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METAL ACCUMULATION AND LOSS BY *CROCOTHEMIS SERVILIA* (DRURY) IN A SMALL LAKE IN SHILLONG, NORTHEASTERN INDIA (ANISOPTERA: LIBELLULIDAE)

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Concentrations and body burdens of copper, cadmium, lead, manganese, zinc, and calcium were measured in the larva and imago. Metal concentrations were greatly affected by size, with the smaller larvae generally having higher concentrations of all metals except copper. Metal body burdens and size, in contrast, were positively correlated. When metal concentrations in different larval size-classes were analyzed, copper levels were not found to exhibit significant changes with increasing weight, while calcium and zinc concentrations levelled off beyond a certain weight. Cadmium, lead, and manganese concentrations, on the other hand, registered steady declines with increasing weight. The body burdens of copper, calcium and zinc increased consistently with increasing weight, while those of cadmium, lead and manganese declined after an initial increase. The concentrations and body burdens of all metals were higher in the final instar larva when compared to those in the adult, except that calcium concentration was higher in the latter. During the final moult, higher proportions of cadmium, lead and manganese were lost along with the exoskeleton than those of copper, calcium and zinc. Thus, the exoskeleton of *C. servilia* might play an important role in sequestration and eventual elimination of excess metals, particularly the non-essential ones.

INTRODUCTION

Freshwater macroinvertebrates are known to accumulate metals and metal concentrations in their tissues are often employed to directly monitor biologically available metal contamination in the environment. However, several factors are known to influence metal accumulation in tissues, one of them being body size. Results obtained from several studies attempting to explain the relationship between body size and metal concentrations in animals (JOHNELS et al., 1967; CROSS et al.,

1973; BOYDEN, 1977; WILLIAMSON, 1980; ZAROOGIAN, 1980; STRONG & LUOMA, 1981; PHELPS et al., 1985; RAINBOW & MOORE, 1986; KRANTZBERG, 1989) indicate that correlations between metal concentration and body size differ among species and among metals, being positively or negatively correlated, or insignificant. Even within a single species, differences between populations are known to occur (reviewed by GREENAWAY, 1974; BOYDEN, 1977; STRONG & LUOMA, 1981). Furthermore, KRANTZBERG (1989) suggested that in addition to concentration, it was equally essential to estimate the metal body burdens, i.e. the total metal content of an individual. Metal concentrations in tissues may change while metal burdens remain constant or vice versa. Therefore, determination of the relationship between body weight and metal concentration alone is likely to mask the dynamics of metal uptake, storage, and accumulation. As most of the previous studies were conducted in temperate latitudes and involved omnivores or herbivore-detritivores, one objective of the present study was to investigate the relationship between body size and metal concentrations and body burdens in a carnivorous insect larva inhabiting a subtropical lentic system. Metal concentrations and body burdens in the adult were also determined to investigate any changes occurring at emergence. On organism's body burden is the total of the metal load on its surface and that which is incorporated internally. Hence, by comparing the adult body burden with that of the final instar larva, the amounts of metals bound to the larval exoskeleton can be determined (KRANTZBERG & STOKES, 1988). Furthermore, RAINBOW & MOORE (1986) showed that while metal concentrations rose sharply with decreasing body weight below about 2 mg, the rate of change of metal concentrations with weight was generally much reduced at weights above 2 mg. Hence the present study also examined the metal concentrations and body burdens in different larval size classes and in the adult.

