day starvation period in order to clear the worms' gut and to acclimate test individuals to experimental conditions preceded experiments. Feeding experiments lasted 28 days. Worms were fed once a day by placing food portions ranging from 0.05 to 0.30 g wet weight into the Petri-dish-housings, according to the size of the individual. These food portions were subject to leaching processes reducing the Cd concentration as shown in Fig. 1. Housings were cleaned every second day from mucus and fecal pellets.

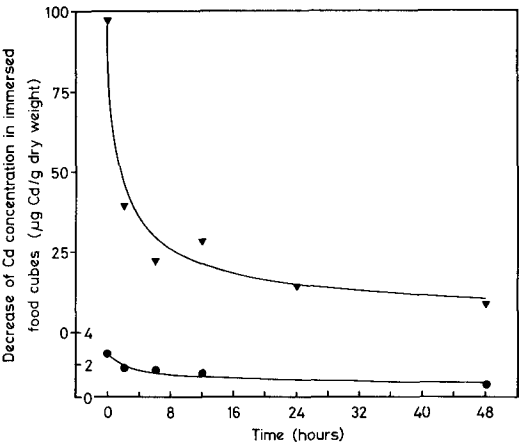

Figure 1. Decrease of Cd concentration in submersed food cubes with time (15 °C and 15 °/oo S).
● Food type I: 3 µg Cd/g dry weight.
▼ Food type II: 97 µg Cd/g dry weight.

Animals were processed 24 h after the last feeding. Wet weight was determined after adhering water was sucked off with blotting paper. Dry weight was determined after lyophilization and subsequent oven drying (60 °C, 20 h). For ash determination dry samples were burned at 500 °C in a muffel furnace (5 mg, 20 h). Methods used for the determination of protein, lipid, glycogen and energy content are described elsewhere (NEUHOFF 1979 b).
- Adenylate energy charge (AEC = (ATP + 1/2 ADP)/(ATP + ADP + AMP); ATKINSON 1968) was assayed according to WITZEL (1979) after an acid extraction of adenylates at cold temperatures (0-4 °C): The whole animal was homogenized (30 s) in 0.6 n sulfuric acid. After centrifugation

(4200 g, 5 min) 50 µg of the supernatant and 50 µl 0.6 n sodium hydroxide were added to 9.9 ml Tris-HCl buffer (pH 7.8). The mixture was kept deep-frozen (-28 °C) until assay. - For Cd determination 5-10 mg dry substance was digested in 2:1 parts of 5 n nitric acid and 70 % perchloric acid (500 µl, 100 °C, 2 h). After dilution, samples were measured with a graphite-furnace atomic absorption spectrophotometer.

Although tests were started with 30 individuals, at the end of some experiments only small numbers were left that could be taken for calculations. All animals with handling damages or those which metamorphosed (especially in spring experiments) were disregarded. To eliminate size dependent differences all data were normalized by means of regression statistics (after SACHS 1974) for a standard worm with 0.11 g organic substance at the end of the 28 day feeding period. Results obtained were compared by t-test with regard to the 5 % level.

RESULTS

Cd contamination (food type II) began to affect production rates and gross energy demand of production after a time lag of one week (Fig. 2). During both seasons, in spring and summer/autumn, Cd-induced decrease of production rates, based on the third feeding week (cf. Tab. 1), was significant and figured -27 and -29 %. Values of gross energy demand of production in experiments with food type II increased 20 and 24 % but not significantly discriminated from control experiments. Comparisons of production parameters were made on the basis of the third feeding week, because animals in the summer/autumn control experiment showed a growth retardation of unknown origin during the fourth feeding week.

Independent of the type of food supplied, a comparison of production parameters during both seasons studied revealed lower production rates (-45 %) and higher gross energy demands of production (64 and 70 %) in summer/autumn. These differences were significant and suggest a seasonal decrease of growth intensity from spring to summer/autumn. While Cd-induced relative decrease of production rates was more or less the same during both seasons, in absolute figures it was smaller in summer/autumn than in spring (cf. Tab. 1).

Field animals showed significantly increasing lipid but decreasing protein and energy values from spring to summer/autumn, while glycogen and Cd were not discriminated (Tab. 2). Feeding was generally followed by a significant increase of lipid and glycogen content or relative decrease of protein content, while energy contents only increased in summer/autumn experiments.

Table 1

Nereis succinea. Seasonal variations in growth parameters of standard worms (with 0.11 g organic substance at the end of the 28-day feeding period) fed with food of different Cd contents. Estimated mean and standard deviation of the estimated mean. P: production rate; C/P: gross energy demand of production; n: number of evaluated individuals. (15 °C, 15 °/oo S)

Season	Food type	Calculated initial weight (g_{ww})	Weight after feeding period of 28 d* (g_{ww})	P/21 d (g_{ww})	n	C/P (21 d ratio)
Spring	I	0.070	0.619 ± 0.019	0.417 ± 0.046	5	2.5 ± 0.3
	II	0.166	0.554 ± 0.012	0.302 ± 0.054	8	3.0 ± 1.0
Summer/ autumn	I	0.293	0.540 ± 0.015	0.234 ± 0.039	16	4.1 ± 1.1
	II	0.341	0.564 ± 0.008	0.165 ± 0.020	13	5.1 ± 1.1

* This weight was calculated for a standard worm with 0.11 g organic substance and gave the basis for the calculation of the initial weight of a standard worm.
ww Wet weight.

