Bull. Environ. Contam. Toxicol. (1989) 43:747–754

Bulletin of Environmental Contamination and Toxicology

Seasonal Changes of Cadmium and Copper Levels in Stem-boring Larvae of *Agapanthia villosoviridescens* (Coleoptera) on Salt Marshes of the Westerschelde Estuary

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Analyses of heavy metals in insects, including their developmental stages, have been widely used to monitor the penetration of these pollutants in various ecosystems, or have been carried out in attempts to make ecological generalizations regarding transfer and accumulation along food chains. In many of these studies all specimens are collected during one sampling effort. Therefore, possible variations in heavy metal contents during the life cycle of the animals as a result of, for instance, a changing physiology of the animals or changing feeding habits and activities, remain undetected. Obviously, this may affect the generality of the conclusions on the usefulness of an insect species in monitoring programs, or regarding the mobility or bioconcentration of heavy metals in food webs.There are few reports dealing with seasonal changes in heavy metal content of insects; That such changes actually may occur is documented in a detailed study of Hunter et al.(1987a) who found a seasonal pattern of cadmium and copper accumulation in the grasshopper Chorthippus brunneus. The seasonal pattern found in this herbivorous insect closely followed seasonal trends in metal contamination levels in the local vegetation. Incidental observations reported by Stary and Kubiznakova (1987) and Mankowska (1987) indicate that heavy metal levels in wood ant workers and in coleopteran imagoes, respectively, also may show seasonal changes. To our knowledge no data are available on seasonal changes in insect larvae.

To obtain more detailed information on seasonal changes of heavy metal levels in insects and their relation with the seasonally changing conditions in the habitat, we studied the time course of cadmium and copper concentrations in larvae of the longhorn beetle Agapanthia villosoviridescens. These live as stem-borers in the salt marsh halophyte Aster tripolium. Details on the life cycle of this insect on estuarine salt marshes have been reported in previous papers (Hemminga et al.1987; Hemminga and van Soelen, 1988). In summer the imagoes pierce the flowering stems of the Aster host plants to deposit a single egg inside the marrow. The larvae which hatch from the eggs remain inside the stems

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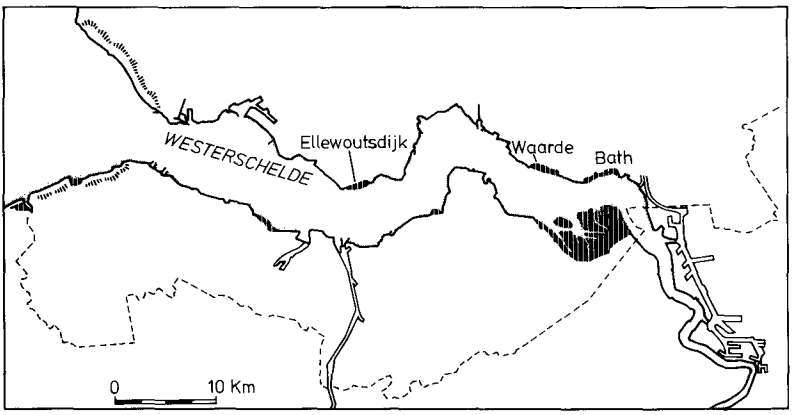

Figure 1. The Westerschelde estuary, with the location of the salt marshes sampled during the investigation.

throughout their larval existence, which lasts until spring of the following year, when pupation ensues. The Aster stems gradually die-back during autumn, but they generally retain their upright position throughout the winter and spring period, depending on the climatic conditions.
Using Agapanthia larvae implied the following advantages for this study: (1) as Agapanthia villosoviridescens has a synchronized development on estuarine salt marshes, the same generation of larvae can be followed in time; (2) this same generation of larvae lives through successive seasons, i.e. from summer to the next spring; (3) on salt marshes their food source is clearly defined, being restricted to the stems of one food plant; (4) the larvae are sedentary and remain in the same stem during the entire larval period; therefore the same population can be followed in time, as there is no exchange of larvae between the sampling site and neighbouring localities.

