Microbial Ecology

A Comparison of the Benthic Bacterial Communities Within and Surrounding *Dreissena* Clusters in Lakes

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Abstract

The impact of *Dreissena* (*Dreissena polymorpha* and *D. bugensis*) on the benthic bacterial community in lakes is largely unknown. Therefore, we quantified differences in the structure and activity of bacterial communities living in sediments (1) associated with *Dreissena* clusters, and (2) unassociated with established clusters (lake bottom sediments). *Dreissena* clusters and sediments were collected from locations in Lake Erie, Lake Ontario, and several inland lakes. Denaturing gradient gel electrophoresis (DGGE) analysis of the benthic bacterial community showed that the bacterial populations selected for by *Dreissena* represent a subset of the bottom communities and are geographically distinct. Community-level physiological profiling (CLPP) showed that overall bacterial activity and metabolic diversity were enhanced by the presence of clusters in all samples, with the exception of those harvested from the two Lake Erie sites. Therefore, *Dreissena* appears to affect both structure and metabolic function of the benthic bacterial community and may have yet unexplored ecosystem and food web consequences.

Introduction

Dreissenid mussels (zebra mussels, *Dreissena polymorpha* and quagga mussels, *D. bugensis*) are nonnative, bottom-attached grazers first introduced to North American lakes via ballast water from Eastern Europe in 1986 [20]. Since their introduction, *Dreissena* has colonized all five of the Laurentian Great Lakes, the surrounding watersheds, and many freshwater bodies east of the Mississippi River, including a large portion of the Mississippi River drainage [3]. *Dreissena*-mediated impacts are of special interest because *Dreissena* are ecosystem engineers, a species that creates or modifies habitats [25, 26, 46]. For example, when *Dreissena* form clusters on lake bottoms, they effectively increase the complexity of the physical habitat and reduce the amount of available, exposed soft substrate. *Dreissena* invasion also impacts ecosystem function by filtering particulates and organic matter from the water column. This filtration facilitates the concentration and sequestration of *Dreissena* waste, both feces and pseudofeces, as well as other detritus that would otherwise be washed into deeper zones by wave action in the nearshore regions [41]. Although many studies have described the impacts of the *Dreissena* invasion on benthic (bottom associated) macrofauna [6, 40, 44, 45], the effects of *Dreissena* on the benthic bacterial community structure and metabolic capacity are currently understudied. The bacterial community of lakes drives the initial degradation of organic material and is a key intermediate in transforming nutrients into forms that are available to plants and algae. Therefore, changes in the bacterial community that occur in response to *Dreissena*-related habitat alteration and grazing are potentially important in aquatic food webs.

Dreissena colonization can have multiple, positive impacts on benthic bacterial communities. *Dreissena* are efficient at filtering the water column [29] and have been shown to improve water clarity and light penetration [31, 32, 38]. Filtered organic material ultimately excreted as feces or pseudofeces [27] might serve as a nutrient source for microorganisms, thereby selecting for a bacterial community that exhibits altered (1) bacterial density, (2) structure, and (3) metabolic capacity when compared to communities inhabiting uncolonized sediments. For example, *Dreissena* have been shown to affect nitrogen dynamics by excreting ammonium and altering food-web

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structure at the sediment–water interface [30]. Furthermore, Hecky et al. [21] developed a conceptual model that predicted that *Dreissena*-mediated changes might be responsible for phosphorous diversion and retention in nearshore areas, resulting in system-wide effects including altered nutrient allocation and bacterial community impacts.

Although *Dreissena* colonization can potentially lead to enhanced bacterial growth and activity, the potential exists for negative interactions as well. For example, an increase in the density of macroinvertebrate consumers in association with *Dreissena* clusters can result in negative effects on the benthic bacterial community. *Dreissena* clusters create interstitial spaces that harbor and protect large numbers of invertebrates [6, 32, 40, 44], whereas organic material imported into the clusters might also provide a source of nutrition [40]. For example, amphipods associated with *Dreissena* clusters have been shown to consume microbes directly or indirectly (attached to particulate material), resulting in decreased bacterial densities [17]. Consequently, *Dreissena* clusters might promote a benthic trophic cascade [7] in which macroinvertebrate predation results in decreased bacterial biomass. Whether *Dreissena* foster positive or negative impacts on benthic bacterial communities, their impact on substratum complexity is likely to be important in channeling energy to higher trophic levels in the Great Lakes [44] and will likely have profound impacts on the food web as the system responds to shifts in energy flow [31].

