



Food selection as a means of Cu intake reduction in the terrestrial isopod *Porcellio scaber* (Crustacea, Isopoda)

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Abstract

The idea that terrestrial isopods regulate copper intake through the state of their copper stores by selection of an optimal copper diet has never been experimentally assessed. We investigated discrimination between untreated and Cu-enriched diets in the isopod *Porcellio scaber* in relation to their copper stores. Animals were pre-exposed to untreated or Cu-enriched poplar leaves. After 14 and 28 days of pre-exposure, two-way food choice experiments were performed for 14 days. Food consumption rates and the amount of copper accumulated due to the copper content of the food and the duration of pre-exposure were compared. Food selection and rejection as ways by which *P. scaber* may reduce or avoid the toxic effects of copper were evaluated. *P. scaber* offered both Cu-enriched food and untreated food preferred the latter, irrespective of the amount of assimilated copper. Copper body burdens increased with copper concentration in the food. In animals offered both Cu-enriched and untreated food, copper body burden also increased, but to a lower extent than in animals fed only a Cu-enriched diet. Therefore, food selection is a way of regulating copper intake, but only to a limited extent. The mortality of experimental animals increased significantly after longer exposure to highly Cu-enriched food. We conclude that in *P. scaber*, the possibility of selecting food of different copper content may mitigate but cannot prevent the toxic effects of copper in copper-polluted environments.

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1. Introduction

Copper is part of the oxygen carrying protein haemocyanin and therefore one of the most important essential metals for all crustaceans (summarised in Vonk, 1960; Goodwin, 1960; Wieser, 1966, 1968). Its deficiency or excess in terrestrial isopods has been the subject of many investigations. In contrast to aquatic crustaceans, terrestrial isopods extract required

copper mainly from their food (Wieser, 1967; Hopkin and Martin, 1984). Leaf litter, which is supposed to be the main food source for terrestrial isopods, contains from 5 mg Cu kg⁻¹ dry weight in uncontaminated environments (Hopkin and Martin, 1982a) to over 15,000 mg Cu kg⁻¹ dry weight in industrially polluted areas (reviewed in Bengtsson and Tranvik, 1989). While 10 mg Cu kg⁻¹ dry weight is hardly enough to cover an isopod's copper needs (Wieser, 1967), concentrations higher than 1000 mg Cu kg⁻¹ dry weight influence isopod mortality (Hopkin et al., 1986; Farcas et al., 1996; Hornung et al., 1998). Two physiological mechanisms of metal dynamics have

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been described in isopods; storage of metals in intracellular granules of the hepatopancreas (Wieser and Klima, 1969; Hopkin and Martin, 1982b; Hopkin, 1989), and complexation of metals by metal-binding proteins (Donker et al., 1990).

An additional mechanism which helps isopods to survive in metal-polluted environments is their ability to discriminate between highly metal contaminated and uncontaminated diet (Van Capelleveen et al., 1986; Drobne et al., 1995; Odendaal and Reinecke, 1998, 1999) or reject metal contaminated food (Joose et al., 1981; Hassall and Rushton, 1982; Drobne and Hopkin, 1994, 1995). Some authors also speculated that terrestrial isopods might be able to select food with an optimal amount of essential copper (Dallinger, 1977).

It is still unknown how terrestrial isopods discriminate between metal contaminated and uncontaminated food. Dallinger (1977) suggested that isopods' food selection might be regulated through their metal body burden. Hopkin (1993) suggested that rejection of metal contaminated food is due to aversion to fungi that concentrate metals while growing on contaminated leaves. Weissenburg and Zimmer (2003) proposed the existence of contact-chemoreception of copper.

The idea that copper intake is regulated by selection of an optimal copper diet controlled through copper reserves was criticised many times, though never experimentally assessed. In this study, the experimental setting was similar to that of Dallinger (1977) but we also measured copper assimilation as a function of dose and time of exposure. The aim of measuring copper body burden was to assess whether or not it is responsible for selection of an optimal copper diet. The effects of copper were determined by food consumption rate and mortality. We discuss food selection and food rejection as ways by which *Porcellio scaber* may reduce or avoid toxic effects of copper contamination.

