

Evaluation of Lake Michigan Sediment For Causes of the Disappearance of *Diporeia* spp. in Southern Lake Michigan

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ABSTRACT. The amphipod, *Diporeia* spp., the dominant benthic macroinvertebrate in the Great Lakes offshore waters, has exhibited a substantial decline in recent years. This decline occurred after the invasion and colonization of the lakes by the zebra mussel, *Dreissena polymorpha*. It has been hypothesized that the decline is a direct result of the competition by the zebra mussel decreasing the amount of food available. This study examined the potential of other stressors, e.g., the presence of toxic materials, as the contributing or main cause of the decline. Bioassays were performed with sediments from stations currently devoid of *Diporeia* (St. Joseph, MI) and with those still having *Diporeia* populations. In 28-d mortality bioassays, no mortality was observed with any of the sediments tested. However, in avoidance/preference tests, sediments from St. Joseph, that are now devoid of *Diporeia*, were avoided compared to sediments from Saugatuck, Grand Haven, and Muskegon, which still have a population. This avoidance was not changed by the addition of either *Spirulina Plus*® or *Tetramin*® flakes to the sediment as a food source but was reversed by a fresh layer of the diatom, *Fragillaria crotonensis*, on the sediment. These studies suggest that despite the high carbon content of the St. Joseph sediment, the nutritional content of the sediment was limited.

INDEX WORDS: *Diporeia* spp., population decline, toxicity bioassay, avoidance/preference testing, Lake Michigan.

INTRODUCTION

The amphipod, *Diporeia* spp., the most widespread and dominant benthic macroinvertebrate in the offshore waters of the Great Lakes, has exhibited a substantial decline in recent years in eastern Lake Erie, southern Lake Michigan, and eastern Lake Ontario (Dermott and Kerec 1997, Nalepa *et al.* 1998). This population decline occurred soon after the invasion and colonization of these areas by the zebra mussel, *Dreissena polymorpha*, and/or the quagga mussel, *Dreissena bugensis*. It was hypothesized that the *Diporeia* decline was the result of a decrease in food availability because of dreissenid mussel filtering activities (Dermott and Kerec 1997, Nalepa *et al.* 1998). In Lake Erie, diatoms have become rare, and cyanobacteria have become more abundant since the introduction of *Dreissena* spp. (Holland 1993, Markarewicz *et al.* 1999). Since *Diporeia* is highly dependent on settling diatoms as a food source (Gardner *et al.* 1989), any decrease in

diatom availability would likely have a negative effect on the population.

Despite the likelihood that a decreased food supply was probably the cause of the *Diporeia* population decline, it seemed prudent to determine whether there might be other factors contributing to the problem such as the presence of some toxicant in the sediments. A decline in available food could certainly lead to a population decline, but it seems unusual to have a total loss of the population in many areas (Nalepa unpublished data). Two sources of such toxicants could contribute to the loss of population. The first could be an unknown anthropogenic contaminant. Despite the general decline in concentrations of frequently monitored persistent organic pollutants over the past decades, as shown by the decline in concentrations in fish (DeVault *et al.* 1996) and herring gull eggs (Hebert *et al.* 1999), many compounds that are released to the environment are not monitored routinely. Among these rarely monitored compounds are those that are not expected to be particularly persistent such as sur-

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factants, many of the newer pesticides and herbicides, and other industrial compounds. The other potential source of toxicants could be from a species of blue-green algae such as *Microcystis*. Large *Microcystis* blooms have developed in many areas of the Great Lakes since the introduction of *Dreissena* (Lavrentyev *et al.* 1995; H.A. Vanderploeg, personal communication, Great Lakes Environmental Research Laboratory, Ann Arbor, MI). Further, blue-green algae in western Lake Erie have increased substantially since the invasion of the zebra mussel, while diatoms have decreased (Makarewicz *et al.* 1999). *Microcystis* can cause mortality in crustaceans (DeMott *et al.* 1991, Reinikainen *et al.* 1994, Smith and Gilbert 1995) and can reduce the productivity and growth of isopods and amphipods (Swiss and Johnson 1976) and the reproduction of *Daphnia* (Shurin and Dodson 1997). Thus, the increased occurrence of *Microcystis* may be one of the contributing reasons for population declines in *Diporeia*, although no large blooms have been reported in Lake Michigan.

