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Effect of feeding regime on the results of sediment bioassays and toxicity tests with chironomids

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Feeding of test organisms during sediment toxicity testing may have a great effect on biological parameters, like the growth and survival of the animals, which are used as toxic endpoints. The nutritional condition of the organisms both before and during toxicity testing affects their fitness, as it does their sensitivity to contaminants. Adding uncontaminated food with high organic carbon and/or lipid content to test vessels possibly changes the partitioning of chemicals and thereby alter their accumulation in animals. Also elimination may be changed by feeding. It has also been observed, that actual toxic responses at population level may be determined by the amount of food available for each organism. In spite of these observations, the effect of feeding has only rarely been extensively studied in sediment ecotoxicology and currently there is no concensus on the feeding regime to be used in toxicity tests.

1. Introduction

The effect of feeding has only rarely been extensively studied in sediment ecotoxicology and currently there is no concensus on the feeding regime to be used in toxicity tests. The term 'feeding regime' describes the quantity of food offered to the test organisms and the manner in which it is presented (e.g. number of feedings per day). In general, feeding of animals during sediment toxicity tests should be avoided (Hill et al. 1993). This is not, however, always possible, especially when chronic tests are conducted in sediments with low nutritional value.

If animals have to be fed, feeding should be kept at a minimum level (Hill et al. 1993). Many commonly used species, like oligochaetes, do not necessarily need additional feeding. Chironomids, however, may starve to death without added food, especially when tests are initiated with young larvae. It is obvious that, without feeding, the risk for false positives (e.g. reduced survival, growth or reproduction due to reasons other than toxicants) is very high (Ankley et al. 1993, Ankley et al. 1994a). Furthermore, even if larvae survive without feeding, food limitation has a great effect on their growth, which is often used as a response to toxicant stress (Day et al. 1994, Rasmussen 1984). In spite of this, both the amount and type of food used in toxicity studies has been very variable (Table 1). It seems that feeding has been fairly abundant but the basis of the feeding regime used has seldom been given.

2. Effect of sediment characteristics on test organisms

Physicochemical characteristics of sediments may have a great effect on the biological responses observed in sediment toxicity tests. Ankley et al. (1994a) studied the effects of physicochemical properties of fifty uncontaminated natural sediments using ten-day exposures with three species, *Hyalella azteca*, *Chironomus tentans* and *Lumbriculus variegatus,* with or without the addition of exogenous food. The range of particle size distribution of the samples was relatively wide. For example, approximately 25% of the samples contained more than 40% of sand or more than 40% clay. Organic carbon content varied from 0.3 to 8.1%. The major factor influencing the results of the tests with all three species was related to nutrition. Survival of unfed *Lumbriculus variegatus*, however, was high and it appears that this species is suitable for tests without feeding. On the contrary, a relatively high percentage (25%) of the *Chironomus tentans* tests would have failed a control survival criterion of 70% without feeding (0.4 mg/larva/d of Tetramin®). Feeding affected growth of midges to a greater extent than it did survival. There was, however, a large variation in survival and growth among both fed and unfed larvae. The variation is suggested to be due to the physicochemical properties of the sediments. Organic carbon content did not have a significant predictive value relative to the nutritive status of the sediments. It appeared, however, that the chironomids grew better in slightly coarser substrates.

Physicochemical characteristics of sediments have to be taken into account especially when field-contaminated sediments are studied. For example, tests with whole sediment dilutions should produce a gradient of contamination that yields quantitative responses of the test organisms. Responses to dilu-

Table 1. Feeding regimes used in toxicity tests and bioassays for *Chironomus tentans* and *Chironomus riparius.*

		test length d	mg/larva/	mg/larva/d	test period
Chironomus tentans	Cerophyl®	until emergence	30	1.5	Nebeker et al. 1984
	Tetra®	(appr. 20 d)	4	0.2	
	Tetra Conditioning Food	14 d	28	2	Adams et al.1985
	Tetrafin®	10 d	60	6	Giesy et al. 1990
	Tetramin®	10 d	4	0.4	West et al. 1994
	Tetrafin®	10 d	4	0.4	Ankley et al. 1994b
Chironomus riparius	Ralston Purina trout chow + dehydrated alfalfa (5:1 w/w)	until emergence (appr. 24 d)			
	commercial dog treats		–	–	Pittinger et al. 1989
	Tetramin®	10 d	5	0.5	Taylor et al. 1991
	Tetramin®	until emergence (20–40 d)	10–20	0.5	McCahon & Pascoe1991
	Cerophyl®	14 d	1.2	0.2	
	Selenastrum capricornutum		$168\times10^{5\bullet}$	$12\times10^{5\bullet}$	
	Hartz® dog treats		2.8	0.2	Nelson et al. 1993