2. Materials and methods

2.1. Collection site and experimental conditions

Porcellio scaber Latr. of both sexes and approximately 20 mg fresh weight were collected in the

Spanderswoud near Hilversum in the Netherlands, a reference area used by the Department of Animal Ecology (Vrije Universiteit, Amsterdam). Before the experiments started, the isopods were kept in a climate room for three weeks at a temperature of 20 ± 0.1 °C and in a 12:12 h light/dark regime. Further experiments were performed under the same conditions. During the acclimation, animals were kept on moist plaster of Paris and fed with uncontaminated poplar leaves.

2.2. Pre-exposure to copper-enriched food

About 1000 animals were divided into six groups: three pre-exposed to untreated poplar leaves or poplar leaves treated with $650 \text{ mg Cu kg}^{-1}$ dry food or $2300 \text{ mg Cu kg}^{-1}$ dry food for 14 days, and three pre-exposed to untreated poplar leaves or poplar leaves treated with $650 \text{ mg Cu kg}^{-1}$ dry food or $2300 \text{ mg Cu kg}^{-1}$ dry food for 28 days. These copper concentrations were selected on the basis of a preliminary experiment where 30 and 70% reductions in food consumption were observed during a 3-week exposure period. Intact leaves were treated with a solution of $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ (Merck, purity 99.5%) in deionized water, by alternate spraying and drying to achieve the appropriate copper concentrations in the leaves. Actual concentrations varied from that desired by <5%. Animals were kept in a climate room in plastic pots (diameter 20 cm), the bottoms of which were filled with moist sand. After 14 and 28 days of pre-exposure eight animals from each group were sacrificed and tissues were prepared for copper analyses ('START' Cu-concentrations).

2.3. Food choice experiments

After 14 and 28 days of pre-exposure, 120 animals from each pre-exposure group were used for food choice experiments. Each of the six experimental groups included 20 animals, caged individually in Petri-dishes (diameter 9 cm), that were offered two poplar leaf pieces (approximately 9 cm^2) of different shape (square and triangular) for 14 days. The two leaf pieces had been treated with copper solutions of different or the same concentrations by the same procedure as described above. Six combinations of nominal copper concentrations (all in mg Cu kg^{-1} dry food)

were used: “0–0”, “0–650”, “0–2300”, “650–650”, “650–2300” and “2300–2300”. The copper concentration in untreated leaves was below 10 mg of Cu kg⁻¹ dry weight. The bottom of the petri-dishes was covered with sand and moistened with deionized water to 50–70% of its water holding capacity.

Animal mortality was recorded three times a week. After 14 days, leaves were dried at 70 °C for 48 h and weighed. The total food consumption rate was calculated as the amount of food consumed (both leaves offered) during 14 days, per dry animal weight. The consumption rate of a single leaf was calculated as the percentage of the total food consumption rate. Faeces were not removed from the dishes during the experiments. Only animals that survived the experiments were used in calculations. At termination of the food choice experiments, the animals' copper body burden was measured.

2.4. Copper concentration analyses

To determine the total body copper concentration, isopods were freeze-dried for 48 h, weighed and digested. Digestion was performed in glass tubes using a nitric and perchloric acid mixture (7:1 (v/v); Ultrapure quality) at increasing temperatures (85, 160 and 185 °C). After evaporation of the acid, the residue was dissolved in 0.1 N HNO₃. Copper body burdens were determined on a Perkin-Elmer 1100B flame atomic absorption spectrometer (AAS) in an air-acetylene flame with deuterium correction of non-specific absorption. The leaves were digested and analysed for copper in the same way. To assure the accuracy of the analytical procedure, samples of plant (poplar leaves) and animal (isopods) material were dried, homogenised and spiked with copper nitrate solution at three different concentration levels (1.0, 2.5 and 4.5 mg l⁻¹, five replicates each) and analysed after digestion as described above. The percentage recoveries of these spiking experiments were 100.1% for plant and 96.3% for animal material.

