Preliminary Investigations for Causes of the Disappearance of *Diporeia* spp. from Lake Ontario

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Abstract

The amphipod *Diporeia* spp. comprised 60-80% of the benthos in offshore Lake Ontario and was an important food for fish. In eastern Lake Ontario, *Diporeia* spp. began disappearing in 1993 just after the arrival of dreissenid mussels. We compared survival of *Diporeia* spp. and *Hyalella azteca* in sediments from areas where *Diporeia* spp. populations had vanished with survival in sediments still inhabited. Survival was also examined in the presence of zebra mussel (*Dreissena polymorpha*) pseudofeces, filtered water from mussel cultures, and added bacteria. The Microtox® test indicated that sediment pore water was not toxic. Sediments from sites with large *Dreissena* spp.

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populations (Lake Erie and western Lake Ontario) lowered *Diporeia* spp. survival. *Diporeia* spp. and *H. azteca* responded differently to test sediments and zebra mussel pseudofeces. Pseudofeces added to Lake Superior sediment greatly reduced *H. azteca* survival but had less effect on *Diporeia* spp. survival. Added bacteria had little effect on the survival of either species. Sediments exposed to dying *Diporeia* spp. caused significant mortality suggesting the presence of a pathogen. *Diporeia* spp. remained common in two inland lakes containing dreissenids indicating that the amphipod can co-exist with the mussels.

Introduction

The biota of the Great Lakes has changed greatly since the arrival of several exotic species over the last two decades. Following the arrival of the zebra mussel (*Dreissena polymorpha)* and quagga mussel (*D. bugensis)*, the deepwater amphipod *Diporeia* spp. (hereafter, diporeia as a common name), formerly *Pontoporeia hoyi,* has declined in all of the Great Lakes except Lake Superior. Since 1992, diporeia has disappeared from suitable habitats in Lakes Michigan, Huron, Erie, and Ontario at depths <70 m where they had been abundant (>3000/m^2) (Dermott and Kerec 1997; Nalepa et al. 1998; Dermott 2001). Maximum densities had occurred at depths of 30-60 m (Nalepa 1989; Sly and Christie 1992) where temperatures remain cold and organic matter accumulates on fine sediments. Settling diatoms and sediment bacteria are the main food of diporeia, and their lipid levels peak after the spring diatom bloom (Gardner et al.1985). This amphipod provided over 20% of the food energy (Flint 1986) for the offshore Lake Ontario fish community (lake whitefish (*Coregonus clupeaformis*, hereafter, whitefish), alewife (*Alosa pseudoharengus*), smelt (*Osmerus mordax*), and Pacific salmon (*Oncorhynchus* spp.)). Commercial catches and reproduction rates of whitefish and smelt have decreased in Lakes Erie and Ontario following the decline in diporeia populations (Dermott et al. 1999; Hoyle et al. 1999; Owens and Dittman 2002). Proposed hypotheses for the loss of diporeia include a decrease in available food due to competition with mussels, increased sediment toxicity due to changing contaminant cycling, biological toxicants associated with zebra mussel pseudofeces or excretions, and pathological bacteria or viruses.

Nutrient levels and algal biomass have declined in the Great Lakes since the 1970s. Between 1981 and 1995, there were significant reductions in phosphorus, algal biomass, and chlorophyll in eastern Lake Ontario, but chlorophyll and algal biomass did not change significantly in the middle of the lake (Johannsson et al. 1998). Since 1990, large mussel populations on shoals and in nearshore regions have further increased water clarity, reduced nutrients and phytoplankton density (Leach 1993; Markarewicz et al. 1999; Millard et al. 1999), and removed a large portion of the algae that had previously settled offshore in diporeia habitat (Dermott and Kerec 1997; Nalepa et al. 1998).

The absence of diporeia near the Niagara River was considered to be a result of high levels of persistent organic compounds in the area (Nalepa 1991) because diporeia are sensitive to sediment contaminants (Gossiaux et al. 1993). Concentrations of the most persistent organic pollutants have declined in the Great Lakes since 1980 (Pierce et al. 1998), but residues of newer compounds such as brominated flame retardants and herbicides have been increasing in the Great Lakes basin (Alaee et al. 1999; Thurman and Cromwell 2000). If these newer compounds are becoming toxic to Great Lakes populations of diporeia, their widespread use likely would also impact diporeia populations in smaller lakes within the Great Lakes basin.

Dreissenids produce large quantities of pseudofeces that accumulate on the bottom near the colonies. During storms, this material is transported offshore and could affect diporeia populations far removed from nearshore colonies. In Lake Erie, a reduction in sediment particle size increased total organic content, and the presence of polycyclic aromatic hydrocarbons in sediments has been associated with biodeposits from the large mussel population (Howell et al. 1996). Blooms of the cyanobacteria *Microcystis* have increased in Lake Erie and Saginaw Bay due to selective rejection of cyanobacteria by filtering dreissenids (Vanderploeg et al. 2001). The toxicant microcystin is known to cause mortality in crustaceans (DeMott et al. 1991) and could be transferred to diporeia via deposited pseudofeces. Biological toxicants and viral, bacterial, or ciliate pathogens may be associated with the mussels or their pseudofeces. There has been a recent increase in botulism in Lake Erie (Getchell et al. 2002) possibly due to bio-magnification of the *Clostridium* toxin by dreissenids or increased spore abundance among the decomposing mussels and pseudofeces in the sediments. In the Bay of Quinte, benthic mats of the filamentous bacterium

Thioploca ingrica appeared in the sediment following the disappearance of diporeia (Dermott and Legner 2002).

The purposes of this study were to determine if sediments from locations where diporeia have disappeared or the presence of mussel pseudofeces, their excretions, or added bacteria would reduce diporeia survival; to determine if the amphipod *Hyalella azteca* also had reduced survival when exposed to such sediments, added pseudofeces, or bacteria; to determine if the Microtox® test exhibited any inhibition when exposed to pore water from these sediments; and to examine trends in diporeia populations in the Bay of Quinte and in two smaller lakes that also have zebra mussel populations.

Methods

Sediments were collected from nine sites in the Great Lakes (Fig. 1, Table 1) mostly in the spring (April-June) and again in the autumn (August-October). Diporeia populations were still present at the Lake Superior, Batchawana Bay, Colpoy's Bay, and mid-Lake Ontario sites but had disappeared at the Lake Erie, western and eastern Lake Ontario, and Bay of Quinte sites (Table 1).

Fig. 1. Location of sediment collections and sampled *Diporeia* spp. populations.

Table 1. Depth, density of *Diporeia* spp. and dreissenid mussels, and relative abundance of *Thioploca ingrica* at sites where sediments were collected. Also given are percent sand, mean particle size (µm), and percent organics (loss on ignition) in sediments.

