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Effects of diet and heavy metals on growth rate and fertility in the deposit-feeding snail *Potamopyrgus jenkinsi* (Smith) (Gastropoda: Hydrobiidae)

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Abstract

Evidence for the influence of food type and heavy metals on shell growth and fertility is presented for a freshwater population of the snail *P. jenkinsi*. When fed an excess of lettuce or lamb heart (protein source), growth rates were higher for lettuce. Highest growth rates occurred at a diet of lettuce plus lamb heart. Fertility was favoured by a diet of lamb heart. When fed an excess of lettuce, the EC_{50} growth values were 16 μg Cd l^{-1}, 13 μg Cu l^{-1}, and 103 μg Zn l^{-1} in lake water; snail fertility was inhibited at 25 μg Cd l^{-1} and 30 μg Cu l^{-1}. A diet of lake detritus spiked with Cd or Cu resulted in a decrease of approximately 50% in growth rates, when compared with growth on non-spiked detritus. Spiked detritus lost metals into lake water. Food type positively interacted with metal stress, both for growth rate and fertility. The assessment of inhibitory effects of detritus contaminated either in the field or, notably, by spiking, and serving as food source for deposit feeders is hampered by sampling problems in the field and by redistribution processes of pollutants between particles and water in laboratory-scale experiments.

Introduction

Organic matter in sediments sustains the detritus food chains, by which sediment-trapped toxicants enter the detrital pathway, becoming available to bottom-dwelling, sediment-ingesting organisms. So, contaminated detrital particles may be an ecotoxicological key factor in the nutritional biology of detritivores (Simkiss, 1990).

Studies on detritus as potential harmful agent for benthic organisms are very scarce. In former papers (Dorgelo, 1988; 1991), it was concluded that growth rates of the detritivorous snail *Potamopyrgus jenkinsi* on natural sediments were positively correlated with the productivity of lakes. Though *P. jenkinsi* exhibits a strong variation in yearly numbers (Dorgelo, 1987), densities up to 72 300 per m^2 have been reported from a Polish lake (Michalkiewicz, 1991), and 88 125 from an English chalk stream (Heywood & Edwards, 1962). By that criterion, this species is an important processor in the detritus cycle. In this study, first, sublethal effects on growth rate and fertility (number of young produced per unit of time) of this snail were determined under long-term exposure to dissolved metals in relation to artificial food types, and, second, the snails were fed spiked natural detritus in order to assess the effects of dietary metal uptake on growth.

Material and methods

Animals and food types

Potamopyrgus jenkinsi is a euryoecious (Dorgelo, 1987), deposit-feeding snail, characterized by parthenogenesis and ovovivipary. Animals were obtained from a laboratory-reared population, originating from the meso-oligotrophic Lake Maarsseveen I (Dorgelo, 1991), lying in the center of The Netherlands. This culture was kept in acid-washed (0.1 N nitric acid; Merck Pro Analysis) 10 l plastic aquaria filled with particle-free (filtered through a 0.45 μm pore size) lake water maintained at 15 °C above 1 cm of sandy Lake Maarsseveen substrate. A depth of 1 cm accommodates the behavioural requirements of *P. jenkinsi* (adults are approximately 2 mm wide and 5 mm long), which live upon the substrate or just beneath its surface. The sand was sieved through a 0.6 mm pore size, treated with hydrochloric acid and thoroughly washed afterwards. The concentrations of Cd, Cu and Zn in unspiked lake water (pH 7.8–8.2) as well as of its major substances are given in Table 1. The aquaria were placed in a room with natural daylength conditions. The snails had a diet of fresh, raw lettuce (*Lactuca sativa*; grown in a greenhouse on the campus in order to level out its metal content), Aufwuchs on the walls of the aquaria and, together with developing detritus, on the substrate.

The experimental analysis of growth and reproduction was performed under natural daylength conditions at 15 °C. The snails were transferred into acid-washed 0.5 l plastic aquaria, or in case of detritus as food type, into 30 ml plastic vials. The sizes of the juveniles and the sexually mature animals are reported in the legends of Figs 1–5.

The food types consisted of lettuce, lamb heart, a combination of both, or lake detritus. They were supplied in excess, since food limitation and metal stress, separately as well as concertedly imposed, affect population parameters (Postma *et al.*, 1994). Lettuce is commonly used for rearing snails, and, by own experience, guarantees growth and reproduction of *P. jenkinsi*. Lamb heart, a protein-rich and relatively metal-poor diet, was used for enrichment of the quality of the food; one lamb heart from an abattoir was freeze-dried and finely pulverized for consumption by the juvenile snails. Detritus is the natural type of food of *P. jenkinsi*. When supplied in combination, lettuce and lamb heart were put in the aquaria in unmixed portions. The Cd, Cu and Zn contents of lettuce, lamb heart and natural detritus are given in Table 1. Many definitions of detritus have been formulated in the aquatic literature (Velimirov, 1991). Several deal with seston particles. Our operational definition results from practical restrictions, and therefore, cannot follow the definition proposed by Velimirov. To obtain natural detritus, we collected material at the water-sediment interface, which, as a mixture of sedimented organic (dead as well as living) and inorganic matter, serves as food for the bottom-dwelling, detritivorous *P. jenkinsi*. A grazer like this snail ingests both organic and inorganic particles, the size of which increases with the size of the animal (Fenchel, 1975). Detritus from Lake Maarsseveen I was collected by means of a hand-operated sampling net with a 0.30 mm mesh and sieved through a 0.60 mm mesh. The suspension obtained still contained many mineral particles. They were partly removed by sedimentation in lake water during one minute after stirring the suspension. The column above the sedimented material was siphoned off. This procedure was repeated three times with the siphoned suspension. The remaining detritus was dried at 80 °C. Further separation of the mineral and organic fractions is too complicated for routine procedures or larger amounts of detritus (Dorgelo & Leonards, 1989). The organic content of the detritus was 27%. The collected amount of detritus allowed weekly renewal during the period of the growth experiment.