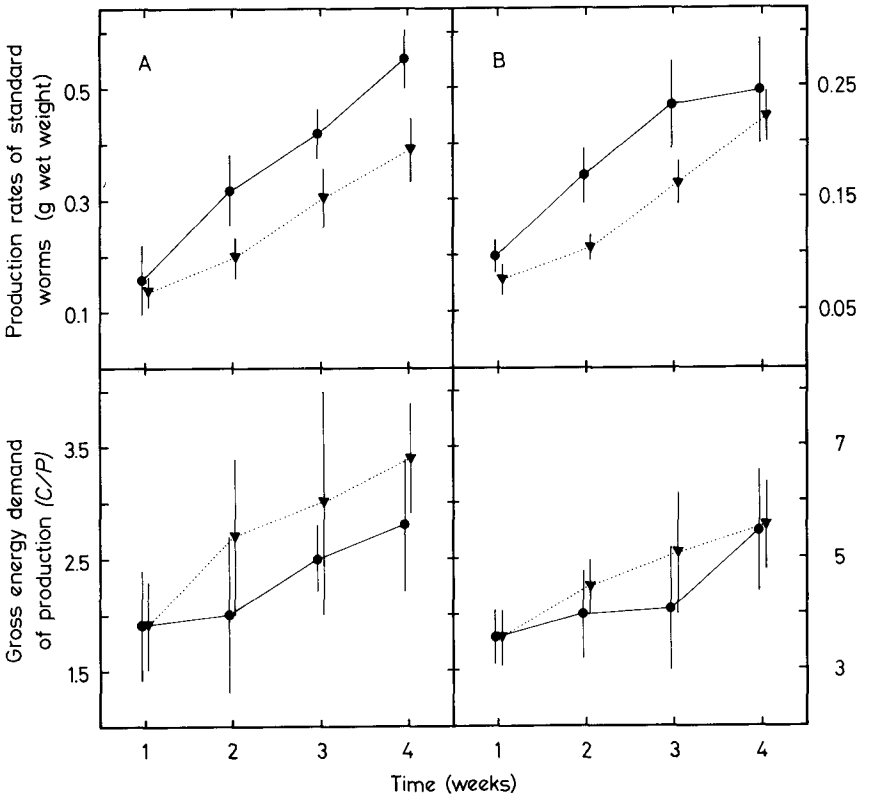

Figure 2. Nereis succinea. Changes of production rate (P) and gross energy demand of production (C/P) of worms with a standard content of 0.11 g organic substance at the end of 28-day feeding experiments (15 °C, 15 °/oo S). For initial weight see Tab. 1. A: Experiments were carried out in spring (April to June). B: Experiments were carried out in summer/autumn (August to October).
●—● Control animals fed with food type I
▼—▼ Worms fed with food type II

Cd contamination of the food had no consequences for standard worm composition or energy content (Tab. 2) but affected adenylate energy charge. Reference worms (0.35-0.55 g wet weight; food type I) showed a mean AEC of 0.91 (sd of the mean: ± 0.01; n: 5), animals fed with food type II had an average AEC of only 0.73 (sd of the mean: ± 0.09; n: 7). Due to high variations in the contamination experiment, AEC-levels were only discriminated at the 10 % level.

Time required by the worms to ingest the food portions provided, normally took up to two hours. Therefore the actual Cd concentration ingested with food type I may

TABLE 2

Nereis succinea. Seasonal variations in body parameters of a standard worm with 0.11 g organic substance fed 28 days with food of different Cd contents. Estimated mean and standard deviation of estimated mean. Experimental conditions: 15 °C, 15 °/oo S.

Season	Food type	Cd content (µg)	Lipid (%)	Glycogen (%)	Protein (%)	Energy (kJ/g)
Spring	Not fed*	0.08 ± 0.01	9.7 ± 0.5	3.6 ± 0.6	50.9 ± 0.6	22.578 ± 0.256
	I	0.13 ± 0.01	12.2 ± 0.4	15.4 ± 1.1	40.5 ± 0.7	22.311 ± 0.235
	II	1.25 ± 0.03	13.3 ± 0.4	16.1 ± 1.1	39.3 ± 1.0	22.827 ± 0.147
Summer/ autumn	Not fed*	0.06 ± 0.01	11.2 ± 0.5	4.4 ± 0.5	44.4 ± 0.8	21.572 ± 0.273
	I**	0.13 ± 0.02	15.9 ± 0.7	12.4 ± 1.3	34.7 ± 0.3	23.023 ± 0.265
	II	0.79 ± 0.03	14.5 ± 0.4	15.9 ± 2.5	41.1 ± 1.0	22.907 ± 0.167

Values are based on 5-10 determinations.
Structural components and energy content are given in relation to the ash-free dry weight.
* In situ worms.
** Repeated experiment (a year later). Protein values of in situ and test worms were considerably lower then.

have ranged from about 3.0 to 1.8 µg/g dry weight and from 97 to 40 µg/g dry weight with food type II (Fig. 1).

