

# Is sediment or pseudofaeces toxicity responsible for the decline of the amphipod *Diporeia hoyi* in Lakes Erie and Ontario?

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The deepwater amphipod *Diporeia hoyi* has disappeared from Lake Erie and much of Lake Ontario at depths <80 m. This amphipod had supplied 20 percent of the fisheries energy budget in the Great Lakes. The exotic mussel *Dreissena bugensis* now forms most of the benthic biomass above 60 m depth, but *Diporeia* is absent over large areas where *Dreissena* are rare. The filamentous bacterium *Thioploca ingrica* is now common at many sites between 30 and 40 m where *Diporeia* has disappeared. Fisheries and Oceans, Canada, investigated the causes of the decline by examining the sediment chemistry, bacterial production and conducted sediment bioassays using *Diporeia*, *Hyalella* and *Microtox*<sup>®</sup>. *Microtox*<sup>®</sup> showed no evidence of toxicity in sediments now devoid of *Diporeia*. Amphipod survival and growth was greatest in sediment that rapidly lost its *Diporeia* population in 1993. Presence of *Thioploca* had no effect on *Diporeia* survival. *Hyalella* was more sensitive than *Diporeia* to test sediments and to filtered water from mussel cultures. Sediment from sites with dense *Dreissena* populations had lower *Diporeia* survival. A diet of mussel pseudofaeces caused significantly lower survival in both *Hyalella* and *Diporeia*. The exact mechanism causing lower survival is currently unknown and may be related to a nutritional problem or associated waste metabolites.

**Keywords:** benthic assays, metals, *Thioploca*, *Dreissena*

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## Introduction

The deep-water amphipod *Diporeia* spp. was the dominant deposit feeding benthic invertebrate in the Great Lakes, and large boreal lakes of glaciated North America between the lower Mackenzie River eastward to Lake Champlain (Dadswell, 1974). The species burrows in mud at temperatures below 14°C, and is usually associated with the presence of lake trout (*Salvelinus namaycus*) and lake whitefish (*Coregonus clupeaformis*). The related species *Monoporeia affinis* and *Pontoporeia femorata* are common in nearshore areas of the Arctic Ocean, the Gulf of St. Lawrence, and are key elements of the Baltic benthos (Elmgren et al., 1990).