Test procedures

The particle-free lake water, enriched with Cd, Cu and Zn from stock solutions of 1000 mg kg^{-1} $CdCl_2$, $CuCl_2$ and $ZnCl_2$, was refreshed weekly, or, in case of the use of lamb heart or lettuce combined with lamb heart, twice a week, to reduce bacterial development. Water samples were taken directly before (detritus) and directly before and after (other diets) water renewal to verify their metal concentrations. Prior to the experiments and the renewal procedure, the aquaria were kept filled with the exposure solutions for two days in order to saturate the walls with the metals, avoiding interference of adsorption to these walls during exposure of the snails. This procedure also prevents the development of Aufwuchs, which would interfere with the food types offered to the snails. The experimental design for the growth experiments on lettuce diet (Fig. 3 and Table 2) consisted of five nominal concentrations of Cd (0 (= particle-free lake water = control), 10, 25, 50 and 100 μg l^{-1}), four nominal concentrations of Cu (0, 5, 10 and 20 μg l^{-1}), and five nominal concentrations of Zn (0, 75, 100, 200 and 400 μg l^{-1}). They resulted in actual concentrations of 0.05 ± 0.04 (S.D.), 6.8 ± 3.1 (second trial: 7.4 ± 2.3), 23.3 ± 4.1, 45 ± 10 and 90 ± 13 μg Cd l^{-1}, of 2.4 ± 1.2, 7.7 ± 3.9, 13.1 ± 2.6 (second trial: 11.7 ± 2.0) and 22.1 ± 3.1 μg Cu l^{-1}, and of 12.3 ± 5.6, 72 ± 16, 115 ± 32, 189 ± 28 and 387 ± 39 μg Zn l^{-1} ($n = 26$).

Shell length increment of juveniles (that did not attain maturity during the experimental periods) was measured every week. Length of the turbinately coiled shells was defined as the maximum anterior to posteri-

Table 1. Concentrations of metals and the major ions in unspiked filtered Lake Maarsseveen water (Timmermans *et al.*, 1989), and of metals in the diets. Water: Cd-Fe in μg l^{-1}, Na-Cl in mg l^{-1}; diets: metals in μg g^{-1}.

Water

Cd	Cu	Zn	Fe	Na	Ca	DOC	PO_4-P	Cl
<0.02	<0.3	<2.0	11.0	18.8	63.6	4.3	<0.1	41

	Lettuce	Lamb heart	Natural detritus
Cd	0.5	0.1	10.7
Cu	155	77	69
Zn	159	83	305

Table 2. Shell length growth rates in μm week^{-1} (± S.D.) of *P. jenkinsi*, fed lettuce, placed in unspiked lake water (control) and in lake water spiked with metals (actual concentrations ± S.D. ($n = 25$) in μg l^{-1}; between brackets: nominal values). Initial shell length 1.7 ± 0.1 mm ($n = 20$). Asterisks: significantly differing from the control. For each trial, starting month and duration (in weeks) are given.

Trial	Control	Cd			Cu			Zn	
		7.4±2.3 (10)	6.8±3.1 (10)	23.3±4.1 (25)	7.7±3.9 (5)	13.1±2.6 (10)	11.7±2.0 (10)	72±16 (75)	115±32 (100)
1. March (12 w)	64±16	39 ±17		20 ±10*	63 ±15				30±17*
2. July (16 w)	66±21		65 ±15			40 ±17*		51±22	
3. Oct. (11 w)	118±35						69 ±22*		

or measurement. The weekly production of young was determined in separate experiments by counting and removing the minuscule juveniles which had left the brood pouch, using a binocular microscope and a plastic pipette, after sieving the contents of the aquaria.

The dried lake detritus used for a growth experiment (Fig. 5) was spiked after resuspension in lake water with a nominal concentration of 2000 μg l^{-1} Cd or Cu (proportion of spiking as well as in the growth experiment: 1 g detritus in 1.25 l lake water). The exposure time was one week during which the detritus was three times per day thoroughly mixed with the water. After that, the water was siphoned off and the detritus was freeze-dried. The amount of spiked detritus was enough for the 8-week period of the experiment. The lake water in this experiment was spiked with a nominal Cd or Cu concentration of 25 μg l^{-1}. The actual concentrations of spiked lake detritus and spiked lake water are given in Table 3. The exchangeable fraction of the total metal content (Tessier *et al.*, 1979; Calmano, 1983; Barbanti & Sighinoffi, 1988; Förstner, 1990; Rubio *et al.*, 1993) in detritus was determined by use of the first step of a sequential extraction technique, *i.e.* the 1 M ammonium acetate (pH = 7) extract after 2 hours shaking. The experimental design for the reproduction experiments (Fig. 4) consisted of three nominal concentrations of Cd (0 (= particle-free lake water = control), 25 and 50 μg l^{-1}), and three nominal concentrations of Cu (0, 30 and 50 μg l^{-1}). They resulted in actual concentrations of 0.06 ± 0.05 (S.D.), 22.8 ± 1.9 and 48.8 ± 3.4 μg Cd l^{-1}, and of 2.5 ± 1.1, 30.0 ± 2.2 (food: lettuce), 29.6 ± 2.0 (food: lettuce plus lamb heart), and 49.0 ± 2.2 μg Cu l^{-1} ($n = 40$).