2.5. Statistics

For statistical analysis of the data, the programme SPSS for Windows was used. Differences in the animals' total food consumption rate and copper accumulation were tested by the use of univariate analysis

of variance (ANOVA) with the Tukey HSD post-hoc test or Student *t*-test where only two samples were compared. The percentage of the single leaf consumption rate was compared to a null-hypothesis of 50% with a two-tailed Student's *t*-test. Standard errors and 95% confidence intervals were calculated where appropriate. Concentrations of copper in animals that died during the experiment were compared with copper concentrations in surviving animals from the same group by the non-parametric Mann–Whitney test. The difference between two binominal probabilities was tested (Pollard, 1977) when the mortality of animals pre-exposed to Cu-enriched food was compared with animals pre-exposed to untreated food.

3. Results

3.1. Food preference in relation to previous exposure to differently Cu-enriched food

Previous exposure to Cu-enriched food had no significant effects on the total food consumption rate in the food choice experiments (Fig. 1). Food consumption rate was highest in the groups offered control and low Cu-enriched leaves, and significantly lower in the groups offered higher copper doses in the food. Decreased consumption of Cu-enriched food was not compensated for by increased consumption of untreated or by less Cu-enriched food (Fig. 1). Only one group of animals out of 12 groups that were offered untreated and Cu-enriched food compensated for decreased consumption of Cu-enriched food by increased consumption of untreated food (Fig. 1E).

Control animals that were offered two untreated leaves (“0–0”) consumed both leaves equally (Fig. 2). Animals offered Cu-enriched (650 mg Cu kg⁻¹) and untreated food preferred the latter (*t*-test, $P < 0.05$) at an average percentage of 60%, irrespective of the copper pre-exposure. The maximal ratio between consumed untreated and Cu-enriched (2300 mg Cu kg⁻¹) food was 75% (untreated food): 25% (Cu-enriched food) (Fig. 2), also irrespective of the copper pre-exposure. In five of six groups, where the animals could choose between food with 650 mg Cu kg⁻¹ and 2300 mg Cu kg⁻¹, they preferred the food with less copper. An exception were the animals 14 days pre-exposed to food enriched with 650 mg Cu

