SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 19, No. 12, pp. 3032–3035, 2000

Printed in the USA
0730-7268/00 $9.00 + .00

SEASONAL CHANGES IN ACUTE TOXICITY OF CADMIUM TO AMPHIPOD *COROPHIUM VOLUTATOR*

BELINDA J. KATER,*† ANDRÉ HANNEWIJK,† JAAP F. POSTMA,‡ and MARCO DUBBELDAM‡
†National Institute for Coastal and Marine Management/RIKZ, Fieldstation Jacobahaven, Jacobaweg 2, 4493 MX, Kamperland, The Netherlands
‡AquaSense Consultance, P.O. Box 95125, 1090 HC Amsterdam, The Netherlands

(*Received* 5 *November* 1999; *Accepted* 10 *May* 2000)

Abstract—In vivo bioassays are frequently used to assess the ecotoxicological risks of contaminated sediments. For quality assurance purposes, these bioassays are accompanied by reference toxicity tests. For the bioassay with *Corophium volutator,* this reference toxicity test is an acute water-phase test with cadmium. Approximately 80 water-phase tests were conducted in the period 1991–1998. *Corophium volutator* shows a significant seasonal variation in response to cadmium, with a high LC50 in the winter period and a low LC50 in the summer period. Same variation can be found if *Corophium* is held in the laboratory instead of freshly collected in the field and if synthetic water is used instead of natural filtered seawater. The observed seasonal variation is not caused by the fact that the organisms are collected in the field or by the variation in seawater used for the water-phase tests.

Keywords—Bioassay *Corophium* Cadmium Season

INTRODUCTION

In addition to chemical analyses, in vivo bioassays are used to assess the ecotoxicological risks of contaminated sediments. For quality assurance purposes, these bioassays are often accompanied by so-called reference toxicity tests. Reference toxicity tests are conducted as a quality control to get an impression of the condition of the test organisms and detect potential changes in sensitivity to contaminants that may influence the interpretation of marine bioassays [1]. In a reference toxicity test, the species sensitivity to a specific toxicant is analyzed and compared to criteria values. If these criteria are exceeded, care should be taken in interpreting the results of the bioassays performed since the organisms used appear to have an increased or decreased sensitivity. Setting the right criteria is therefore essential, especially considering the financial consequences that might arise if a sediment is judged to form a possible ecotoxicological hazard.

Numerous factors are known to affect species sensitivity to stressors like toxicants. Verriopoulos and Moraitou-Apostolopoulou [2], for example, found that environmental factors like dissolved oxygen concentration, temperature, salinity, and population density affected the response of the copepod *Tisbe holothuriae* to cadmium. DeLisle and Roberts [3] showed an effect of salinity on the response of the mysid *Mysidopsis bahia* to cadmium, while McCahon and Pascoe [4] showed differences in sensitivity of the freshwater amphipod *Gammarus pulex* from different age-groups to cadmium. An increased sensitivity of young animals of the marine amphipod *Marinogammarus obtusatus* as compared to adults was found by Wright and Frain [5]. McGee et al. [6] at last established that size, reproductive status, and molting cycle significantly affected the acute toxicity of cadmium to the estuarine amphipod *Leptocheirus plumulosus.* Most of the factors mentioned here can be controlled if bioassays are performed with laboratory reared animals but might affect bioassay results when the bioassays are performed using field-collected animals.

Little is known about seasonal variations in sensitivity to toxicants. McGee et al. [6] compared the response of the field-collected amphipod *Leptocheirus plumulosus* in May, August, and November. They concluded that field-collected organisms appeared to exhibit a seasonal variation in cadmium sensitivity. Other organisms have shown seasonal variation in laboratory water-phase tests, like the grass shrimp *Palaemonetes pugio* with sodium dodecyl sulfate [7] and the copepod *Acartia tonsa* with copper [8].