The metal concentrations of the (acidified) water samples and the food types were analyzed by flame or furnace AAS, following Timmermans *et al.* (1989). The food samples were freeze-dried, weighed and dissolved by wet digestion using nitric acid and hydrogen peroxide. For quality control of the metal analysis, digestion blanks and reference material (IAEA MAA-3/TM shrimp homogenate) were used.

To test for significance of differences with the controls, Bartlett's test for homogeneity of variances, ANOVA and Sheffé's test for a posterior comparison

Table 3. Measured metal concentrations (± S.D.) during the growth experiment with spiked lake water (metals in $\mu g\ l^{-1}$; $n = 32$) and spiked natural detritus ($\mu g\ g^{-1}$, $n = 16$) (see also Fig. 3). Cd a. natural detritus plus lake water (control); b. detritus plus spiked lake water; c. spiked detritus plus lake water; d. spiked detritus plus spiked lake water. Cu a - d: idem.

Detritus					Water
Metal	Treatment	Total	Exchangeable fraction		
Cd	a	10.7 ± 6.4	4.2 ± 2.4	(39.3%)	0.05 ± 0.04
	b	25.3 ± 8.0	6.2 ± 2.8	(24.5)	21.3 ± 12.7
	c	1598 ± 149	485 ± 194	(30.4)	54 ± 19
	d	1762 ± 182	552 ± 177	(31.3)	44 ± 9
Cu	a	69 ± 6	5.4 ± 0.9	(7.8)	2.4 ± 1.1
	b	86 ± 10	7.4 ± 0.5	(8.6)	12.6 ± 2.6
	c	948 ± 29	68 ± 11	(7.2)	62 ± 4
	d	973 ± 15	75 ± 11	(7.7)	63 ± 5

of means were used. From the dose-response relationships the EC_{50} values were calculated by probit analysis (Finney, 1971) and the NOEC values according to Williams (1971).

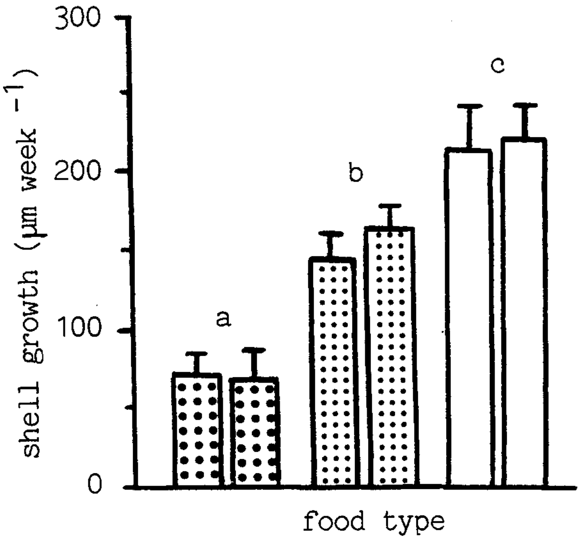

Fig. 1. Shell growth rates in μm week^{-1} (± S.D.) of *P. jenkinsi* (presented in duplicate) during 10 weeks (start: end of November), placed in filtered lake water and fed different types of food; a = lamb heart; b = lettuce; c = lettuce plus lamb heart. Initial shell length 1.8 ± 0.2 mm ($n = 10$). a significantly differs from b and c, and b from c ($P<0.01$).

Results

Effect of type of diet on shell length growth and fertility

In Fig. 1, the effect of the food type on shell growth rate in *P. jenkinsi* is demonstrated. The replicates per food type did not differ significantly ($P>0.05$). Highest growth rates occurred when the snails were fed a combination of both constituents (Fig. 1c).

In Fig. 2, the effect of type of diet on fertility in *P. jenkinsi* is demonstrated by comparing the effect of lettuce and lettuce plus lamb heart. The combination had proved to increase shell growth rate (Fig. 1). With an exclusively lettuce diet, the production of young decreased from the start, but with a lettuce plus lamb heart diet, reproduction increased during the fifth to the tenth week. The total number of young produced on the lettuce diet was 55, and 889 on the lettuce plus lamb heart diet.

Effect of metals on shell length growth and fertility

Table 2 shows the inhibitory effect of different concentrations of Cd, Cu and Zn, compared to the control, on the growth rates of *P. jenkinsi*, feeding upon lettuce. Exposure to 10 μg Cd l^{-1} was performed in duplicate. The growth rates in the control experiments (64 ± 16 and 66 ± 21 μm week^{-1}, respectively) did not significantly differ ($P<0.001$). During the first trial (starting in March), the snails showed a significant lower growth rate ($P<0.001$) at 10 μg Cd l^{-1} when compared to that of the control; however, during the second trial (starting in July), growth rate was not sig-