An additional potential cause of the *Diporeia* population decline could be the introduction of a disease vector such as a bacteria, fungus, or virus. Such disease vectors could have been introduced with the zebra mussel or subsequent to its introduction. There is likely some association with the presence of dreissenids since the *Diporeia* decline did not occur until after dreissenids became established.

The objectives of this study were to test whether there was evidence of a toxicant in the sediment that was contributing to the disappearance of *Diporeia*, and to examine the hypothesis that food was limiting the population.

MATERIALS AND METHODS

Sediment Characterization

Lake sediment was collected in July and August 1998 at four locations from the southern Lake Michigan basin, St. Joseph (42° 12.43' N, 86° 37.89' W); Saugatuck (42° 41.14' N, 86° 18.90' W); Grand Haven (43° 02.47' N, 86° 18.90' W), and Muskegon (43° 11.27' N, 86° 25.78' W). All sites were at a 45 m water depth. Silt was the dominant substrate at St. Joseph, Saugatuck, and Grand Haven, while silty sand was the dominant substrate at Muskegon. These sites were selected because of widely varying *Diporeia* densities as determined by other and ongoing studies. Mean densities of *Diporeia* at these or nearby sites in 1992 and 1993 at St. Joseph, Saugatuck, and Grand Haven were 2,900,

16,400, and 10,400/m², respectively, and mean densities in 1997 at these same sites and at Muskegon were 0, 3,000, 6,800, and 10,600/m² respectively (Nalepa *et al.* 1998, Nalepa unpublished data). Sediments were also collected in September 1999 from the St. Joseph and Saugatuck sites and from another station off Muskegon, MI (43° 10.92' N, 86° 26.96' W) at 60 m water depth. The new Muskegon station was chosen because *Diporeia* populations were declining at the 45-m Muskegon site that was sampled in 1998. It was felt that the 60-m site was more representative of sediments with abundant amphipods, based on the ease of collection of *Diporeia* from this site. Sediments were collected by Ponar grab and the top 2 cm of sediment were placed in acetone rinsed jars, sealed, transported to the laboratory on ice, and placed in the dark at 4°C until the initiation of the bioassays.

Within a month of collection, sediment organic carbon content was determined using a Perkin-Elmer 2400 CHN Elemental analyzer. The sediment (100 mg of dry sediment) was pretreated by acidification with 2 mL of 1 N HCl to remove inorganic carbon. The samples were shaken for 24 h then dried at 90°C. The analyzer was calibrated using acetanilide, and the calibration was confirmed using a low organic carbon source, Florissant, MO soil (Table 1). The Florissant, MO soil was collected from a site in Missouri that was known to have no pesticide application and was provided by the Columbia Environmental Research Center, USGS, Columbia, MO. Prior to analysis the sediments were sieved through a 1 mm mesh screen to remove any macroinvertebrate organisms.

Diporeia for the assays were initially collected by Ponar grab at a third site off Muskegon, MI at 35 m depth (43° 13.12' N, 86° 27.02' W) in July and August 1998 and at the 60 m Muskegon site in September 1999. The collection site was changed because the *Diporeia* population had essentially disappeared from the shallower site by September 1999. The animals were gently screened from the sediment, placed into plastic bags with cool lake water, and transported to the laboratory on ice. The animals were housed at 4°C in a shallow Plexiglas aquarium with lake sediment and water. The culture water was changed weekly. The amphipods were fed weekly a dietary supplement of TetraMin®, a flaked fish food (approximately 0.5 to 1 g per aquarium per week). This food has routinely been used to feed *Diporeia* in long-term culturing at our

TABLE 1. Characteristics of sediments collected in July, 1998.

