

RESPONSE OF THE AMPHIPOD *DIPOREIA* SPP. TO VARIOUS STRESSORS: CADMIUM, SALINITY, AND TEMPERATURE

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ABSTRACT. The tolerance of the amphipod *Diporeia* spp. (formerly *Pontoporeia hoyi*) to salinity and temperature and the acute toxicity of cadmium were determined by laboratory tests. No mortality occurred during 28 d exposure at a salinity of 20 g sea salt L⁻¹. Slower swimming and mortality occurred when the salinity was increased to 25 g sea salt L⁻¹. In freshwater, *Diporeia* tolerate a temperature of 26°C but exhibit 82% mortality within 24 h at 28°C. Thus, *Diporeia* collected from southern Lake Michigan demonstrate a significant salinity and thermal tolerance. Sensitivity to cadmium depends on both the temperature and the salinity of the exposure solution. At 4°C in fresh water, the 96 h LC₅₀ for Cd, administered as CdCl₂, was 0.78 mg Cd·L⁻¹ (95% C.I. 0.40-1.02 mg Cd·L⁻¹). At 15°C, it decreased to 0.065 mg Cd·L⁻¹ (95% C.I. 0.051-0.074 mg Cd·L⁻¹). In salt water, the tolerance to Cd at 4°C [96 h LC₅₀ of 49.4 mg Cd·L⁻¹ (95% C.I. 45.3-52.7 mg Cd·L⁻¹)] was much greater than in fresh water and decreased with increasing temperature, 96 h LC₅₀ of 6.02 mg Cd·L⁻¹ (95% C.I. 6.02-7.32 mg Cd·L⁻¹) at 15°C. Overall sensitivity to cadmium increases with increasing temperature and decreases with increasing salinity within the salinity tolerance of the organism. *Diporeia* are important Great Lakes benthos exposed to a wide range of contaminants. While *Diporeia*'s sensitivity to contaminants has been implied, this work provides supportive data on the sensitivity of *Diporeia* to selected stressors.

INDEX WORDS: Cadmium, *Diporeia*, osmoregulation, thermal regulation.

INTRODUCTION

Diporeia spp. constitute approximately 65% of the benthic macroinvertebrate biomass at depths greater than 30 m in Lake Michigan (Nalepa 1989) and are an important food chain component for most of the fish in the Great Lakes (Mosely and Howmiller 1977). *Diporeia* are considered "glacial relicts" that are thought to have descended from the marine amphipod, *Pontoporeia femorata* (Vainola and Varvio 1989). The taxonomic identity of these Great Lakes amphipods has undergone several name changes from *Pontoporeia affinis* to *Pontoporeia hoyi* (Segerstrale 1977) and most recently to *Diporeia* spp. (Bousfield 1989).

The disappearance of *Diporeia* from polluted environments (Nalepa and Landrum 1988) suggests they are sensitive to contaminants. Only a

few specific data are available to substantiate this suggestion, including acute toxicity to carbaryl and pentachlorophenol (Landrum and Dupuis 1990) and thermal tolerance with Lake Superior collected organisms (Smith 1972). This work expands the data base on the sensitivity of *Diporeia* by examining the response to temperature, salinity, and the acute toxicity of cadmium (Cd)—a common heavy metal pollutant.

MATERIALS AND METHODS

Diporeia Collection and Culture

Diporeia were collected by Ponar grab at a depth of 24-29 m from Lake Michigan approximately 4.8 km southwest of Grand Haven, Michigan. The *Diporeia* were gently screened from the sediment, placed in lake water, and transported on ice to the laboratory. At the laboratory, *Diporeia* were housed in shallow aquaria with 4-5 cm of 29 m Lake Michigan sediment and approximately 10 cm

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of Lake Michigan water at 4°C. *Diporeia* used for testing were actively swimming juveniles ranging from 1.39 to 6.34 mg wet weight (3.28 ± 1.3 , $n = 100$, mean \pm sd) that were selected randomly, specifically excluding fertile females.

Salinity Tolerance

Exposure solutions, from 5 to 35 g sea salt·L⁻¹, were made by diluting a stock artificial seawater solution composed of 1.4 kg of granular Instant Ocean (Aquaria Systems) with carbon-free distilled water. The salinity concentrations of the exposure solutions were determined by measuring the solution densities at 4°C and extrapolating the concentrations from a standard curve of concentration versus solution density.