MATERIAL AND METHODS

Crocothemis servilia (Drury) larvae were collected from Ward Lake, a small artificial lake in Shillong (25°34'N; 91°52'E), Meghalaya State, northeastern India, with a hand net (mesh size 60 μm) on 4 occasions within a one-week period during June 1993. They were kept in filtered lake water for two days to allow defaecation and were then sorted into size classes of 2 mm interval (total size range: 2--18 mm). Four to seven larvae of each size class were pooled and dried at 60°C to constant weight. A total of 33 weight classes were obtained from the dried samples, their mean individual dry weights ranging from 0.28 mg to 24 mg. The samples were then digested to dryness in 10 ml boiling concentrated HNO_3. The residues were then re-dissolved in 20 ml 10% HNO_3 and analyzed for Cu, Cd, Pb, Zn, Ca and Mn in a Perkin-Elmer 2380 atomic absorption spectrophotometer by the flame method. Several final instar larvae were reared in the laboratory to obtain adults; these were similarly processed and analyzed. Procedural blanks, acid washed glassware, analytical grade reagents and double--distilled deionized water were used to reduce contamination errors. Replicate lake water samples were collected and analyzed for pH, conductivity, alkalinity and oxygen content using standard methods (MICHAEL, 1984). Concentrations of Cu, Cd, Pb, Zn, Ca and Mn in the lake water were also determined by atomic absorption spectrophotometry after collecting water samples in clean, acid--washed PVC bottles and acidifying them in the field with 1 ml concentrated HNO_3.

Regression and Correlation analyses were carried out according to BHATTACHARYA & JOHNSON (1977).

RESULTS

Data on water quality, including Cu, Cd, Pb, Zn, Ca and Mn concentrations in the lake water, are given in Table I. Alkalinity and conductivity were both low, while the pH was slightly acidic. Metal concentrations followed the order Ca > Cu > Zn > Pb > Mn > Cd.

Table I

Data on water quality, including metal concentrations (mean ± standard deviation), in Ward Lake, Shillong, India, during June 1993

pH	Conductivity ($\mu s\ cm^{-1}$)	Dissolved oxygen (mgl^{-1})	Alkalinity (mgl^{-1})	Concentration (gl^{-1}) Cu	Cd	Pb	Zn	Ca	Mn
6.2±0.25	83.3±14.2	6.9±0.26	39±4.2	101±8	<0.5	14±5	59±25	592±50	10.8±3.7

Details of double-log regressions between metal concentrations and dry weight (Tab. II) revealed a clear size effect for all metals except copper, with smaller larvae having higher metal concentrations. When a wide range of weights was considered, relationships between body weight and metal concentrations were negatively significant for all metals except copper. In contrast, regressions of weight versus body burdens (content) were positively significant for all metals, indicating a gain in body burdens with increasing weight (Tab. II).

The larval samples were further sorted into five size classes (i.e. 0-5 mg, 5-10 mg, 10-15 mg, 15-20 mg, and 20-25 mg; mean dry weights 2.7, 6.6, 12.7, 17.4 and 23 mg respectively). The largest weight class contained final instar nymphs only. The mean metal concentrations ($\mu g\ g^{-1}$) and metal body burdens (ng individual^{-1}) in

Table II

Details of the relationship Log C = Log a + b Log W for *C. servilia* larvae analysed for Cu, Cd, Pb, Zn, Ca and Mn where C is metal concentration ($\mu g\ g^{-1}$ dry weight)/content(ng), W is dry weight (mg); a and b are constants – [SIG = significance of regression; – NS = not significant; – **P<0.01; – ***P<0.001]

	Copper log a	b	SIG	Cadmium log a	b	SIG	Lead log a	b	SIG	Zinc log a	b	SIG	Calcium log a	b	SIG	Manganese log a	b	SIG
Concentration	1.52	0.094	NS	1.11	-0.479	***	2.8	-0.452	***	2.38	-0.229	**	2.68	-0.289	**	2.21	-0.412	***
Content (body burden)	1.25	1.29	***	0.924	0.501	***	1.72	0.783	***	1.99	1.09	***	2.36	0.958	***	1.84	0.872	***

these five classes and in the adults are presented in Figure 1. In the larvae mean copper concentrations fluctuated around 35-50 μg g^{-1} dry weight without showing pronounced differences among the size classes, while zinc and calcium concentrations after an initial decline, more or less levelled off beyond size-class 3 (mean dry weight 12.7 mg). In contrast, cadmium, lead and manganese concentrations consistently decreased till the final instar, although cadmium concentration in size