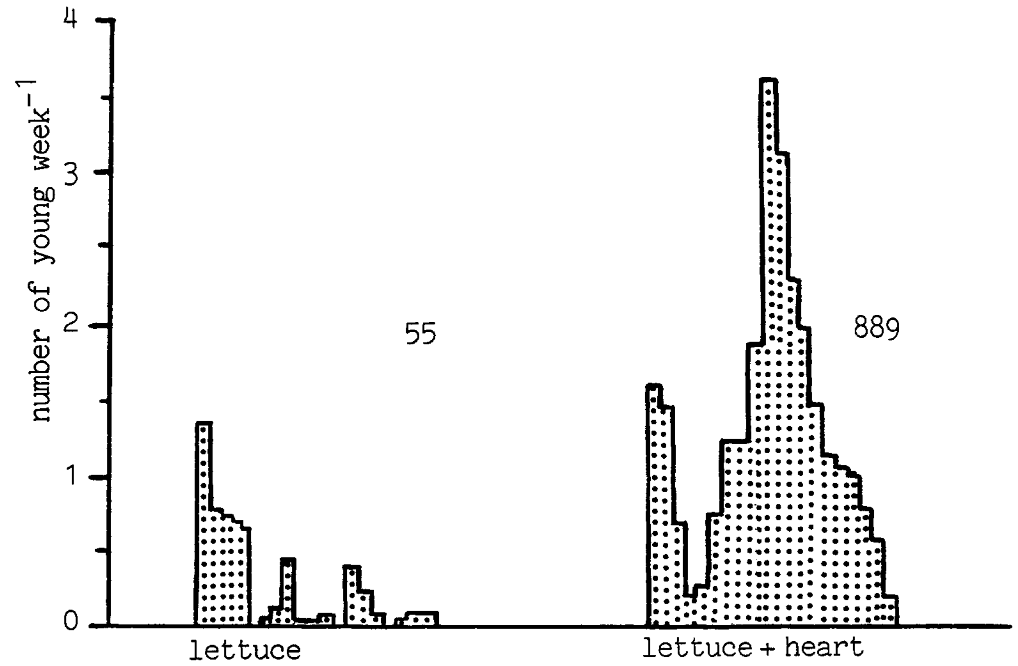

Fig. 2. Mean numbers of young produced by *P. jenkinsi*, fed either lettuce or lettuce and lamb heart per individual per week during 20 weeks. Shell length 4.1 ± 0.3 mm ($n = 40$). Inserted figures: total numbers of young produced.

nificantly lower than that of the control. Exposure of snails to 25 μg Cd l^{-1} resulted in a significant growth inhibition ($P<0.001$). At 5 μg Cu l^{-1}, no significant effect occurred ($P>0.05$), contrary to the result at 10 μg Cu l^{-1} ($P<0.001$). A second trial at 10 μg Cu l^{-1} was tested later in the season (October–December) against a separate control. It resulted in a growth rate of 69 ± 22 μm week^{-1}, again a significant difference ($P<0.001$) from 118 ± 35 μm week^{-1} of the control. In the case of Zn (Table 2), growth inhibition was significant ($P<0.001$) at 100 μg l^{-1}, but not at 75 μg l^{-1} ($P>0.05$). Additional results from preliminary experiments (not inserted into Table 2) showed an almost complete suppression of growth at 100 and 50 μg Cu l^{-1} and 400 and 200 μg Zn l^{-1}. The actual concentrations for the nominal values of 400 and 200 μg Zn 1-l were 387 ± 39 and 189 ± 28 μg l^{-1}. All results of the growth rates under metal stress are illustrated in Fig. 3 as percentages of growth inhibition, when compared to the controls (= 100%).

The EC_{50} growth values were 16 μg Cd l^{-1}, 13 μg Cu l^{-1} and 103 μg Zn l^{-1}, and the NOEC growth values 7 μg Cd l^{-1}, 5 μg Cu l^{-1} and between 50<75 μg Zn l^{-1}; the LOEC growth values were 25 μg Cd l^{-1} (actual concentration 23.3 ± 4.1 μg l^{-1}), 10 μg Cu l^{-1} (11.7 ± 2.0), and 75 μg Zn l^{-1} (71.5 ± 16.1 μg l^{-1}). These figures indicate the highest sensitivity of *P. jenkinsi* to copper.

Figure 4 shows the inhibitory effect, assessed in autumn, of different concentrations of Cd and Cu, compared with the control (snails in unspiked lake water), on fertility in *P. jenkinsi*, fed either lettuce or lettuce plus lamb heart. When fed lettuce (Fig. 4A), the production of young came to an end after 5–6 weeks at exposure to 25 and 50 μg Cd l^{-1}, contrary to the production in the control. The total numbers produced were 164 and, significantly differing from 164 young ($P<0.01$; *t*-test), 86 and 109 (Fig. 4A). At exposure to 30 μg Cu l^{-1}, the production of young came to an end after 6 weeks, contrary to the control; the total number produced was 67, being significantly ($P<0.01$) lower than that in the control (Fig. 4A). When fed lettuce plus lamb heart (Fig. 4B), the production of young in the control increased after 4 weeks, whereas at exposure to 30 and 50 μg Cu l^{-1}, hardly any young were produced after 5 weeks. The total numbers produced were 523, 154 and 114 (Fig. 4B). The numbers of 154 and 114 are significantly lower ($P<0.01$) than that of the control, and 114 significantly differs from 154. The results at 30 μg Cu l^{-1} at both diets demonstrate that the addition of lamb heart to lettuce increased fertility. In case of 0, 25 and 50 μg Cd l^{-1} (results assessed in winter,