We collected larvae from three salt marshes along the Westerschelde estuary. This estuary is severely polluted by heavy metals originating mainly from upstream sources (van der Kooij, 1982); a large fraction of these metals is retained within the estuary (Salomons et al. 1981). The fringing salt marsh soils, which are a sink for trace metals, show a gradient in pollution, with levels of heavy metals generally increasing in upstream direction (Beeftink et al.1982). Salt marsh halophytes growing on these marshes show uptake of metals from the soil (Beeftink et al.1982; Beeftink and Nieuwenhuize, 1986). Further transfers of heavy metals through the natural food chains on these salt marshes have not been investigated sofar.

MATERIALS AND METHODS

The location of the three estuarine salt marshes sampled during the investigation is shown in Fig.1. In each marsh, a 100X25 m part of the middle marsh area was selected as sampling site. Flowering stems of Aster tripolium, which showed circular marks

due to ovipository activity of Agapanthia females, were collected periodically from each sampling site (30-100 stems per sampling site, per location) from late summer 1986 until the beginning of spring 1987. In the laboratory, the stems were cut open longitudinally; living Agapanthia larvae, when present, were collected. The larvae were freeze-dried, weighted and analyzed for Cd and Cu as described in Nieuwenhuize et al. (1988).
For each metal the data were analysed in a two-way analysis of variance, where sampling time was one treatment and sampling station the other. The number of replicates (13) for the statistical procedure was equalized by deleting randomly assigned data. To relate Cd and Cu body burdens to body weight, a logarithmic transformation of the data was carried out, as the regression lines computed from the transformed data fitted the data better than without transformation. Covariance analysis was subsequently carried out on the various regressions.

RESULTS AND DISCUSSION

A detailed account of the growth and development of the Agapanthia larvae during the 1986-1987 season has been given in Hemminga and van Soelen (1988). However, for the sake of clarity of the present results, a few observations will be repeated here. After hatching of the larvae in August they rapidly gain weight: the autumn months are a period of growth. In upstream direction the larvae show a better growth performance, which is probably due to the changing host plant quality along the salinity gradient of the estuary. During winter and spring mean larval weights fluctuate. The mean larval dry weights attain their peak values in this period; this period, however, is probably more characterized by mortality than by growth: selective mortality of small larvae instead of actual growth may well cause the (moderate) increase in mean weights.

Fig. 2 shows the time course of the Cd and Cu levels in the larvae collected on the three marshes between 1 September and 24 March. The results of the analyses of variance are summarized in Table 1. The time course of the Cd and Cu levels shows a conspicuous difference: whereas there is no effect of time with respect to the Cu levels, the effect of time on Cd levels is highly significant. In autumn the Cd levels show a sharp decline, remaining relatively low thereafter. Within the same generation of larvae, Cd levels may be found that differ by a factor of more than ten, depending on the sampling date.

The differences between Cd and Cu may be due to a changing bioavailability of these metals in the food ingested, or, alternatively, to different physiological mechanisms involved in metabolism of Cd and Cu in the larvae. A changing bioavailability of the metals in the marrow tissue of the Aster stems, the food of the larvae, as such is not unlikely. In autumn, after flowering of the sea asters, the stems die-back. The ensuing slow decay process undoubtedly will change the chemical characteristics of the stem tissues. Temperatures, however, also decrease steadily in this