The present study focused on a benthic estuarine amphipod, *Corophium volutator.* This field-collected organism is one of the standard organisms used in the Netherlands, United Kingdom, and other European countries in bioassays to assess the toxicity of marine sediments. An acute water-phase test with cadmium is used as a reference toxicity test in this bioassay. The sensitivity of *Corophium* to cadmium in water-phase tests has been thoroughly investigated by our laboratory in the past nine years. Besides analyzing seasonal variation by using the available data set, additional experiments were performed to get a better understanding of possible factors influencing sensitivity to toxicants by comparing field-collected animals (tested within 10 d after collection) with organisms that had been maintained in the laboratory for three to seven months as well as comparing the response to cadmium in artificial seawater with that in natural seawater.

MATERIALS AND METHODS

Corophium volutator

The amphipods used in the present study were collected at the Oesterput, a relatively unpolluted site located in the Eastern Scheldt (The Netherlands). Seawater temperature varies in this area between an average of 5°C in January and 20°C in August. Seawater pH varied between 8.0 and 8.2 and salinity between

* To whom correspondence may be addressed (b.j.kater@rikz.rws.minvenw.nl).

28‰ and 32‰. Animals were collected by sieving sediment from the intertidal zone with seawater over a 500-μm mesh. The remaining sediment fraction, containing the animals, was transported to the laboratory, where the *Corophium* was acclimatized to the standard temperature of 15°C for at least 2 d. *Corophium* were not fed. Only animals not able to pass through a 500-μm mesh were used in the experiments. All standard reference toxicity tests were performed within 10 d after collecting the animals in the field. For the additional experiments, amphipods were kept in the laboratory for a prolonged period up to five months.

Standard acute water-phase toxicity assay

Acute water-phase toxicity assays with cadmium chloride ($CdCl_2$) dissolved in water were performed to evaluate the response during the year. *Corophium* was exposed for 72 h to the following nominal concentrations; 0, 1, 1.8, 3.6, 7.5, and 11 mg Cd/L. Cadmium chloride was diluted in seawater from the Eastern Scheldt filtered over a sand-bed filter removing particles larger than 10 μm. In selected experiments ($n = 11$) performed throughout 1994 and 1995, the actual cadmium concentration was measured in each concentration at the end of the experiment using atomic absorption spectroscopy.

Experiments were performed in 1-L glass beakers. Each beaker contained 20 randomly selected *Corophium* and was aerated continuously during the experiment. At the beginning and end of each experiment, salinity, oxygen saturation, pH, and temperature were measured. After 72 h (70–76 h), the number of surviving *Corophium* in each beaker was counted. Experiments were conducted regularly from 1991 onward, and the result of a total of 80 experiments are used for the present study.

Response of laboratory-kept animals

The first few years of standard experiments indicated a decrease in sensitivity during the spring. Additional experiments were performed during the spring of 1998 using animals that had been maintained in the laboratory for a prolonged period (three to seven months). The aim of these experiments was to get an impression of the possible effects of variation in field conditions on the response of *Corophium*. For these experiments, a large number of animals was collected in December 1997. *Corophium* was held at constant laboratory conditions (15°C, 16:8-h light:dark cycle, and daily refreshment of overlying water). Five water-phase toxicity tests with laboratory-maintained animals were performed in the period March 1998–July 1998 simultaneously with the experiments using animals freshly collected from the field.

Response in artificial seawater

The standard experiments were performed using natural seawater, and seasonal changes in its composition might affect toxicity. Additional experiments were therefore performed in which the response of *Corophium* to cadmium in natural seawater was compared to the response in artificial seawater. This artificial seawater was made by mixing deionized water with sea salt (Meersalz Professional, Krefeld, Weigandt, Germany) to a salinity of 32‰. Five experiments were performed in the period March 1998–July 1998. The addition of cadmium and performance of the test was as in the standard water-phase test.