STATION	% Carbon	S.D.	% Nitrogen	S.D.	n = 3	Diporeia Abundance no./m ²	
						July 98	Aug 98
St. Joseph	1.67	0.01	0.24	0.02	n = 3	0	0
Saugatuck	0.59	0.12	0.09	0.01	n = 3	43	7
Grand Haven	0.53	0.03	0.07	0.01	n = 3	428	885
Muskegon	0.54	0.03	0.08	0.01	n = 3	6,205	4,620
Florissant Soil	1.17	0.01	0.18	0.005	n = 3	NA	NA

NA = Not Applicable

laboratory. Bioassays were initiated within 1 month after collection.

28 d Mortality Bioassay

Sediments (50 g wet sediment) from each of the Lake Michigan sites were added to each of ten 250-mL beakers along with Lake Michigan water (150 mL). Florissant, MO soil was used as a control. After a 24-h settling period, 10 juvenile *Diporeia* (weight range approximately 3 to 8 mg wet weight per organism based on observed size) were added to each beaker. The animals were examined twice a day (morning and afternoon), during the first 2 days and daily after. Any occasional *Diporeia* trapped by the surface tension at the water-air interface was submerged. Part of the water was exchanged in the beakers daily throughout the 28-d assay period by an auto/manual technique, where 1,000 mL of water was added to a holding tank, which equally distributed water to eight beakers simultaneously (Zumwalt *et al.* 1994). Upon termination of the bioassay, the contents of the beakers were poured onto a 1-mm-mesh screen, the sediment was rinsed away, and the number of live and dead animals recorded. This bioassay was only performed with sediments collected in July 1998.

120 h Avoidance/Preference Assay

In addition to the 28-d mortality bioassay, a sediment avoidance/preference bioassay was performed as a measure of a more subtle response to the various sediments. This bioassay records the number of animals that choose and burrow into a particular sediment (Gossiaux *et al.* 1993). This bioassay was performed on sediments collected on all three sampling dates and within one month of collection.

Sediments from each of the sites were added to each of five labeled petri dishes (47 mm diameter × 1.5 cm depth, 30 g sediments in each). The 20 petri dishes (4 sediments × 5 replicates) containing lake sediments, along with petri dishes containing Florissant, MO soil, and/or combusted sand as controls, were placed randomly into a single rectangular aquarium containing 6 L of lake water (approximately 10-cm depth). In some of the studies, the number of replicates per sediment was raised to 6. The aquarium was then placed into an environmental chamber at 4°C and illuminated with a red darkroom light. The sediments were allowed to settle for 24 h, after which 200 *Diporeia* were added to the aquarium. As before, organisms trapped at the surface were submerged. After 120 h, each dish was removed, the sediments sieved through a 1 mm mesh screen, and the number of animals that burrowed into each sediment was recorded. Difference in the number of animals in each of the sediment samples was tested using a Kruskal-Wallis One Way Analysis of Variance. Differences were considered significant at $p < 0.05$.

Additional, separate avoidance/preference bioassays were conducted as above, but various organic food supplements, Spirulina Plus[®] flakes, TetaMin[®] flakes, and the diatom *Fragillaria crotonensis*, were added to the sediments from each site. These studies were conducted immediately after the tests on the sediments without amendment. The Spirulina Plus[®] flakes or TetaMin[®] flakes were homogenized into each sediment to obtain an organic carbon content of 2%. TetaMin[®] is a general aquatic fish food and is used successfully in our culturing of *Hyalella azteca*, thus it is expected to be adequate for *Diporeia*. Further, it has been used as a supplement in culturing *Diporeia* at our laboratory for years. Spirulina Plus[®] flakes have been

used in a separate feeding study, and shown to be utilized as a food source by *Diporeia* for long periods (Quigley, Personal Communication, Great Lakes Environmental Research Laboratory, Ann Arbor). In another assay, *Fragillaria crotonensis*, ~ 3,000 cells, were settled onto the surface of each sediment as a food supplement. The control in each case remained combusted sand with no amendment. Statistical tests were conducted as given above.

RESULTS

28 d Mortality Bioassay

No significant mortality was observed in any of the sediments tested (Table 2). This suggests that there were no acute toxicants in the sediments contributing to the disappearance of *Diporeia*. This bioassay, however, does not rule out the potential impact of a chronic toxicant such as one that would affect reproduction in these organisms or the long-term health of these organisms.