(• = cells)

tion gradients can identify the degree of necessary remediation of sediments to reduce toxicity. Selection of the appropriate diluent for the contaminated sediment, however, may influence the results of the study. Diluting sediments may reduce the amount of food available to the organisms, requiring them to spend more energy on foraging. Therefore, if contaminants come primarily from food, dilution may not reduce the total exposure but increase energetic stress, which could result in an increased toxicity of diluted sediments. Furthermore, test organisms that feed on sediment organic matter, with little or no associated contaminants, may be food limited if the dilutions reduce the content of organic carbon the in sediments (Nelson et al. 1993). Also, when the toxicity of field-collected sediment samples is tested, special attention should be paid to the selection of a suitable reference sediment. The physicochemical characteristics (at a minimum, particle size and organic matter) of the reference sediment should be as similar as possible to those of test sediments (Hill et al. 1993). This might be difficult to achieve, but the possible effects of the differences on biological responses should be evaluated when the results are discussed.

3. Effect of feeding on fitness of test organisms

The nutritional status of an aquatic organism, both prior to and during testing, can significantly modify the apparent toxicity of a chemical. In order to decrease the variability of toxicity test results, feeding regimes and food types used should be routinely reported. Monitoring the pre-experimental nutritional status of test organisms also allows the researcher to assure that the organisms are in the acceptable condition for toxicity tests.

The nutritional status of an organism is concerned with the quantity and quality of the organism's diet, as well as the levels of contaminants present in the diet. The quality of diet refers to its proximate composition (percentage of protein, lipid, carbohydrate, water, ash), the specific composition of these various components (amino acid profiles, amount of essential fatty acids) and the presence of adequate amounts of micronutrients (vitamins and minerals) (Lanno et al. 1989). The nutritional status of an animal affects its metabolic rate. Feeding an organism will increase its metabolic rate (heat increment of feeding; Jobling 1981) and hence the uptake, metabolism and depuration rates of a toxicant. For example, it has been observed, that feeding decreased the toxicity of copper to copepods (*Acartia tonsa*) (Sosnowski et al. 1979), as well as the toxicity of linear alkylbenzene sulfonate to *Daphnia magna* (Taylor 1985).

The composition of a diet will affect the composition of an organism feeding upon that diet. For example, high levels of dietary lipid will result in an increased deposition of depot fat in rainbow trout (Watanabe 1982). An increase in the lipid compartment of an organism will increase bioaccumulation of lipophilic toxicants. A diet that increases lipid content in animals may also alter the toxicity of some lipophilic toxicants (Hickie & Dixon 1987).

Pre-experimental nutritional status can also have a marked effect on toxicant impact. A increased quantity and availability of protein increased the tolerance of rainbow trout and bluegills to chlordane and the PCB product, Arochlor 1254 (Mehrle et al. 1977). Even different geographical strains of a brine shrimp, *Artemia*, have different nutritional values, which can affect the outcome of a toxicity test in which they are used as food (Bengtson et al. 1994). The effect of food quality on the toxicity of copper to *Daphnia magna* has also been observed. Daphnids fed vitamin-enriched algae were less sensitive to chronic copper stress than animals fed a trout-granule diet. Food quality did not, however, affect the acute toxicity of copper (Winner et al. 1977). There are very few studies available in the literature where the effect of food quality on benthic species is extensively examined (see Ankley et al. 1993).

Nutrition has probably been a major factor influencing the results of many studies which examined growth, survival and reproduction of species exposed to sediments differing in organic carbon content, particle size distribution and chemical composition (de Wolf et al. 1992, Sijm et al. 1993, Ankley et al. 1994a). These studies demonstrated lower growth rates, reproduction and survival in nutritionally poor substrates such as sediments from oligotrophic lakes. Phipps et al. (1993) suggest, that food should be added to nutritionally poor sediments during bioassays in order to avoid results that are due to nutrition rather than contaminant toxicity. Some other results indicate, however, that possible differences in the growth of midge larvae, caused by sediment quality, can not be compensated by feeding (Ristola et al. 1994). In the study of Harkey et al. (1994), feeding appeared to have little effect on growth and lipid content of the *Chironomus riparius* in sediments with low organic carbon content (about 0.47%) at 10°C. This test, however, was initiated with fourth instar midge larvae, which probably are less sensitive to starvation than first instar larvae. Also, at 10°C the metabolic rate is considerably decreased compared with exposure at 20°C (Cairns et al. 1975), which probably also lowers the nutritional demands of organisms. When 40-d emergence tests, starting with first instar larvae were conducted with four uncontaminated sediments (OC% 0.5–59) and three feeding levels (no feeding, 5 mg/larva or 10 mg/larva during the whole test), most of the midges died without feeding. Only in the most organic sediment some of the larvae (73%) survived, but their growth was extremely slow. Increasing the amount of added food enhanced larval development (Ristola et al. 1994).

