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Chronic Toxicity (Mortality) of Freshwater Amphipod *Diporeia* spp. for Zn in Sediment Microcosm

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Abstract - Sediment microcosm experiments were conducted for 14 and 28 days using Zn spiked sediment to examine chronic toxicity (mortality) of *Diporeia* spp. as a function of density and time. Mean cumulative *Diporeia* mortality in 28 day sediment microcosms was 25% at 1,800 μg g^{-1} total Zn in sediment. Although a certain fraction (20~40%) of *Diporeia* was dead, its mortality was attributed by handling stress within 4 days and was not significantly encreased with increasing within the range of Zn concentrations examined in this study. These results suggest that *Diporeia* can tolerate Zn contaminated sediment and may be useful as a biomonitor for Zn contamination in freshwater environments.

Key words : toxicity, mortality, amphipod, metal

INTRODUCTION

The amphipod *Diporeia* spp. which was known as *Pontoporeia hoyi* (=*affinis*) is generally considered as one group (spp.) because taxonomic differences have not been resolved (Smith 1972; Segerstrale 1977; Landrum and Nalepa 1998; Song 2003). The amphipod *Diporeia* is the most widespread benthic macroinvertebrate not only in North American freshwater, especially in the Great Lakes, arctic and subarctic lakes and rivers but also in brackish water in the Baltic Sea (Mozley and Alley 1973; Moore 1979; Nalepa *et al.* 1985). *Diporeia* is more abundant in the deeper and colder regions of lakes, where the average temperature is less than 20°C unlike other freshwater amphipods (i.e. *Hyalella azteca, Gammarus fasciatus*) (Bousfield 1958; Smith 1972).

As a detritivore, *Diporeia* utilizes freshly settled algae as a primary food source and it is fed upon by many species of Great Lakes fish (Gardner *et al.* 1990). Therefore, *Diporeia* plays a major role in the movement of not ony energy, nutrients but also contaminants through the food web between benthic and pelagic production and upper trophic levels (Landrum and Nalepa 1998; Song 2003).

Due to these kinds of important roles, many studies have examined the dynamics of contaminant accumulation in this species, including body burdens, bioaccumulation, and toxicokinetics (Landrum and Faust 1994; Landrum *et al.* 1997; Song 2000).

In recent studies, *Diporeia* have been exposed to contaminants in the laboratory, both water and sediments, to verify these field observations and further evaluate toxicological responses in this organism. Toxic response studies of *Diporeia* have used concentration measures for both the environmental compartment and organism tissue to describe the mortality due to exposure to selected toxicants. A review of field data indicates that *Diporeia* were very sensitive to toxicants. Especially several toxicity studies suggested that the sensitivity of *Diporeia* was comparable or similar to that of other amphipods to organic matter contaminants (Spehar *et al.* 1985; Kierstead and Barlocher 1989). However, in matched bioassays to field collected sediment, *Diporeia* was less sensitive to toxicants than *Hyalella azteca* (Burton *et al.* 1996). In contrast to lots of studies for organ-

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ic matter toxicities of *Diporeia*, laboratory studies examining trace metal toxicity to *Diporeia* were relatively few compared to other freshwater invertebrates (e.g. *Hyalella azteca*).

The purpose of this study is to examine the mortality of *Diporeia* spp. as a chronic toxicity for sediment bound Zn and the possibility of using it as a biomonitor to contaminant metal in freshwater sediments. For the purpose *Diporeia* is cultured in Zn spiked (manipulated) sediments during 14 day and 28 days in sediment microcosm. Zinc is selected as an appropriate metal in this study because it is abundant in sediments and usually accumulated in *Diporeia* spp. (Song and Breslin 1998).