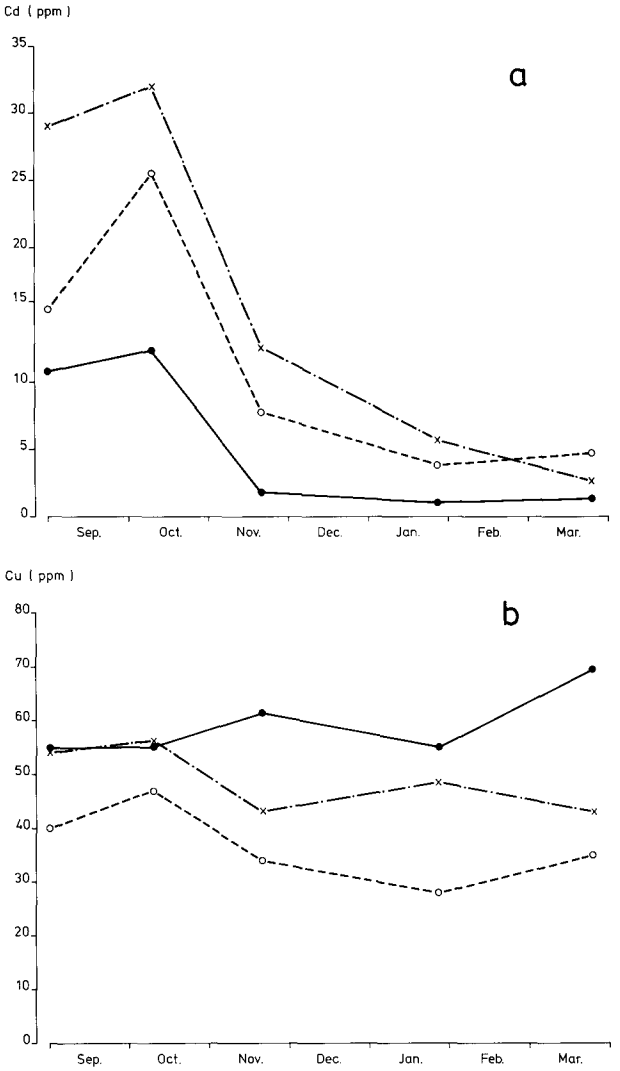

Figure 2. Time course of (a) cadmium and (b) copper levels (on dry weight basis) in larvae of Agapanthia villosoviridescens from three salt marshes of the Westerschelde estuary. Each point represents the mean value of 13 observations.
Bath: ———— ; Waarde: - · - · - · - ; Ellewoutsdijk: -------

period. After the intense feeding in the late summer and early autumn period, food consumption of the larvae in the stems probably will diminish and ultimately will stop as a result of these falling temperatures. That cessation of ingestion of marrow tissue actually does occur in the course of the autumn period is suggested by observations carried out on October 9th and November 20th; these demonstrated that the length of the cavity in the stems (caused by the feeding activity of the larvae) did not significantly increase in this period (results not shown). If food consumption is resumed at the end of the larval period, in

spring, is uncertain: the stem marrow tissue by then has completely disappeared as a result of decomposition processes. Larval activity in this period results in the accumulation, on both ends of the larval cavity, of scrapings from the wood cylinder; we do not know if this material also is consumed. If we assume, in view of the considerations regarding the diminishing food consumption in winter given above, that a potentially changing bioavailability of the metals in the food source is not relevant, it follows that (1) different physiological mechanisms operating in the larvae cause differences in the time course of Cd and Cu levels and (2) that fluctuations of heavy metal levels in the larvae may be caused by an accumulation-elimination cycle, i.e. accumulation coinciding with food consumption until mid autumn, and elimination after cessation of food intake during the late autumn and winter period.

The decreasing Cd levels in autumn and winter suggest that Cd is rapidly eliminated from the larval body between the sampling in October and November. The decrease in Cd concentrations levels off towards the end of the year and from January onwards further excretion of Cd apparently does not occur, or only at a slow rate. From an accumulation-elimination experiment with lead in Orchesella cincta (Collembola), van Straalen and van Meerendonk (1987) concluded that this heavy metal was present in three compartments, each showing different elimination rates. Cd in Agapanthia may show a comparable compartmented behaviour. In this respect it is interesting that according to Sumi et al. (1984) Cd accumulated in the fat body in the larva of the midge Chironomus yoshimatsui was eliminated slowly relative to Cd present in the digestive tract contents. The field data shown in the present study are difficult to interpret in terms of different compartments, as also changing temperatures during the

Table 1. Analysis of variance of Cd and Cu levels in larvae of Agapanthia villosoviridescens, studied from Sept. 1986 - March 1987 on three salt marshes of the Westerschelde. ** $p < 0.01$; *** $p < 0.001$.

	SS	DF	MS	F	p
CADMIUM					
Time	13490.6	4	3372.65	34.24	***
Marsh	3877.3	2	1938.65	19.68	***
Time * Marsh	2046.8	8	255.85	2.60	**
Error	17732	180	98.51		
COPPER					
Time	1811.7	4	452.92	1.01	n.s.
Marsh	14142.7	2	7071.35	15.75	***
Time * Marsh	4669.1	8	583.63	1.3	n.s.
Error	80800	180	448.89		

observation period will have affected elimination rates. The important point to stress here, however, is the indication given by the data that under field conditions Cd levels may change profoundly during larval life, probably as a result of Cd accumulation-elimination phases which are related to changing environmental conditions experienced by the larvae. At the same time, elimination of Cu apparently does not occur after the cessation of feeding activity, as levels remain constant throughout the winter period. A possible explanation for this phenomenon might be found in the fact that, in contrast to Cd, Cu is an element of importance in biological processes. The levels of Cu in the larvae are in the same order of magnitude as the levels reported for various insect species living under (relatively) unpolluted conditions (e.g. van Straalen et al.1987; Stary and Kubiznakova, 1987; Hunter et al. 1987a,b). Cu in the larvae of these salt marshes may not have reached surplus levels, and it is possible that it is therefore retained in the body as physiologically useful. This point remains unresolved here.