4. Effect of feeding on toxicity and accumulation of chemicals

Feeding of test organisms during a bioassay is often expected to reduce contaminant accumulation. When feeding is the dominant accumulation route, changes in organism health, in response to the contaminant, can enhance or reduce ingestion and/or elimination rates. For example, stress placed on the test organism due to handling or to an unfamiliar exposure environment may reduce its feeding rate. Conversely, the feeding rate, as well as overall metabolic rate, may be increased as a stimulatory response to contaminant exposure. If an animal feeds selectively on uncontaminated particles, the concentration of contaminants in its food is less than in bulk sediment. If uncontaminated food is preferentially ingested, elimination may be enhanced because of differential partitioning of the contaminants to fecal material. Thus, it is possible to see a decrease in contaminant accumulation when organisms are fed uncontaminated food during a bioassay (Harkey et al. 1994).

Postma et al. (1994) have studied the interacting effects of cadmium toxicity and food limitation on the midge, *Chironomus riparius,* during chronic exposure in the laboratory. If the food was supplied *ad libitum,* both larval developmental time and mortality of the larvae were negatively affected by cadmium concentrations of 2.0–16.2 μg/l. Food limitation of unexposed larvae at high population densities reduced fitness, judging from on all parameters studied, and consequently reduced the population growth rate (by 85%). The effects on larvae, which were exposed to both cadmium and food limitation, differed considerably from the response to the individual stress factors. Exposure to cadmium increased mortality among food-limited first and second instar larvae. Consequently, the amount of food available for each surviving larva increased. At low cadmium concentrations (2.0 and 5.6 μg/l), these indirect positive effects overruled the direct negative effects, and caused an increase in the fitness of the food-limited exposed larvae, compared with the food-limited unexposed controls. At a concentration of 16.2 μg Cd/l, the negative effects of cadmium on food-limited midges balanced the positive effects of reduced food limitation. At this concentration, the population growth rate did not differ significantly from the food-limited control. The authors concluded that the indirect positive effects of cadmium on food limitation could eliminate direct negative effects of low cadmium concentrations on food-limited chironomid populations. For this reason, it is less likely that negative effects of low toxicant concentrations would be assessed in the field. The amount of food available may determine, if chemical stress has negative effects on population level (Hallam et al. 1989).

To determine the potential change in exposure brought about by adding uncontaminated food, whole sediment contaminant bioaccumulation by the midge, *Chironomus riparius*, was determined in the presence and absence of added food (Harkey et al. 1994). When fourth instar larvae of *C. riparius* were exposed to polycyclic aromatic hydrocarbons (PAHs) and/or DDT, and *trans*-chlordane in different feeding levels (one or two mg Cerophyl® to each larva every third day, controls were not fed), highly variable responses were observed. Individual larvae were analyzed for contaminant concentration, mass and total lipid content after two-, four-, seven- and ten-day exposures. Accumulation of pyrene and benzo(a)pyrene was significantly higher with feeding while accumulation of chrysene was significantly less as compared with the controls. The increased bioavailability of pyrene and BaP with feeding may be due to enhanced sorption onto food particles, which would have overridden any reductions in contaminant ingestion resulting from decreased feeding rates, or increases in elimination due to contaminant effects. No feeding-related differences were observed for the two insecticides. The variation between exposure intervals was high and contaminant accumulation was far from consistent.

Adding uncontaminated food with high organic carbon and/or lipid content to test vessels may change the partitioning of a chemical, and thereby, alter considerably its accumulation to animals. This process may be important especially in water-only tests but it can affect also the results in sediment tests. However, there is very little information on this subject regarding sediment testing. Pascoe et al. (1990) have studied the acute toxicity of cadmium to *Chironomus riparius* under four different sets of conditions: 1) no food, no artificial sediment, 2) food provided (Tetramin®), no artificial sediment, 3) no food, artificial sediment provided (homogenized filter paper), or 4) food and artificial sediment provided. The presence of food clearly increased the observed toxicity of cadmium, while the presence of an artificial sediment decreased toxicity. It was demonstrated that cadmium was rapidly sorbed from the test solution onto the food, which increased its accumulation to larvae feeding on that food.