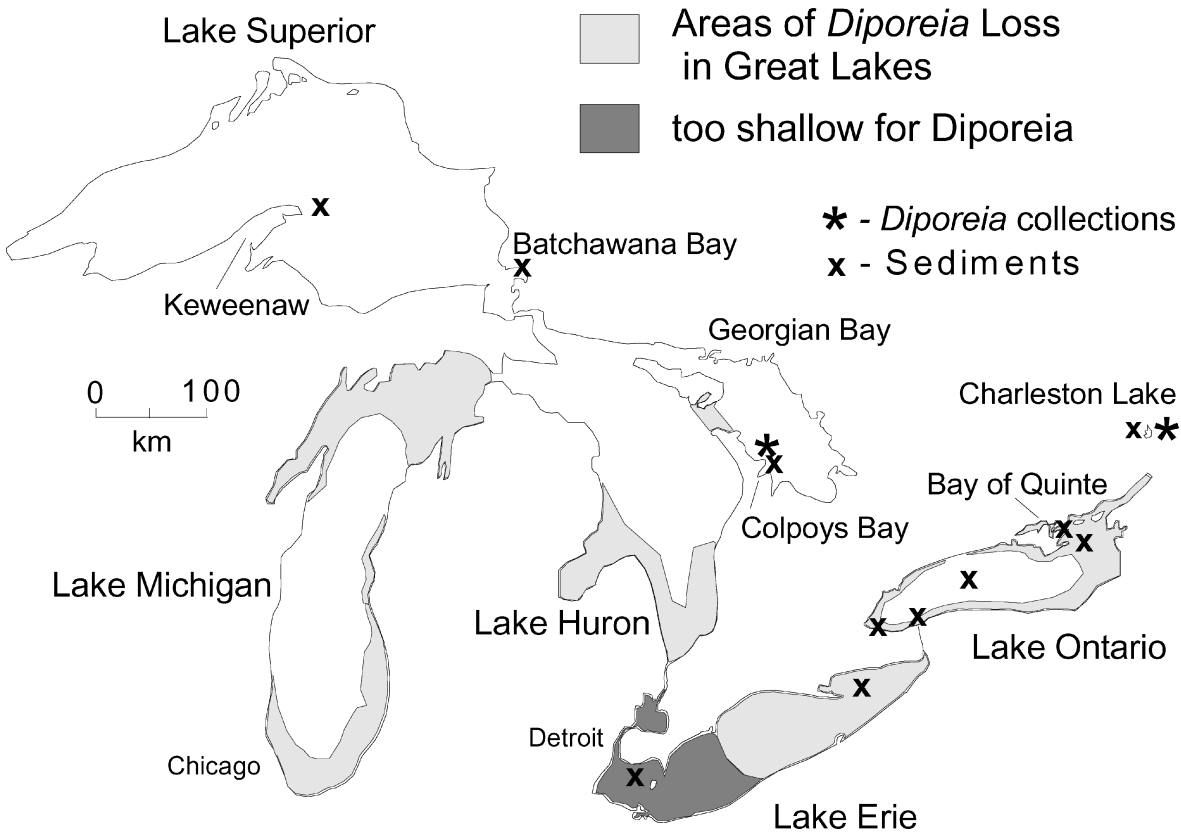
In the Great Lakes, *Diporeia* formed 60 to 80% of benthic biomass, except in shallow western Lake Erie. Before 1990, amphipod densities were typically >3000 m<sup>-2</sup>, with maximum densities of 12,000 m<sup>-2</sup> (Nalepa et al., 1998) and dry biomass of 3 g m<sup>-2</sup> existing at depths between 30 and 60 m. Their high lipid levels (to 50% of their dry weight; Gauvin et al., 1989), and high assimilation of deposited diatoms (Fitzgerald and Gardner, 1993) made *Diporeia* an important pelagic—benthic link between the spring algal blooms and the commercial fisheries (lake whitefish and rainbow smelt (*Osmerus mordax*) and the forage fish: alewife (*Alosa pseudoharengus*), and sculpins (*Cottus* spp.) in the lakes (Flint, 1986; McDonald et al., 1990).

In the 1990s, zebra mussels (*Dreissena polymorpha*) invaded Lakes Erie and Ontario, with *D. bugensis* colonizing down to 60 m depth (Mills et al., 1993). Filtering by the mussels in nearshore areas increased water clarity and changed phytoplankton, benthic populations and food-webs (Nalepa and Fahnenstiel, 1995; Stewart et al., 1998). After the arrival of the mussels, *Diporeia* decreased in eastern Lake Erie beginning in 1992 (Dermott and Kerec, 1997) and eastern Lake Ontario in 1993 (Dermott, 2001). The amphipod has progressively disappeared from large areas at depths <80 m in lakes Ontario, Erie, Michigan and Huron as shown in Figure 1 (Nalepa et al., 1998; Lozano et al., 2001; T. Nalepa, NOAA, personal communications). Declines in commercial catches and reproduction rate of lake whitefish and rainbow smelt have since occurred (Hoyle et al., 1999).

Hypothesized causes for the loss of *Diporeia* include, decreased food availability (*i.e.* diatoms) due to the intense filtering by nearshore dreissenid mussels populations, toxic sediments due to changing contaminant cycling, and toxic excretions or associated

disease from the mussels. Between 1981 and 1995 there were significant reductions in phosphorus levels, algal biomass and chlorophyll in shallow eastern Lake Ontario but not in mid-lake (Johannsson et al., 1998). However, the 50% reduction of algae biomass is not sufficient to account for the complete disappearance of *Diporeia* from most of eastern Lake Ontario.

Altered food-webs may make sediment-bound contaminants more available, or toxicants may be associated with the large quantities of pseudofaeces that are deposited by the exotic mussels. During storms this material could be transported offshore to the *Diporeia* populations. Blooms of the Cyanobacteria *Microcystis* have increased as *Dreissena* selectively reject small-sized blue green algae (Vanderploeg et al., 2001). The toxicant microcystin present could be transferred offshore to *Diporeia* via deposited pseudofaeces. In addition, mats of the filamentous sulphur bacteria *Thioploca ingrica* (Beggiatoaceae) became common in parts of eastern Lake Ontario after *Diporeia* disappeared (Dermott and Legner, 2002). *Thioploca* form



**Figure 1.** Map of the Great Lakes showing areas where *Diporeia* populations have disappeared (shaded) and locations where sediments were collected (x) and sites where live *Diporeia* were collected for the assays (\*).

droplets of sulphur by reacting  $H_2S$  in the sediment with  $NO_3$  from the water; thus, the presence of *Thioploca* could make the sediments a less favourable medium for *Diporeia*.

