Bull. Environ. Contam. Toxicol. (1993) 50:883–890

Environmental Contamination and Toxicology

Effects of Dietary Copper and Zinc Concentrations on Feeding Rates of Two Species of Talitrid Amphipods (Crustacea)

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Semi-terrestrial, high shore talitrid amphipods are generally indiscriminate in their choice of food, consuming items as diverse as decaying macroalgae, dead companions and terrestrial leaf-litter (and have on occasion been observed consuming old plastic bags, JMW pers. obs.), provided that victuals are neither too hard nor too dry (Moore & Francis 1985). The choice to ingest or reject food is governed by stimuli from receptors sensitive to taste, smell, moisture content, texture and/or visual appearance. The animal will only eat the food if the sensory information from the receptors is in the correct combination. Dallinger (1977) suggested that terrestrial invertebrates may be able to select food containing optimum amounts of essential metals. This would require the presence of complex feedback mechanisms in the animal, which would control feeding. No physiological/biochemical mechanism for any such system controlling metal regulation has, however, yet been identified in any organism (Hopkin 1989). It is unlikely that in their daily lives talitrid amphipods would encounter atypically high concentrations of metals in their food, except at heavily polluted locations.

In laboratory experiments, terrestrial isopods have been shown to be reluctant to ingest food heavily contaminated with copper salts (Hassell & Rushton 1982), eventually succumbing to starvation rather than to the toxic effects caused by excessive copper accumulation. Often in metal accumulation studies, the chosen experimental animals are fed food substrates previously exposed to a range of increasing metal concentrations, invariably in the form of a deposit of the metal salt on the surface of the food item. Such surface deposits may change the texture and taste of

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the food by killing surface microorganisms and so bring about an indirect reduction in feeding, not necessarily as a result of the increased metal concentrations.

There is considerable inherent chemical variability between individual food types, and samples from the same food type; for instance regions of a particular leaf or algal frond (Moore & Francis 1985). Food status will also change, particularly with respect to the state of decay. Such natural variability might also affect the palatability of a particular food type to consuming animals, posing a problem of quality control of offered food in replicated experiments.

This study describes experiments that were undertaken to determine feeding rates and preferences of amphipods feeding on variably metal-enriched algal discs, in order to quantify feeding differences both within and amongst treatments.

MATERIALS AND METHODS

Orchestia gammarellus (Pallas) were collected by hand from beneath seaweed cast-up in the supralittoral zone and Orchestia mediterranea Costa from beneath littoral boulders on the same shore at Farland Bight, near Millport, Great Cumbrae Island, Firth of Clyde, Scotland (OS grid reference NS 17325415)(for details of their biology see Spicer et al. 1987). Replicate groups of five O. gammarellus and five O. mediterranea (previously starved for 24hr) were placed in individual Petri dishes (suitably humidified with a strip of filter paper moistened in artificial seawater 33 of ppt salinity (Tropic Marin Neu, TMN) held in place by capillarity to the inside of the lid). Replicate groups of amphipods were selected for experiments such that each group contained amphipods of both sexes and of a similar size range (10 - 14 mm in length, ca 10 mg dry weight in both species).

Stranded Laminaria digitata Hudson (decayed until green) was collected from the same location as experimental amphipods (see above) and rinsed briefly in double-distilled water. Discs (23 mm diameter) having suitably low concentrations of copper and zinc (8.7 μg Cu g^{-1}, SD 1.4 and 63.2 μg Zn g^{-1}, SD 5.4), were cut from regions of the blade which were free of epiphytes, using a stainless steel cork-borer previously rinsed in double-distilled water. Sets of ten discs were exposed in 50 cm^3 of TMN (at 10°C) to a range of dissolved copper or zinc concentrations for 24hr. Additional metal was added as required from stock solutions of Analar grade chloride salt (BDH Ltd., U.K.) in double-distilled water.

Exposures were carried out simultaneously, for the following series of metal concentrations: a) Copper; groups of ten discs were exposed to concentrations of 20 (control), 100, 316, 1000, 1749 or 3162 μg Cu l^{-1} in TMN. Zinc: groups of ten discs were exposed to the same range of concentrations in TMN.