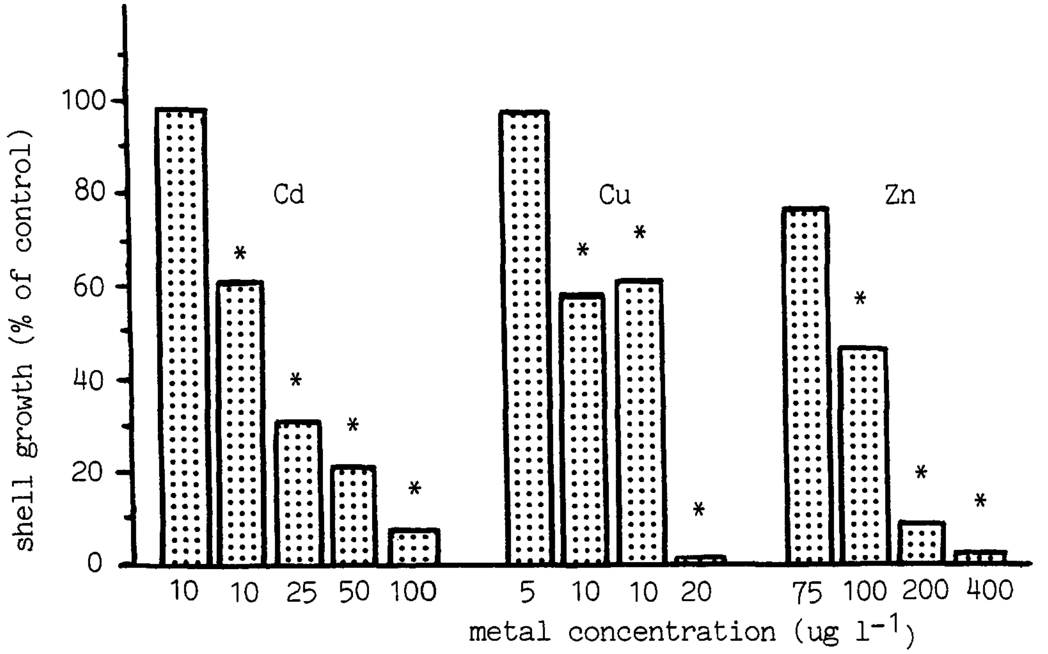

Fig. 3. Shell growth rates of *P. jenkinsi* as a percentage of those of the controls (= 100%) at various metal concentrations during 11–16 weeks. Snails were fed lettuce; initial shell length 1.7 ± 0.1 mm (n = 10–20). Asterisks: significantly ($P<$ 0.01) differing from the control (see also Table 2).

not included in Fig. 4), the production of young came to an end after 5 weeks and the low numbers of young were 30, and, significantly differing from this control ($P<0.01$), 17 and 17, respectively. Preceding experiments demonstrated that the production of young at 5, 10 and 15 μg Cu l^{-1} did not differ from that of the control. Tested at 75 and 100 μg l^{-1} Cd as well as Cu, high mortality of the parents caused a premature end of the experiment.

Effect of spiked detritus on shell length growth

Spiking lake detritus with 2000 μg g^{-1} Cd or Cu, or lake water with 25 μg g^{-1} Cd or Cu, resulted in the metal concentrations of detritus and water as given in Table 3. The detritus adsorbed much more Cd than Cu. The exchangeable fraction of Cd is much larger than that of Cu. The values of the dissolved Cd and Cu concentrations indicate desorption of both metals from the spiked detritus into solution (Table 3; compare c and d with a and b).

Figure 5 shows the significant growth inhibitory effects ($P<0.001$) of lake detritus spiked with 2000 μg g^{-1} Cd or Cu in lake water (Fig. 5c, d), compared with growth rates on natural detritus in lake water (controls: Fig. 5a). Growth rates in lake water spiked with 25 μg l^{-1} Cd or Cu (Fig. 5b) did not significantly differ ($P>0.05$) from the controls. Growth rates on spiked detritus (Fig. 5c) and on spiked detritus in spiked water (Fig. 5d) did not differ significantly ($P>0.05$) as well.

Discussion

Diet

Growth rates of *P. jenkinsi* fed lettuce varied between approximately 70 μm week^{-1} (controls in Table 2; start in March, July and October) and 150 μm week^{-1} (Fig. 1b; start: end of November). This difference might be related to season, with higher growth rates in the laboratory in winter at 15 °C. The intermediate value of 118 μm week^{-1} in October/November (Table 2) as well as the value of approximately 150 μm week^{-1} (Fig. 1b; also observed in winter on lettuce diet) support this explanation.

The addition of lamb heart (protein) to the lettuce favoured snail fertility (Fig. 2), which may naturally follow from the growth rates presented in Fig. 1c. Diets based on a single food item as compared to a variety of food items have also been demonstrated to be deficient