MATERIALS AND METHODS

1. Sampling and handling of amphipod and sediments

Diporeia and sediments were collected using a PONAR grab sampler from surficial sediment at a water depth of about 60 m in Lake Michigan in cooperation with P.F. Landrum (Great Lake Research Laboratory, NOAA). The amphipods were isolated from the surficial sediments (0 ~ 3 cm) using gentle screening with cool lake water (4 ~ 6°C). *Diporeia* were then placed in a cooler (4 ~ 6°C, dark) with unsieved sediments and lake water. Detail methods of sampling and handling of amphipod and sediments are described in Song and Breslin (2004).

2. Analysis of Zn in sediments and statistics

To determine concentration of Zn in sediments, approximately 0.2 g of dried sediments was digested with 1 ~ 3 mL of HNO_3, $HClO_4$, HF according to the same process in Song and Breslin (1998). Statistical analysis of significant differences for wet weight and mortality of amphipod before and after experiments was performed by analysis of variance (ANOVA).

3. Microcosm experiments

Six different Zn concentrations were designed by spiking of Zn in sediment to prepare microcosms. The lowest concentration (C0) of Zn in the sediments was defined as the concentration of Zn in the collected and sieved sediment and was referred to as the unspiked control sediment. The method of spiking and design of Zn concentration ranges (C1 ~ C4) in sediments were according to previous literatures with considering adsorption rates of Zn on sediments (ASTM 1995; Berry *et al.* 1996; Peterson *et al.* 1996) (Table 1).

Table 1. Spiked Zn concentrations in five different treatment microcosms

Conc. (No.)	C0[a]	C1	C2	C3	C4
Spiked [Zn] (μg g dry sed^{-1})	–	154	308	769	1539

[a]Control treatment without Zn spiking

Fourteen (for C0, C2 and C4) and eight (for C1 and C3) replicated treatment microcosms (500 mL glass jar) were prepared with different time and density for this experiments. Six replicates among the fourteen replicates for C0, C2 and C4 were used as reference microcosms without organisms at 0, 14 and 28 days. Six replicate treatments for C0, C2 and C4 were used in low-density microcosms (12 amphipods in a jar) for 14 days and 28 days, respectively. The remaining two replicates in treatments C0, C2 and C4 were used in high density microcosms (24 amphipods in a jar). For C1 and C3 treatments, eight replicate microcosms were prepared. Six of these were used for 14 day and 28 day microcosms while two replicates were used for reference treatments without organisms.

Three mature (> 6 mm) and 9 immature (< 6 mm) sized amphipods were placed into 500 mL glass jars as low density microcosms (12 amphipods in a jar: 1,500 individuals m^{-2}), while 6 mature and 18 immature sized amphipods were placed into a glass jars as high density microcosms (24 amphipods in a jar: 4,500 individuals m^{-2}), respectively. After that all microcosms were maintained inside of a dark and cold (5°C) room. Approximately half of the overlying water in each treatment was replaced every four days with filtered lake water. When water was replaced, aeration was stopped for 1 day. Food was not supplied during microcosm experiments because *Diporeia* can survive in sediment for several months without added food (Landrum 1989; Landrum and Faust 1994). At the end of each exposure, 1 ~ 3 amphipods were collected and the individual wet weight was determined. After each experiment live and dead amphipods were counted to examine their mortality. Detail configuration of sediment microcosm experiments was presented in Song and Breslin, 2004.