Sediment source	Depth (m)	*Diporeia* spp. (no./m^2)	Dreissenids (no./m^2)	*Thioploca ingrica**	Sand (%)	Size (µm)	Organics (%)
Lake Superior							
Lake Superior (composite)	104/ 164	96	0	-	66	65.6	1.7
Batchawana Bay	32	548	0	+	1	7.3	4.3
Lake Huron							
Colpoy's Bay	33	2000	+	-	19	5.8	2.4
Lake Erie							
Western	10	0	500	-	5	7.1	6.2
Eastern	38	0	4590	+	0	3.2	3.4
Lake Ontario							
Western	45	0	2320	-	22	21.8	3.2
Mid-lake	125	987	0	-	0	3.9	7.9
Eastern	35	0	0	+	2	9.7	10.6
Bay of Quinte	32	0	420	+++	0	5.3	9.7

*Degree of abundance: - absent; + low; +++ high

Diporeia were collected during cold epilimnion conditions in spring and autumn from Colpoy's Bay on Georgian Bay. The amphipods were maintained at 5ºC in the dark for up to four months in large plastic bags with native sediments and 12 L of water. Initially, each bag contained a minimum of 100 amphipods. Every month, half of the water in each bag was replaced with cooled, dechlorinated tap water, and the amphipods were fed about 0.25 g of freeze-dried diatoms. Assays were conducted for up to 90 days to allow chronic effects to appear during this short part of their two-year generation time. Methods were adapted from 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 (6-cm ID x 7-cm high) resulting in a density of about 1800 m^{-2} and a dry biomass of 1.7 g m^{-2}. The amphipods were captured and transferred to the jars using 6-mm ID pipettes to reduce exposure to air. Each jar was filled with water and covered with a 1-mm-mesh screen held on by an elastic band. Six replicate jars were placed in a 12-L aquarium containing 3 L of water. Aquaria were transferred to an incubator set at 10ºC with a 12-h light/dark photoperiod to match the August to October conditions at a 35-m depth in Lake Ontario. A bubbler was placed in each aquarium, and the water level was gently adjusted to leave the jars 1 cm below the water surface. The sediment surfaces in the jars were monitored at 24 and 48 h after setup, and any dead or injured diporeia seen on the surface during the first 48 hours were replaced. Once per week, the water in the aquarium was drained, 2 mg of freeze-dried diatoms were added to each jar, and fresh 10ºC dechlorinated water was added to each aquarium to bring the final volume back up to 3 L. This feeding provided slightly more than the consumption rate of diporeia in Lake Ontario (0.03 mg organics $individual^{-1}day^{-1}$) (Dermott and Corning 1988). At 30 and 60 days, the sediment in each jar was screened through a 1-mm-mesh sieve, and the surviving amphipods were counted and placed back into the original sediment in the same jar. At the end of 90 days, the surviving amphipods were counted, and specimens were either frozen for lipid analysis or preserved for pathological analysis. For most sediments, two replicated trials were conducted, and each trial was run within three months of the month of sample collection (Table 2).

Table 2. Sediments assayed for *Diporeia spp.* and *Hyalella azteca* survival and for Microtox® tests. N = number of replicates in all trials. nt = not tested.

Sediment source	Collection month	*Diporeia* spp. replicates N	*Hyalella azteca* N	Microtox® test N
Lake Superior				
Lake Superior (composite)	May	6	8	2
Batchawana Bay	July	12	16	2
Lake Huron				
Colpoy's Bay	June	6	8	nt
Lake Erie				
Western Lake Erie	April	6	8	2
Eastern Lake Erie	April	6	8	2
	October	6	8	2
Lake Ontario				
Western	June	6	8	nt
	October	6	8	2
Mid-lake	April	6	8	nt
	October	6	8	2
Eastern	April	12	12	2
Bay of Quinte	April	6	8	2
	October	6	8	2

Selected diporeia were measured, placed into Durham tubes (6 x 50 mm), dried under nitrogen for 24 hours at 60ºC, and frozen at -80ºC in preparation for total lipid analysis. In some assays, diporeia were sampled for lipids after only 60 days exposure. For comparison, lipids were also determined for diporeia (4-7 mm) collected in field samples from Colpoy's Bay and mid-Lake Ontario. Lipids were determined on a per dry-weight basis using lipid extraction with chloroform:methanol (2:1, v/v) and quantified gravimetrically (Cavaletto et al. 1996).

In addition to conducting assays on sediments collected from various regions of the Great Lakes, test assays were also conducted where either dreissenid pseudofeces, filtered water from dreissenid cultures, dead or paralyzed diporeia, or the bacterium *Bacillus thuringiensis* (commercially *B.t.*) were added to a control sediment (Table 3). The sediments used as controls were from the Lake Superior composite, Batchawana Bay, or Colpoy's Bay (Georgian Bay), and mid-Lake Ontario (for *H. azteca*), all of which supported stable diporeia populations. The *B.t.* was used as a surrogate for bacteria that could colonize pseudofeces and interfere with either the gut flora or gut enzymes of diporeia. To each jar, 0.2 mg of *B.t.* was added in weeks 1 and 3.

In the pseudofeces assays each week, 0.3 or 0.4 mL of pseudofeces slurry, a food source in place of diatoms or TetraMin® flakes, was added to each replicate jar or cone. Assuming pseudofeces were much less nutritional than diatoms, the volume of added pseudofeces was adjusted to provide double the organic matter in the regular weekly diet (2 mg of diatoms for diporeia or 2.5 mg TetraMin® for *H. azteca*). Pseudofeces were collected from laboratory cultures of dreissenids held in 30-L aquaria and fed frozen diatoms collected from Lake Ontario during the spring bloom (March-April). The pseudofeces were siphoned onto a 28-µ mesh, placed in centrifuge tubes, and allowed to settle for 24 h. The ratio of water was then adjusted to 12 mL pseudofeces:8 mL water. Replicate, 1-mL aliquots of this pseudofeces slurry were dried and ashed to estimate organic content. From these calculations, we calculated the required feeding volume of slurry that had double the organic content of the diatom diet. The pseudofeces were stored for a minimum of two weeks at 5ºC before use to represent a transport time from nearshore to below the thermocline.

Table 3. Number of test assays in all trials for *Diporeia* spp. and *Hyalella azteca* survival using dreissenid pseudofeces, filtered water, or added *Thioploca ingrica* or *(B.t.)* bacteria. Control sediments for *Diporeia* spp. were from Lake Superior or Batchawana Bay. Control substrates for *H. azteca* were mid-Lake Ontario sediment and gauze only. nt = not tested.

Assay	***Diporeia* spp. replicates**	***Hyalella azteca* replicates**
Batchawana Bay	12	12
Mid-Lake Ontario	6	8
Pseudofeces		
Superior	6	8
Batchawana Bay	12	8
Filtered water/Batchawana Bay	12	nt
Zebra mussels		
Batchawana Bay	6	nt
Colpoy's Bay	6	nt
Thioploca ingrica/Bay of Quinte	6	nt
Bacillus thuringiensis (B.t.)		
Batchawana Bay	6	nt
Colpoy's Bay	nt	4
Bay of Quinte	nt	4
Dying amphipods/Batchawana Bay	12	nt
Pseudofeces/gauze	nt	8
Filtered water/gauze	nt	8
TetraMin®/gauze	nt	12

Water from dreissenid cultures was put through a 0.45-μ filter to remove all but viral-sized particles and dissolved chemicals. The 3 L of water in each assay aquarium or 1 L in each Imhoff cone (*H. azteca*) was replaced every week with freshly filtered water at the appropriate temperature. Diporeia were also exposed to clean control sediment (Batchawana Bay) on which dying diporeia were left for 48 h. We exposed diporeia directly to competition with dreissenids by adding two mussels (17-mm length) to each

jar containing the control sediment (Batchawana Bay). The feeding regime for this assay was 6 mg diatoms/week (standard diet plus 2 mg for each mussel).

We also measured survival of diporeia and the sulfur bacterium *T. ingrica* in one experiment. *T. ingrica* filaments were collected from Bay of Quinte sediment by screening them through a 0.5-mm mesh. Approximately 0.12 g of bacteria was added to jars containing screened Bay of Quinte sediment, resulting in a biomass of bacteria about one-third that at the collection site. Five diporeia were added to each of five jars; one jar had no diporeia. At 30 and 60 days the jars were screened on a 0.5-mm mesh and the number of surviving diporeia and the wet weight of surviving *T. ingrica* filaments were tallied.