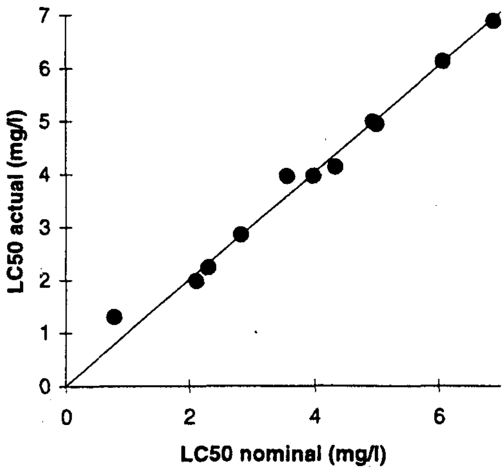

Fig. 1. Relation between LC50 values calculated using actual and nominal concentrations (line: y = x).

Statistical analyses

From each test, the LC50 was computed, after logarithmic transformation of the concentration data, using the trimmed Karber–Spearman method [9]. For some tests, LC10 and LC90 were calculated. Seasonal variation in LC50 was analyzed with an analysis of variance if the prerequisites for this test were fulfilled. If there was a significant difference between months, a post hoc test (Tukey's HSD) was used to test which months differed significantly from one another. Differences between LC50 derived from actual and nominal concentrations were analyzed with the Wilcoxon sign-rank test on pairs. For all statistical analyses, a probability level of 0.05 was used.

Comparing LC50 values with other data

To research possible explanations for observed variations in LC50 values, these values were correlated with other data on *Corophium*. If all prerequisites were fulfilled, a Pearson correlation was used. If prerequisites could not be fulfilled, a Kentall–Tau correlation was used, both with a probability level of 0.05. The average length of *Corophium* in the water-phase test was estimated by using length–frequency histograms of the *Corophium* Oesterput population [10]. Because there is no description of the reproduction of the Oesterput population, data of a *Corophium* population in the Dovey Estuary [11] are used.

RESULTS

Nominal versus actual concentration

The actual cadmium concentrations, measured in 11 experiments, were generally in agreement with the nominal values. On basis of the actual data, LC50 values were calculated and compared with LC50 values derived from the nominal concentrations in the same experiment (Fig. 1). The Wilcoxon sign-rank test on pairs showed no significant difference between both LC50s ($p = 0.594$). Further analyses were performed using LC50 values based on the nominal cadmium concentration.

Seasonal variation

The average LC50 for each month showed a seasonal pattern, with highest LC50 (= reduced sensitivity) in April and lowest LC50 (= high sensitivity) in August (Fig. 2). After logarithmic transformation, the data were analyzed with an

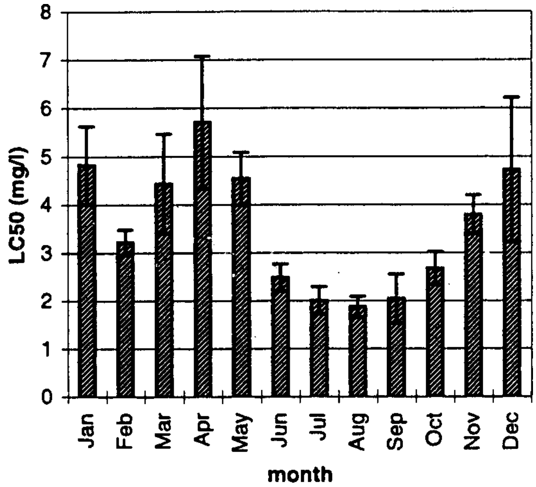

Fig. 2. Average LC50 of each month (1991–1998), with standard error, of the response of *Corophium volutator* to cadmium in an acute water-phase test.