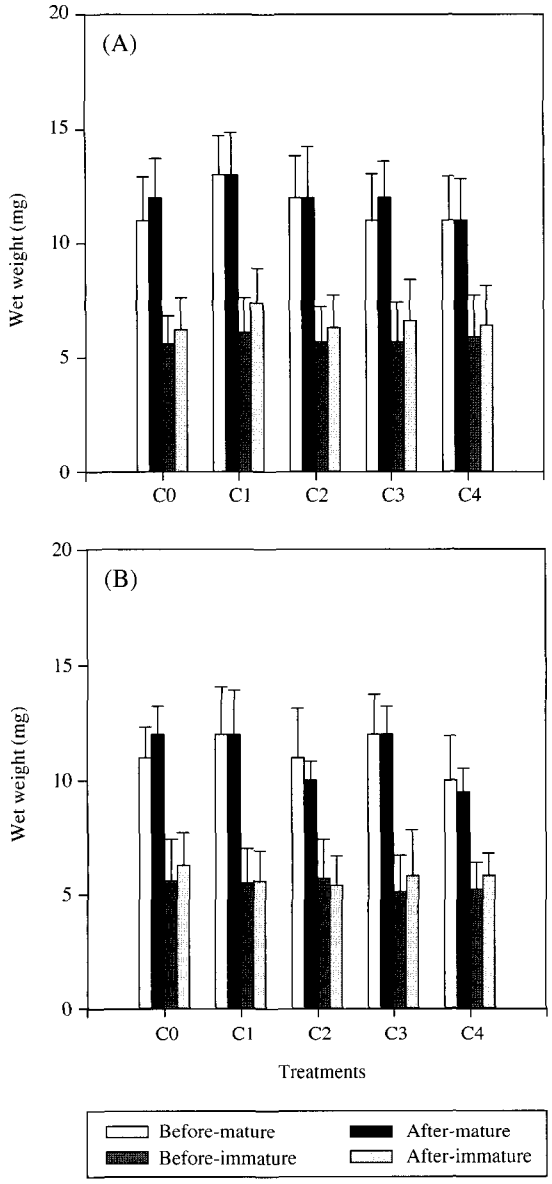

Fig. 1. Comparison of *Diporeia* wet weight before and after 14 day (A) and 28 day (B) microcosm experiments.

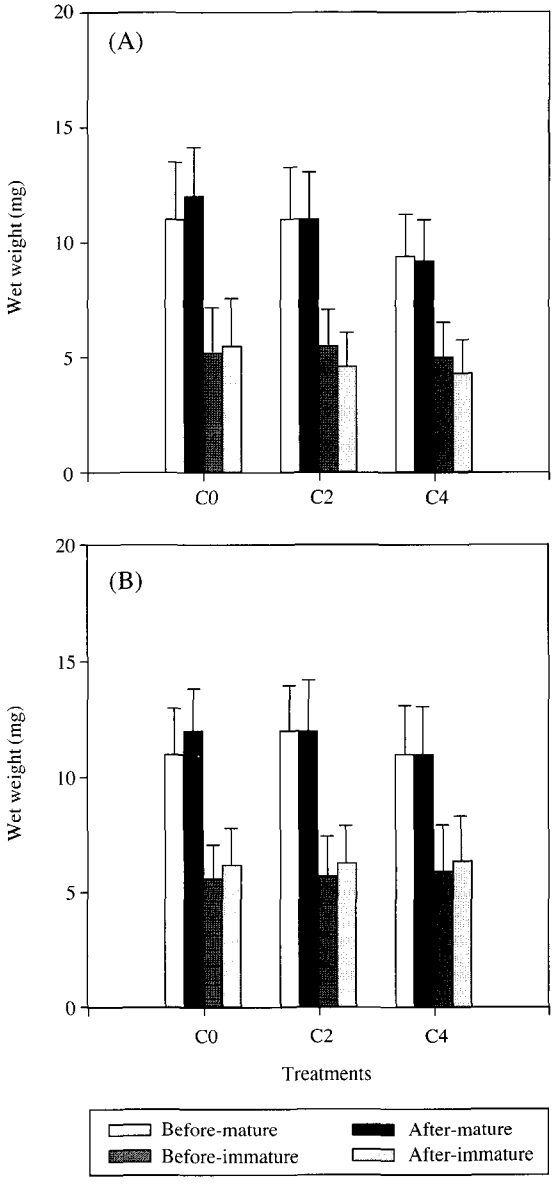

Fig. 2. Comparison of *Diporeia* wet weight before and after 14 day microcosm experiments at high (A) and low (B) densities.