Assays using *H. azteca* were conducted for 28 days (four weeks) following the procedure of Borgmann and Norwood (1999) who used Imhoff settling cones with 1 L of water overlying 15 mL of sediment. Young amphipods (0-10 days old; <0.01 mg ind^{-1}) were obtained from laboratory cultures grown at room temperature with a 16-h-light:8-h-dark photoperiod. Fifteen young amphipods were added to each cone and fed 2.5 mg TetraMin® fish flakes weekly. The amphipods were exposed to nine different sediments and five treatments including sediment with either added pseudofeces or the insecticidal bacterium *B.t*. In addition, amphipods were exposed to three treatments using cotton gauze as a substrate instead of sediment. They were exposed to either gauze alone (control), filtered water from an aquarium containing zebra mussels, or zebra mussel pseudofeces (food source) in place of the TetraMin® (Table 3). Two trial runs of four replicates were conducted for each treatment with sediments from Lakes Erie and Ontario collected in spring and autumn. Sediment was sieved through a 1-mm mesh prior to use to remove macroinvertebrates, mussels, shell fragments, and coarse particles.

Dissolved oxygen, ammonia concentration, pH, and temperature were measured prior to adding the amphipods and at the end of the 28-day period. After 28 days, animals were counted and then allowed to clear their guts in a 50-µM solution of the chelator ethylenediamine tetraacetic acid (EDTA) for 24 hours. After gut clearing and drying for 48 hours at 60ºC, dry weights of *H. azteca* were measured. The animals were then frozen for future calculations of body burdens and lipid levels. Statistical comparisons were

done on the average number surviving in each test using one-way analysis of variance (ANOVA) and treatment comparisons were made using the *F*-test.

The Microtox® liquid-phase acute-toxicity test was conducted using pore water extracted from sediments (Table 1). Pore water was isolated by centrifuging approximately 240 mL of sediment at 6000 rpm for 60 min at 4ºC (Giesy et al. 1988) and filtering the supernatant through glass-microfiber filters (GF/C) under a 10-psi vacuum. Pore water was stored at 4ºC and used within three days. Two replicate tests were performed using the Beckman Microtox® model 500 analyzer following the standard 100% test protocol (Microtox Omni Software, Azur Environmental, Strategic Diagnostics, 111 Pencader Dr., Newark, Delaware, 19702-3322). Control standards of saline were prepared to measure the photo-luminescence of the bacteria. As a positive test of toxicity, solutions of phenol and $ZnSO_4$ were prepared to measure photo-inhibition of the photobacterium after 5-min and 15-min exposures.

Changes in diporeia populations in the lower Bay of Quinte were examined using published and unpublished data from 1967-2000. Trends in diporeia populations in two lakes with dreissenid populations were also examined: Cayuga Lake (upper New York State) and South Bay (Manitoulin Island, Lake Huron). In Cayuga Lake, two sites at 43- and 63-m depths (42º 28.93'N: 076º 31.30'W and 42º 29.31'N: 76º 31.41'W, respectively) were sampled, and these data were compared to data from 1994 (E. L. Mills, Cornell University Biological Field Station, 900 Shackelton Point Road, Bridgeport, New York, 13030, unpubl. data). Data from five sites in South Bay were compared to the data of Cooper (1964) and Johnson (1988). At each site, a 22-cm Ekman grab was used to collect five replicate samples, which were sieved through a 0.58-mm screen (#30 mesh) and preserved in 10% formalin following the methods of Johnson (1988). The diporeia and dreissenids were later removed, identified, enumerated, and weighed (blotted wet biomass).

Results

Diporeia had significantly lower survival (ANOVA, $P < .005$) in sediments from eastern Lake Erie and western Lake Ontario than in Lake Superior sediment (Fig. 2). Both the eastern Lake Erie and western Lake Ontario sites

had high *D. bugensis* densities (>2300 m^{-2}; Table 1). Survival in sediments from western Lake Erie and eastern Lake Ontario was not significantly less than in Lake Superior sediment ($P > 0.5$). Survival was less variable in the Lake Superior composite sediment than in sediment from Batchawana, Colpoy's Bay, or mid-Lake Ontario. Survival in Bay of Quinte sediment was the same as in Lake Superior sediment despite the absence of diporeia in the bay.

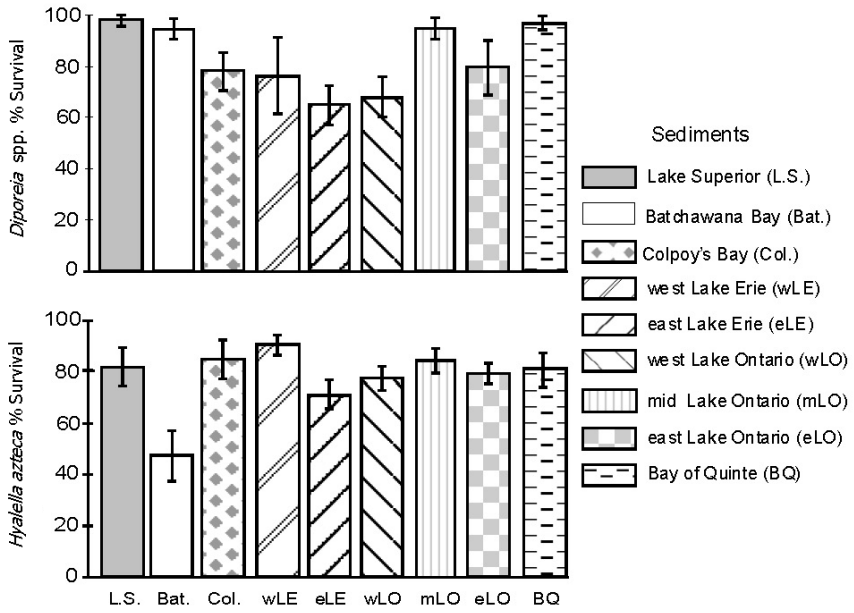

Fig. 2. Average percent survival and standard error bars for *Diporeia* spp. (upper panel) and *Hyalella azteca* (lower panel) when exposed to Great Lakes sediments. Data from all trials; *Diporeia* spp. experiments lasted 90 days, and *H. azteca* experiments lasted 28 days.

Diporeia survival was significantly lower (ANOVA, $P < 0005$) in the control sediment to which zebra mussel pseudofeces were added than in Lake Superior sediment (Fig. 3). Addition of pseudofeces reduced survival 24%

below that in the control sediment. Diporeia survived better in Batchawana Bay sediment when exposed directly to zebra mussels in the same jar than when exposed to either pseudofeces ($P < 0.5$) or filtered water from mussel aquaria ($P > 0.5$). Mussel density in the jars was about 600 m^{-2} with a dry shell-free biomass of 10.0 g m^{-2}, almost six times the biomass of the added amphipods. Diporeia was not sensitive to the bacterium *B.t.* added to the control sediment ($P > 0.1$). They were, however, very sensitive to Batchawana Bay sediment onto which paralyzed and dying diporeia had been placed for 48 hours and then removed ($P < 0.001$) (Fig. 3). Dead animals from this assay were preserved for pathological examination to identify causes. Microsporidian and a rickettsia-like parasite were found in amphipods from some of the assays.