A standard curve of salinity versus density at 4°C was generated by weighing out appropriate amounts of artificial sea salts for each of the seven solutions to cover the range of 5 to 35 g sea salt·L⁻¹. The solutions were cooled to 4°C and the density of each solution measured to generate a standard curve.

The exposure solutions (in triplicate) were placed in 400-mL plastic beakers and held at 4°C. Dissolved oxygen (DO) and pH were determined in initial samples of exposure water (test) and Lake Michigan water (control). The pH was measured on a Beckman pH meter, and DO was determined by Winkler titration (Grasshoff 1983).

Twenty *Diporeia* were added to each of three beakers containing salt water (5 g sea salt·L⁻¹) at 4°C. Controls in 4°C Lake Michigan water were also run in triplicate. Dead *Diporeia* were removed after 24 h and the remaining animals were gently placed into beakers containing the next higher concentration of sea salt. The activity of the animals was also examined visually every 24 h. The concentration of sea salt was increased 5 g sea salt·L⁻¹ every 24 h. This process was repeated until the organisms had been exposed to 35 g sea salt·L⁻¹. The exposure to the 35 g sea salt·L⁻¹ continued for 72 h (216 h total experiment duration). This procedure allowed the *Diporeia* to acclimate to a maximum tolerated concentration. At the end of the experiment, the pH and DO of the water were again measured. No substrate was used in this portion of the experiment so that the animals could be easily observed and transferred between solutions.

In a second bioassay, the 96 h tolerance of *Diporeia* to salinity was examined with the exposure to full osmotic shock. Salinity concentrations ranging

from 5 to 35 g sea salt·L⁻¹ with 5 g sea salt·L⁻¹ increments were cooled to 4°C, as before. Twenty animals were then added to each of three replicate beakers at each concentration, including a lake water sample for control, and held at 4°C. The animals were examined for mortality every 24 h out to 96 h. The dead animals were removed every 24 h. The LC₅₀, concentration that results in 50% mortality, was then calculated for each 24 h interval using the SAS probit procedure (SAS 1985).

In a third bioassay, *Diporeia* were acclimated to 20 g sea salt·L⁻¹, an apparent no effect level, and placed in beakers containing 2 cm of sediment that had been mixed with salt water containing 20 g sea salt·L⁻¹ and 600 mL saltwater (20 g sea salt·L⁻¹) at 4°C. The animals were screened from the sediment and percent mortality was determined after 28 d.

Thermal Tolerance

Twenty five beakers, each beaker containing 1 cm of sediment and 400 mL of lake water, were cooled to 4°C. Twenty *Diporeia* were then placed into each beaker. After every 24 h, one beaker was removed, the contents screened to determine mortality, and the temperature increased by 2°C/day. The remaining beakers were removed and examined for dead animals on the sediment surface, then returned to the incubator. When the temperature reached 24°C, replicates of three beakers were removed and examined for mortality by sieving out the animals. The remaining beakers were again examined for surface mortality and returned to the incubator. This bioassay ran until the temperature reached 30°C. The remaining beakers were removed, the organisms sieved from the sediment, and the mortality determined.

Cadmium Toxicity

The acute toxicity of CdCl₂ to *Diporeia* was determined using a 96 h static exposure in either lake water or in water containing 20 g sea salt·L⁻¹. The test solutions were made by diluting a stock CdCl₂ solution containing ¹⁰⁹Cd so that the actual concentrations of the dilutions could be determined from ¹⁰⁹Cd activity (Table 1). Three replicates of each concentration were placed in plastic beakers. Water samples (2 mL) from each replicate were removed and the ¹⁰⁹Cd activity determined. Samples of the stock solution were also analyzed for ¹⁰⁹Cd activity.

After cooling to 4°C, twenty *Diporeia* were then added to each beaker. Every 24 h, dead animals

TABLE 1. Actual concentrations for cadmium toxicity bioassays (mean mg Cd/L \pm SD, n = 3).