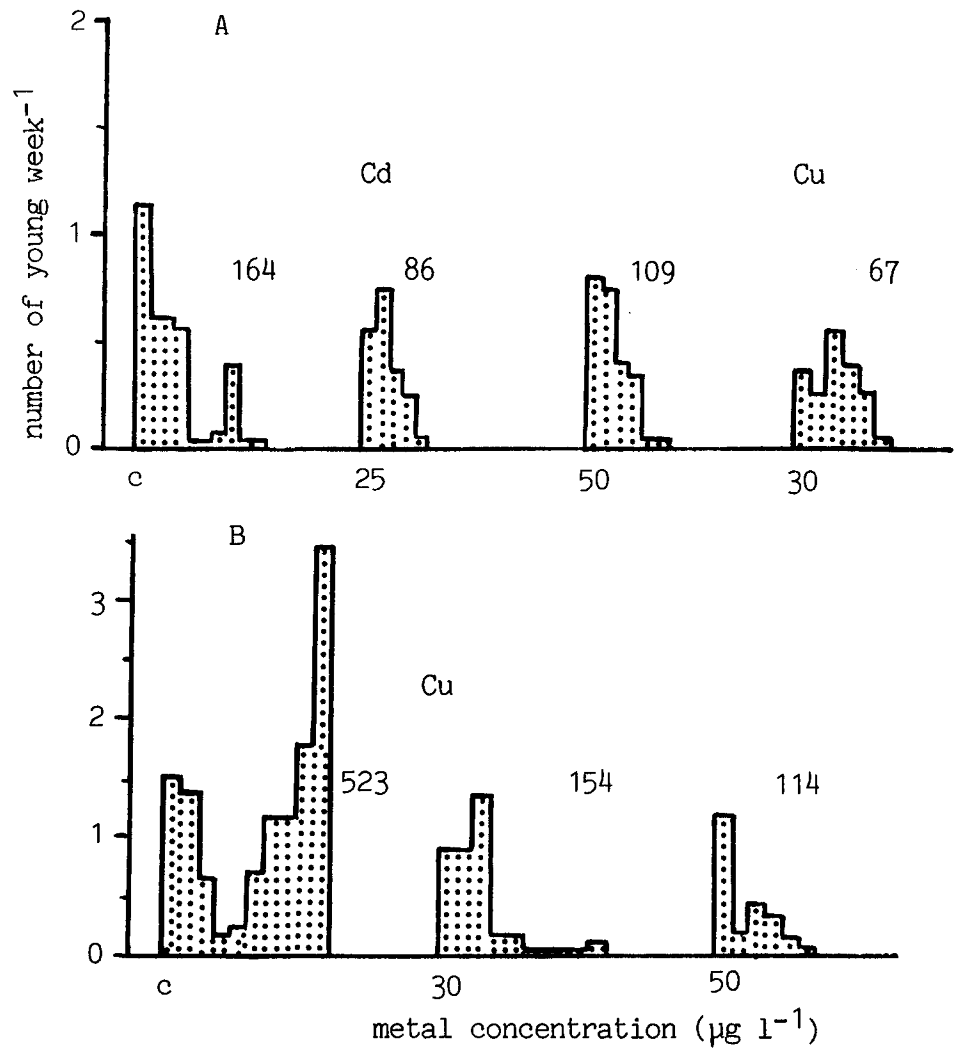

Fig. 4. Mean numbers of young produced by *P. jenkinsi* per individual per week during 10 weeks at different metal concentrations (c = control) and different types of food. Shell length 4.1 ± 0.3 mm ($n = 40$). A = lettuce; B = lettuce plus lamb heart. Inserted figures: total numbers of young produced.

in case of our laboratory-reared population of *P. jenkinsi*. Analysis of the brood pouch of adult snails (fed lettuce) from the culture and from Lake Maarsseveen I in September ($n = 20$) and October 1991 ($n = 10$) revealed an average number of pre-neonatal stages per snail of 3.2 and 0.2 in the culture, and 13.2 and 13.1 in the lake. Natural detritus consists of a mixture of various dead and live organic matter (Dorgelo & Leonards, 1989) with a high chemical diversity, as compared to lettuce. A single diet component also appeared to be deficient in bivalve mollusks (Dorgelo, 1993).

The production of young (Fig. 2) came to an end after 20 weeks. In *P. jenkinsi*, different embryonic stages of development can be found in the brood pouch during the whole year, but in the winter the production of eggs is suppressed (unpubl. data). The experiments were started in autumn at 15°C, but under natural daylength conditions, so it is assumed that the minimum fertility after 20 weeks (in January) was a natural phenomenon.

Metals

The growth rates of *P. jenkinsi* at 10 μg Cd l^{-1}, determined in two trials, whether or not significantly differed from the control (Table 2). These trials started in

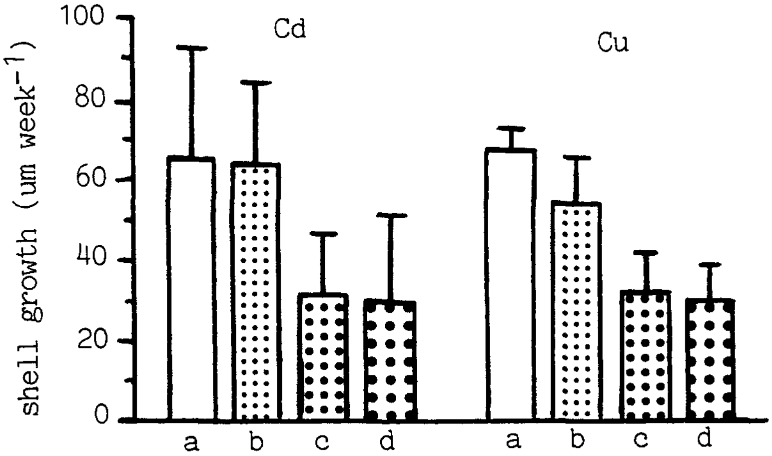

Fig. 5. Shell growth rates in μm week^{-1} ($\pm$ S.D.) of *P. jenkinsi* during 8 weeks; initial shell length 2.0 ± 0.1 mm ($n = 20$). a = lake detritus plus filtered lake water (control; different for Cd and Cu); b = lake detritus plus spiked lake water; c = spiked lake detritus plus filtered lake water; d = spiked lake detritus plus spiked lake water.