The objectives of this study were to examine the questions: 1) Are Great Lakes sediments that have lost their *Diporeia* populations now toxic to the amphipods *Diporeia* and *Hyaella*, or display toxicity in the Microtox<sup>®</sup> pore water test? 2) Does the presence of the sediment bacterium *Thioploca* affect *Diporeia* survival? 3) Are dreissenid pseudofaeces or the excretions of *Dreissena* toxic to the amphipods?

## Methods

Sediments were collected from sites where *Diporeia* have disappeared (Figure 1), sites where *Diporeia* are still present, and sediment containing bacterial mats of *Thioploca* (Bay of Quinte). Sediments were collected using an Ekman or PONAR grab. The top 4 cm of sediment was removed and stored in plastic bags at 5°C until used (within 3 months of collection). Sediment sub-samples were freeze dried for particle size and chemical analysis by the National Laboratory for Environmental Testing (Environment Canada, 1995) for major metals, loss on ignition, total organic carbon and neutral herbicides. Particle size was done by the sedge/sieve method. Bacterial production in the sediments was measured in the sediment-water interface layer from settled sediments by modification of the <sup>3</sup>H Leucine uptake method (Hwang and Heath, 1997).

### Microtox<sup>®</sup> porewater test

The bioluminescence or Microtox<sup>®</sup> test is used to quantify toxic effects of substances in aqueous solutions or pore waters. The Microtox<sup>®</sup> liquid phase acute toxicity assay was conducted using pore water extracted from sediment by centrifuging 240 ml of sediment at 6,000 rpm for 60 min at 4°C (Giesy et al., 1988; Munawar et al., 1999). The supernatant was filtered through glass microfibre filters (Whatman GF/C) under 10 psi vacuum. Pore water was maintained at 4°C until used. This assay was performed using a Beckman Microtox<sup>®</sup> model 500 toxicity analyzer following the standard 90% test protocol (Microtox Omni Software, Azur Environmental). Control standards of saline were prepared, as well as solutions of phenol and  $ZnSO_4$  used as a positive test of photo-inhibition of the photo-bacterium after 5-min and 15-min exposures.

### Sediment assays with *Hyaella*

Sediment assays using *Hyaella azteca* were conducted for 28 d following the procedure by Borgmann and Norwood (1999) using Imhoff settling cones with 1 l of water overlaying 15 ml of sediment. *Hyaella* were grown in laboratory cultures at room temperature with a 16 h light: 8 h dark photo period. Fifteen young *Hyaella* (0–10 day old) were added to each cone. The *Hyaella* were fed 2.5 mg of TetraMin<sup>®</sup> fish flakes added weekly to each cone. The *Hyaella* were exposed to the collected sediments, and to three treatments which used cotton gauze instead of sediment as a substrate. Control sediments used in the *Hyaella* assays were a composite from Lake Superior near the Keweenaw Peninsula, which combined sediments from two depths (104 and 164 m).

Two trial runs of four replicates were conducted for all treatments and in the case of the sediments there were two trials per sampling season (spring and fall). Prior to use, sediment was sieved through 1 mm mesh to remove macro-invertebrates, mussels, shell fragments and coarse particles. Temperature and pH were monitored throughout the incubation period, dissolved oxygen and ammonia concentrations were measured prior to the addition of the organisms and at the end of the 28 d period.