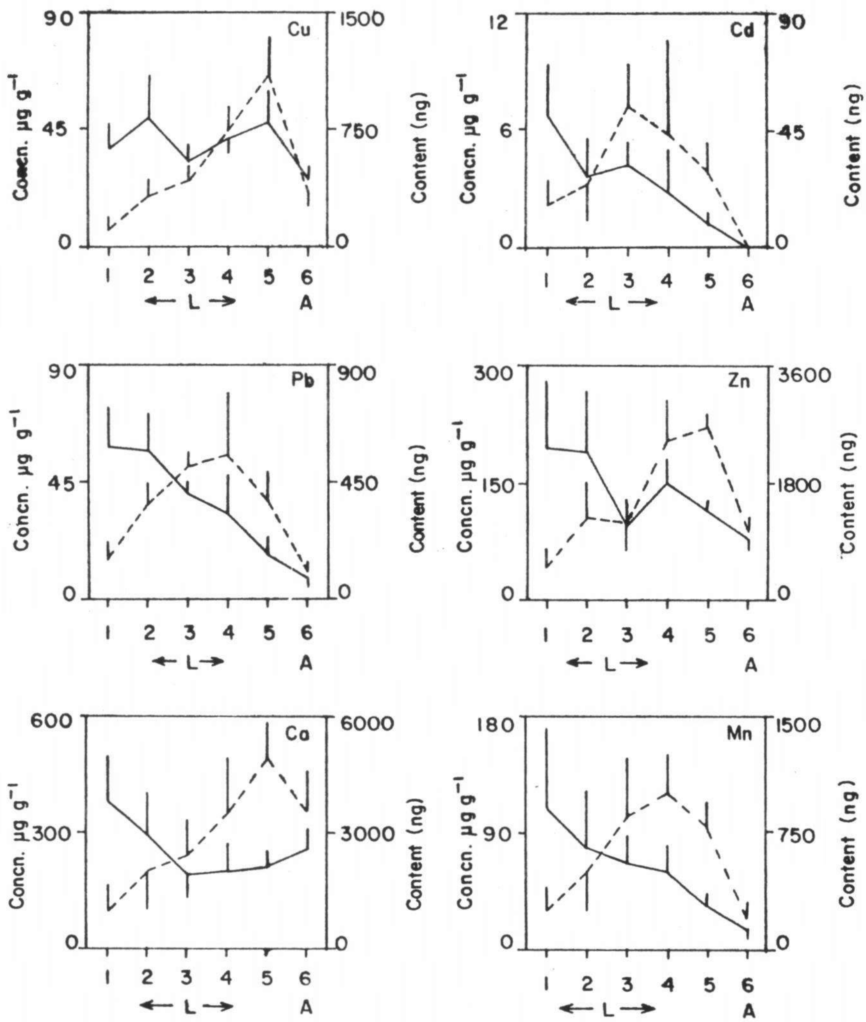

Fig. 1. Concentrations (solid lines) in μg g^{-1} dry weight and body burdens/contents (broken lines) in ng $individual^{-1}$ of Cu, Cd, Pb, Zn, Ca and Mn in the larvae and adults of *Crocothemis servilia* – [L 1--5 = larval size classes; – A6 = adult stage]

classes 2 and 3 (mean weight 6.6 and 12.7 mg respectively) did not differ appreciably from each other. Body burdens of copper, zinc and calcium increased with increasing weight, while those of cadmium, lead and manganese, after an initial increase, decline beyond size class 3 or 4 (Fig. 1).

Table III
Proportions (%) of metal burdens in the cuticle of *C. servilia*

Metal burden in cuticle (%)					
Cu	Cd	Pb	Zn	Ca	Mn
67.8	100	74.1	59.3	28.4	75.8

Both concentrations and body burdens of metals registered decreases in the adult, except that calcium concentrations were relatively higher in the adult than in the final instar larva (Fig. 1). The proportions of metals bound to the cuticle of the final instar larva are shown in Table III and followed the order Cd>Mn>Pb>Cu>Zn>Ca.