Freshwater Concentrations			Saltwater Concentrations		
4°C	10°C	15°C	4°C	10°C	15°C
0.43 \pm 0.05	0.20 \pm 0.01	0.068 \pm 0.010	47.4 \pm 4.5	15.9 \pm 0.78	5.3 \pm 0.6
0.89 \pm 0.07	0.31 \pm 0.01	0.096 \pm 0.004	52.1 \pm 2.4	22.8 \pm 0.40	9.7 \pm 0.5
1.00 \pm 0.03	0.48 \pm 0.01	0.105 \pm 0.003	73.1 \pm 7.1	29.3 \pm 1.34	14.1 \pm 0.6
1.18 \pm 0.07	0.52 \pm 0.02	0.207 \pm 0.005	84.2 \pm 15.7	35.9 \pm 2.12	16.4 \pm 0.2
1.50 \pm 0.16	0.65 \pm 0.07	0.393 \pm 0.020	109.4 \pm 4.5	44.3 \pm 1.55	22.9 \pm 0.7

were removed, the mortality recorded, and the beakers were returned to the incubator. Additionally, water samples (2 mL) were taken every 24 h and analyzed for ^{109}Cd activity. After 96 h the beakers were removed, mortality recorded, and LC₅₀ values calculated.

The ^{109}Cd activity was measured in a fixed geometry to obtain relative counts, using a lithium-drifted germanium detector coupled to a Nuclear Data multichannel analyzer. The high energy resolution permits unambiguous determination of the ^{109}Cd activity. Because the geometry was identical for each sample, including the stock solution, relative counts could be compared directly and it was not necessary to determine absolute activity. The actual concentrations for the bioassays were calculated from the ^{109}Cd activity of the exposure solutions and the specific activity of the stock solution (Table 1).

Four additional bioassays were performed, two in lake water and two in 20 g sea salt·L⁻¹, at 10 and 15°C. The organisms were acclimated to temperature and salinity prior to the assay by increasing the temperature of the holding aquaria by 2°C/day and the salinity by 5 g sea salt·L⁻¹/day respectively. The animals were first acclimated to salinity and then to temperature. The animals were then used in the assays 24 h after the acclimation process was completed. The acute toxicity tests were performed as above and the concentrations determined from the ^{109}Cd activity (Table 1).

In addition the ^{109}Cd activity was measured in the dead organisms at all time points and the live organisms at the end of each test.

Statistics

LC₅₀ values were determined using the SAS probit procedure (SAS 1985). Means and slopes of regression lines were compared using Student's t test. Data were considered significant at $p \leq 0.05$.

RESULTS

Salinity Tolerance

Diporeia tolerated a gradual salinity increase to 20 g sea salt·L⁻¹ at 4°C before any behavioral changes were observed. At 25 g sea salt·L⁻¹, *Diporeia* began to exhibit stress in the form of slower swimming which deviated from the normal vigorous patterns that were usually observed. Mortality was first observed at 30 g sea salt·L⁻¹ (15% \pm 10%, n = 3, mean \pm sd) and increased to 57% \pm 16% (n = 3) mortality after 48 h at 35 g sea salt L⁻¹. When gradually acclimated to 20 g sea salt·L⁻¹, *Diporeia* did not exhibit any mortality for the 28 d exposure.

When *Diporeia* were placed directly into different concentrations of sea salts, some mortality was observed at 20 g sea salt·L⁻¹ after the first 24 h and at 15 g sea salt·L⁻¹ after 48 h, but this was not significantly different from control mortality in Lake Michigan water (Table 2). Significant mortality was observed at 25 g sea salt·L⁻¹ (Table 2). The LC₅₀ values for each 24 h period were not statistically different over the 96 h exposure, 24 h LC₅₀ 26.7 g sea salts·L⁻¹ (95% C.I., 16.7–36.6 g sea salts·L⁻¹). This value suggests that the mortality was due to the initial osmotic shock. At the beginning of the experiment, the pH of the water was 8.05 (mean, n = 2) and the DO was 12.10 \pm 0.22 $\mu\text{g O}_2\cdot\text{mL}^{-1}$ (mean \pm SD, n = 3), neither value changed over the course of the experiment. Therefore, *Diporeia* mortality did not result from oxygen depletion. The pH of the saltwater was essentially the same as that measured for Lake Michigan water, 8.13 (mean, n = 2).