### Sediment assays with *Diporeia*

Live *Diporeia* were collected during isothermal conditions from Colpoys Bay on Georgian Bay, or Charleston Lake near Kingston. The *Diporeia* were maintained in sediments from the collection location at 5°C in the dark. Half the water in the cultures was replaced every month, and they were fed freeze dried diatoms (0.8 g) every month. Assays were conducted for 90 d adapted from the methods of Gossiaux et al., 1993, Jackson et al., 1995 and Munawar et al., 1999. Five adult sized *Diporeia* (4–7 mm) were added to 40 ml of sediment in 250 ml jars (density about 1500 m<sup>-2</sup>). The amphipods were captured and transferred to the jars using 6 mm I.D. pipettes to reduce exposure to air. Each jar was covered with a 1 mm mesh held on by an elastic band. Six replicate jars were placed in a 12-litre aquarium containing 3 l of water leaving the jars 1 cm below the water surface. A bubbler was placed in each aquarium. Assays were done at 10°C, with a 12 h light/dark photo period. Once per week, 2 mg of freeze dried diatoms were added to each jar to exceed the feeding rate of *Diporeia* living in mid Lake Ontario (Dermott and Corning, 1988). At 30 and 60 days, the

mud in the jars was screened through 1 mm mesh, the surviving amphipods counted, then replaced into the original mud in the same jars. At the end of 90 days, the mud was re-screened and the surviving amphipods removed, counted and examined before being preserved. Control sediments used in the *Diporeia* assays were a composite from Lake Superior near the Keweenaw Peninsula, or from Batchawana Bay on eastern Lake Superior.

### Assays with bacteria or pseudofaeces

Assays of sediment from the Bay of Quinte exposed *Hyalella* and *Diporeia* to the sulphur bacterium *Thioploca ingrica*. In addition, survival of both *Diporeia* and the *Thioploca* was measured in one experiment. *Thioploca* filaments were collected from Bay of Quinte sediment by screening it through a 0.5 mm mesh. Approximately 0.12 g of bacteria was added to each of the jars containing pre-screened Bay of Quinte sediment. Five *Diporeia* were added to each of 5 jars, one jar had no *Diporeia*. At 30 and 60 days the jars were screened on a 0.5-mm sieve and the number of surviving *Diporeia* and the wet weight of surviving *Thioploca* filaments tallied.

Toxicity of the bacterium *Bacillus thuringiensis* (*Bt*) was tested on *Hyalella* and *Diporeia* by adding 5  $\mu$ l of a 4000 I.U. per mg solution (Thuricide) of the natural insecticide to the control sediments in each cone or jar. Sediments used for the added *Bt* assays were Batchawana Bay for *Diporeia*, and Colpoys Bay and the Bay of Quinte sediment for *Hyalella*.

Toxicity of pseudofaeces and *Dreissena* excretions was also tested. Laboratory cultures of mostly zebra mussels (*Dreissena polymorpha*) were fed frozen diatoms collected from Lake Ontario during the spring bloom (March–April). Pseudofaeces was collected from the aquaria by siphon onto a 28  $\mu$  mesh, placed in graduated tubes and allowed to settle for 24 h. After settling, the ratio of water was adjusted to 12 ml pseudofaeces: 8 ml water. One ml aliquots of this pseudofaeces slurry were dried and ashed to estimate average organic content. The organic content of the pseudofaeces used in the assays averaged 0.013 g ml<sup>-1</sup> (S.E. 0.0014), or 14.62% (S.E. 1.28%) of its dry weight. The required volume of the 12:8 slurry that had double the organic content as compared to the diatom or TetraMin<sup>®</sup> diet was calculated, and this volume added to each jar once per week in place of the normal diet. The pseudofaeces were stored for a minimum of 2 wk at 5°C before use to represent a transport time from the nearshore to below the thermocline.

Filtered water from the aquarium containing *Dreissena* was prepared by filtering the water through a 0.45  $\mu$  filter to remove all but viral-sized particles and dissolved chemicals. The 3 l of water in each assay aquarium (*Diporeia*) or 1 l in each Imhoff cone (*Hyalella*) was replaced every week with freshly filtered water cooled to the appropriate temperature. Control sediments for the *Hyalella* in the assays with added pseudofaeces or filtered water were from mid Lake Ontario, because of better survival than in the Lake Superior sediments. Assays with *Hyalella* were also done using cotton gauze as the substrate, exposure to filtered water, or mussel pseudofaeces added to the gauze.

Mean values and standard errors were determined for the amphipod assays. Significant differences ( $p < 0.05$ ) between test samples and control sediment were determined by Analysis of Variance (ANOVA) of the number alive at the end of the assays, followed by Bonferroni comparisons.