In an attempt to clarify a component of the large-scale, system-wide impacts of this invasive species, we analyzed differences in the benthic bacterial community in response to *Dreissena* by assessing the structure and activity of bacterial communities living in sediments (1) associated with established *Dreissena* clusters, and (2) unassociated with established clusters (lake bottom sediments). Community fingerprinting methods have become popular to characterize how bacterial communities respond to ecological changes [35, 37]. For example, denaturing gradient gel electrophoresis (DGGE) provides highly reproducible fingerprints of complex bacterial communities [11] by separating polymerase chain reaction (PCR)-generated DNA fragments that vary in nucleotide sequence by as little as one in several hundred base pairs [13]. Characteristics of community activity can be monitored with community-level physiological profiling (CLPP). CLPP provides an estimate of the metabolic ability of a bacterial population by assessing its ability to degrade an array of carbon sources in the presence of a tetrazolium dye. Commonly used in a microplate format, respiration of any carbon source by a member of the community results in a concomitant reduction of the dye, and the formation of purple coloration in the microplate well that contained the utilized carbon source. Patterns and intensity of color formation have been used to monitor changes in bacterial communities resulting from environmental impacts [16] and to compare bacterial communities in freshwater sediment environments [5]. Despite the demonstrated potential of DGGE and CLPP to characterize the structure and activity of bacterial communities, the use of these methods to determine the impact of *Dreissena* on the benthic habitat has yet to be performed.

The goal of this study was to assess the impact of *Dreissena* colonization on benthic bacterial community structure and metabolism. Because *Dreissena* redirect carbon-rich compounds from pelagic to benthic zones, create protected interstitial spaces, and increase the bottom surface area colonizable by microorganisms, our expectations were to find altered bacterial community structure and activity in sediments associated with mussel clusters when compared to nearby, uncolonized sediments.

Methods

Sites and Sampling. Samples were collected from six sites within the lower Great Lakes watershed (Fig. 1) including eastern Lake Erie (Erie, PA), western Lake Erie (Maumee Bay), Onondaga Lake, Lake Ontario, Oneida Lake, and Owasco Lake. At each site, three *Dreissena* clusters were collected by hand and three samples of sediment (free of clusters) from within 1 m of each cluster were collected by scraping a 50-mL Falcon tube across the lake bottom. Both types of samples were collected from 1–2 m of water depth and transported to the laboratory on ice. Materials (sediments, feces, and pseudofeces) within the interstitial spaces of the clusters were collected by breaking open the cluster and retrieving the materials with a sterile spatula. The

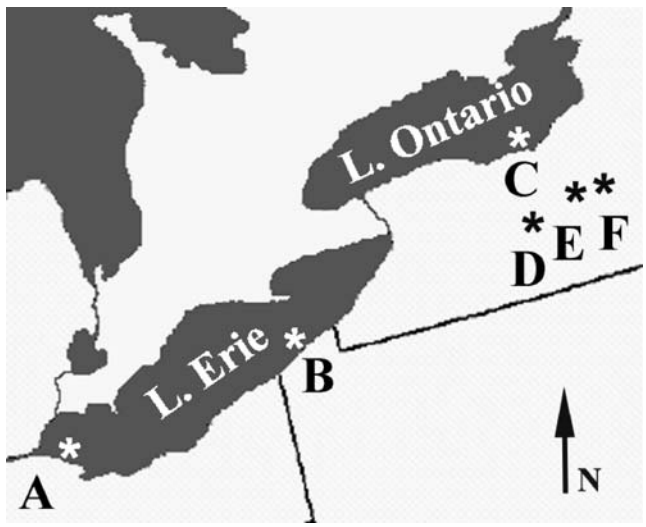

Figure 1. Map of the lakes showing the collection sites (*), Maumee Bay (A) Erie, PA (B), Ontario (C), Owasco (D), Onondaga (E), and Oneida (F).