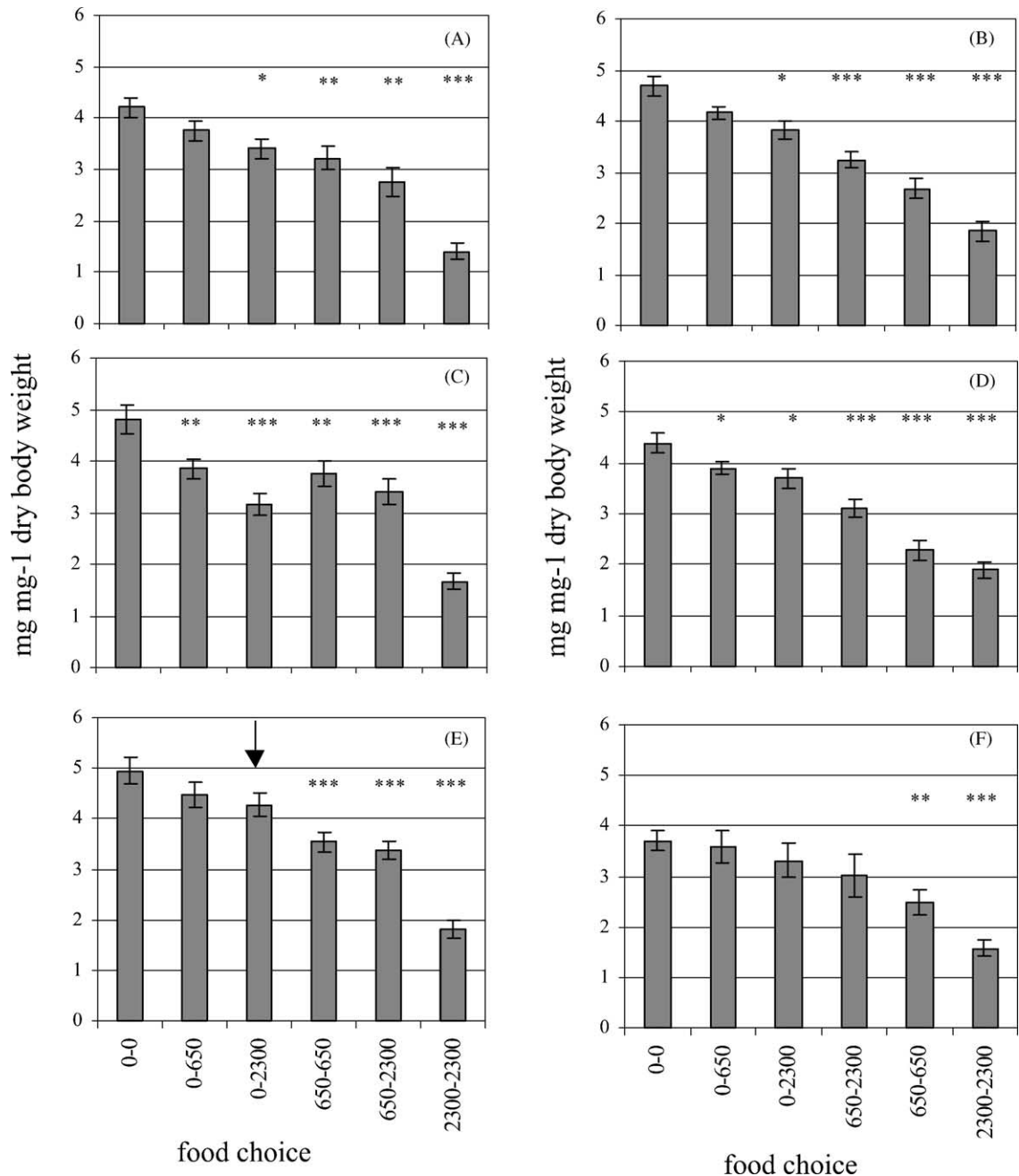


Fig. 1. Total food consumption rate (mean \pm S.E.) of *Porcellio scaber* during 14 days of food choice experiments in differently pre-exposed animals: (A) pre-exposure to untreated food, 14 days; (B) pre-exposure to untreated food, 28 days; (C) pre-exposure to 650 mg Cu kg⁻¹, 14 days; (D) pre-exposure to 650 mg Cu kg⁻¹, 28 days; (E) pre-exposure to 2300 mg Cu kg⁻¹, 14 days (a significant (ANOVA, $P < 0.05$) increase in consumption of untreated leaves in group '0-2300' compared to untreated leaves in control group ('0-0'), is marked with an arrow); (F) pre-exposure to 2300 mg Cu kg⁻¹, 28 days. Mean values that differ from the control group ('0-0') are labelled with asterisks (Student's t -test: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