Thermal Tolerance

Increasing temperature had no effect on survival until 24°C, and then only 6.5% \pm 1.53% (mean \pm sd, n = 3) mortality was observed. At 26°C no

TABLE 2. Cumulative percent mortality when exposed to full osmotic shock (mean \pm SD, n = 3).

Sea Salt Concentration (g/L)	24 h	48 h	72 h	96 h
Control	0	8.3 \pm 7.6	8.3 \pm 7.6	8.3 \pm 7.6
5	0	0	0	0
10	0	0	0	0
15	0	1.6 \pm 0.6	1.6 \pm 0.6	1.6 \pm 0.6
20	5.0 \pm 5.0	8.3 \pm 7.6	8.3 \pm 7.6	8.3 \pm 7.6
25	50.0 \pm 22.9	56.7 \pm 22.5	66.6 \pm 12.6	71.7 \pm 10.4
30	100.0 \pm 0.00	100.0 \pm 0.00	100.0 \pm 0.00	100.0 \pm 0.00
35	100.0 \pm 0.00	100.0 \pm 0.00	100.0 \pm 0.00	100.0 \pm 0.00

additional animals were affected. At 28°C, more than 50% of the animals screened from the sediment were dead (Table 3).

Cadmium Toxicity

At all three temperatures, cadmium's acute toxicity to *Diporeia* was lower in salt water than in lake water (Table 4). The accumulation of Cd by *Diporeia* also differed among the toxicity tests. The range of Cd concentrations in live *Diporeia* at 96 h and 4°C in salt water was 60–117 ng Cd·mg⁻¹ wet weight compared to 15–28 ng Cd·mg⁻¹ wet weight in lake water over the range of Cd concentrations studied. The dry to wet weight ratio is 0.269 \pm 0.052 (Landrum 1988). The ratios of the *Diporeia* Cd concentration to the water concentration for the 4°C exposures were 33 \pm 11 (mean \pm SD, n = 4) for lake water and 1.2 \pm 0.3 (n = 6) for salt water after 96 h exposures across the range of exposures. Thus, the amount of Cd in the water that was available to *Diporeia* was significantly lower in salt water than in lake water. The body burden data were too variable to calculate, lethal body burden for 50% mortality.

TABLE 3. Mortality of *Diporeia* exposed gradually to increasing temperature^a.

Temperature °C	% Mortality (Mean \pm SD, n = 3)	Elapsed Time d
4–22	0 \pm 0.00	10
24	6 \pm 1.53	11
26	0 \pm 0.00	12
28	82 \pm 0.58	13
30	100 \pm 0.00	14

^aThe temperature was elevated at 2°C every 24 h.

The toxicity of Cd progressively increased as the temperature increased over the range of 4 to 15°C for both salt water and lake water (Table 4). However, at 15°C, Cd was still less toxic in salt water than when measured at 4°C in lake water. The relationships between 96 h LC₅₀ for Cd toxicity and temperature in fresh water and salt water were not linear with temperature.

Fresh water:

$$\text{Log LC}_{50} = 0.291 (\pm 0.147) - 0.093 (\pm 0.014) \cdot T$$

where T is temperature in °C, r² = -0.978, n = 3.

Salt water:

$$\text{Log LC}_{50} = 2.036 (\pm 0.133) - 0.078 (\pm 0.012) \cdot T$$

where T is temperature in °C, r² = -0.974, n = 3.

The slopes of the regression lines, the response to temperature, are not statistically different in spite of the higher concentrations of Cd required to produce mortality in salt water as represented by the differences in the intercepts of the two regression lines.

DISCUSSION

Because of their importance in the benthic food web and benthic coupling to the pelagic food web, developing a data base of ecological and toxicological information about this species is important both for understanding their productivity and tolerance to anthropogenic stress and the function of the Great Lakes ecosystem. Few previous studies have directly measured the response of *Diporeia* to potential environmental and anthropogenic stressors. Previous studies suggested that *Diporeia* were

TABLE 4. LC_{50} values (mg Cd/L) with 95% confidence interval for *Diporeia* exposed to cadmium.