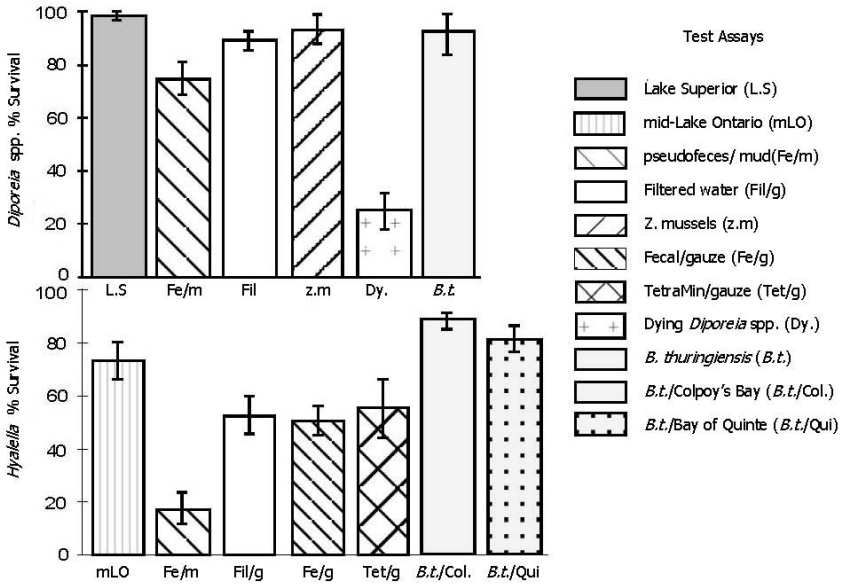

Fig. 3. Average percent survival of *Diporeia* spp. and *Hyalella azteca* in various test assays. Bars are ±1 standard error.

The addition of the bacterium *T. ingrica* to the Bay of Quinte sediment had no effect on diporeia survival (97%) when compared to either Lake Superior or mid-Lake Ontario sediments ($P > 0.7$). The amount of *T. ingrica* in the sediment was reduced by the presence of diporeia due to burrowing or grazing on the bacterial filaments (Fig. 4).

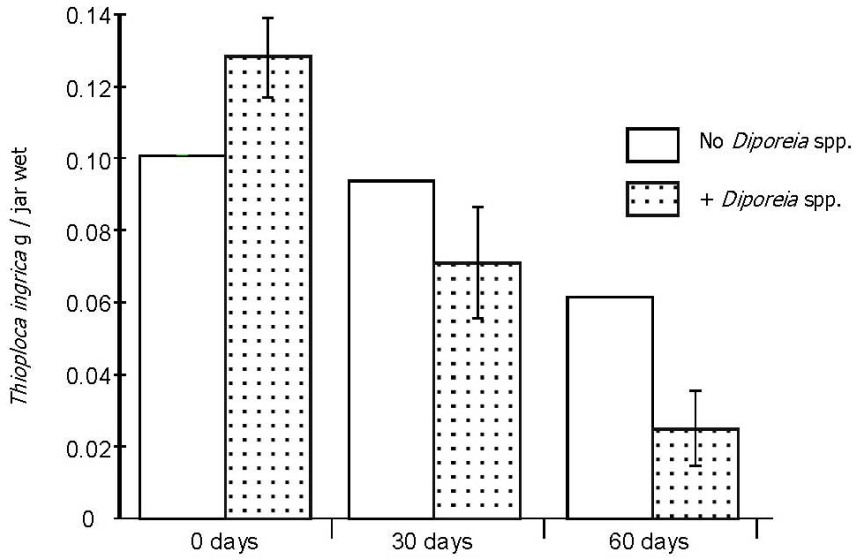

Fig. 4. Wet weight of *Thioploca ingrica* filaments after 30 and 60 days in sediment with or without *Diporeia* spp. Bars are ±1 standard error.

Sediment surfaces in jars with healthy diporeia were well tilled, typically having no visible burrows. Only when a few amphipods remained in the jar was the sediment surface smooth with visible burrow openings. Dead or moribund amphipods were often seen on top of such sediments. Active amphipods burrowed into the sediment and fed on algal remains, bacteria, and detritus, which appeared as a dark band in their guts. However, when diatoms were added, the amphipods would often orient vertically in the sediment with only their eyes and antennae exposed. Then the diporeia would sweep the sediment surface with their antenna, drawing the recently settled diatoms toward their mouths. After a few minutes of sweeping, they

would burrow to re-emerge about one to two body-lengths away and then begin sweeping the surface again.

Lipid levels were highest in diporeia collected from mid-Lake Ontario in June but were not significantly higher in amphipods collected from Colpoy's Bay (Table 4). For most assays, there was no difference in lipids after 90-days exposure compared to 60 days, indicating that the feeding regime of 2 mg per week was sufficient. Lipid levels were highest in diporeia exposed to *T. ingrica* or in direct competition with zebra mussels in the same jars, indicating that the added *T. ingrica* filaments and increased ration (6 mg) for jars with mussels provided diporeia with more calories. Lipid levels in diporeia fed only pseudofeces were equal to those fed diatoms on Lake Superior sediment (Table 4).

Survival of *H. azteca* was high when exposed to the Lake Superior composite, Colpoy's Bay, western Lake Erie, and mid-Lake Ontario sediments (Fig. 2). Low survival was observed after exposure to sediment from Batchawana Bay (ANOVA, $P > 0.01$). Survival of *H. azteca* was not significantly less ($P > 0.3$) in sediments from sites with high densities of dreissenids (eastern Lake Erie and western Lake Ontario, Table 1). Survival in western Lake Erie sediments was the highest of all assays and was significantly higher than in eastern Lake Erie sediment ($P > 0.02$). The survival of *H. azteca* tended to be high in sediments with higher organic content (Table 1).

Table 4. Mean length and percent lipid of *Diporeia* spp. from collection sites in Lake Ontario and Colpoy's Bay during 2001 and values for *Diporeia* spp. exposed to various control/test sediments and assays. S.E. given in parentheses.

Sediment/assay	N (repl.)	Exposure (days)	Length (mm)	Total lipid (% dry mass)
Sediment*				
Mid-Lake Ontario				
April	15	0	5.15 (0.42)	12.33 (2.59)
June	14	0	6.05 (0.30)	20.34 (2.41)
Oct	14	0	5.39 (0.16)	13.76 (1.83)
Colpoy's Bay (June)	30	0	5.39 (0.16)	15.53 (2.06)
Assays				
Lake Superior (composite)	14	60	6.32 (0.31)	12.05 (1.04)
	14	90	6.33 (0.23)	10.57 (1.42)
Eastern Lake Erie	14	90	6.29 (0.32)	8.64 (0.91)
Western Lake Ontario	14	60	6.83 (0.08)	15.02 (1.86)
	22	90	5.75 (0.20)	5.06 (1.10)
Mid-Lake Ontario	15	60	6.95 (0.15)	8.58 (1.33)
	12	90	6.55 (0.24)	8.36 (1.87)
Bay of Quinte	14	90	6.64 (0.23)	10.28 (1.42)
Bay of Quinte + *Thioploca ingrica*	14	90	5.83 (0.15)	16.11 (1.19)
Filtered water + control	12	60	6.71 (0.18)	12.18 (1.79)
	33	90	5.97 (0.12)	14.87 (1.13)
Z. mussels + control	8	60	6.59 (0.29)	17.57 (0.89)
	18	90	5.96 (0.16)	18.35 (1.31)
Pseudofeces + control	19	60	6.60 (0.19)	11.39 (1.21)
	26	90	6.11 (0.18)	10.57 (1.55)

***** Lipids prepared within one week of collection.

Survival of *H. azteca* on cotton gauze as a sediment-free control with TetraMin® as food (Tet/g in Fig. 3) was lower in all but one of the sediments (ANOVA, $P < 0.05$; Fig. 3). The *H. azteca* survival on gauze exposed to either filtered water (Fil/g) from the mussel cultures or pseudofeces (Fe/g) was similar to survival on gauze alone (Tet/g; $P > 0.5$). Pseudofeces clearly had a negative effect on *H. azteca* survival when added to Lake Superior composite sediment (Fe/m; $P < 0.001$), in which only 17% of the *H. azteca* survived the 28-day exposure (Fig. 3). In all 16 trials of pseudofeces added onto sediment, survival was less than in any other treatment. Presence of the bacterium *B.t.* had no effect on *H. azteca* survival in either Colpoy's Bay or Bay of Quinte sediments ($P > 0.5$; Fig. 3). Survival in these two *B.t.* treatments was better than in mid-Lake Ontario sediment ($P > 0.7$).