## Results and discussion

The sediments assayed, depth of collection, particle size and organic content are listed in Table 1. At all sites analyzed, levels of neutral herbicides were below detection limits (atrazine 18 ng g<sup>-1</sup>; simazine and D-simazine 10 ng g<sup>-1</sup>; benzoylpropethyl 2.7 ng g<sup>-1</sup>; butylate 10 ng g<sup>-1</sup>; diclofomethyl 6.2 ng g<sup>-1</sup>; metolachlor 17 ng g<sup>-1</sup>). This would be expected as only the Bay of Quinte site was close to tributary sources of agricultural chemicals. The concentrations of selected metals are listed in Table 2. Metal levels were greatest in sediments from mid Lake Ontario and western Lake Erie, but none of the sites had sediments that exceeded the Severe Effects Levels guidelines (SEL; Persaud et al., 1993). Both Batchawana Bay and Charleston Lake had elevated levels of Cr, Pb and Zn in spite of being in forested watersheds with relatively few inhabitants.

The west and east Lake Erie, and west Lake Ontario sites had the highest density of *Dreissena* in the sediments examined (500, 4590, 2320 m<sup>-2</sup> respectively, Table 3). No mussels were collected at the mid or east Lake Ontario sites. *Dreissena* were present in Colpoys Bay at < 10 m<sup>-2</sup> but not quantified. A negative association between *Diporeia* and *Dreissena* densities existed above densities of about 500 m<sup>-2</sup> (Figure 2). This vivid negative relationship between the two species has also been shown in both Lake Erie and Ontario (Dermott and Kerec, 1997; Lozano and Nalepa, 2003), and suggests *Dreissena* densities of > 500 m<sup>-2</sup> are detrimental to *Diporeia*.

**Table 1.** Depth (m), percent sand, mean particle size ( $\mu\text{m}$ ) and percent organics (loss on ignition) in the collected sediments.

Station	depth m	% Dry	% sand	% clay	Mean	% Organic loss % of Dry	Organic C %	Organic N %	Herbicides
					particle micron				
L.Superior-comp.	101	54.4	65.7	12.3	102.2	1.7	0.85	0.18	<d.l.
Batchawana Bay	32	34.2	1.5	31.2	7.7	4.3	1.71	0.17	<d.l.
Colpoy's Bay	38	—	22.8	27.8	5.2	2.4			<d.l.
L. Erie-west	10	34.5	4.9	34.6	7.7	6.2	3.43	0.17	<d.l.
L. Erie-east	38	43.4	0.3	60.7	2.7	3.4	2.45	0.11	<d.l.
L. Ontario-west	45	41.8	22.1	17.9	17.8	3.2	2.60	0.22	<d.l.
L. Ontario-mid	125	15.9	0.6	47.5	4.3	7.9	2.98	0.35	<d.l.
L. Ontario-east	35	22.6	2.1	26.1	9.8	10.62	6.08	0.68	<d.l.
Bay of Quinte	32	20.4	0	39.2	6.7	9.7	7.10	0.82	<d.l.
Charleston Lake	40	9.8	1.2	37.6	7.3	25.29	12.1	1.36	<d.l.

\*Detection limits (d.l. ng g<sup>-1</sup>): Atrazine 18.0; Simazine 10.0; Metolachlor 17.7; Benzoylpropethyl 2.7.

Bacterial production at the sediment-water interface layer in the collected sediments was greatest in sediments from eastern Lake Erie, western and eastern Lake Ontario and lowest in Batchawana Bay (Table 3). Bacterial production reflected the increased organic content of the sediments from deposited material, and may be partly due to the pseudofaeces deposited by the mussels. In Charleston Lake much of the organic content was from refractory plant debris from the forest in the watershed (Table 1) and did not support high bacterial production.