After 24hr the discs were drained and damp-dried with tissue paper. For each soak, three discs from each exposure were dried to constant weight (60°C), digested in conc. HNO_3 (Aristar grade, BDH Ltd., U.K.) at 100°C and made up to known volume with double-distilled water. Digests or dilutions thereof were analyzed for total copper and zinc content by flame AAS using an IL-157 spectrophotometer with flame atomization and background correction where appropriate. The remaining algal discs were used for the feeding rate studies.

Two experimental series were undertaken. In the first series, feeding rates were determined for amphipods (O. gammarellus) feeding for 48hr on a single disc of previously copper- or zinc-exposed alga (see below for the range of concentrations). Amphipods were left to feed for 48hr after which time dry weights of algal discs and of amphipods were obtained (48hr, 60°C).

In the second series groups of amphipods were allowed to feed for 20 days on algal discs containing one of a number of copper or zinc concentrations; the derived metal concentrations in the algal discs having resulted from a 24hr exposure to a log series of dissolved metal concentrations (see above). In the case of copper the metal concentrations in the treated algal discs were measured to be: 9.0 (control), 17.0, 68.0, 145, 688 or 817 μg Cu g^{-1}; and for zinc 50.0 (control), 63.0, 94.0, 202, 275, or 458 μg Zn g^{-1}.

Ten discs (23 mm diameter) of L. digitata which had been allowed to decay until green were damp-dried, weighed and subsequently dried (60°C) to obtain an estimate of the dry weight to wet weight ratio. A control Petri dish had no animals, but contained an individual disc of L. digitata in order to assess weight changes of the L. digitata throughout the experimental period, in the absence of amphipod grazing. Two discs of pre-weighed algae previously exposed to dissolved metals were presented to each respective amphipod group. Discs were changed every 48h and weight loss (i.e. feeding rate) determined. Changes in weight of algal discs not due to amphipod grazing (but due to changes in water content) were corrected for with reference to the controls. Feeding rates of the amphipods are expressed as milligrams (dry wt. alga eaten) per gram (dry wt. of amphipods) per hour (mg g^{-1} h^{-1}).

RESULTS AND DISCUSSION

Table 1 shows the mean consumption rates per hour for *O. gammarellus* from the first experimental series allowed to feed for 48hr on the decaying discs of *L. digitata* of a particular copper or zinc dietary concentration, respectively. Analysis of the data by ANOVA

Table 1. Experiment 1. Mean (± SD) rates of consumption by *Orchestia gammarellus* of a disc of decaying *Laminaria digitata* frond material after exposure of the discs to one of a log series of dissolved (a) copper and (b) zinc concentrations for 48hr.

a) Cu conc. (μg/g)	Feeding rate (mg g^{-1} h^{-1})
9(control)	1.89 (0.055)
17	1.75 (0.057)
68	1.60 (0.086)
144	1.65 (0.050)
688	0.60 (0.051)
817	0.43 (0.070)
b) Zn conc. (μg/g)	**Feeding rate (mg g^{-1} h^{-1})**
50(control)	1.81 (0.041)
63	1.63 (0.057)
94	1.36 (0.202)
202	1.46 (0.057)
275	0.60 (0.091)
458	0.59 (0.081)

(adapted from Sokal & Rohlf 1981) revealed a significant ($P<0.001$) reduction in consumption rate of the amphipods with increasing dietary metal concentration for both copper and zinc. Subsequent two-way ANOVA revealed a significant ($P<0.01$) difference between consumption rates of copper-rich and zinc-rich food substrates, with greater amphipod consumption rates when feeding upon copper-rich algae. Further *a posteriori* ANOVA revealed no significant differences ($P>0.05$) between the consumption rates of *O. gammarellus* when feeding upon the lower four algal copper concentrations but a significant reduction in feeding occurred at higher food copper concentrations, i.e., at and above 688 μg Cu g^{-1}. This was not the case for zinc in which there was a significant ($P<0.05$) reduction in consumption rates at all dietary zinc concentrations above the control.

Table 2 shows the mean consumption rates by *O. gammarellus* upon copper- and zinc-treated algae during the second experiment (i.e., the 20 day exposure). ANOVA revealed no significant changes ($P>0.05$) in consumption rates throughout the 20-day period for each dietary metal concentration for both copper and zinc. There were, however, significant (P<0.001) differences in amphipod consumption rates between dietary metal concentrations for both copper and zinc enrichment. Generally, there was a reduction in amphipod consumption at higher concentrations of each metal.