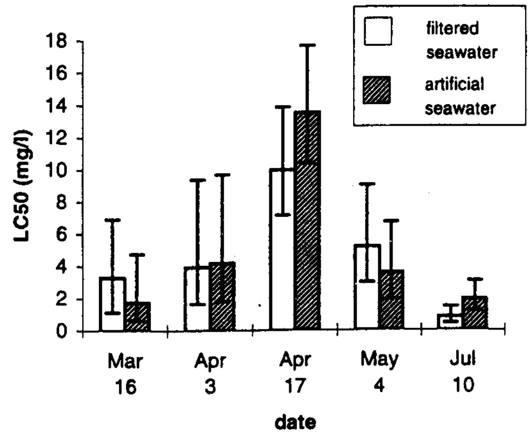

Fig. 4. LC50 values of the response of *Corophium volutator* to cadmium dissolved in filtered natural seawater and in artificial seawater with 95% confidence intervals. All experiments were performed in 1998.

analysis of variance, demonstrating significant differences between the different months. A Tukey HSD post hoc test showed that April, with the high LC50 value, differed significantly from the months in the period with a low average LC50 value (July, August, and September). The low LC50 value in August, on the other hand, differed significantly from the months with the high values (November, December, January, April, and May). Furthermore, the low LC50 values recorded in September showed significant difference with April, May, and November values.

In addition to these seasonal variations based on the LC50 values, results obtained during 1998 were used to calculate LC10 and LC90 values. Generally, these values showed the same patterns as compared to the LC50 values, and these results are therefore not presented.

Origin of the amphipods

To investigate whether changes in the field population might add to increasing LC50 values in April, toxicity experiments with newly collected animals were compared to experiments using animals that were collected the previous December. Based on the results (Fig. 3), this seemed not to be the case since the increase in LC50 values in April was observed for both newly collected animals as well as for animals that had been maintained in the laboratory at 15°C since collection. No significant differences were detected between both groups of animals (Wilcoxon sign-rank test on pairs, $p = 0.686$). Unfortunately, *Corophium* collected in December and held in the laboratory did not reproduce. Therefore, in July all amphipods died without a new generation, and it was impossible to conduct any further tests with this group of organisms.

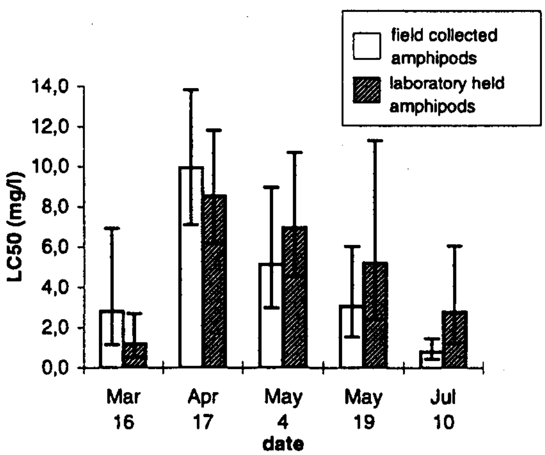

Fig. 3. Cadmium LC50 values of the response of laboratory-kept and freshly field-collected *Corophium volutator*, with 95% confidence intervals. All experiments were performed in 1998.

Artificial versus natural seawater

Based on the five experiments performed, no significant differences (Wilcoxon sign-rank test on pairs, $p = 0.686$) were detected in LC50 values for animals exposed in natural seawater as compared to animals exposed in artificial seawater with the same salinity (Fig. 4). The experiments therefore demonstrated that the increase in LC50 values as observed in April is not likely to be due to the quality of the seawater since this increase was also observed in the test using artificial seawater.

Effects of length and percentage gravid females

The average length of Oesterput *Corophium* larger than 5 mm varied between 5.8 mm (July) and 7.1 mm (May). There was no significant correlation between the average monthly length and average monthly LC50 value ($r^2 = 0.401$, $p = 0.197$, $n = 12$). There was also no significant relation between the percentage gravid female (data from Dovey Estuary) and the average monthly LC50 value ($\tau = -0.240$, $p > 0.05$, $n = 12$).