Water Temp. (°C)	24 h	48 h	72 h	96 h
Lake Water				
4	*	1.6 (1.5 - 1.8)	1.0 (0.5 - 1.3)	0.8 (0.4 - 1.0)
10	*	0.74 (0.62 - 1.03)	0.41 (0.36 - 0.46)	0.28 (0.24 - 0.32)
15	0.37 (0.31 - 0.49)	0.16 (0.14 - 0.19)	0.08 (0.07 - 0.09)	0.06 (0.05 - 0.07)
Salt Water (20 g sea salt·L ⁻¹)				
4	*	69.1 (64.3 - 74.2)	57.4 (52.4 - 61.6)	49.4 (45.3 - 52.7)
10	*	46.5 (40.0 - 61.7)	23.6 (14.8 - 29.8)	17.5 (0.2 - 23.8)
15	*	15.4 (14.1 - 17.1)	8.3 (4.5 - 11.7)	6.7 (6.0 - 7.3)

*No LC_{50} could be calculated because of insignificant mortality.

less sensitive to a cholinesterase inhibitor, carbaryl, and a respiratory inhibitor, pentachlorophenol, than other crustaceans when tested at 4°C (Lan-drum and Dupuis 1990). However, the amphipods were found to be extremely sensitive to thermal shock (Smith 1972). Our study extends the current understanding of *Diporeia* response to thermal stress and introduces data on the resistance to osmotic shock and cadmium acute toxicity.

Salinity Tolerance

The salinity tolerance for *Diporeia* ranges from fresh to brackish water. They easily tolerated 20 g sea salt·L⁻¹ for 28 d. When *Diporeia* were acclimated, a salt concentration equivalent to full strength sea water (35 g sea salt·L⁻¹) was required to produce 50% mortality. Nonacclimated organisms exposed to full osmotic shock exhibited a 24 h LC_{50} at 26.7 g sea salt·L⁻¹. The high salinity tolerance suggests that *Diporeia* have good osmoregulatory capability at 4°C, the nominal temperature that these organisms experience in the environment. This high salinity tolerance is not unusual among the Amphipoda. In fact, *Diporeia*'s survival during exposure to high salinity by osmotic shock was similar to that of *Hyaella azteca* which exhibit a 96 h LC_{50} between 22.5 and 25.0 g NaCl·L⁻¹ at 20°C (Nebeker and Miller 1988).

The recent interest in the impact of contaminated sediments requires the development of bioassays that are relatively insensitive to the physical environment such as salinity while still responding to the contaminants. The range of osmoregulation represented by the 28 d survival at 20 g sea salt L⁻¹ suggests that *Diporeia* would be a suitable bioassay

species for examining the toxicity of sediments collected along a salinity gradient, such as an estuary. Most of the marine or estuarine amphipods currently recommended for sediment testing require a minimum of 25 g sea salt·L⁻¹ (ASTM 1991). The most frequently used amphipod for estuarine/marine sediment evaluations of toxic contaminants is *Rhepoxynius abronius*. This amphipod is extremely sensitive to changes in salinity and sediment composition during bioassays and cannot be used below 25 g sea salt·L⁻¹ (DeWitt *et al.* 1988). In contrast, *Diporeia* is relatively insensitive to moderate salinity stress. It is also insensitive to sediment composition, since it is found in sediment types ranging from coarse sand to silty muck (Nalepa *et al.* 1985). Thus, *Diporeia* will provide an alternative organism for evaluation of a wide range of sediments because of their tolerance to salinity and sediment composition.

Thermal Tolerance

The apparent temperature tolerance observed for *Diporeia* (26°C) was more than two times greater than the 96 h LT_{50} of 10.8°C previously described by Smith (1972). The difference between the results may be explained in part by the environmental conditions to which the animals are naturally adapted and by experimental design. The *Diporeia* tested by Smith (1972) were collected from Lake Superior at a depth of 10–12 m and a temperature of 6°C. Also, the experimental method used by Smith transferred the amphipods directly from 6°C to each test temperature producing a maximum thermal shock. In the experiments reported here, the temperature was increased gradually to

reduce thermal shock. However, Lake Michigan *Diporeia* are naturally exposed to temperatures that exceed 15°C during the summer, and most survive thermal shocks of 10°C or more due to upwelling and downwelling events which rapidly alter the temperature. The organisms collected for this study may be genetically adapted to tolerate wider changes in environmental temperatures than found in Lake Superior.