triplicate samples of material from within the cluster and bottom sediment from each site were pooled to form a composite sample that represented pooled variability at each site. This collection strategy allowed us to examine the impact of mussel colonization on benthic bacterial communities by separating bacterial communities associated with the mussel clusters (cluster communities) from those associated with uncolonized sediments (bottom communities). CLPP was conducted within 24 h of sample collection, and samples used for DNA analyses were frozen for later processing.

Heterotrophic plate counts. Five grams of material and 10 g of 5-mm diameter glass beads were shaken in 45 mL of 10 mM phosphate buffer (pH 7) for 1 h. Serial dilutions were plated (100 µl) in triplicate onto 0.1X tryptic soy agar, and incubated at 25°C for 72 h to determine the number of colony forming units (cfu) in each pooled sample.

DNA isolation and PCR. DNA was isolated and purified from cluster and bottom communities according to the soil DNA isolation method of Sigler and Zeyer [43]. Additional purification was performed by eluting the DNA through a microcentrifuge spin filter containing sterile polyvinylpyrrolidone (PVPP). A portion of the 16S rRNA gene was PCR-amplified from the DNA samples with primers BAC 968 F (5′-AACGCGAAGAACCTTAC-3′) and BAC 1401 R (5′-CGCTGTGTACAAGACCC-3′) according to the protocol of Felske et al. [12]. A gc-clamp [34] was attached to the 5′ end of primer BAC 968 F to facilitate DGGE-based separation of the resulting PCR products. A negative PCR control containing no DNA was included in each set of reactions. Amplification of the proper gene fragment (~430 bp) was confirmed by comparison with a DNA size ladder after agarose gel electrophoresis.

Community structure via denaturing gradient gel electrophoresis (DGGE). DGGE of the PCR products was performed according to the method of Sigler et al. [42]. After staining with a 1:10,000 dilution of GelStar nucleic acid stain (20 min), fingerprints were visualized with a Kodak Gel Logic 200 imaging system and image analysis was performed with GelCompar II software (version 3.5, Applied Maths). DNA markers were loaded such that a maximum of five samples separated each marker lane. DGGE fingerprints were normalized using a custom marker of PCR products that were distributed along the length of the DGGE gel after electrophoresis. The similarity of DGGE fingerprints was calculated using the Pearson's product moment correlation coefficient [24] and optimization (3%) was used in the GelCompar II software to account for electrophoretic shifts between any two identical bands/patterns to provide the most appropriate fingerprint comparison. Cophentic correlation coefficients were calculated in GelComparII software to validate relational inferences represented by dendrogram clustering.

Community metabolism via community-level physiological profiling (CLPP). Based on the results of heterotrophic plate counts, approximately 10^5 cfu (in 100 µl of inoculum) from each pooled sample were inoculated into each well of a BIOLOG GN microtiter plate, resulting in one plate for each habitat type at each of the six sites sampled (n=12). Plates were placed into a humidified container and incubated aerobically at 25°C for 7 days during which the optical density (OD at 490 nm) of each well was measured every 12 h with a Model 680 (Bio-Rad) microplate reader. After subtraction of the absorbance value of the control well from each of the remaining 95 wells, two parameters were calculated to describe the metabolic activity of the sampled communities: (1) the average well color development (AWCD) [15] and (2) the community metabolic diversity (S) [47]. The AWCD estimates the overall respiration of the heterotrophic community and is proportional to the total dehydrogenase activity in an environmental sample amended with additional carbon [2]. The AWCD was calculated for each sample as the average OD_{490} of all 95 carbon-source-containing wells at each time point. *S*, estimates the overall metabolic diversity of the bacterial community, expressed as the total number of carbon sources utilized by the community at each time point. It was calculated by summing the number of wells at each time point that exhibited an OD_{490} exceeding a minimum threshold OD of 0.25 [15].