RESULTS AND DISCUSSION

1. *Diporeia* condition index in microcosm experiments

As a measure of *Diporeia* condition, wet weights (mg) of mature and immature amphipod were measured and compared before and after each microcosm experiment. Results of experiments showed that *Diporeia* wet weights were not significantly changed (mature, ANOVA, P=0.67; immature, ANOVA, P=0.20) in sediment microcosms as a function of time and density (Figs. 1 and 2). It may be due to the low metabolic conditions of *Diporeia* (i.e. temperature, food quality in sediment) in the sediment microcosms. In this study, *Diporeia* was cultured at 4~6°C without added food. These temperatures were colder than their estimated optimum temperature (i.e. 8~12°C) for maximal growth (Gordeev 1952; Bousfield 1989). Based on the *Diporeia* growth rate (0.33 mm month^{-1}) in colder season (November~April) nearshore (Winnell and White, 1984), their growth rate may

Table 2. Mortality of *Diporeia* spp. in 14 day and 28 day sediment microcosm experiments

Day	Treatment	Initial no. of amp.		No. of dead amp.				No. of live amp.		Mortality (%)	
				Within 4 days		Total					
		Im	M.	Im.	M.	Im.	M.	Im.	M.	Im.	M.
14	C0	18	6	2(1.4)	1(1.4)	5.5(2.1)	1.5(0.7)	12(0.7)	5(0.7)	31(6)	25(6)
	C2	18	6	3(2.8)	0.5(0.7)	5.5(2.1)	1(1.4)	11(0.7)	6(2.1)	31(6)	17(7)
	C4	18	6	3.5(0.7)	1(1.4)	6.5(0.7)	1.5(0.7)	12(0.7)	5(0.6)	36(4)	25(5)
14	C0	9	3	0.7(1.2)	0.3(0.6)	2(1)	0.3(0.6)	7.3(0.6)	2.7(0.6)	22(5)	11(6)
	C1	9	3	1(1)	0.7(0.6)	1.7(0.6)	1(0)	7.3(0.6)	2(0)	19(6)	33(0)
	C2	9	3	0(0)	0(0)	1.7(2.1)	0(0)	7.3(2)	2.7(0.6)	19(3)	0(0)
	C3	9	3	0.3(0.6)	0(0)	2.3(1.5)	0.3(0.6)	6.7(1.5)	2.7(0.6)	26(7)	11(5)
	C4	9	3	0.3(0.6)	0(0)	1(1)	0.7(0.6)	8(1)	2.3(0.6)	11(4)	22(5)
28	C0	9	3	0.7(0.6)	0.3(0.6)	2.3(1.2)	0.7(0.6)	6.7(1.1)	2.3(1.1)	26(3)	22(6)
	C1	9	3	0.7(0.6)	0.3(0.6)	1.7(0.6)	0.7(0.6)	7.5(0.7)	2.3(0.6)	19(3)	22(5)
	C2	9	3	0.7(0.6)	0(0)	2(1)	1(0)	7(1)	2(0)	22(4)	33(0)
	C3	9	3	0.3(0.6)	0(0)	1.7(0.6)	1.3(0.6)	7.5(0.7)	1.7(0.6)	19(6)	44(4)
	C4	9	3	1(1.7)	0.3(0.6)	1.3(0.6)	1(1)	7.7(0.6)	1.3(0.6)	15(6)	33(3)

be lower than 0.33 mm month^{-1} due to the microcosm conditions (i.e. low temperature and lower food quality). This growth rate may not be measurable due to errors in obtaining mean wet weight based on wet weight-body length relationships (Song 2003).

2. Mortality of *Diporeia* in microcosm experiments

Mortality (%) of *Diporeia* was determined by total number of dead animals divided by initial number of animals in each treatment for 14 day and 28 day microcosms (Table 2). *Diporeia* mortality was not significantly changed (ANOVA, P=0.53) between mature and immature individuals following 14 days, however, it was significantly higher (ANOVA, P=0.046) for mature compared to immature individuals following 28 days (Table 2).