DISCUSSION

The response of *Corophium volutator* shows a significant seasonal variation with a period of a low sensitivity (high LC50) for cadmium in the winter period (November–April) and a high sensitivity (low LC50) in the summer period (July–September). Such seasonal variation has also been demonstrated for the estuarine amphipod *Leptocheirus plumulosus* [6], where the response in August was significantly lower than in May and November. The additional experiments performed in the present study indicate that the quality of the filtered seawater is not directly responsible for the observed seasonal pattern because synthetic water shows the same pattern compared to seawater. Neither of the natural fluctuations in the field (temperature and photoperiod) influences the seasonal variation because the response of laboratory held amphipods that are not confronted with seasonal fluctuations in the field show the same response pattern compared to the field-collected animals.

A possible factor explaining seasonal variation is the size of the animals. McGee et al. [6] found an increasing LC50 for cadmium in *Leptocheirus plumulosus* with an increase in size. The same relation was found in *Gammarus pulex* by McCahon and Pascoe [4]. The size of the amphipod used in the present study was limited to amphipods larger than 5 mm. Based on the record length–frequencies, there was no significant correlation between the average size present in the field and the sensitivity to cadmium. In addition, Ciarelli et al. [10] did not find a relation between the size of the amphipods and the responses on lindane-spiked sediment.

Besides size, the reproduction cycle is another factor that could affect the sensitivity of *Corophium* to cadmium. McGee et al. [6] found that gravid females of *Leptocheirus plumulosus* were more sensitive to cadmium than males or nongravid females. In the case of *C. volutator,* a biannual reproductive period is expected, with a winter generation breeding from February to July and dying during the summer and a summer-breeding generation that produces the next winter generation. This is recorded for both the Oesterput population [10] and the Dovey Estuary population [11]. In the Dovey Estuary, the number of gravid females is recorded each month. When the number of gravid females is compared to the seasonal variations in sensitivity to cadmium demonstrated in the present study, no significant correlation is noted. It therefore seems unlikely that the reproductive cycle or the number of gravid females might be the sole causative factor for the observed seasonal variations.

Meador [12] found that amphipods held in the laboratory showed a lower LC50 compared to amphipods freshly collected from the field. In that study, the increased sensitivity of laboratory-kept amphipods correlated with reduced lipid levels. An increased sensitivity of laboratory-bred animals was also found for *Palaemonetes pugio* [7] and *Repoxynius abronius* [13]. In our study, however, no difference was found between the LC50 values of organisms originating from a subpopulation held in the laboratory and organisms directly collected from the field. This indicates that the variation found in LC50 values during the year is not caused by a field-dependent factor like length of days or temperature.

In conclusion, the present study demonstrated a significant seasonal variation in the sensitivity of the amphipod *C. volutator* to cadmium. The results further indicated that fluctuations in the quality of the seawater, the natural water temperature, the size of the animals, or the reproductive cycle of the animals do not play an important role in this phenomenon in the animals collected and tested in our laboratory.

Attention should therefore focus on other explanations (e.g., the molting cycle) since it is known that there is a link between the molt cycle of crustaceans and cadmium sensitivity [14]. Postmolt crustaceans, which are actively taking up calcium ions in the recalcifaction process, showed an enhanced uptake and sensitivity to cadmium compared to pre- or intermolt animals [5,6]. Although many authors have described the biology of *Corophium* [15–17], the molt of the amphipod *C. volutator* has never been studied in detail. Future research will therefore focus on bioaccumulation of cadmium, which can be affected by the biological activity of the animals in relation to the need for calcium. Requirements for skeletal calcium are particularly high during the crustacean molt cycle and probably rely on active calcium pumps. Even in saline environments where calcium is relatively plentiful, rapid calcification is essential for postmolt crustaceans to feed, move, and avoid predation [18].

Besides future research on the influence of molt and bioaccumulation on the seasonal variation in LC50, research will be focused on the question whether this seasonal variation is a specific feature of cadmium or can be observed for other toxicants or other stress factors (e.g., pH, salinity). If the seasonal variation in sensitivity is indeed a general feature, attention should be paid to the guidelines for sediment toxicity testing. In that case, it might be necessary to set different limits on the acceptable LC50 values for positive control tests depending on the season.