To determine the impact of clusters on the functional diversity of the benthic bacterial community, we assessed the community's use of the 95 carbon sources in the context of six substrate guilds (amines/amides, amino acids, carbohydrates, carboxylic acids, polymers, and miscellaneous) [47]. The proportion of utilized wells in each carbon source guild was calculated (there is an unequal number of wells representing each of the six guilds in BIOLOG plates) and compared with a two-factor analysis of variance (ANOVA) with carbon-source guild and habitat (cluster vs bottom community) as main effects. The interaction term indicated whether guild usage differed between cluster and bottom communities. The data were arcsin-square root transformed to stabilize variance [48].

Results

Heterotrophic plate counts. Plate count data revealed that cluster communities harbored higher densities of heterotrophic bacteria than bottom communities (Fig. 2). In most lakes the number of colony forming units (cfu) was at least 10-fold higher within cluster communities (mean across lakes 2.0×10^8 cfu) compared to bottom

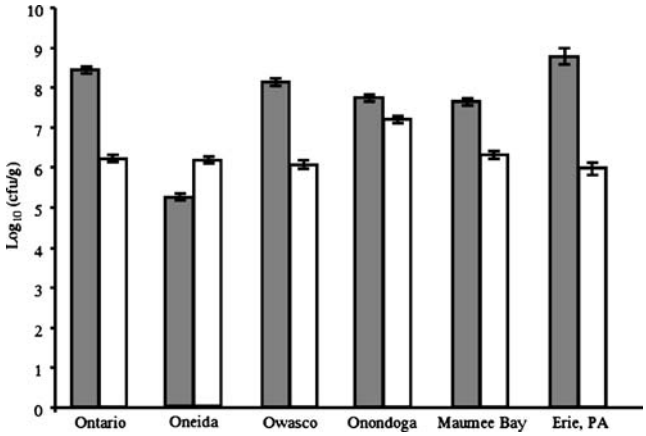

Figure 2. Heterotrophic plate counts of bacteria isolated from cluster sediment communities (gray bars) and bottom sediment communities (open bars) harvested from each lake. Error bars indicate the standard error among three replicates.

communities (mean across lakes 3.9×10^6 cfu), with the exception of Oneida Lake in which bottom community bacterial density was higher than cluster community bacterial density.

Bacterial community structure. DGGE fingerprints were successfully generated from all DNA samples with the exception of those isolated from Erie, PA. Fingerprints contained between 11 and 28 dominant bands in addition to many diffuse minor bands, indicating that both cluster sediment and bottom sediment harbored complex bacterial communities. Dendrogram analysis revealed high bacterial community similarity within the triplicate samples collected from either cluster or bottom sediments from each lake, suggesting high homogeneity within sites. With the exception of one branch node (64%), all cophentic correlation coefficients exceeded 78%, indicating that the relationships represented by the dendrogram were appropriate. Within a given site, cluster community fingerprint replicates were between 72% and 98% similar (mean 91%, Fig. 3, Table 2), whereas bottom communities were between 88% and 98% similar (mean 93%, Fig. 3, Table 2). Although within-site heterogeneity was limited, when all sites were considered together, the presence of *Dreissena* clusters impacted the structure of the bacterial communities. Specifically, communities in cluster sediments exhibited a significantly decreased number of bands in the DGGE fingerprints compared to bottom communities (p=0.02, one tailed t test). In addition, on a per site basis, we observed clear dendrogram separation of the cluster- and bottom communities from four of the five sites examined. The exception was one replicate of the bottom community from Onondaga Lake (Fig. 3).