Toxicity of Cadmium

Cadmium is one of the more frequently reported contaminants in the Great Lakes (Rathke and McRae 1989). Regardless of its entry route to the lakes, Cd rapidly partitions to particles and becomes deposited in the sediments resulting in exposure of benthic organisms. The levels of Cd in *Diporeia* are reported to range from 0.68 to 2.75 $\mu\text{g}\cdot\text{g}^{-1}$ dry weight in different areas of Lakes Huron and Ontario (Rathke and McRae 1989). These concentrations are 30 to 100 times lower than the concentrations found in the live *Diporeia* after 96 h exposure to Cd in our experiments. Thus, the Cd exposures from both water and sediment sources are not high enough to produce acute mortality (96 h toxicity). This does not suggest that the levels of Cd found in *Diporeia* in the Great Lakes are safe levels. Chronic effects may occur based on concentrations found in some parts of the Great Lakes (2–3 $\mu\text{g}\cdot\text{L}^{-1}$, Rathke and McRae 1989), particularly since chronic effects have been reported for other amphipods exposed to Cd in low, 1–5 $\mu\text{g}\cdot\text{L}^{-1}$ concentrations (Borgmann *et al.* 1989). However, we expect exposures to contaminated sediments to be much more important for these organisms than exposures from the low levels (generally > 0.05 $\mu\text{g}\cdot\text{L}^{-1}$ dissolved Cd: Rathke and McRae 1989) found in the open waters of the Great Lakes.

Comparisons of *Diporeia* sensitivity to other freshwater amphipods for Cd toxicity are limited. A 96 h LC_{50} for the freshwater amphipod, *Gammarus pulex* (sexually mature males only) was 0.03 mg Cd·L⁻¹ in lake water at 11°C (McCahon and Pascoe 1988) compared to 0.282 mg Cd·L⁻¹ for *Diporeia* at 10°C. The difference between the two organisms may be explained in part by the sexual maturity of the organisms. The *G. pulex* were sexually mature males while the *Diporeia* were sexually immature females. Alternatively, *Diporeia* may be a less sensitive species than *G. pulex*.

While much work needs to be done to determine Cd toxicity effects on freshwater amphipods,

marine amphipods have been used to assess Cd toxicity associated with sediments and/or in salt water. In our saltwater bioassays, the 96 h LC_{50} for *Diporeia* was much higher than for the marine amphipods tested, although slight differences exist in the respective experimental designs. The marine amphipod, *R. abronius*, was tested at 25 g sea salt·L⁻¹, 15°C, and with sediment to yield an LC_{50} of 1.61 mg Cd·L⁻¹ (Swartz *et al.* 1985). *Allorchestes compressa* was tested at 34 g sea salt·L⁻¹ at 20°C without sediment, and exhibited an LC_{50} at 0.78 mg Cd·L⁻¹ (Ahsanullah *et al.* 1988), while *Diporeia*, in our study, was tested at 20 g sea salt·L⁻¹, 10 and 15°C without sediment to yield LC_{50} 's of 17.5 and 6.7 mg Cd·L⁻¹, respectively. In another study where the experimental conditions were more comparable to this study, 96 h LC_{50} 's for Cd toxicity were determined in 20 g sea salt·L⁻¹ using 13 different marine species (Eisler 1971). The LC_{50} 's ranged from 21 to 55 mg Cd·L⁻¹ for teleosts, which were the most resistant organisms, while the most sensitive organisms were the crustaceans with LC_{50} 's between 0.32–4.1 mg Cd·L⁻¹. In comparing *Diporeia* with these organisms tested at 20 g·L⁻¹, *Diporeia* is apparently more tolerant to Cd than the marine crustaceans and nearly as tolerant as the teleosts, although *Diporeia* was tested at a lower temperature which enhances its Cd tolerance.

Temperature and salinity greatly influence the toxicity of Cd in a variety of marine species (Sundra *et al.* 1978, Fischer 1986, Voyer and Modica 1990). Our data confirm the general findings that Cd toxicity increases with both increasing temperature and decreasing salinity. Understanding the role of these variables is necessary because variations in the environment can alter acute toxicity. Temperature is one of the most important ecological parameters. Temperature changes in shallower regions of the Great Lakes where *Diporeia* may be found can easily be 15°C over the course of a season which would increase Cd toxicity by nearly a factor of ten.