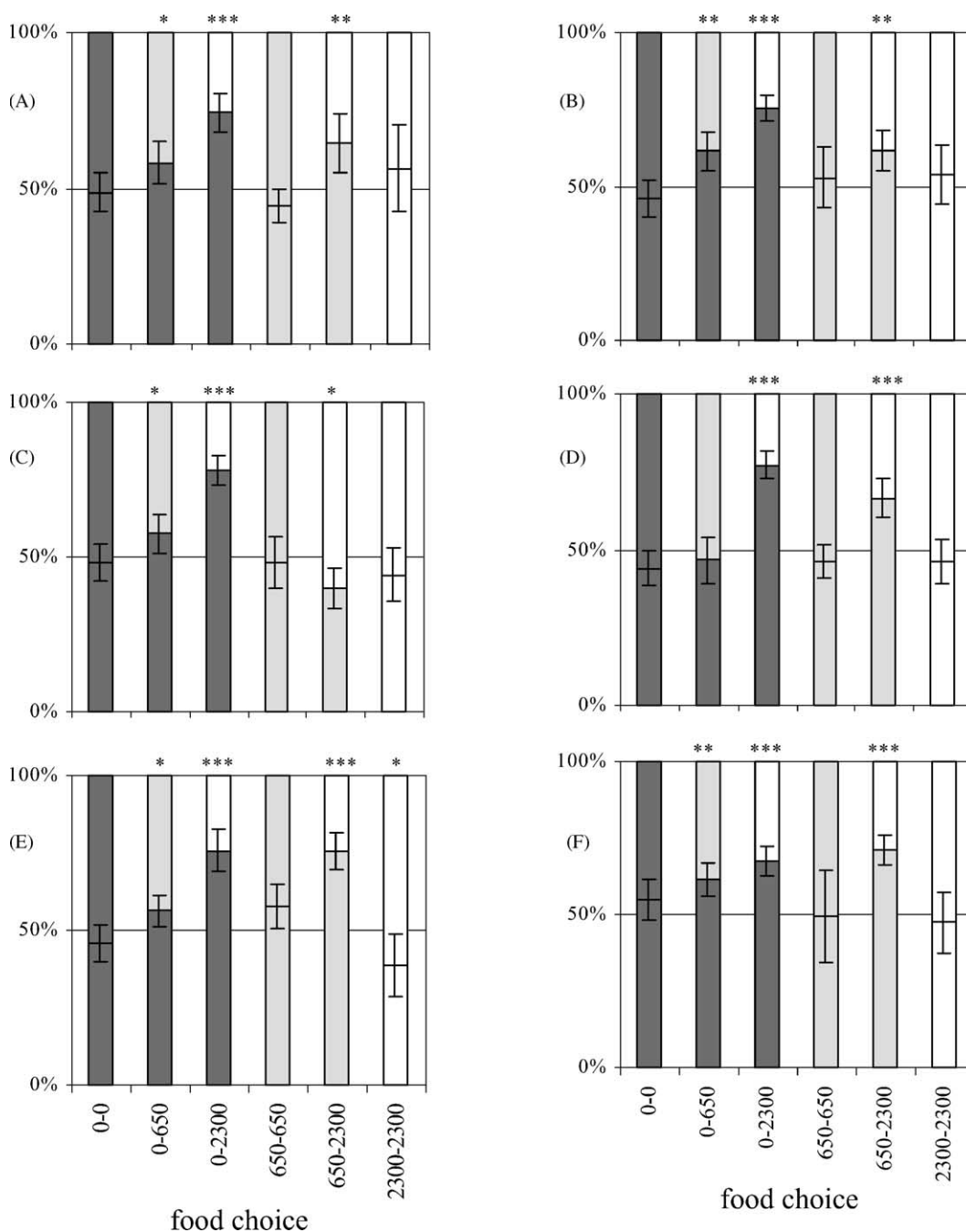


Fig. 2. The ratios between consumption rates of the two offered leaves (with 95% confidence intervals) in differently pre-exposed animals. (A) pre-exposure to untreated food, 14 days; (B) pre-exposure to untreated food, 28 days; (C) pre-exposure to 650 mg Cu kg⁻¹, 14 days; (D) pre-exposure to 650 mg Cu kg⁻¹, 28 days; (E) pre-exposure to 2300 mg Cu kg⁻¹, 14 days; (F) pre-exposure to 2300 mg Cu kg⁻¹, 28 days. Asterisks above bars represent significant differences between consumption rates of offered leaves and 50% (horizontal line) (Student t-test: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Dark grey bars - untreated food; light grey bars - food with 650 mg Cu kg⁻¹ dry weight; white bars - food with 2300 mg Cu kg⁻¹ dry weight.