The lake bacterial communities exhibited well-defined differences in structure (Fig. 3), regardless of sediment type (cluster vs bottom). Specifically, no lake was more than 50% similar to any other lake and several lake-to-lake

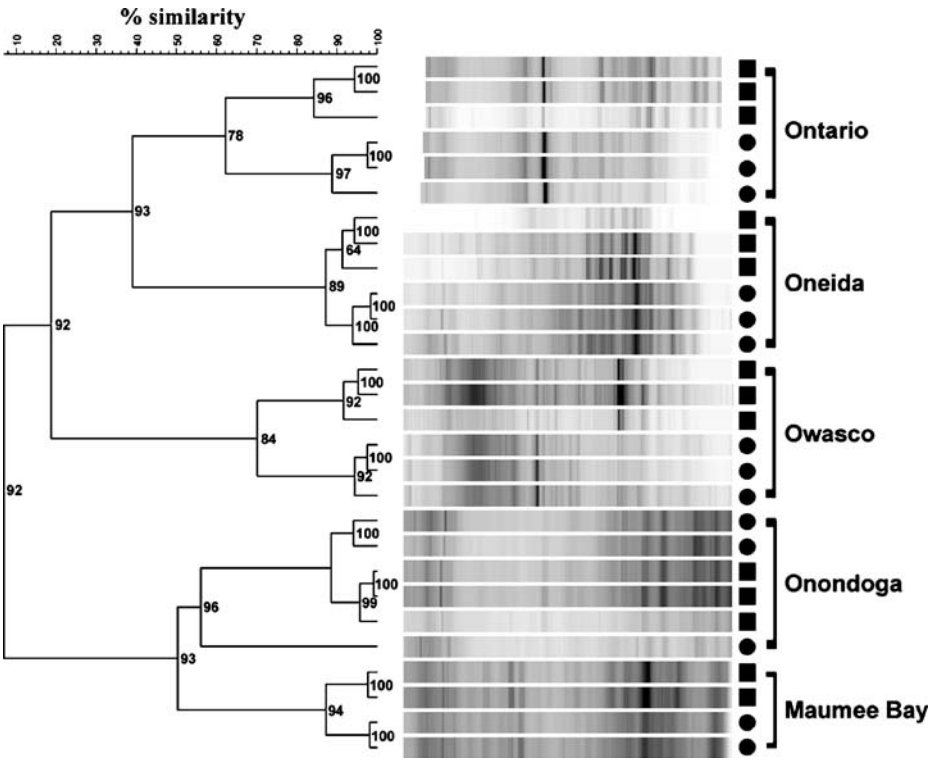

Figure 3. Dendrogram analysis of DGGE fingerprints of bacterial communities inhabiting cluster sediment (■) and bottom sediment (●) in each of the investigated lakes. Numeric values at branch nodes indicate the cophenetic correlation coefficient associated with each cluster.

Table 1. Comparison of overall bacterial community structure (percent fingerprint similarity based on Pearson's correlation coefficient) for each site and mean percent similarities among all sites and within each site

	Ontario	*Oneida*	*Owasco*	*Onondaga*	*Maumee Bay*	*Among all lakes*	*Within each lake*
Ontario	*X*	39	22	6	17	21	81
Oneida		*X*	16	0	26	20	92
Owasco			*X*	0	0	9	86
Onondaga				*X*	50	14	85
Maumee Bay					*X*	23	94

comparisons yielded similarity indices less than 1% (Fig. 3, Table 1). Owasco Lake exhibited the least similarity to the other lakes with a mean similarity index of 9% (Fig. 3, Table 1), whereas Onondaga Lake yielded the highest similarity index to any other lake, exhibiting 50% similarity to Maumee Bay (Fig. 3, Table 1).

Bacterial activity. Although OD_{490} was measured every 12 h, all CLPP data are presented with respect to the incubation endpoint (i.e., after 185 h). The bacterial cluster communities exhibited greater AWCD than bottom communities at all locations with the exception of the two Lake Erie sites (Fig. 4). Specifically, the AWCD of cluster communities from Owasco Lake, Lake Ontario, and Onondaga Lake were higher (OD_{490} of 0.35, 0.38, and 0.38, respectively) than the AWCD of bottom communities (0.25, 0.16, and 0.09, respectively; Fig. 4). The AWCD of Oneida Lake communities followed the same trend; however, the difference between cluster communities (OD_{490} of 1.86) and bottom communities (OD_{490} of 0.20) was more pronounced (Fig. 4). In contrast, the two Lake Erie sites exhibited a pattern opposite of the other four lakes, as a higher AWCD was observed in bottom communities than in the cluster communities. The mean AWCD of cluster communities was 0.33 at Erie, PA (eastern Lake Erie) and 1.54 at Maumee Bay (western Lake Erie), whereas bottom community AWCD was 0.66 at Erie, PA and 1.73 at Maumee Bay (Fig. 4).