Cumulative mortalities of *Diporeia* (mature plus immature as a function of time) were similar (23~25%) in the C0~C4 treatments in the 28 day microcosm experiment (Fig. 3C, 3D). In the 14 day high density microcosm, 43~56% of the total cumulative mortality (30~34%) occurred within 4 days (Fig. 3C). While 10~43% and 56~100% of the total cumulative mortality (23~25%) in the C0~C4 treatments occurred within 4 days and 14 days, respectively, in the 28 day low density microcosm (Fig. 3D). In 14 day exposure, cumulative mortality of it was significantly higher (ANOVA, P=0.0035) at high density compared to low density (Fig. 3C and 3D).

Relatively high initial mortality of *Diporeia* (30% in 4 days and 80 % in 14 days of total 28 day cumulative mortality) suggests that *Diporeia* were affected by stresses of handling processes and/or other harsh sediment conditions. In these experiments, each *Diporeia* was handled to determine the wet weight prior to insertion into the sediment microcosms. For the variation of Zn concentration, *Diporeia* cumulative mortalities (23~25%) were not significantly changed with increasing Zn concentration in sediment following 28 days which means that over 75% of *Diporeia* survived at 1,800 μg g^{-1} total Zn (corresponded with spiked Zn concentration of 1,539 μg g^{-1}) in sediment. These results suggest that *Diporeia* can tolerate for the highly Zn contaminated sediment. In short-term (7 day) bioassay experiments for Hamilton Habor sediment, Jackson *et al.* (1995) found that *Diporeia* mortality exceeded 64% in hypolimnion mud contained the high concentration of Zn (1,500~2,000 μg g^{-1}). However they did not figure it out what kind of material contribute to its high mortality among particularly high Zn, total PCBs and PAHs in sediments.

Several literatures also suggested that *Diporeia* mortality was affected by high other metal concentrations in sediment environment. Results of field studies showed that *Diporeia* densities decrease with increasing sediment Cu (Kraft 1979). In long-term (460 days) laboratory soft-bottom microcosms, tissue cadmium (Cd) concentrations in *Diporeia* gradually increased up to about 3,500 times compared to dissolved Cd concentrations (6.5 μg L^{-1}) in the overlying water and their mortality was about 70% for adults and 100% for juveniles (Sundelin 1983). However, *Diporeia* accumulated Zn in its body up to 2.7 μmol g^{-1} at 23~25% cumulative mortalities following 28 days in this