It is notable that amphipods in the 20 d experimental series (Table 2) had higher consumption rates per hour than those depicted in Table 1, at all comparable metal concentrations.

Table 2. Experiment 2. Mean (± SD) rates of consumption by *Orchestia gammarellus* of discs of decaying *Laminaria digitata* frond variously enriched with (a) copper or (b) zinc for 20 days.

	Feeding rate (mg g^{-1} h^{-1})				
	Day				
	4	8	12	16	20
a) Cu conc. (μg/g)					
9(control)	3.31	4.33	3.57	5.00	3.03
	(0.19)	(0.11)	(0.70)	(0.37)	(0.26)
17	5.53	6.05	5.36	6.32	3.17
	(0.33)	(0.26)	(0.87)	(0.48)	(0.20)
68	6.32	5.34	4.71	8.37	5.51
	(0.21)	(0.81)	(0.71)	(0.51)	(0.55)
145	5.02	6.83	4.88	3.10	5.39
	(0.22)	(0.92)	(0.22)	(0.73)	(0.72)
688	5.30	-0.70*	3.25	4.35	3.75
	(0.05)	(0.74)	(0.32)	(0.81)	(0.11)
817	1.12	2.00	2.70	1.72	1.22
	(0.02)	(0.11)	(0.03)	(0.21)	(0.07)
b) Zn conc. (μg/g)					
50(control)	4.22	3.25	4.23	5.76	6.33
	(0.11)	(0.82)	(0.81)	(0.22)	(0.80)
63	4.73	5.32	4.17	5.27	3.35
	(0.18)	(0.77)	(0.83)	(0.12)	(0.32)
94	5.55	4.73	4.22	-0.62*	3.71
	(0.22)	(0.56)	(0.79)	(1.10)	(0.17)
202	3.76	3.55	4.08	4.72	3.38
	(0.37)	(0.24)	(0.61)	(0.18)	(0.27)
275	3.62	4.22	3.29	4.12	5.27
	(0.16)	(0.21)	(0.12)	(0.11)	(0.76)
458	2.10	2.01	1.80	1.63	0.90
	(0.08)	(0.11)	(0.17)	(0.22)	(0.20)

* negative value indicates weight gain by the algal disc

Similarly, Table 3 shows the mean rates of consumption by *O. mediterranea* for an identical copper- and zinc-exposure series to those in Table 2. ANOVA revealed no significant differences between feeding rates of the two amphipod species at the same dietary copper or zinc concentration. There were also no significant changes in consumption rate with time but significant ($P<0.01$) reductions in consumption with increasing exposure to elevated dietary metal sources for both copper and zinc.

Table 3. Experiment 2. Mean (± SD) rates of consumption by <u>Orchestia mediterranea</u> of discs of decaying <u>Laminaria digitata</u> frond variously enriched with (a) copper or (b) zinc for 20 days.

Feeding rate ($mg\ g^{-1}\ h^{-1}$)					
	Day				
	4	8	12	16	20
a) Cu conc. (µg/g)					
9(control)	5.23	4.85	4.10	3.71	4.02
	(0.66)	(0.42)	(0.33)	(0.11)	(0.17)
17	6.21	5.90	5.88	5.56	5.67
	(0.15)	(0.17)	(0.11)	(0.48)	(0.89)
68	4.11	4.23	4.33	4.45	5.07
	(0.26)	(0.13)	(0.15)	(0.51)	(0.58)
145	3.27	3.10	3.52	3.71	3.88
	(0.37)	(0.33)	(0.51)	(0.17)	(0.45)
688	2.28	2.12	2.57	3.57	3.72
	(0.52)	(0.37)	(0.47)	(0.16)	(0.32)
817	0.89	0.91	1.11	1.37	0.92
	(0.05)	(0.16)	(0.08)	(0.09)	(0.02)
b) Zn conc. (µg/g)					
50(control)	3.30	5.72	2.76	3.75	3.57
	(0.12)	(0.21)	(0.22)	(0.09)	(0.08)
63	3.71	3.55	3.22	3.16	4.30
	(0.66)	(0.45)	(0.31)	(0.31)	(0.11)
94	4.13	4.87	3.10	3.55	3.61
	(0.37)	(0.39)	(0.52)	(0.27)	(0.23)
202	4.14	4.01	3.08	3.03	3.28
	(0.28)	(0.14)	(0.16)	(0.43)	(0.51)
275	2.82	2.13	1.81	1.92	1.11
	(0.19)	(0.12)	(0.16)	(0.08)	(0.31)
458	0.82	0.90	0.83	0.67	0.12
	(0.15)	(0.18)	(0.07)	(0.11)	(0.27)