S, an index of community metabolic diversity, was calculated as the number of carbon sources utilized by the bacterial communities (OD_{490} greater than 0.25) and represents potential metabolic diversity. At all six sites, *S* followed a pattern similar to that exhibited by AWCD. For example, in Owasco Lake, Onondaga Lake, and Lake Ontario, the cluster communities utilized a greater number of carbon sources than did the bottom communities. Cluster communities utilized 38%, 41%, and 36% of the available carbon sources, whereas bottom communities utilized 31%, 18%, and 8% of the available carbon sources in Owasco Lake, Onondaga Lake, and Lake Ontario, respectively (Fig. 5). As was observed with the AWCD, *S* in Oneida Lake showed the greatest difference between cluster and bottom communities, as cluster communities utilized 94% of the carbon sources, whereas bottom communities utilized 18% (Fig. 5). In contrast, the two Lake Erie sites displayed an opposite trend, as *S* in the bottom sediment was higher than the *S* measured in the cluster sediment. In Erie, PA 40% and 57% of the carbon sources were utilized by cluster communities and bottom communities, respectively, whereas in Maumee Bay 85% and 86% of available sources were utilized by cluster communities and bottom communities, respectively (Fig. 5).

Substrate guild utilization. *S* was also calculated in the context of substrate guild usage (amides/amines, amino acids, carbohydrates, carboxylic acids, polymers, and other carbon sources) for cluster and bottom communities at each site. The pattern of carbon guild usage between cluster and bottom communities mirrored the relationships observed in the AWCD and *S* analyses (Fig. 6). Specifically, the proportion of carbon sources within a guild utilized by the cluster communities

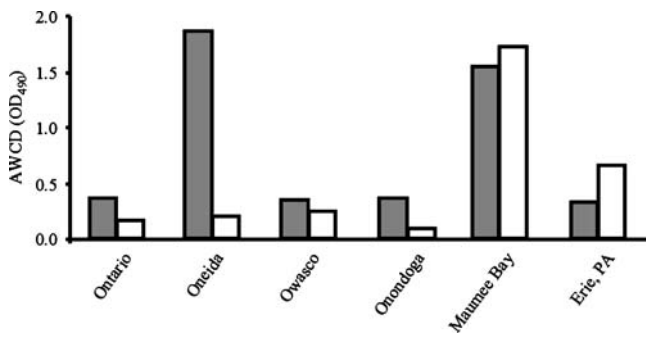

Figure 4. Bacterial community activity expressed in terms of average well color development (AWCD) for cluster communities (gray bars) and bottom communities (open bars) at each site.

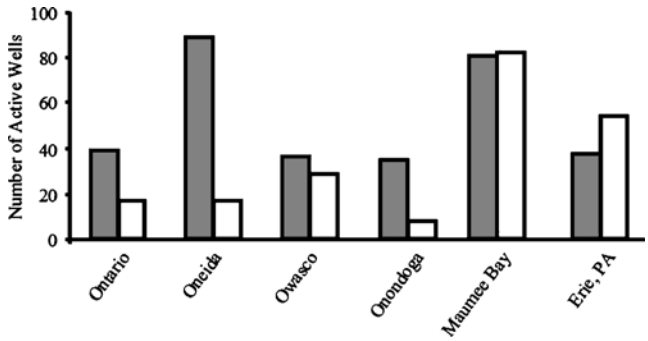

Figure 5. Bacterial community metabolic diversity expressed in terms of the number of active wells for cluster communities (gray bars) and bottom communities (open bars) at each site.