Acknowledgement—Peter Schout and Johan Jol assisted during field sampling. Dick Vethaak, Tonny Wijte, and Hannie Maas provided useful comments on the manuscript, and Frank Vijverberg provided water-quality data.

REFERENCES

1. American Society for Testing and Materials. 1980. Reference toxicant in quality control of aquatic bioassays. In Buikema AL, Cairns J Jr, eds, *Aquatic Invertebrate Bioassays.* STP 715. Philadelphia, PA.
2. Verriopoulos G, Moraitou-Apostolopoulou M. 1981. Effects of some environmental factors on the toxicity of cadmium to the copepod *Tisbe holothuriae. Arch Hydrobiol* 91:287–293.
3. DeLisle PF, Roberts MH Jr. 1994. The effect of salinity on cadmium toxicity to the estuarine mysid *Mysidopsis bahia*: Roles of chemical speciation. *Mar Environ Res* 37:47–62.
4. McCahon CP, Pascoe D. 1988. Use of *Gammarus pulex* (L) in safety evaluation test-culture and selection of a sensitive life stage. *Ecotoxicol Environ Saf* 15:245–252.
5. Wright DA, Frain JW. 1981. Cadmium toxicity in *Marinogammarus obtusatus*: Effect of external calcium. *Environ Res* 24: 338–344.
6. McGee BL, Wright DA, Fisher DJ. 1998. Biotic factors modifying acute toxicity of aqueous cadmium to estuarine amphipod *Leptocheirus plumulosus. Arch Environ Contam Toxicol* 34:34–40.
7. Tatem HE, Anderson JW, Neff JM. 1976. Seasonal and laboratory variations in the health of grass shrimp *Palaemonetes pugio*: Dodecyl sodium sulfate bioassay. *Bull Environ Contam Toxicol* 16:368–375.
8. Sosnowski SL, Germond DJ, Gentile JH. 1979. The effect of nutrition on the response of field populations of the calanoid copepod *Acartia tonsa* to copper. *Water Res* 13:449–452.
9. Hamilton MA, Russo RC, Thurston RV. 1977. Trimmed Spearman–Karber method for estimating median lethal concentrations in toxicity bioassays. *Environ Sci Technol* 12:714–719.
10. Ciarelli S, Vonck APMA, van Straalen NM. 1997. Reproducibility of spiked-sediment bioassays using marine benthic amphipod, *Corophium volutator. Mar Environ Res* 43:329–343.
11. Fish JD, Mills A. 1979. The reproductive biology of *Corophium volutator* and *C. arenarium* (Crustacea: Amphipoda). *J Mar Biol Assoc UK* 59:355–368.
12. Meador JP. 1993. The effect of laboratory holding on the toxicity response of marine infaunal amphipods to cadmium and tributyltin. *J Exp Mar Biol Ecol* 174:227–242.
13. Robinson AM, Lamberson JO, Cole FA, Swartz RC. 1988. Effects of culture conditions on the sensitivity of a phoxocephalid amphipod, *Rhepoxynius abronius,* to cadmium in sediment. *Environ Toxicol Chem* 7:953–959.
14. Wright DA, Welbourn PM. 1994. Cadmium in the aquatic environment: A review of ecological, physiological, and toxicological effects on biota. *Environ Rev* 2:187–214.
15. Meadows PS, Reid A. 1966. The behaviour of *Corophium volutator* (Crustacea: Amphipoda). *J Zool Lond* 150:387–399.
16. Watkin EE. 1941. The yearly life cycle of the amphipod, *Corophium volutator. J Anim Ecol* 10:77–93.
17. Meissner K, Bick A. 1997. Population dynamics and ecoparasitological surveys of *Corophium volutator* in coastal waters in the Bay of Mecklenburg (southern Baltic Sea). *Dis Aquat Org* 29:169–179.
18. Wright DA. 1995. Trace metal and major ion interaction in aquatic animals. *Mar Pollut Bull* 31:8–18.