March and July, respectively, but since the growth rates in the controls did not significantly differ (66 ± 21 and 64 ± 16 μm week^{-1}), a seasonally determined factor seems not plausible. We have no explanation for this difference in growth inhibition. Figure 3 shows that *P. jenkinsi* is more sensitive to Cu than to Cd. This sensitivity to Cu was also reported by Brown (1980) and Watton & Hawkes (1984). Arthur & Leonard (1970) found that the growth of *Physa integra* stopped at 14.8 and 28.0 μg Cu l^{-1}; no growth inhibition was observed at 8 μg Cu l^{-1}. These results correspond with those for *P. jenkinsi*. Other gastropod data refer to exposure to unnaturally higher dissolved metal concentrations (Münzinger, 1987; Münzinger & Guarducci, 1988; Forbes, 1991).

In *P. jenkinsi*, the inhibition of growth occurred at a lower Cu concentration (10 ppb) than the inhibition of reproduction (30 μg l^{-1} or somewhat lower). Disorder of gastropod reproduction by metals was also reported before but partly again at exposure to high dissolved concentrations (Ravera, 1977; Münzinger, 1987; Münzinger & Guarducci, 1988). In *Physa integra* at 8 μg Cu l^{-1} and less, a second generation was observed to be produced (Arthur & Leonard, 1970). Willis (1988) found that the reproductive capacity of *Ancylus fluviatilis* was reduced at 180 μg Zn l^{-1}; no effect was observed at 100 μg Zn l^{-1}.

When fed spiked lake detritus, shell growth rates of *P. jenkinsi* distinctly decreased in water that was enriched by Cd or Cu from the detritus (Fig. 5c, d; Table 3). It remains uncertain whether the decreased growth rates were caused by the intake of spiked detritus or by exposure to the increased metal concentration of the lake water, or by both. Winger *et al.* (1984) concluded that Cu associated with detritus (up to 150 μg g^{-1}) had no effect on the growth of apple snails, but they were also fed lettuce during exposure to the spiked detritus. So, detritus as a food may have been ignored by these snails.

When lake detritus was spiked, the adsorption as well as the exchangeable fraction were substantially higher for Cd as compared with Cu (Table 3c, d). Lake detritus also adsorbed more Cd than Cu from the (slightly) spiked lake water (Table 3b), but the initial Cd concentration of the detritus was much lower than that of Cu (Table 3a). The amount of Cd desorbing from spiked detritus into solution in unspiked lake water was 3.4% of the uptake and much smaller than the exchangeable fraction; in case of Cu, this amount was 6.3%, approximately equalling its exchangeable fraction (Table 3c). These results for detritus may be connected with the findings for sediments in general, indicating that Cd, as well as Zn and Mn, are easily, and Cu as well as Pb and Cr, are moderately reducible components (Salomons & Förstner, 1984).

Interaction between diet and metal stress

Comparison of the mean growth rates of *P. jenkinsi* fed lettuce at a concentration of dissolved Cd of 23.3 ± 4.1 μg l^{-1} (growth rate 20 ± 10 μm week^{-1}; Table 2) and fed detritus at 21.3 ± 12.7 μg Cd l^{-1}

(63 ± 23 μm week^{-1}; Table 3b; Fig. 5b) shows a significant (P<0.01; t-test) higher growth rate in case of the detritus diet. The same holds for Cu (P<0.05) when comparing the growth rates at 13.1 ± 2.6 μg l^{-1} (40 ± 17 μm week^{-1}; Table 2) and at 12.6 ± 2.6 μg l^{-1} (58 ± 12 μm week^{-1}; Table 3b; Fig. 5b). The growth rates of the controls (during March–May and November–February, respectively) did not significantly differ under both food regimes (Table 2; Fig. 5a). So, detritus mitigated the growth suppressing effect of Cd and Cu, which demonstrates positive interaction between this type of diet and metal stress. It is uncertain whether, compared with lettuce, the lower Cu content of detritus enhances its quality, since, on the other hand, its Cd content is much higher (Tables 1 and 3). The positive interaction tended to show up also in the experiments with spiked detritus. When fed detritus spiked with Cd (Fig. 5c, d), growth rates were 30 ± 16 μm week^{-1} at 54 ± 19 μg Cd l^{-1} (actual dissolved fraction; Fig. 5c; Table 3c) and 31 ± 20 μm week^{-1} at $44 \pm 9 \mu$g Cd l^{-1} (Fig. 5d; Table 3d). These values are higher than that at 23.3 ± 4.1 μg Cd l^{-1} (diet of lettuce; 20 ± 10 m week 1; Table 2), but not significantly (P>0.05). The growth rates at 62 ± 4 μg Cu l^{-1} and 63 ± 4 μg Cu l^{-1} (Table 3c, d) were 33 ± 10 (Fig. 5c) and 31 ± 8 μm week^{-1} (Fig. 5d), *i.e.* close to the growth rate at 13.1 ± 2.6 μg Cu l^{-1} (diet of lettuce; 40 ± 17 μm week^{-1}; Table 2). These growth rates were observed while the metal contents of the detritus were much higher than those of the lettuce (compare Tables 1 and 3). Therefore, a negative effect caused by the metals ingested with (spiked) detritus is unlikely, or was dominated by the positive effect of the detritus diet. It is supposed that the lettuce diet represents a nutritional stress that increases the susceptibility of *P. jenkinsi* to Cd and Cu.

Figure 4 illustrates that the suppressing effect of 30 μg Cu l^{-1} on fertility was mitigated by the addition of lamb heart to the lettuce diet. This demonstrates again positive interaction between type of diet and Cu stress.