### Microtox<sup>®</sup> testing

Only the pore water from eastern Lake Ontario sediment caused a positive response in the Microtox<sup>®</sup> assay of less than 30% after 15 min exposure. Other sediments tested caused stimulation of photoactivity as compared to the saline controls (Table 4). The lack of significant difference in Microtox<sup>®</sup> activity before and after exposure to the sediment pore water indicated metal levels in the sediments (Table 2) were not sufficient to cause inhibition. Likewise, pore water from

**Table 2.** Metal values as mg kg<sup>-1</sup> in tested sediments.

Sediment	As	Ca	Cd	Cr	Cu	Fe	Ni	Pb	Zn
Lake Superior									
Composite	6	23800	0.3	25	25	16400	19.7	12.5	40
Batchawana Bay	7	7660	1.5	65	38	13713	31.7	30.5	111
Lake Huron									
Colpoys Bay	5	79456	1.9	48	25	25917	51.8	40.6	69
Lake Erie									
Western	4	35600	1.8	62	40	28900	46.9	48.8	159
Eastern	6	81300	0.4	49	26	32700	38.2	18.3	95
Lake Ontario									
Western	5	15600	0.4	38	27	27200	28.9	22.6	95
Mid-lake	20	16900	2.1	74	75	46200	61.9	88.1	293
Eastern	7	64100	1.2	42	47	20600	38.1	41.3	141
Bay of Quinte	10	8440	1.0	63	42	43700	50.4	27.6	143
Charleston lake	9	11400	2.2	64	41	43900	40.7	172	298
SEL	33	—	10	110	110	—	75	250	820

Also given are the Ontario Ministry of Environment's severe effect level (SEL) which is considered heavily polluted and affects the health of sediment-dwelling organisms (Persaud et al., 1993).

**Table 3.** Depth (m), density of *Diporeia* and dreissenid mussels (number m<sup>-2</sup>), and relative abundance of *Thioploca* at sites where sediments were collected.

Sediment	Depth (m)	<i>Diporeia</i> (no. m <sup>-2</sup> )	<i>Dreissena</i> (no. m <sup>-2</sup> )	<i>Thioploca</i> (g m <sup>-2</sup> Wet)	Bacterial production (mg C hr <sup>-1</sup> .)
Lake Superior					
Composite	101	96	0	—	
Batchawana Bay	32	548	0	10	0.394
Lake Huron					
Colpoys Bay	33	2000	+	—	
Lake Erie					
Western	10	0	500	—	0.400
Eastern	38	0	4590	12	0.999
Lake Ontario					
Western	45	0	2320	—	0.872
Mid-lake	125	987	0	—	0.397
Eastern	35	0	0	83	0.790
Bay of Quinte	32	0	420	206	0.576
Charleston Lake	40	3740	0	0	0.398

Degree of abundance: — = not seen, + = low.

sediments with high *Dreissena* densities did not result in photo-inhibition. Thus, the absence of *Diporeia* in sediments from Lake Ontario or eastern Lake Erie does not appear to be due to toxic chemicals in the pore water.

**Table 4.** Percent effect of various sediments in Microtox<sup>®</sup> assays, using a concentration of 90% pore water plus saline.

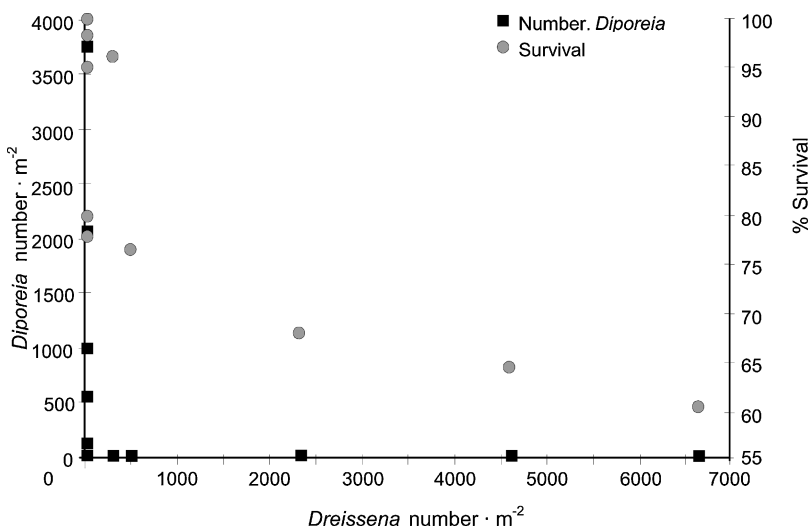
Sediment	5 min.	15 min.
Lake Superior		
Composite	9.5	14.8
Batchawana Bay	8.5	4.8
Lake Huron		
Colpoy's Bay	n.t.	n.t.
Lake Erie		
Western	−16.3	−24.9
Eastern	−22.5	−30.8
Lake Ontario		
Western	−26.1	−38.5
Mid lake	−32.5	−25.9
Eastern	43.7	29.3
Bay of Quinte	0.3	−14.5
Charleton Lake	n.t.	n.t.
Saline Control	0.0	0.0
Phenol (5 mg L <sup>-1</sup> )	68.8	66.2
ZnSO4 (5 mg L <sup>-1</sup> )	32.0	70.0

Two trials were done with measurements of photoactivity made at 5 and 15 minutes.  
n.t = not tested.