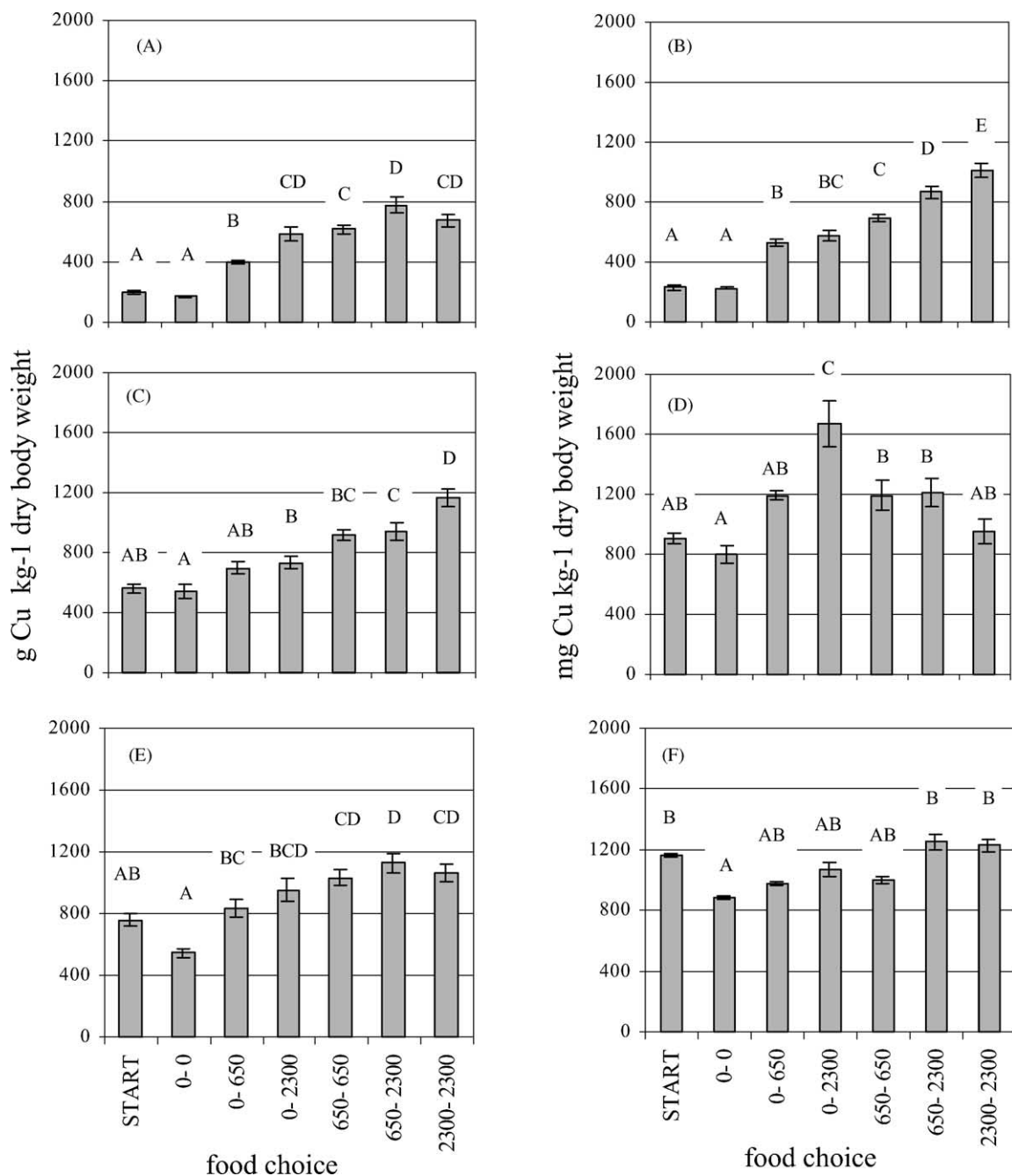


Fig. 3. Mean (\pm S.E.) concentrations of copper in *Porcellio scaber* after different pre-exposures (“START”) and 14 days of food choice experiments. (A) pre-exposure to untreated food, 14 days; (B) pre-exposure to untreated food, 28 days; (C) pre-exposure to 650 mg Cu kg⁻¹, 14 days; (D) pre-exposure to 650 mg Cu kg⁻¹, 28 days; (E) pre-exposure to 2300 mg Cu kg⁻¹, 14 days; (F) pre-exposure to 2300 mg Cu kg⁻¹, 28 days. Mean values that differ significantly (ANOVA, Tukey test, $P < 0.05$) from each other are labelled with different letters.

Table 1
Mortality during food choice experiments with *Porcellio scaber* according to type and duration of pre-exposure to Cu-enriched food^a

Food choice (mg Cu kg ⁻¹ –mg Cu kg ⁻¹)	Pre-exposure (mg Cu kg ⁻¹ dry food weight)					
	0		650		2300	
	14 days	28 days	14 days	28 days	14 days	28 days
0–0	0	1	1	1	2*	4*
0–650	0	0	1	2	4	8
0–2300	1	1	3	1	2	4
650–650	0	3	1	5	2	9**
650–2300	1	1	3	2	3	9*
2300–2300	2	1	1	1	5	2
SUM	4	7	10	12	18 ^b	36 ^c

Letter a indicates number of animals in each food choice group was 20. Asterisks indicate when the body copper concentrations of dead animals significantly (Mann–Whitney test) exceeded copper concentrations in surviving animals from the same group, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Letters b, c indicates significantly higher mortality ($P < 0.01$, <0.001 , respectively) from animals pre-exposed to 0 mg Cu kg⁻¹ dry food weight.

kg⁻¹, which consumed significantly more food with higher amounts of copper (Fig. 2C).

3.2. Copper accumulation during pre-exposure and during food choice experiments