A further point of interest is whether the relation between Cd and Cu body burden and larval weight changes in time. This relation is often used in the analysis of environmental pollution of different localities (Martin and Coughtrey, 1982). We investigated this matter using the data series of the Bath marsh. Analyses of covariance were carried out on the regressions between body burden and larval weights computed for each time point. The differences among adjusted means are highly significant both for Cd ($p<0.001$) and for Cu ($p<0.01$). There are no significant differences, however, between slopes, neither for the Cd nor for the Cu series. Thus larvae of similar weight may differ in their body burden on

Table 2. Cd and Cu levels in Westerschelde salt marsh soils and in Aster tripolium and larvae of Agapanthia villosoviridescens.
* Data from Beeftink et al. (1982)

CADMIUM (ppb)	Ellewoutsdijk	Waarde	Bath
* Marsh soil	690	1595	3095
* Aster tripolium (middle marsh)	1200	5400	4240
Larvae (October)	25570	32260	12370
Larvae (March)	4840	2670	1420

COPPER (ppm)	Ellewoutsdijk	Waarde	Bath
* Marsh soil	21.3	27.8	35.2
* Aster tripolium (middle marsh)	8.1	3.7	11.7
Larvae (October)	47.82	51.37	54.95
Larvae (March)	35.57	43.55	69.74

different time points during the larval period, which can be explained, at least with respect to Cd, to accumulation and elimination phenomena mentioned above. The analysis, moreover, shows that the increase of body burden with larval weight is independent of larval age, indicating that the various physiological mechanisms by which this relation is brought about remains unchanged during the larval period.

In Table 2 Cd and Cu levels in the larvae are compared to literature data on the concentrations of these metals in the soils of the salt marshes of this study and in the shoots of Aster tripolium collected from these marshes. Cd and Cu levels in the salt marsh soil increase in upstream direction (Beeftink and Nieuwenhuize, 1982). The metal levels in Aster tripolium, measured by the same authors, do not show this trend; there is a lack of correlation between metal levels in sediment and plants of the various marshes: apparently the metals are sequestered partly in forms unavailable to the plants. A lack of correlation is also obvious on the next level of transfer, from plants to larvae. The data show that, at least during the feeding period in autumn, the larvae concentrate Cd and Cu in their tissues to levels well above those reported for their host plant, a phenomenon also observed for Cd in larvae of bark-feeding beetles (Vogel, 1986). These findings indicate that in the food chain plant-insect larva, bioconcentration may occur with respect to this metal. A cautionary remark must be made here, as the data on levels in Aster pertain to the complete plant, while the larvae feed only in the flowering stems. According to Beeftink and Nieuwenhuize (1986),however, differences in Cd and Cu levels between various above-ground parts of Aster, including the stems, are only limited. For Cd, the levels in the larvae are high as compared to the Aster levels only in autumn; when levels have decreased in the winter period, the Cd concentrations in the larvae are of the same order of magnitude as values in the host plants during their growth period. It is obvious that the sampling moment in this case will determine the outcome of questions on transfer and bioconcentration.

In conclusion, the present study shows that within larval populations of Agapanthia villosoviridescens, Cd levels strongly fluctuate in time. This phenomenon was not found for Cu. The variable Cd level is interpreted as being the result of changing environmental conditions in the field during the larval period, i.e. lowering temperatures causing the cessation of feeding activity. The present study illustrates the importance of knowledge of the life history of organisms and of seasonal sampling to draw correct conclusions when animals are used in biomonitoring programs or when food chain transfer or bioconcentration phenomena are studied.

Acknowledgments. We thank Dr. P.M.J.Herman for advice on statistical analysis and Drs. C.H.R.Heip and A.H.L.Huiskes for valuable comments on the manuscript.

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Received January 6, 1989; accepted May 14, 1989.