Avoidance/Preference Bioassay

On average 177 ± 21 (out of 200) *Diporeia* burrowed into the non-amended sediments and in the sediments with the diatom treatment per test, while only 80 amphipods burrowed into the sediments for the two tests with the Spirulina Plus® or TetraMin® flakes added to the sediment. When natural sediments were tested for preference/avoidance by *Diporeia* with no amendments, there was a strong avoidance of the St. Joseph sediment and the controls; numbers of *Diporeia* found in sediments from the St. Joseph site were significantly lower than in sediments from the Saugatuck, Grand Haven, and Muskegon sites in both the 1998 and the 1999 bioassays. Numbers of *Diporeia* in sediment from the latter three sites were not significantly different from each other in the 1998 bioassays. However, in

September 1999, with the use of sediment from the new Muskegon site, both the St. Joseph and Saugatuck sites had significantly lower numbers of *Diporeia* than the Muskegon site (Table 3). (Note: When comparing data among sediments, it is only appropriate to compare down columns and not across columns as the tests were performed at different times and with different collections of sediment and *Diporeia*.) This indicated that the Saugatuck site was avoided relative to the new Muskegon site that had an abundant population of *Diporeia* based on ease of organism collection. The September 1999 sediment collection was performed primarily to test whether the Saugatuck site, that was by then also completely devoid of *Diporeia*, would be avoided relative to a site with abundant amphipods.

The controls in the July 1998 bioassays, combusted sand and Florissant, MO soil, were also avoided relative to the three equally preferred stations in Lake Michigan (Table 3). The avoidance of the two controls compared to the Grand Haven sediment is consistent with previous tests (Gossiaux *et al.* 1993).

The response of *Diporeia* to food supplements varied. Adding Spirulina Plus® or TetraMin® to the sediments resulted in all the sediments from the sites being equally avoided relative to the non-amended sand control (Table 3). However, adding approximately 3,000 *Fragillaria crotonensis* cells to the sediment surface increased the number of *Diporeia* in the St. Joseph sediments such that there was no difference between the numbers found in St. Joseph, Saugatuck, and Grand Haven sediments (Table 3). This result supports the hypothesis that food limitation or perhaps essential nutrient limitation at the St. Joseph site is partly or largely responsible for the observed disappearance of *Diporeia* from these sediments.

DISCUSSION

The absence of an acute response, no mortality in 28 d of exposure, to any of the sediments, does not completely discount the involvement of a toxicant, either naturally occurring or anthropogenic, in the disappearance of *Diporeia* from the southeastern portion of the lake. For instance, a chronic exposure may impact reproduction, which may lead to a decline in the population; the 28-d bioassay is inadequate to test for reproductive or growth effects on a species that has a generation time of at least two years at these depths in Lake Michigan (Winnell

TABLE 2. Percent mortality of *Diporeia* after 28 d exposure to sediments from each of four sampling sites in Lake Michigan for samples collected in July 1998.

Sediment	% Mortality \pm S.D.
St. Joseph	0 \pm 0
Saugatuck	1 \pm 3.2
Grand Haven	1 \pm 3.2
Muskegon	3 \pm 6.7
Florissant	0 \pm 0

TABLE 3. The number of *Diporeia* found burrowed in sediment from each of the sites after 120-h period.

Station	July 1998	July 1998	August 1998	September 1999	August 1998	August 1998	August 1998
	No Amendments				Spirulina Plus® Flakes	TetraMin® Flakes	Diatoms
	1 st Assay, n = 5	2 nd Assay, n = 5	n = 5	n = 6	n = 6	n = 5	n = 6
St. Joseph	1.2 ^a ± 1.1	2.8 ^a ± 2.9	0.5 ^a ± 0.5	0.5 ^a ± 0.8	0 ^a ± 0	0 ^a ± 0	8.5 ^a ± 4.9
Saugatuck	9.6 ^b ± 3.6	8.2 ^b ± 2.2	12.7 ^b ± 8.1	4.8 ^b ± 2.4	3.9 ^a ± 5.5	0 ^a ± 0	11.8 ^a ± 2.6
Grand Haven	12 ^b ± 6.3	15.2 ^b ± 2.5	10.7 ^b ± 5.9	NT	0 ^a ± 0	0 ^a ± 0	10.2 ^a ± 4.6
Muskegon ¹	11.4 ^b ± 9.1	7.6 ^b ± 2.7	5.3 ^b ± 3.6	29 ^c ± 25	NT	NT	NT
Sand (control)	0.7 ^a ± 0.7	0.7 ^a ± 0.7	1.1 ^a ± 1.1	0 ^a ± 0	8.6 ^b ± 7.6	14.2 ^b ± 11.9	0.5 ^b ± 0.5
Florissant Soil	2.2 ^a ± 3.4	3.6 ^a ± 3.3	NT	NT	NT	NT	NT