Average individual dry weights of *H. azteca* on sediments or gauze was related to observed survival rates (Fig. 5). Body weight after 28 days was greatest on mid-Lake Ontario or Bay of Quinte sediments and was lowest on Batchawana Bay sediment or Lake Superior sediment with added pseudofeces. Although *H. azteca* in treatments with gauze and pseudofeces (Fe/g) or gauze and filtered water (Fil/g) had much better survival than the treatment with pseudofeces added to the sediment (Fe/m), their final average body weights (as growth) in the gauze treatments were very low (<1.5 mg) and similar to final body weight in pseudofeces on sediment (Fe/m; Figs. 3 and 5). Final weights of *H. azteca* exposed to the gauze alone as a control were double the final weight in the other gauze treatments and more similar to that in the sediment treatments.

Levels of neutral herbicides, including atrazine, were below detection limits in all the sediments tested (<18 $\mu g\ kg^{-1}$). Metal levels were greatest in sediments from mid- and eastern Lake Ontario (Table 5), both of which had higher amphipod survival than the Lake Erie sediments. Metal levels in Batchawana Bay (Lake Superior) sediments were higher than eastern Lake Erie sediments. Metal levels in the Lake Ontario sediments were well below the estimated Severe Effect Levels (SEL) which are levels above which acute toxicity to biota are likely (Persaud et al. 1992) (Table 5). However, the chelating ability of the higher Ca, Fe, and organic content (Table 1) in the Lake Ontario sediment may make its metals less available to aquatic organisms compared to levels in the Batchawana Bay sediment.

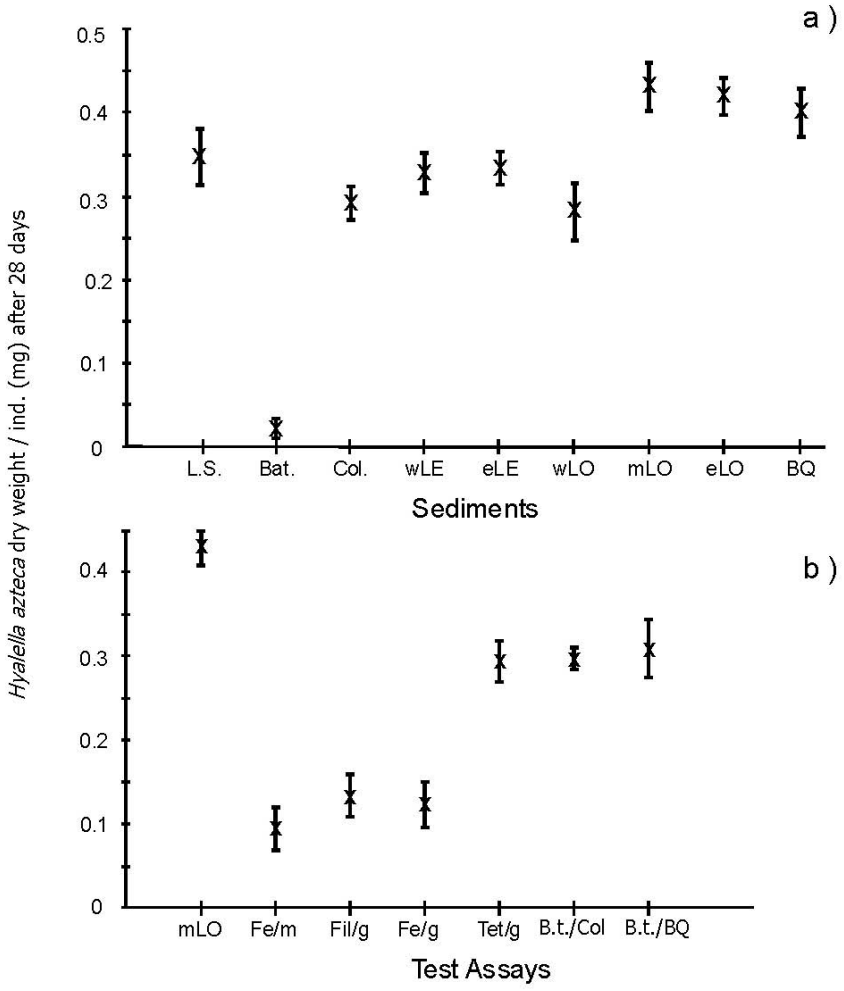

Fig. 5. Average individual dry weight (mg) and standard errors of *Hyalella azteca* after 28-day exposures to: (a) sediments or (b) various test assays. Abbreviations as in Figs. 2 and 3.

Table 5. Metal values as mg/kg in tested sediments. Also given is the Ontario Ministry of Environment's Severe Effect Levels (SEL), which are considered levels that acutely affect the health of sediment-dwelling organisms (Persaud et al. 1992).

Sediment Source	Ca	Cd	Cr	Cu	Fe	Ni	Pb	Zn
Lake Superior								
Lake Superior (composite)	23800	0.3	25	25	16400	19.7	12.5	40
Batchawana Bay	7660	1.5	65	38	13713	31.7	30.5	111
Lake Huron								
Colpoy's Bay	n.a.	1.9	48	25	25917	51.8	40.6	69
Lake Erie								
Western	35600	1.8	62	40	28900	46.9	48.8	159
Eastern	81300	0.4	49	26	32700	38.2	18.3	95
Lake Ontario								
Western	15600	0.4	38	27	27200	28.9	22.6	95
Mid-lake	16900	2.1	74	75	46200	61.9	88.1	293
Eastern	64100	1.2	42	47	20600	38.1	41.3	141
Bay of Quinte	8440	1.0	63	42	43700	50.4	27.6	143
SEL	-	10	110	110	-	75	250	820

None of the sediments caused a sufficient response in the Microtox® test to allow calculation of an EC_{50} (effective concentration) from a pore-water dilution series. The test with 90% pore water indicated that only sediment from eastern Lake Ontario caused a response (Table 6). A negative percent-effect indicated stimulation rather than inhibition as shown by the 32 to 70% inhibition in the standard solutions of phenol and $ZnS0_4$. The Lake Superior composite sediment caused the second greatest inhibition of the photobacteria (14.8%) in the sediment pore-waters tested. Western Lake Ontario sediment caused the greatest photoactivity (-39%) compared to the saline control. Thus, the absence of diporeia at the eastern Lake Erie and Ontario sites was apparently not due to acute toxicity of a chemical pollutant in their sediments.

We compared diporeia densities from two sites in the Bay of Quinte (Glenora at 21-m depth and Conway at 32-m depth) with chlorophyll *a* levels (Fig. 6). Following improvements in sewage treatment during 1977, chlorophyll decreased steadily between 1985 and 1998 to about 40% of 1977 levels (Fig. 6). A large die-off of white perch (*Morone americana*), a winter predator of diporeia, occurred in the bay during the winter of 1977-1978 (marked as W.P. in Fig. 6). As water quality improved and chlorophyll decreased in the bay, the diporeia population increased until 1990, especially at the shallow 21-m site. Dreissenid mussels became common in the lower bay during 1993 (marked Z.m. in Fig. 6), after which diporeia disappeared at the 32-m site. The population persisted until 1998 at the less favorable 21-m site, which experiences temperatures above its optimum.

Table 6. Average percent effect in Microtox® tests on various sediments using a concentration of 90% pore-water plus saline. Two trials were done with measurements of percent-effect taken at 5 and 15 minutes. nt = not tested.