The decreased LC_{50} for Cd at higher temperatures in fresh water and salt water may be due to the increased metabolism by the organism, resulting in increased Cd uptake. When the slopes from the regression equations between LC_{50} and temperature for freshwater and saltwater exposures are compared, the degree to which temperature affects the toxicity of Cd in *Diporeia* is not significantly different between the two water types. Thus, the response to changes in toxicity with changing temperature suggests a common physiological

response whether the organisms are in fresh water or salt water.

The decrease in toxicity with increased salinity results in part from changes in the speciation of Cd. In salt water, Cd-inorganic or -organic ligand complexes increase as salinity increases thus, proportionately less free Cd is available for accumulation (Sundra *et al.* 1978). This decreased toxicity in salt water could also result, in part, from competition between the other divalent metal ions in the salt solution for uptake sites for Cd, and from competition at the receptor sites within the animal, thereby altering *Diporeia's* resistance to Cd. Both mechanisms, reduced bioavailability and increased resistance, seem to be operating in the presence of salt water. The ratio of the amount of Cd accumulated after 96 h to the concentration in the water was 33 ± 11 ($n = 4$) for fresh water compared to 1.2 ± 0.7 ($n = 6$) in salt water demonstrating a reduced bioavailability of Cd in salt water. Additionally, the concentrations of Cd in the organisms at 96 h were approximately five times greater in saltwater than in the freshwater exposures, indicating that seawater also imparts some increased resistance to Cd. The amount of Cd required to produce toxicity in a related marine amphipod, *Pontoporeia affinis*, (Sundelin 1983) is approximately the same as that found for the *Diporeia* exposed in fresh water. The increased resistance to Cd toxicity, based on larger amounts of Cd in living organisms, in salt water, and the somewhat greater resistance to Cd in salt water at elevated temperatures, suggests that *Diporeia* are less stressed, at least over the 96 h exposure, in salt water than in fresh water.

SUMMARY

Diporeia collected from southern Lake Michigan can be described as a euryhaline species with a moderate tolerance to thermal stress. The sensitivity to Cd toxicity was somewhat lower than for other crustaceans of both freshwater and saltwater origin. These studies add to the developing information base necessary to understand the relationship between this important Great Lakes organism and both the potential pollutant impact from anthropogenic contaminants and environmental stresses.

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REFERENCES

- Ahsanullah, M., Mobley, M. C., and Rankin, P. 1988. Individual and combined effects of zinc, cadmium and copper on the marine amphipod, *Allorchestes compressa*. *Aust. J. Mar. Freshwater Res.* 39:33-37.
- ASTM. 1991. Standard Guide for conducting 10-day static sediment toxicity tests with marine and estuarine amphipods. E1367-90. In *1991 Annual Book of ASTM Standards, Volume 11.04 Pesticides, Resource Recovery, Hazardous Substances and Oil spill Responses, Waste Disposal, Biological Effects*, pp. 1105-1119. American Society for Testing and Materials. Philadelphia, PA.
- Borgmann, U., Ralph, K. M., and Norwood, W. P. 1989. Toxicity test procedures for *Hyaella azteca* and chronic toxicity of cadmium and pentachlorophenol to *H. azteca*, *Gammarus fasciatus* and *Daphnia magna*. *Arch. Environ. Contam. Toxicol.* 18:756-764.
- Bousfield, E. L. 1989. Revised morphological relationships within the amphipod genera *Pontoporeia* and *Gammaracanthus* and the "glacial relict" significance of their postglacial distributions. *Can. J. Fish. Aquat. Sci.* 46:1714-1725.
- DeWitt, T. H., Ditworth, G. R., and Swartz, R. C. 1988. Effects of natural sediment features on survival of the phoxocephalid amphipod, *Rhepoxynius abronius*. *Mar. Environ. Res.* 25:99-124.
- Eisler, R. 1971. Cadmium poisoning in *Fundulus heteroclitus* (Pisces: Cyprinodontidae) and other marine organisms. *J. Fish. Res. Board Can.* 28: 1225-1234.
- Fischer, H. 1986. Influence of temperature, salinity, and oxygen on the cadmium balance of mussels *Mytilus edulis*. *Marine Ecology* 32:265-278.
- Grasshoff, K. 1983. Determination of oxygen. In *Methods of Seawater Analysis*, K. Grasshoff, M. Ehrhardt and K. Kremling, eds. pp. 203-229. Verlag Chemie, Weinheim, Federal Republic of Germany.
- Landrum, P. F. 1988. Toxicokinetics of organic xenobiotics in the amphipod, *Pontoporeia hoyi*: role of physiological and environmental variables. *Aquatic Toxicol.* 12:245-271.