Samples with the same letter are not statistically different within each column. Note: When comparing data among sediments, it is only appropriate to compare down column and not across columns as the tests were performed at different times and with different collections of sediment and *Diporeia*

NT = Not Tested

1. The Muskegon station was changed for the September 1999 bioassay.

and White 1984). The absence of an acute response also does not preclude the potential for impacts by a disease vector such as a fungus, bacteria, or virus.

It has been observed that blooms of blue-green algae, such as *Microcystis*, have increased since the establishment of zebra mussels (Makarewicz *et al.* 1999). *Microcystis* produces a toxin that causes reproductive and growth problems in other crustaceans (Swiss and Johnson 1976, Shurin and Dodson 1997). There are no reports of *Microcystis* blooms in Lake Michigan as observed in Lake Erie, where declines in *Diporeia* populations have also been observed (Dermott and Kerec 1997). There may be a contribution to the *Diporeia* decline that the zebra mussel has mediated through the naturally occurring *Microcystis* toxin or other blue-green algal toxin. Thus, further work to demonstrate that the sediments are not chronically toxic to amphipods would be a logical next step.

The avoidance/preference test is one of the most sensitive bioassays for detecting contaminated sites (Burton *et al.* 1996). However, it is not clear whether *Diporeia* are responding positively to a preferred sediment, or actively avoiding an unpreferred sediment. The results of this study suggest that the sediment from the St. Joseph site is truly avoided since there is approximately an equal preference for sediments from the other sites. The Saugatuck sediment in the September 1999 bioassay now appears to be avoided compared to the new Muskegon site,

further suggesting that sites devoid of *Diporeia* have some less favorable characteristic compared to sites with an abundant *Diporeia*. Thus, there appears to either be something missing from the St. Joseph sediment and more recently the Saugatuck sediment to attract the *Diporeia*, or something that is sending a chemosensory signal that the amphipods avoid. It is clearly not the apparent overt nutritional character of the sediments since St. Joseph sediment contains the highest organic carbon content. Further, adding organic carbon in the form of TetraMin® and to a lesser degree the Spirulina® which should be nutritionally adequate, caused all the amended sediments to be avoided. The selection of 2% organic carbon for this material may have resulted in an over-enrichment condition that caused the *Diporeia* to avoid all the sediments.

Finally, results from sediments with the diatom addition provide some support to the hypothesis that there is a nutritional limitation. Because diatoms are nutritionally important for the energetics of *Diporeia* (Gardner *et al.* 1989), their presence may produce a chemosensory material that the amphipods can detect and to which they are attracted. Thus, when diatoms were added to the sediments, all sediments become equally preferred, and St. Joseph sediment was no longer avoided. While this suggests that a food resource is limiting and results in the disappearance of *Diporeia* from the southern basin of Lake Michigan, it is not positive proof. Of

course, inconsistent with this hypothesis is the finding that diatom-additions to the Saugatuck and Grand Haven sediment did not increase the number of *Diporeia* burrowing in these sediments compared to sediments without the diatom additions. Also, populations at the Saugatuck and Grand Haven sites in 1998 had declined dramatically from previous years, but preference/avoidance bioassays did not show any avoidance when tested in 1998.

In summary, if there is a toxicant in the sediments that is contributing to the disappearance of *Diporeia*, it appears that it would have to be acting through a chronic mechanism of action. Testing for a chronic response would be an appropriate next step.

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