DISCUSSION

Log metal concentrations was negatively correlated with log body weight for all metals except copper (Tab. II). RAINBOW & MOORE (1986) observed similar size effects for copper, iron, zinc and lead in several species of amphipods collected from a wide range of habitats. They advanced the view that with decrease in animal size and concomitant increase in surface area: volume ratio, larger proportions of metals were passively adsorbed onto the cuticle, thereby producing the size effect observed. It was also suggested that growth might dilute metal concentrations in an organism if tissue was added faster than metal (AOYAMA et al., 1978; STRONG & LUOMA, 1981). In any case, the exact nature of the relationship between metal concentrations and body weight is known to differ widely among species, among metals, and even among conspecific populations (BOYDEN, 1977; STRONG & LUOMA, 1981). Nevertheless, results obtained so far suggest that it would be meaningless to compare mean metal concentrations among aquatic invertebrates without allowance for size/biomass- dependent variations. Whenever such variations influence metal concentrations, it is difficult to compare metal concentrations in a given species in different habitats or seasons, because uniformly sized individuals may not be available, a situation often met with during field-based biomonitoring (KRANTZBERG, 1989). However, in tropical and subtropical systems, where individuals of all size-classes are usually present almost throughout the year, metal analysis of randomly collected samples are expected to generate less of such size-induced errors.

Regression analyses (Tab. II) as well as metal concentrations in different size-classes (Fig. 1) revealed that copper concentrations did not show any definite trends, whereas zinc and calcium concentrations, after an initial decrease with increasing body weight, more or less levelled off beyond a mean dry weight of c 12 mg. This probably indicates that *C. servilia* larvae have some ability to regulate the body concentrations of all these metals. Such regulatory ability is known to be a feature of a wide range of organisms, with annelids known to regulate zinc and manganese (IRELAND, 1975; ROBERTS & JOHNSON, 1978), decapod crustaceans copper and zinc (RAINBOW & MOORE, 1986), and chironomids zinc, nickel, copper and manganese (KRANTZBERG, 1989). In contrast, continuous decline in the concentrations of cadmium, lead and manganese with increasing body weight (Fig. 1) and the highly significant and negative regression coefficients for these metals (Tab. II) suggest that they are not likely to be under homoeostatic control in *C. servilia*. Greater cadmium concentrations in smaller individuals were found in chironomid larvae (HEMELRAAD et al., 1986; KRANTZBERG, 1989), copper and cadmium in *Baetis rhodani* Pict. (SOECHTIG, 1989), and copper and silver in certain populations of bivalves (STRONG & LUOMA, 1981).

The relationships between metal body burdens and dry weight, in contrast to those between concentration and weight, were positively significant (Tab. II). Figure 1 also reveals that there was a progressive increase in copper, zinc and calcium burdens with increasing body weight. This would imply a net uptake of these metals throughout the life of a species (CROSS et al., 1973; GIESY & WIENER, 1977; WILLIAMSON, 1980), and the results obtained in the present study reflect sustained requirements of copper, zinc and calcium throughout the larval life span of *C. servilia*. However, cadmium, lead and manganese body burdens were found to decline after an initial increase upto the 3rd or 4th size classes. A decline in metal body burdens could possibly occur as a result of either or both of two major mechanisms: either they were excreted out at a higher rate than they were accumulated, or were sequestered in the exoskeleton and then eliminated during ecdysis, as has been observed in crustaceans (ALIKHAN et al., 1990; BARDEGGIA & ALIKHAN, 1991). Although the amounts of metals sequestered in the exoskeleton during the earlier larval moults were not investigated in the present study, the proportions of cadmium, lead and manganese lost along with the exoskeleton during the final moult in *C. servilia* were higher than those of copper, zinc and calcium (Tab. III). In Canadian lakes with pH above 5.1 (as in Ward Lake), KRANTZBERG & STOKES (1988) observed that larger proportions of cadmium and lead as compared to copper, zinc and calcium were lost along with the exoskeleton during emergence in chironomids. Hence, the exoskeleton appears to play an important role in eliminating excess metals, especially the non-essential ones, in aquatic insects, as has also been observed in certain crustaceans (ALIKHAN et al., 1990; BARDEGGIA & ALIKHAN, 1991).

The concentrations of all metals except calcium were relatively low in the adult. Calcium concentrations registered a steady rise from the 3rd size class onwards, and this trend was continued in the terrestrial adult phase. Higher calcium levels in the larger larvae and in the adult could indicate an increased metabolic demand for this metal, especially in the adult, the reasons for which, however, are not clearly understood at present.

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