- _____, and W. S. Dupuis. 1990. Toxicity and toxicokinetics of pentachlorophenol and carbaryl to *Pontoporeia hoyi* and *Mysis relicta*. In *Aquatic Toxicology and Risk Assessment: thirteenth Volume*, ASTM STP 1096, W. G. Landis and W. H. van der Schalie, eds., pp. 278-289. American Society for Testing and Materials, Philadelphia, PA.
- McCahon, C. P., and Pascoe, D. 1988. Increased sensitivity to cadmium of the freshwater amphipod *Gammarus pulex* (L.) during the reproductive period. *Aquatic Toxicology* 13:183-194.
- Mosley, S. C., and Howmiller, R. P. 1977. Zoobenthos of Lake Michigan. In *Environmental Status of the Lake Michigan Region*. Volume 6, pp. 37-381. Argonne National Laboratory, ANL/IES-40, pp. 37-381.
- Nalepa, T. F. 1989. Estimates of macroinvertebrate biomass in Lake Michigan. *J. Great Lakes Res.* 15:437-443.
- _____, and Landrum, P. F. 1988. Benthic invertebrates and contaminant levels in the Great Lakes: effects, fates and roles in cycling. In *Toxic Contaminants and Ecosystem Health: A Great Lakes Perspective*. M. S. Evans, ed., pp. 77-102. John Wiley & Sons, NY.
- _____, Quigley, M. A., Childs, K. F., Gauvin, J. F., Heatlie, T. S., Parker, M. P., and Vanover, L. 1985. *The macrobenthos of southern Lake Michigan*. NOAA Data Report ERL GLERL 28, Great Lakes Environmental Research Laboratory, NOAA, Ann Arbor, MI.
- Nebeker, A. V., and Miller, C. E. 1988. Use of the amphipod crustacean *Hyalella azteca* in freshwater and estuarine sediment toxicity test. *Environ. Toxicol. Chem.* 7:1027-1033.
- Rathke, D. E., and McRae, G. 1989. *1987 Report on Great Lakes Water Quality, Appendix B, Great Lakes Surveillance, Volume I*. International Joint Commission, Windsor, Ontario.
- SAS Institute Inc. 1985. *Users Guide to Statistics*, 5th ed., pp. 639-646. Cary, NC.
- Segerstrale, S. G. 1977. The taxonomic status and pre-history of the glacial relict *Pontoporeia* (Crustacea, Amphipoda) living in North American lakes. *Continental Biol. Soc. Sci. Finn.* 89:1-18.
- Smith, W. E. 1972. Culture, reproduction and temperature tolerance of *Pontoporeia affinis* in the laboratory. *Trans. Amer. Fish. Soc.* 2:253-257.
- Sundelin, B. 1983. Effects of cadmium on *Pontoporeia affinis* (Crustacea: Amphipoda) in laboratory soft-bottom microcosms. *Mar. Biol.* 74:203-212.
- Sundra, W. G., Engle, D. W., and Thoutte, R. M. 1978. Effect of chemical speciation on toxicity of cadmium to grass shrimp *Palaemonetes pugio*: importance of free cadmium ion. *Environ. Sci. Tech.* 12:409-413.
- Swartz, R. C., Ditsworth, G. R., Schults, D. W., and Lamberson, J. O. 1985. Sediment toxicity to a marine infaunal amphipod: cadmium and its interaction with sewage sludge. *Marine Environ. Res.* 18: 133-153.
- Vainola, R., and Varvio, S. L. 1989. Molecular divergence and evolutionary relationships in *Pontoporeia* (crustacea: amphipoda). *Can. J. Fish. Aquat. Sci.* 46:1705-1713.
- Voyer, R. A., and Modica, G. 1990. Influence of salinity and temperature on acute toxicity of cadmium to *Mysidopsis bahia* Molenock. *Arch. Environ. Contam. Toxicol.* 19:124-131.

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