Kosalwat and Knight (1987) have observed a different effect of substrate-binding to copper. They exposed fourth instar larvae of *Chironomus decorus* to copper in water and/or copper in Cerophyl®, which was used as food and substrate. Copper concentration in the midges increased with copper concentration in Cerophyl®. The results suggested, however, that the concentration determined from midges might reflect mostly Cu bound to food and fecal matter in the midge gut. The low BCF values seemed to confirm that only a small amount of Cu in the food substrate was actually adsorbed from the gut. Midges were found to be very tolerant of copper-spiked food substrates. Copper was more toxic to midges when they were xposed to it in water (aqueous forms) than when they were exposed also to the food substrate with Cu (organically complexed forms). The authors hypothesize, that in natural conditions, sediment-bound copper is unlikely to cause immediate toxic effects unless the concentration reaches an extremely high level (several thousand parts per million). Over an extended period, however, lower levels might produce chronic toxic effects on aquatic organisms.

Landrum et al. (1992) have studied the relative importance of water and ingested food as possible routes of benzo(a)pyrene (BaP) and 2,2′,4,4′,5,5′-hexachlorobiphenyl (HCB) accumulation in *Mysis relicta*. The accumulation was examined in the presence or absence of two food types, *Daphnia pulex* and *Tabellaria flocculosa*. The availability of either food enhanced HCB accumulation approximately two times compared with accumulation from a water source alone, but reduced BaP accumulation about 50%. Feeding did not affect HCB elimination but enchanced BaP elimination. The elimination rate constants were considerably larger when *Mysis* fed on *T. flocculosa*, which most likely resulted from the increased mass feeding rates for *Mysis* feeding on the diatom, compared with feeding on *Daphnia*. The fraction of accumulation via ingestion of contaminated food was greater when mysids fed on *Daphnia* than *Tabellaria,* and was greater for HCB than BaP. The more rapid elimination rate for animals feeding on the diatom may be partially related to the rate of food movement through the gut, coupled with the increased sorptive capacity of diatom frustules, as compared with cladoceran carapaces.

Enhanced elimination with feeding has been reported also in other studies. For example, the, consumption of a sediment accelerated anthracene elimination in the amphipod *Hyalella azteca* (Landrum & Scavia 1983), and Leversee et al. (1982) observed that *Chironomus riparius* exhibited increased elimination of BaP while feeding on a paper towel substrate.

5. How to select a suitable feeding regime

A feeding regime has to be modified according to the goals of the study, the nutritional value of the sediments and the demands of test animals used. Ankley et al. (1993) have studied the effects of feeding and flow rate on water quality and exposure conditions using sediment tests with various benthic organisms, among them *Chironomus tentans*. The main goal was to determine a feeding regime that filled the following criteria: 1) acceptable control survival, i.e. ≥ 70%, 2) a mean dry weight of at least 0.8 mg/test organism in a 10-d test when performed in clean quartz sand or an uncontaminated sediment with low (ca. 1%) organic carbon content, and 3) ≥ 60% saturation of dissolved oxygen in overlying water when an uncontaminated sediment with high (ca. 8%) organic carbon content was used. The test was initiated with second instar larvae. Also various food materials (trout starter, Tetrafin®, and a yeast-cerophyll-trout chow (YCT) mixture) were used. The survival and growth of *C. tentans,* held in quartz sand, for ten days without feeding was relatively poor. When larvae were fed YCT, survival of larvae increased markedly, but their growth was less than 0.8 mg/organism at all feeding levels. At feeding rates greater than or equal to 0.4 mg/Tetrafin®/larva/d, the criteria concerning larval survival and growth were met. To maintain the concentration of dissolved oxygen at an acceptable level, the overlying water had to be renewed at a rate of four volumes/d. There is very little information available on the minimum feeding needed by *Chironomus riparius*, but there are some observations, that its nutritional requirements are lower than those of *C. tentans* (G.A. Burton pers. comm.). Acceptable larval survival in a 10-d test has been achieved even with a feeding level of 0.3 mg Tetramin®/larva during the whole test period (Ristola et al. 1994).

A constant feeding regime makes it easier to compare the results between various toxicity studies but, on the other hand, the prediction of ecological consequences on the basis of laboratory tests becomes more difficult. In natural conditions, *Chironomus riparius* usually inhabits very eutrophic environments (Gower & Buckland 1978), where its density may be several thousand individuals per square meter (Frank 1982, Kerkum et al. 1994). In principle, eutrophic sediments have a high nutritional value, but competition for food (and space) among first and second instar larvae probably occurs at least during spring and early summer, when the larval density is at its peak. First and second instar larvae are not only exposed to limited food resources, but they have also been observed to be more sensitive, at least to the effects of cadmium, than the third or fourth instar larvae (Williams et al. 1986). There are, however, some observations that differences in sensitivity between instars depend on toxicant type (Norberg–King et al. 1994). The amount of food available during the life span of an imago may vary from *ad libitum* to weak, and even to starvation, which probably affects its sensitivity to xenobiotics. From both acute and chronic experiments on a range of species, it has become clear that well-fed animals are usually less susceptible to toxicants than food-limited ones (see Postma et al. 1994).

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