Sediment source	% effect @ 5 min.	% effect @ 15 min.
Controls		
Saline	0.0	0.0
Phenol (5 mg/L)	68.8	66.2
$ZnSO_4$ (5 mg/L)	32.0	70.0
Lake Superior		
Lake Superior (composite)	9.5	14.8
Batchawana Bay	8.5	4.8
Lake Huron		
Colpoy's Bay	nt	nt
Lake Erie		
Western Lake Erie	-16.3	-24.9
Eastern Lake Erie	-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

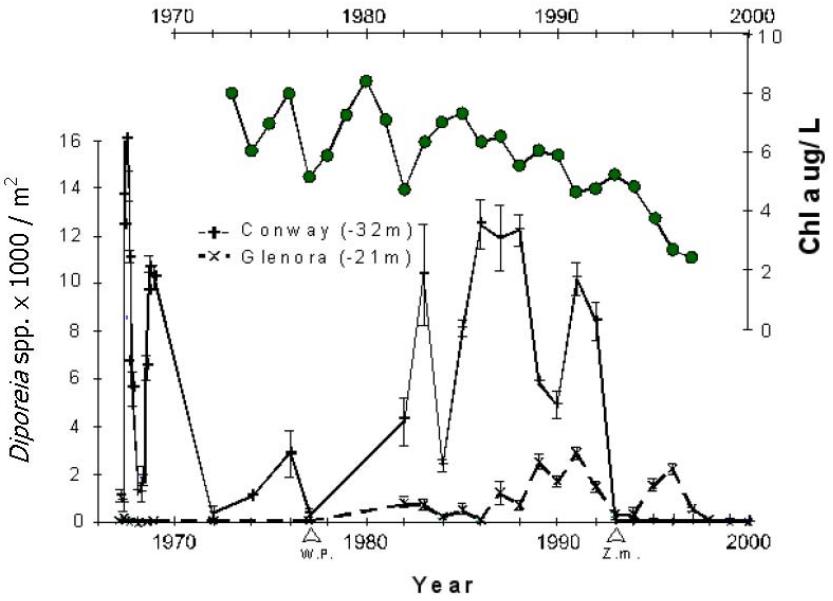

Fig. 6. Density of *Diporeia* spp. at two sites in the lower Bay of Quinte (1967-2000) and chlorophyll *a* levels at the 32-m site (1973-1997) (K. Nichols, Ontario Ministry of Environment and Energy, RR #2, Sutton West, ON L0E 1R0, Canada, unpubl. data). Bars are ±1 standard error. W.P. marks date of a white perch die-off and the start of phosphate controls. Z.m. marks when zebra mussels became common in the bay.

Diporeia remained common in South Bay, Lake Huron, and in Cayuga Lake, NY (Fig. 7), in the presence of moderate zebra mussel populations. In South Bay, zebra mussel density was 766 m^{-2} (SE = 344.6) above a 20-m depth and 7.5 m^{-2} (SE = 5.2) between 30 and 37 m. In Cayuga Lake, zebra mussel density in 2000 averaged 1452 m^{-2} (SE = 456) at 43-m depth and 200 m^{-2} (SE = 120) at 64-m depth. Mussel densities were 1640 m^{-2} (SE = 646) and 700 m^{-2} (SE = 588), respectively, at these depths in 2001. The diporeia population decreased in South Bay but increased in Cayuga Lake after the arrival of zebra mussels (Fig. 7).

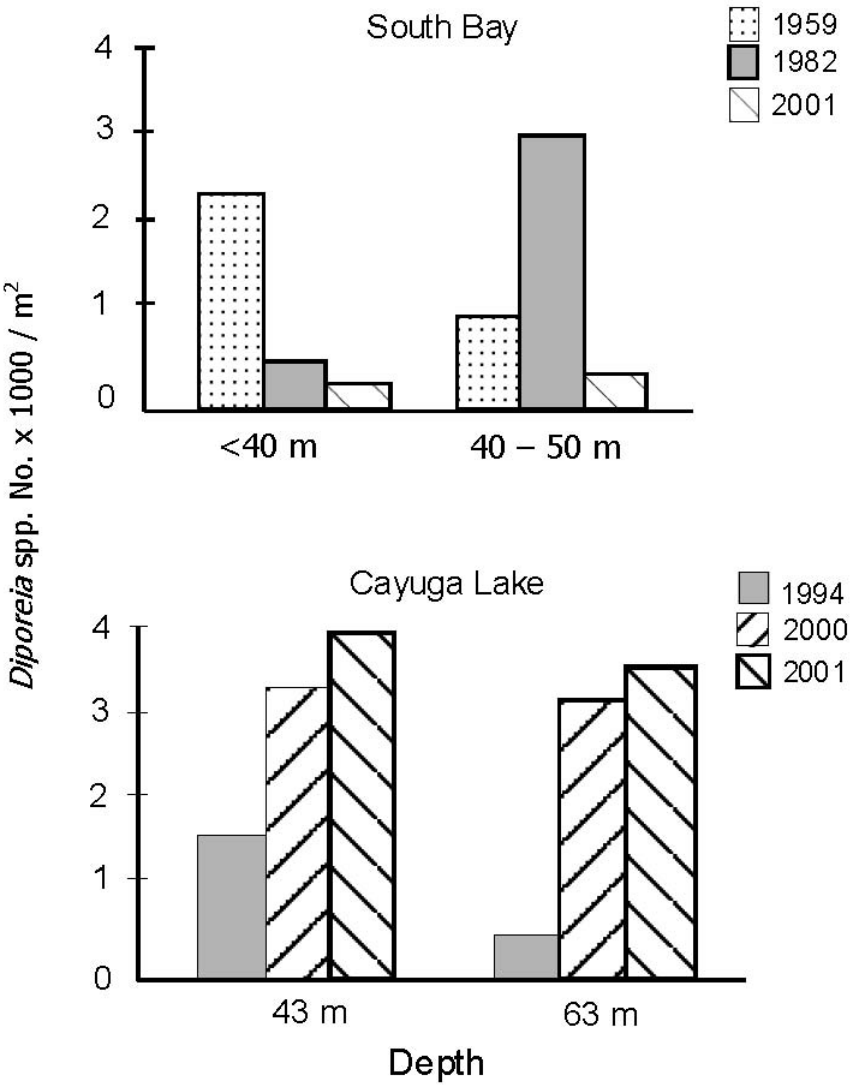

Fig. 7. Trends in *Diporeia* spp. density in South Bay, Lake Huron, and Cayuga Lake, upper New York State.

Discussion

Nalepa et al. (1998) theorized that the diporeia population decline in offshore regions of the Great Lakes was due to food scarcity caused by the filtering of large nearshore dreissenid populations. Direct competition for settling algae had occurred in eastern Lake Erie where *D. bugensis* density averaged over 4000 m^{-2} at depths between 30 and 64 m (Dermott et al. 1998). At mussel densities >1000 m^{-2}, diporeia and dreissenids rarely occurred together in the same samples from Lake Erie (Dermott and Kerec 1997) and Lakes Ontario and Michigan (Nalepa et al. 2005). The extended mussel siphons intercept settling detritus 1-2 cm above the sediment surface before it can settle onto the sediment where the diporeia feed. This direct competition for space and food may have been the cause of the disappearance of diporeia from Lake Erie after the mussels had colonized most of the bottom of the eastern basin (Dermott et. al. 1998). The extant diporeia population in Cayuga Lake is an exception to this concept.