DISCUSSION

Sediments may function as traps for precipitating trace elements and thus can contain high levels of heavy metals. Ingestion of sediment particles, e.g. by burrowing polychaetes, however, can provide a route of uptake for trace metals, which may be mobilized in the digestive tract due to a lower pH of the gastric fluid (MOORE 1981). In experiments Cd may have been taken up from food as well as from the medium because leaching processes should have increased Cd content of the water to a certain extent. However, according to PENTREATH (1973) and YOUNG (1977) heavy metal uptake from the water is relatively less in comparison to uptake from the food. Higher Cd contents of N. succinea at the end of control experiments indicate that the food in the natural environment was less Cd contaminated than the Mytilus tissue used for food preparation.

A decrease of productivity towards summer/autumn, which was independent of Cd contamination, revealed the influence of the season, which, in spring, was found in high productivity rates in the laboratory corresponding to a steep increase of biomass and reserve compounds of N. succinea in the field prior to spawning (NEUHOFF 1979 b).

Cd-induced growth retardation can be found in larvae of M. edulis and various Crassostrea species (LEHNBERG and THEEDE 1979; WATLING 1982). Growth of fish is Cd-affected as well (KOYAMA and ITAZAWA 1977; WESTERNHAGEN et al. 1978). Young M. edulis (15-18 mm), however, may reach Cd concentrations of 150 µg/g dry weight without any harmful effects (POULSEN et al. 1982) while a much lower Cd uptake (7-12 µg/g dry weight) reduced the production rate of immature N. succinea on the order of -30 % and increased the gross energy demand of production.

While production of N. succinea was thus clearly affected, it is astonishing that the major compounds of the animals showed no effects which could be attributed to the Cd contamination of the food. Adenylate energy-charge values, however, were reduced to a level which, according to IVANOVICI (1980), indicates stress. This may either mean an over-proportional energy demand e.g. of Cd-immobilizing activities or it may represent consequences of Cd-inhibition of enzymes (EICHHORN 1975) involved in ATP production. A depleting ATP pool, however, may lead to a decrease of production rates because there is less energy available for anabolism.

ACKNOWLEDGEMENTS

The author thanks Prof H. Theede for his permanent interest in this work, for stimulating discussions and critical reading of the manuscript. The research was carried out with financial support by the 'Deutsche Forschungsgemeinschaft' to H. Theede (grant: Th 158/13).

REFERENCES

ATKINSON, D.E.: Biochem. 7, 4030 (1968).
EICHHORN, G.L.: Active sites of biological macromolecules and their interaction with heavy metals. Pages 123-142 in A.D. MCINTYRE and C.F. MILLS (eds.), Ecological Toxicology Research. New York: Plenum Press (1975).
ERLENKEUSER, H., E. SUESS, H. WILLKOMM: Geochim. et Cosmoch. Acta 38, 365 (1974).
GOERKE, H.: Veröff. Inst. Meeresforsch. Bremerh. 13, 1 (1971).
IVANOVICI, A.M.: Helgoländer Meeresunters. 33, 556 (1980).
KINNE, O.: Zool. Anz. 153, 114 (1954).
KOYAMA, J. and Y. ITAZAWA: Bull. Jpn. Soc. Sci. Fish. 43, 527 (1977).
LEHNBERG, W. and H. THEEDE: Helgoländer wiss. Meeresunters. 32, 179 (1979).
MANGUM, C.P.: Chesapeak Sci. 10, 64 (1969).
MOORE, M.N.: Elemental accumulation in organisms and food chains. Pages 535-569 in A.R. LONGHURST (ed.), Analysis of Marine Ecosystems. London: Academic Press (1981).
NEUHOFF, H.-G.: Mar. Ecol. Prog. Ser. 1, 255 (1979 a).
NEUHOFF, H.-G.: Mar. Ecol. Prog. Ser. 1, 263 (1979 b).
PENTREATH, R.J.: J. mar. biol. Ass. U.K. 53, 127 (1973).
POULSEN, E., H.U. RIISGARD, F. MØHLENBERG: Mar. Biol. 68, 25 (1982).
REISH, D.J. and R.S. CARR: Mar. Poll. Bull. 9, 24 (1978).
SACHS, L.: Angewandte Statistik. 4 ed. Berlin: Springer (1974).
THEEDE, H.: Helgoländer Meeresunters. 33, 26 (1980).
WATLING, H.R.: Bull. Environm. Contam. Toxicology 28, 195 (1982).
WESTERNHAGEN, H.von, V. DETHLEFSEN, H. ROSENTHAL, G. FÜRSTENBERG, J. KLINCKMANN: Helgoländer wiss. Meeresunters. 31, 471 (1978).
WITZEL, K.-P.: Arch. Hydrobiol. Beih. Ergebn. Limnol. 12, 146 (1979).
YOUNG, M.L.: J. exp. mar. Biol. Ecol. 30, 315 (1977).
Accepted June 27, 1983