Weeks & Rainbow (in press) provide complimentary data with regard to the accumulation of body copper and zinc by these two species of amphipods during this experiment. Therefore, only aspects of the feeding rate shall be discussed here. Food chanced upon by an amphipod in the field is unlikely to contain an excess of toxic metals. In most instances, therefore, it is unlikely that amphipods would benefit from an ability to detect the levels of metals in their food. Dallinger (1977), however, has suggested that terrestrial invertebrates may be able to select food of an optimum concentration of essential trace metals. Such an ability would require complex positive/negative feedback mechanisms in order to control feeding behaviour. Under such a system an internal deficit of an essential metal would result in the animal selecting food with a high concentration of that metal, whereas an excess of body metal would stimulate feeding on food containing low metal concentrations. Such a concept is complex and there is no physiological or biochemical evidence for the operation of such a system controlling dietary intake in any organism. Moreover there are no known chemoreceptors which respond to specific metals other than the major ions, either in the mouth or gut of any animal (Hopkin 1989).

Amphipods grazing upon a copper-enriched diet appeared to reduce their feeding rate at a threshold copper value (in this instance including and upwards of 688 μg Cu g^{-1} dry wt.). Amphipods grazing on a zinc-enriched diet, however, showed only a gradual reduction in consumption rate with increasing dietary zinc concentrations.

O. gammarellus ate proportionately more algal material with low concentrations of copper or zinc than algal material with higher concentrations of copper or zinc. It is not proven, however, that the observed lowering of consumption rate of _O. gammarellus_ is caused by a discrimination between algal material which differed apparently only in their metal concentrations. Care should be taken in interpreting such results. It is possible that the amphipods were not discriminating on the basis of copper or zinc concentration in the food but that their change in consumption rate was a response to a difference in concentration of the anion (in this case chloride, from the metal salt) which they were able to taste. Moore & Francis (1985) have shown that _O. gammarellus_ consumed more food with slightly higher salt content than that with lower proportions of salt, but not food with such hypersaline chloride concentrations as in this study. Applications of metals as salts to food material may also change the texture and taste of the food by killing surface microorganisms. Amphipods may merely be rejecting food which tastes unusual.

It is of interest that for both talitrid species the duration of feeding upon a metal enriched diet, whether of 48hr or 20 d resulted in the same pattern of disruption in consumption rates, i.e., a pronounced reduction at the highest dietary metal concentrations. Such an observation would suggest that continued exposure to elevated dietary metal concentrations did not result in the animals becoming acclimatized to elevated dietary metal concentrations and did not result in the resumption of normal feeding rates with increasing exposure time.

The observed differences between the consumption rates of O. gammarellus from the two experimental series (Tables 1 and 2) are probably indicative of the inherent variability of different algal fronds as a food source palatable to amphipods. The consumption rates for O. gammarellus presented in Tables 1 and 2 are comparable with those calculated by Moore & Francis (1985), 5.7 mg g^{-1} h^{-1}. The lower rates occurring in Table 1 may be a feature of the reduced palatability of the L. digitata offered to experimental amphipods in this group.

It is suggested that the inherent natural variability in feeding rates of terrestrial invertebrates is further complicated when feeding on metal-enriched diets. Generally, increased metal concentrations in the diet resulted in a reduction in feeding rate (i.e., consumption rate), although this was further complicated by the natural variability or the "taste" of the food substrate.

Acknowledgments. I thank Dr Philip S. Rainbow for reading drafts of the manuscript and Dr P. Geoffrey Moore for the provision of the Scottish talitrids.

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Received July 20, 1992; accepted December 17, 1992.