A few amphipods were always present in the Bay of Quinte during the 1970s when diporeia densities were kept low by poor water quality and high fish predation (Johnson and McNeil 1986). As water quality improved, the diporeia population increased as the density of algae in the Bay of Quinte declined from 30-50% prior to 1990. Dreissenids were only established for one summer in the lower Bay of Quinte before diporeia disappeared at the 32-m site in 1993 (Dermott 2001). The persistence of diporeia among a moderate zebra mussel population in Cayuga Lake weakens our argument that food competition is the sole cause of the decline. At many locations in the Great Lakes where diporeia are disappearing, dreissenids are rare or limited to a narrow depth zone along the shore. In other areas, mussel clumps occur sporadically at depths of 40-70 m (Lozano et al. 2001; Nalepa et al. 2005). Algal production in nearshore areas can be greatly reduced by dreissenids (Millard et al. 1999). Dense dreissenid populations on the slopes of lakes can reduce the amount of suspended food before it moves offshore to the depositional zones inhabited by diporeia. Total lipid levels in diporeia collected from mid-Lake Ontario in 1989 (32-37%) (Cavaletto et al. 1996) were greater than the mid-lake average of 11-20% during 2001, which may reflect a declining food supply. We estimate that the decline in algal biomass in the Great Lakes since the start of phosphate control and the arrival of dreissenids can account for a reduction between 40% to as much as 70% of the diporeia population. If reduced algal production were the only cause, a

slow decline to densities typical of ultra-oligotrophic lakes (Superior, Great Bear) would be expected. Instead, a rapid and complete elimination of diporeia occurred across large areas of the Great Lakes within 2-5 years after the arrival of zebra mussels.

The Microtox® tests failed to show that any of the tested sediments were acutely toxic indicating that chemical toxicity in the sediments is not the cause of the diporeia decline. Likewise, Landrum et al. (2000) found no acute toxicity to diporeia in Lake Michigan sediments that are now devoid of diporeia. Concentrations of most persistent organic chemicals, including most agricultural chemicals, have decreased in the Lake Ontario drainage basin (Pierce et al. 1998; J. Struger, Environmental Conservation Branch, Environment Canada, 867 Lakeshore Rd., Burlington, ON L7R 4A6, Canada, personal communication). Exceptions are increased use of glyphosate on modified soya and corn crops, increased residues of atrazine and brominated compounds, and the occurrence of endocrine disrupters in nearshore waters (Alaee et al. 1999; Thurman and Cromwell, 2000). It is unknown if these compounds, at the levels reported, affect diporeia survival and reproduction. Other substances, such as cadmium and dump wastes, have been shown to create reproductive disorders and asynchronous maturation in the Baltic amphipod *Monoporeia affinis* (Breitholtz et al. 2001).

Diporeia survival in sediments from areas with high dreissenid populations was consistently lower in our 90-day experiments. We do not know whether exposure to these sediments over the two-year lifespan of diporeia would also reduce reproductive success. The high survival and lipid levels in the diporeia in water filtered from mussel cultures suggests that mussel excretory products are not detrimental. Pseudofeces added to the Lake Superior composite or Batchawana Bay (Lake Superior) sediment caused very low survival and growth of *H. azteca* but reduced diporeia survival by only 24%. Feeding on pseudofeces may reduce amphipod fitness, as shown by the low growth of *H. azteca* when exposed to pseudofeces, as pseudofeces would be less nutritional than the same amount of diatoms or green algae. Landrum et al. (2000) suggested that sediments that had lost their diporeia populations may now be nutritionally limited to diporeia. In their experiments, diporeia were attracted to sediments following the addition of diatoms but avoided the same sediments without added diatoms. In our experiments, lipid levels in amphipods exposed to sediments that had

lost their populations were not lower than in amphipods exposed to control sediments suggesting that the nutritional quality was adequate.

An unknown agent was found to be transmissible to diporeia when dying amphipods were left on Batchawana Bay sediment for 48 h and then removed. The dying amphipods were originally isolated from assays of western Lake Ontario sediment. Also, during the same time period, our diporeia cultures from Colpoy's Bay also had high mortality. This mortality could be the result of a pathogen that came from the amphipods collected from Colpoy's Bay or from the western Lake Ontario sediments. Additional work to identify the pathogen causing the mortality and the role these agents play in the decline of diporeia is ongoing. Messick et al. (2004) identified a rickettsia-like organism and a haplosporidian in diporeia from Lakes Huron and Michigan.

Conclusions

This study indicated that the loss of diporeia from suitable habitats is not due to the presence of toxic chemicals or the bacterium *T. ingrica* in sediments. Mussel pseudofeces significantly reduced diporeia survival compared to those fed diatoms, although *H. azteca* was more sensitive to the pseudofeces. Tests with the bacterium *B.t.* showed no effect on the survival of either diporeia or *H. azteca*. This study was unable to rule out bacterial, viral, or ciliate pathogens associated with dreissenids or their pseudofeces as factors in the diporeia population decline. Pseudofeces apparently reduce the fitness of diporeia, perhaps by interfering with metabolism, or they are a source for a protozoan infection. The high survival in our filtered water assay (0.45-µ filter) indicates the agent is not a chemical or virus which would pass through the filter. If a pathogen is the cause of the demise of diporeia, the coevolution of dreissenids and *Echinogammarus ischnus*, a recently introduced shallow-water amphipod, opens the possibility that dreissenids act as a secondary host in transmitting a parasite to diporeia.

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References

Alaee, M., Sergeant, D.B., Muir, D.C.G., Whittle, D.M., and Solomon, K. 1999. Distribution of polybrominated diphenyl ethers in the Canadian environment. Organohalogen Compounds. **40**: 347-350.

Borgmann, U., and Norwood, W.P. 1999. Sediment toxicity testing using large water-sediment ratios: an alternative to water renewal. Environ. Pollut. **106**: 333-339.

Breitholtz, M., Hill, C., and Bengtsson, B.E. 2001. Toxic substances and reproductive disorders in Baltic fish and crustaceans. Ambio **30**: 210-216.

Cavaletto, J.F., Nalepa, T.F., Dermott, R., Gardner, W.S., Quigley, M.A., and Lang, G.A. 1996. Seasonal variation of lipid composition, weight and length in juvenile *Diporeia* spp. (Amphipods) from lakes Michigan and Ontario. Can. J. Fish. Aquat. Sci. **53**: 2044-2051.

Cooper, J.E. 1964. The bottom fauna of South Bay Lake Huron. M.A. thesis, University. of Toronto.

DeMott, W.R., Zhang, Q.X., and Carmichael, W.W. 1991. Effects of toxic cyanobacteria and purified toxins on the survival and feeding of a copepod and three species of *Daphnia*. Limnol. Oceanogr. **36**: 1346-1357.

Dermott, R. 2001. Sudden disappearance of the amphipod *Diporeia* from eastern Lake Ontario: 1993 - 1995. J. Great Lakes Res. **27**: 423-433.

Dermott, R.M., and Corning, K. 1988. Seasonal ingestion rates of *Pontoporeia hoyi* (Amphipoda) in Lake Ontario. Can. J. Fish. Aquat. Sci. **45**: 1886-1895.

Dermott, R., and Kerec, D. 1997. Changes to the deep-water benthos of eastern Lake Erie since the invasion of *Dreissena*: 1979 to 1993. Can. J. Fish. Aquat. Sci. **54**: 922-930.

Dermott, R., and Legner, M. 2002. Dense mat-forming bacterium *Thioploca ingrica* (Beggiatoaceae) in eastern Lake Ontario: implications to the benthic food web. J. Great Lakes Res. **28**: 688-697.

Dermott, R., Munawar, M., Witzel, L., and Ryan, P. 1999. An assessment of food-web changes in eastern Lake Erie: impact of *Dreissena* spp. and phosphorus management on rainbow smelt, *Osmerus mordax*. *In* The State of Lake Erie Ecosystem (SOLE)—past, present and future. *Edited by* M. Munawar, T. Edsall, and I. Munawar. Backhuys Publishers, The Netherlands. pp. 367-385.

Dermott, R., Munawar, M., and Lorimer, J. 1998. Submersible confirmation of the profuse abundance of *Dreissena* on sediments in Lake Erie: implications to food web. Verh. Internat. Verein. Limnol. **26**: 2044-2047.