Problems in detritus-oriented, ecotoxicological studies

The sampling, handling and operational definition of natural detritus demand careful descriptions for comparison of biological as well as physicochemical studies. Wilhm (1970) and Winger *et al.* (1984), also involving detritus in ecotoxicological, macroinvertebrate studies, gave no details about detritus sampling and treatment. Nutritional value of detritus relates to its organic content but both organic and inorganic matter are ingested by detritivores, and both constitute ligands for metal adsorption. So, uptake of metals from these two compartments of natural sediment cannot be separated.

Usually, organisms are simultaneously exposed to contaminated water (dissolved metal fraction) and contaminated food (particulate fraction). Kay (1985), reviewing the movements of Cd in food webs, concluded that 'the uptake of Cd from food appears to be significantly less efficient than bioconcentration from water by gill-breathing aquatic animals'. The relative contributions of dietary metal intake and direct absorption from the water can only be determined by experiment in case of carnivory if the predator is offered contaminated prey that is swallowed entirely (e.g. fish fed spiked insect larvae). Mess making predators, grazers on spiked microalgae, and in particular deposit feeders offered 'naturally' polluted or spiked detritus (or sediment) or spiked artificial food (e.g. Tetraphyll or other fish food flakes), are also exposed to dissolved metals due to desorption from the food into the water (e.g. see Table 3c). The relatively small amount of water above polluted particles in experimental aquaria favours relatively high dissolved fractions, contrary to the situation in the field. For that reason, Absil (1993) applied EDTA to neutralize leaking from Cu-spiked diatoms. Literature on metal uptake from food often deals with bioaccumulation (e.g. Schulz-Baldes, 1974; Amiard & Amiard-Triquet, 1979; Kay, 1985; Kosalwat & Knight, 1987; Amiard *et al.*, 1988; Luoma, 1989; Timmermans & Davids, 1989; Van Hattum *et al.*, 1989; Timmermans *et al.*, 1992; Absil, 1993). In general, soluble sources appeared to be more important for accumulation than food. But, bio-accumulated metals can, within limits, be immobilized by binding proteins (Brown, 1982; Hamer, 1986; Carpene, 1993) preventing harmful effects. Data on sublethal effects of metals via food are far less numerous. Weis & Weis (1992; 1993) observed in static tests that contaminated macroalgae were toxic to the snail *Nassarius obsoletus* grazing upon them (1992), and that the carnivorous snail *Thais haemastoma floridana* feeding in flow-through aquaria on oysters with elevated tissue metal levels grew less than snails fed reference oysters over an 8-week period (1993). Cairns *et al.* (1984) repeatedly rinsed Cu-spiked sediments to lower the aqueous Cu concentrations to equilibrium levels before performing toxicity tests. Their conclusion that 'little, if any, of the toxicity can be attributed to sediment-bound copper' is not surprising, since, by washing, the metal fraction with the smallest bonding strength is excluded from the organism's exposure. The dynamics of sorption are also evident from the results reported by Pascoe *et al.* (1990) who found in acute Cd toxicity tests that fourth instar chironomid larvae showed a decreased LT_{50} in the presence of food (Tetramin flakes) due to an increased toxicity by rapid transfer of Cd from the test solution onto the food. We also found this transfer from spiked lake water onto lake detritus (Table 3b). Hatakeyama (1987; 1988) reported a reduced emergence success in a chironomid fed a mixture of dried yeast and Tetramin E contaminated with 220 μg Cd g^{-1} or 1770 μg Cu g^{-1}.

Another aspect of time as indirect agent includes aging of detritus. First, this is important in nutritional biology, as illustrated by Tenore (1977) and Tenore & Hanson (1980) who found that the net incorportation of detritus in the polychaete *Capitella capitata* was related to biochemical changes associated with the decomposition of detritus. Second, and this refers to particulates in general, the (scarce) data on aging effects and other diagenetic processes taking place after deposition suggest to cause a stronger binding of metals to the sedimentary particles (Salomons & Förstner, 1984; p. 43). Förstner (1990) distinguished between high-energy and lower-energy adsorption sites and refers to the, here again, scarce literature that mentions a two-step metal uptake process, consisting of a very rapid (1 hr) adsorption, followed by a slower process (perhaps lasting days or possibly months), called adsorbate diffusion into the solid substrate. Reversely, this can explain the reduced effect on organisms after washing spiked particles. Absil (1993) aged metal-spiked sediments for 5 months in stagnant, weekly refreshed water and dim light 'in order to reach equilibrium conditions that would be comparable to the field situation' (although natural sediments are regularly supplied with freshly contaminated detrital particles). She found that the aged sediments were much less toxic to the deposit feeder *Macoma balthica* than freshly spiked sediments.

A positive relationship between the weakly adsorbed sediment metal fraction of the different geochemical phases and the metal content of benthic animals could for some organism-specific metals be demonstrated, either with roughly sieved sediment (Diks & Allen, 1983; Van Hattum *et al.*, 1988; Ying *et al.*, 1992), or with the <70 μm (Tessier *et al.*, 1984) or <63 μm granulometric fraction (Absil, 1993). However, these results deal again with bioaccumulation. Analysis of the relation between easily extractable metal fractions and sublethal effects on e.g. growth and reproduction would make more ecological sense by enabling clarification of the complicated notion of bio-availability (Luoma, 1989).

In summary, in studies on deposit feeders, (a) the operational definition of detritus, (b) the time-dependent sorption dynamics between (ingestible) particles and water under experimental conditions, and (c) the weakly bound fractions, are all important determinants to be known for understanding fate and sublethal effects following coupled ingestion of contaminated, organic and inorganic particles.

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