The total copper body burden of the animals collected from the field was approximately 200 mg Cu kg⁻¹ dry body weight, and increased after 14 or 28 days of pre-exposure to Cu-enriched food (Fig. 3 “START”). In the main experiment, copper concentrations in animals increased further with copper concentrations in the food (Fig. 3). The copper body burden reached a “saturation” level of around 1200 mg Cu kg⁻¹ dry body weight. As reported in the literature, the copper body burden rarely exceeds 1000 mg Cu/kg dry weight (Wieser and Büchel, 1976; Hopkin et al., 1986; Hopkin and Hames, 1994). In one group of animals the average copper body burden was around 1600 mg/kg dry body weight (Fig. 3D). These animals were pre-exposed for 28 days to 650 mg/kg Cu-enriched food and fed for 14 days with both untreated and 2300 mg kg⁻¹ Cu-enriched food.

In animals offered both Cu-enriched and untreated food, the copper body burden also increased, though to a lower extent than in animals fed only a Cu-enriched diet. Copper concentrations in animals pre-exposed to 2300 mg kg⁻¹ Cu-enriched food decreased after feeding on untreated food (Fig. 3E and F). This indicates that copper loss may occur after feeding on uncontaminated food.

3.3. Mortality during food choice experiments

Mortality increased in animals pre-exposed for 14 days to 2300 mg Cu kg⁻¹ food, and was especially pronounced in animals pre-exposed to this copper concentration in the food for 28 days (Table 1). In 22 out of the 36 animals (pre-exposed for 28 days to 2300 mg Cu kg⁻¹) that died during the main experiment, the copper body burden was significantly higher (Mann–Whitney test, $P < 0.05$) than in surviving animals measured at the end of the experiment. In the majority of dead animals the copper body burden did not exceed that of surviving animals.