Flint, R.W. 1986. Hypothesized carbon flow through the deepwater Lake Ontario food web. J. Great Lakes Res. **12**: 344-354.

Gardner, W.S., Nalepa, T.F., Frez, W.A., Cichocki, E.A., and Landrum, P.F. 1985. Seasonal patterns in lipid content of Lake Michigan macroinvertebrates. Can. J. Fish. Aquat. Sci. **42**: 1827-1832.

Getchell, R.G., Wooster, G.A., Topic-Popovic, N., Culligan, W.J., Bowser, P.R. 2002. Prevalence of type E botulism in fish in the Lower Great Lakes. 4th International Symposium on Aquatic Animal Health, New Orleans. Sept. 2-6, 2002.

Giesy, J.P., Graney, R.L., Newsted, J.L., Rosiu, C.J., and Benda, A. 1988. Comparison of three sediment bioassay methods using Detroit River sediments. Environ. Toxicol. Chem. **7**: 483-498.

Gossiaux, D.C., Landrum, P.F., and Tsymbal, V.N. 1993. A survey of Saginaw River and Saginaw Bay, Lake Huron, sediments using two bioassays with the amphipod *Diporeia* spp. J. Great Lakes Res. **19**: 322-332.

Howell, E.T., Marvin, C.H., Bilyea, R.W., Kauss, P.B., and Sommers, K. 1996. Changes in environmental conditions during *Dreissena* colonization of a monitoring station in eastern Lake Erie. J. Great Lakes Res. **22**: 744-756.

Hoyle, J.A., Shaner, T., Casselman, J.M., and Dermott, R. 1999. Changes in lake whitefish (*Coregonus clupeaformis*) stocks in eastern Lake Ontario following *Dreissena* mussel invasion. Great Lakes Res. Rev. **4**: 5-10.

Jackson, M., Milne, J., Johnston, H., and Dermott, R. 1995. Assays of Hamilton Harbour sediments using *Diporeia hoyi* (Amphipoda) and *Chironomus plumosus* (Diptera). Can. Tech. Rep. Fish. Aquat. Sci. No. 2039.

Johannsson, O.E., Millard, E.S., Ralph, K.M., Myles, D.D., Graham, D.M., Taylor, W.D., Giles, B.G., and Allen, R.E. 1998. The changing pelagia of Lake Ontario (1981 to 1995): A report of the DFO long-term biological monitoring program. Can. Tech. Rep. Fish. Aquat. Sci. No. 2243.

Johnson, M.G. 1988. Production by the amphipod *Pontoporeia hoyi* in South Bay, Lake Huron. Can. J. Fish. Aquat. Sci. **45**: 617-624.

Johnson, M.G., and McNeil, O.C. 1986. Changes in abundance and species composition in benthic macroinvertebrate communities of the Bay of Quinte, 1966-84. Can. Spec. Publ. Fish. Aquat. Sci. **86**: 177-189.

Landrum, P.F., Gossiaux, D.C., Nalepa, T.F., and Fanslow, D.L. 2000. Evaluation of Lake Michigan sediment for causes of the disappearance of *Diporeia* spp. in southern Lake Michigan. J. Great Lakes Res. **26**: 402-407.

Leach, J.H. 1993. Impacts of the zebra mussel, (*Dreissena polymorph*) on water quality and spawning reefs in western Lake Erie. *In:* Zebra mussels: biology, impacts and control. *Edited by* T. Nalepa and D. Scholesser. Lewis Publishers, Boca Raton, Florida. pp. 381-397.

Lozano, S.J., Sharold, J.V., and Nalepa, T.F. 2001. Recent declines in benthic macroinvertebrate densities in Lake Ontario. Can. J. Fish. Aquat. Sci. **58**: 518-529

Markarewicz, J.C., Lewis, T.W., and Bertram, P. 1999. Phytoplankton composition and biomass in the offshore waters of Lake Erie: pre- and post-*Dreissena* introduction (1983-1993). J. Great Lakes Res. **25**: 135-148.

Messick, G., Curtis, S., Burreson, E., and Tyler, S. 2004. A Rickettsia-like organism and a Haplosporidian found in declining populations of *Diporeia* spp. amphipods from Lakes Michigan and Huron. Presentation at the Conference of the World Aquaculture Society, 1-5 March 2004, Honolulu.

Millard, E.S., Fee, E.J., Myles, D.D., and Dahl, J.A. 1999. Comparison of phytoplankton photosynthesis methodology in Lakes Erie, Ontario, the Bay of Quinte and the Northwest Ontario Lake Size Series. *In* The State of Lake Erie Ecosystem (SOLE)—past, present and future. *Edited by* M. Munawar, T. Edsall, and I., Munawar. Backhuys Publishers, The Netherlands. pp. 441-468.

Munawar, M., Dermott, R., McCarthy, L.H., Munawar, I.F., and van Stam, H.A. 1999. A comparative bioassessment of sediment toxicity in lentic and lotic ecosystems of the North American Great Lakes. Aquat. Ecosys. Health Manag. **2**: 367-378.

Nalepa, T.F. 1989. Estimates of macroinvertebrate biomass in Lake Michigan. Can. J. Fish. Aquat. Sci. **44**: 515-524.

Nalepa, T.F. 1991. Status and trends of the Lake Ontario macrobenthos. Can. J. Fish. Aquat. Sci. **48**: 1558-1567.

Nalepa, T.F., Fanslow, D.L., and Messick, G. 2005. Characteristics and potential causes of declining *Diporeia* spp. populations in southern Lake Michigan and Saginaw Bay, Lake Huron. *In* Proceedings of a workshop on the dynamics of lake whitefish (*Coregonus clupeaformis*) and the amphipod *Diporeia* spp. in the Great Lakes. Great Lakes Fish. Comm. Tech. Rep. 66 (this issue).

Nalepa, T.F., Hartson, D.J., Fanslow, D.L., Lang, G.A., and Lozano, S.J. 1998. Declines in benthic macroinvertebrate populations in southern Lake Michigan, 1980-1993. Can. J. Fish. Aquat. Sci. **55**: 2402-2413.

Owens, R.W., and Dittman D.E. 2002. Disruption of the benthic food-web in Lake Ontario: Consequences on native fishes. Conference proceedings, Great Lakes of the World (GLOWIII), Arusha, Tanzania. February 18-20, 2002.

Persaud, D., Jaagumagi, R., and Hayton, A. 1992. Guidelines for the protection and management of aquatic sediment quality in Ontario. ISBN 0-7729-9248-7. Ontario Ministry of the Environment, Water Resources Branch, Toronto, Ontario, Canada.

Pierce, R.C., Whittle, D.M., and Bramwell, J.B. 1998. Chemical contaminants in Canadian aquatic ecosystems. Public Works Government Services Publishing. ISBN 0-660-17475-8.

Sly, P.G., and Christie, W.J. 1992. Factors influencing densities and distributions of *Pontoporeia hoyi* in Lake Ontario. Hydrobiologia **235/236**: 321-352.

Thurman, E.M., and Cromwell, A.E. 2000. Atmospheric transport, deposition, and fate of triazine herbicides and their metabolites in pristine areas of Isle Royal National Park. Environ. Sci. Technol. **34**: 3079-3085.

Vanderploeg, H.A., Liebig, J.R., Carmichael, W.W., Agy, M.A., Johengen, T.H., Fahnenstiel, G.L., and Nalepa, T.F. 2001. Zebra mussel (*Dreissena polymorpha*) selective filtration promoted toxic *Microcystis* blooms in Saginaw Bay (Lake Huron) and Lake Erie. Can. J. Fish. Aquat. Sci. **58**: 1208-1221.