Sediment assays

*Diporeia* survival was greatest in the pristine muds from Lake Superior and Charleston Lake (Table 5). Survival was lowest in sediment from the Niagara Bar, off the mouth of the Niagara River. Low amphipod populations in this area were considered a result of chemical inputs from the river (Nalepa and Thomas, 1976). Unfortunately, the Microtox<sup>®</sup> test was not done with the sediment from the Niagara Bar to determine if chemical contaminants are still present. There was lower *Diporeia* survival in sediments from Lake Erie and west Lake Ontario (64 to 68%), all of which have high *Dreissena* populations (>2000 m<sup>-2</sup>). Survival was high in sediment from east Ontario and the Bay of Quinte which has been devoid of *Diporeia* since 1993 but still had only a few mussels present (Table 3; Dermott, 2001). Landrum et al. (2000) found that survival of *Diporeia* was not reduced in Lake Michigan sediments which had lost their amphipod populations, but suspected the low nutritional content caused the amphipods to avoid those sediments.

*Hyalella* was very sensitive to the mud from Batchawana Bay, in eastern Lake Superior (Table 5), an area with little human activity. The low concentrations of the chelators: calcium, iron and organic matter in the sediment from this site may make the metals in the igneous rocks around Batchawana Bay more bioavailable, especially the relatively higher levels of Cr and Zn (Table 2). *Hyalella* survival in sediment from



**Figure 2.** Relationship between *Dreissena* density and *Diporeia* density and survival in the sediments assayed.

eastern Lake Erie (71%) or Lake Ontario (77%) with high *Dreissena* densities was not statistically significantly lower than that in the Lake Superior composite sediment (81.7%; Table 5).

## Bacterial assays

Neither *Diporeia* nor *Hyaella* had reduced survival in Bay of Quinte sediments that contained *Thioploca* (Table 6). The added *Thioploca* represented less than 1/4

of the amount that occurred naturally in these sediments (Table 3; Dermott and Legner, 2002). The amount of *Thioploca* decreased in jars with added *Diporeia* over 60 d (Figure 3), indicating the filamentous bacteria was consumed or destroyed by the amphipods. The recent increase of *Thioploca* in eastern Lake Ontario was likely a result of the bacteria taking advantage of increased sediment organic content following the decline of the *Diporeia* population. Survival of *Hyaella* was not reduced when exposed to the bacteria *Bt* on either

**Table 5.** Percent survival, Standard Error (S.E.) of *Hyaella* and *Diporeia* in the assayed sediments (n = number of trials).

	<i>Hyaella</i> % Survival	S.E.	n	<i>Diporeia</i> % Survival	S.E.	n
L. Superior						
Composite	81.7	7.4	8	98.4	1.8	12
Batchawana Bay	47.5*	9.1	16	95.0	3.6	12
Lake Huron						
Colpoys Bay	85.0	7.7	8	78.0*	7.2	6
Lake Erie						
Western	90.8	3.1	8	76.6	15.0	12
Eastern	71.0	5.7	20	64.4**	7.6	18
Lake Ontario						
Western	77.0	3.9	20	68.0**	7.8	18
Niagara Bar	n.t.			60.0**	13.6	6
Mid-lake	84.1	3.9	16	95.0	4.2	12
Eastern	79.4	7.2	16	80.0	9.6	12
Bay of Quinte	79.1	3.8	20	96.6	2.6	12
Charleston Lake	72.4	11.9	8	100	0	6

Significant difference compared to control sediment, \* =  $p < 0.05$ , \*\* =  $p < 0.001$ .

n.t. = not tested.