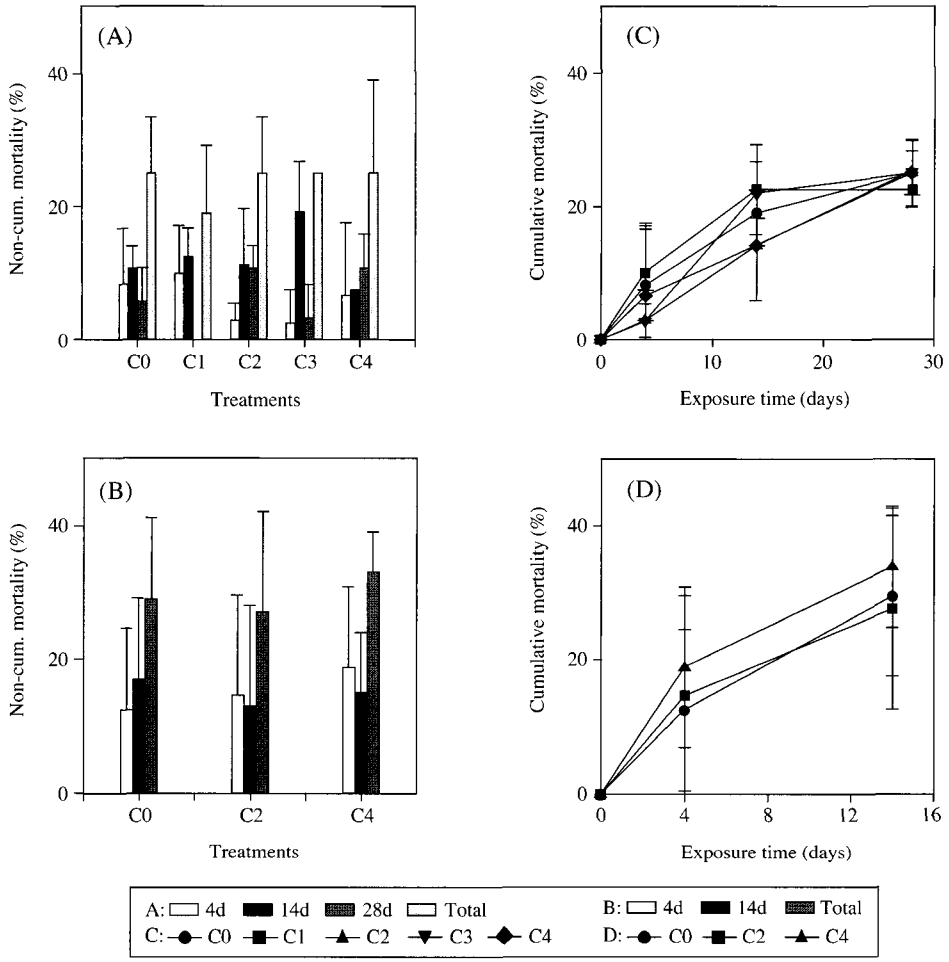

Fig. 3. Non-cumulative (A and B) and cumulative (C and D) *Diporeia* (mature plus immature) mortality (%) as a function of treatment (A and B). exposure time (C and D) and density (A and C: low density, B and D: high density).

experiment. It was comparable with LBC25 (Lethal Body Concentration resulting in 25% mortality; 3.08 μmol g^{-1}) of *Hyalella azteca* in similar sediment microcosm experiments by Borgmann and Norwood (1997). Although *Diporeia* LBC25 for sediment-bound Zn was not obtained directly in these studies, results of experiments suggest that *Diporeia* LBC25 is higher than the LBC25 for *Hyalella azteca* as most (30~80%) of the observed *Diporeia* mortality was occurred due to handling stress rather than Zn toxicity. These results may suggest that *Diporeia* is more tolerant than *Hyalella azteca* of Zn contaminated sediments.

In particular, *Diporeia* is considered as an appropriate organism to examine the accumulation of contaminant metals from freshwater sediments because it actively feeds on sediment both in aerobic and anaerobic (i.e. hypolimnion) (Dermott and Corning 1988; Lopez and Elmgren 1989; Jackson *et al.* 1995; Song and Breslin 1998; Song 2003). Based on its tolerance for high concentration of Zn in this study and accumulation of Zn in sediment, *Diporeia* may be used as a potential biomonitor for contaminant metals in freshwater sediment.

CONCLUTION

Following 14 day and 28 day sediment microcosms for *Diporeia*, wet weight (mg) of it did not significantly changed before and after experiments. It may be due to the low metabolic conditions of *Diporeia* (i.e. temperature, food quality in sediment) in the sediment microcosms. Based on the *Diporeia* growth rate (0.33 mm $month^{-1}$), the growth rate may not be measurable due to errors in obtaining mean wet

weight based on wet weight-body length relationships. Relatively high initial *Diporeia* mortality within 4 days suggests that *Diporeia* were affected by stress from the handling process and/or other harsh sediment conditions.

Diporeia cumulative mortalities (23 ~ 25%) were not significantly changed with increasing Zn spiking concentration in sediment and over 75% of *Diporeia* survived at total Zn (1,800 μg g^{-1}) in sediment following 28 day microcosm experiments. These results suggest that *Diporeia* can tolerate high Zn contaminated sediment.

The tolerance and accumulation ability of *Diporeia* for high concentration of Zn in sediment, *Diporeia* can be considered as a potential biomonitor for contaminant metals in freshwater sediments.

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