4. Discussion

The results reported in this paper confirm those obtained by Dallinger (1977) that *Porcellio scaber* prefers food with no copper added if offered together with food enriched with high concentration of copper. Contrary to Dallinger’s findings (1977), in our experiments discrimination against a Cu-enriched diet was dependent neither on previous exposure to copper nor on copper body burden. Therefore, our data do not support the speculation of Dallinger (1977) that isopods can monitor the state of their copper stores by a unique homeostatic mechanism, which regulates copper intake. Dallinger (1977) also reported that reduced consumption of Cu-enriched food was

compensated for by consumption of untreated food. This was not observed in our study.

In our food choice experiments, animals pre-exposed to untreated food as well as copper pre-exposed animals consumed some Cu-enriched food even when it was offered along with the untreated food. This indicates that animals might not discriminate against Cu-enriched food prior to ingestion, but the reduced consumption rate of Cu-enriched food could be due to adverse metabolic effects of ingested copper. Copper ions directly generate oxyradicals and enhance lipid peroxidation (Scott-Fordsmand et al., 2000) and can therefore exert their toxic action immediately after entering the cells in high amounts. On the other hand, isopods could detect copper-rich food by chemoreception as was proposed by Weissenburg and Zimmer (2003). Nevertheless, animals might consume some Cu-enriched food ‘willingly’, to compensate for eliminated copper, as demonstrated by Weissenburg and Zimmer (2003).

The discrimination against Cu-enriched food by chemoreceptors is not necessarily directly related to copper. It is known that *P. scaber* is strongly attracted by the odour of metabolites released by microorganisms that colonise food particles (Zimmer et al., 1996). It was suggested by Hassall and Rushton (1982) that isopods respond to differences in microbial populations of the litter brought about by different copper treatments. Weissenburg and Zimmer (2003) found significantly lower cellulase activity in copper-contaminated litter, which indicates microbial population changes. As stated by Zimmer et al. (1996) some by-products of cellulolytical activity attract isopods. Based on the literature data and personal observations, isopods strongly preferred fungal-colonised food (Gunnarsson, 1987; Zidar et al., 2003). Hopkin (1993) suggested that discrimination against copper-rich food may be due to aversion to fungi. He found that copper concentrations in fungal hyphae might exceed by several times the copper concentrations in the leaves on which they are growing. Therefore, with the ingestion of fungal hyphae, the dose of ingested copper may increase greatly. In that case, the discrimination against copper-enriched diet would be related to both the “bad taste” of food, that can be recognised by chemoreception, and the adverse metabolic effects of copper that make food unacceptable.

In our experiments, a reduced consumption of Cu-enriched food was not compensated for by an increased ingestion of untreated food. Altogether, isopods consumed lower amounts of food per unit time. On longer exposure, this eventually leads to reduced growth rates, as was demonstrated in the feeding experiments of Hassall and Rushton (1982) and Farcas et al. (1996). The other effect of a copper-enriched diet was increased mortality, as was also reported by Farcas et al. (1996). High mortality was evident in animals pre-exposed to highly Cu-enriched food, although these animals had access to untreated food after pre-exposure. It seems that in some of these animals copper stores were overloaded. Also, the animals’ death is not necessarily related to their copper storage capacities.

When food selection is discussed as a means by which *P. scaber* may avoid the toxic effects of copper in a polluted environment, a ‘safety factor’ as described by Hopkin and Hames (1994) for zinc should be taken into account. Namely, concentrations of metals in laboratory experiments that cause the animals’ response can be several times lower than those in the field. By reason of the higher extractability of copper from artificially Cu-enriched food than from naturally contaminated food, copper “detectability” can also increase in laboratory experiments. We might conclude that in *P. scaber* copper-enriched food avoidance may mitigate but probably cannot prevent toxic effects of copper in heavily copper-polluted environments.

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