**Table 6.** Assays of *Diporeia* and *Hyaella* survival using mussel pseudofaeces, filtered water, or added bacteria.

	<i>Hyaella</i> % Survival	S.E.	n	<i>Diporeia</i> % Survival	S.E.	n
L. Superior Composite	81.7	7.4	8	98.4	1.8	12
Mid-Lake Ontario	84.1	3.9	16	95.0	4.2	12
Pseudofaeces/control mud	17.5**	3.2	16	74.4**	5.2	24
Filtered water/control mud	65.0*	10.3	8	89.6	3.4	24
<i>Thioploca</i> /Bay of Quinte	79.1	3.8	20	100	0	5
Bt/Batchawana	n.t.	—		93.4	9.4	6
Bt/Colpoys Bay	88.7	3.3	8	n.t.	—	
Bt/Bay of Quinte	81.3	4.7	8	n.t.	—	
TetraMin®/gauze	55.0**	11.0	8	n.t.	—	
Filtered water/gauze	52.5**	7.0	8	n.t.	—	
Pseudofaeces/gauze	50.8*	10.1	8	n.t.	—	

Control sediments for *Diporeia* were from Lake Superior or Batchawana Bay. Control substrates for *Hyaella* were mid-Lake Ontario sediment and gauze-only. Symbols are as in Table 5, n.t = not tested.

Colpoys Bay or Bay of Quinte sediment (Table 6). Survival of *Diporeia* was over 93% when exposed to this natural insecticide, indicating *Bt* is not toxic to amphipods.

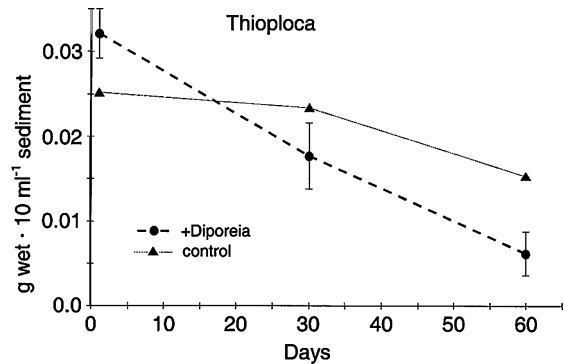
Pseudofaeces assays

Mussel pseudofaeces caused significantly less survival of *Diporeia* as compared to the control sediments (Table 6), in spite of the organic content of the added pseudofaeces being double that of the diatom diet in the controls. A diet of pseudofaeces would be less nutritional than a diet of diatoms, and would be expected to reduce growth rate but not short term survival. Mussel excretions, as filtered H<sub>2</sub>O from mussel cultures, reduced *Diporeia* survival only slightly (89%). This suggests that it is the consumption of the pseudofaeces

and not the excretions of the mussels that is responsible for the reduced survival. *Hyaella* was very sensitive to mussel pseudofaeces on mud resulting in a survival of only 17.5%. *Hyaella* was less sensitive to pseudofaeces when it was added to assays using cotton gauze as the substrate. However, survival of *Hyaella* was similar in all the assays that used gauze as the substrate (Table 6). *Hyaella* was also more sensitive to the filtered water from the mussel cultures (65% survival) than was *Diporeia*. Biomagnification of contaminants in the mussel pseudofaeces would not be expected as the lab cultures of the *Dreissena* were fed the same diatoms as were the *Diporeia* living in the control sediments. This suggests either a waste product, a bacterium or a pathogen in the pseudofaeces may be responsible for the reduced survival of *Diporeia*.

Conclusions

- There was no evidence of toxicity in the Microtox® test. This suggests the loss of *Diporeia* was not due to the presence of toxic chemicals in the sediment pore water.
- Presence of *Thioploca* in the sediment (Bay of Quinte) did not reduce survival of either amphipod.
- Exposure to *Dreissena* excretions or minute sized particles in the water inhabited by the mussels did not reduce survival of *Diporeia*.
- *Diporeia* survival was reduced by 30% in sediments from sites with high mussel densities and their pseudofaeces also significantly reduced survival of both *Hyaella* and *Diporeia*.



**Figure 3.** Wet weight of *Thioploca* filaments surviving in sediment with (circle) or without (triangle) *Diporeia*. Bars are 1 standard error.



- The exact mechanisms explaining the loss of *Diporeia* is still unknown and may be related to nutritional problems, waste metabolites or diseases that effect fecundity.

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