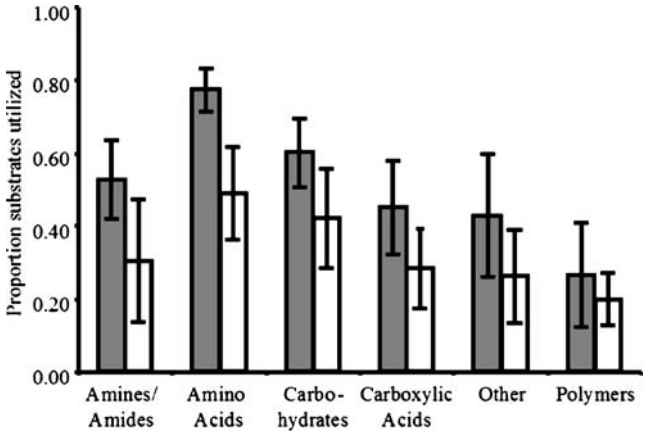

Figure 6. Mean proportion of substrates utilized for each substrate guild from cluster communities (gray bars) and bottom communities (open bars). Error bars indicate the standard error among three replicates.

significantly correlated with that of bottom communities ($r = 0.85$, $p = 0.029$) and the interaction term of the two-factor ANOVA comparing guild usage by habitat type (cluster vs bottom community) was not significant (ANOVA, $F_{df\ 11,\ 60} = 1.98$, $p = 0.05$, interaction $F_{df\ 5} = 0.28$ $p = 0.92$), indicating that substrate guilds were used similarly by communities from both habitats.

Discussion

Ecological disturbances caused by introduced species such as *Dreissena* spp. are complex and generate great environmental uncertainty and unpredictability. This uncertainty is magnified when the introduced species is an ecosystem engineer that modifies habitat and promotes numerous direct and indirect system-wide effects [26]. As ecosystem engineers, *Dreissena* drive impacts that are not fully understood, mainly because studies that explore more than one component of a system simultaneously are rare. In the current study, we showed that *Dreissena* clusters affected bacterial density, community structure, and metabolic activity of the benthic bacterial community. Plate counts revealed increased heterotrophic bacterial density within *Dreissena* clusters, whereas DGGE analysis revealed that the bacterial community structure was clearly altered by the presence of *Dreissena*. Furthermore, CLPP revealed that bacterial activity and potential metabolic diversity were enhanced by the presence of *Dreissena* in all sites, with the exception of the two Lake Erie sites. Samples from these two sites exhibited higher diversity and activity in sediment without cluster colonization. Regardless of the direction of the change, our results clearly demonstrate that *Dreissena* impact the benthic community on multiple levels.

The geographic separation of the investigated sites appeared to have a large impact on observed differences in bacterial community structure. Specifically, site-to-site similarity (combining cluster- and bottom sediment communities) was never greater than 50%, suggesting that although bacterial communities were sampled from similar environmental matrices (cluster sediment or bottom), geography and site-specific characteristics impacted the bacterial community structure to a greater degree than did the presence of *Dreissena* clusters. Nonetheless, our data suggest that within each lake *Dreissena* indeed impacted the composition of the bacterial community inhabiting the cluster sediment. Frischer et al. [14] observed similar *Dreissena*–bacteria interactions, as bacterial communities inhabiting pseudofecal material exhibited significantly lower diversity and evenness, suggesting that *Dreissena* clusters altered the sediment environment allowing certain populations of bacteria to dominate. In the current study, this impact was reflected first by differences between bacterial communities in cluster and bottom sediment within each site (Table 2), and second by a significantly lower DGGE band number in cluster sediment compared to bottom sediment, each suggesting the presence of discrete bacterial communities associated with either *Dreissena* clusters or sediment. Although DGGE band number cannot serve as a proxy for bacterial diversity, it can serve as an index of community structural complex-

Table 2. Among- and within-lakes comparison of community structure similarity (mean percent) based on Pearson's correlation coefficient

	Ontario		*Oneida*		*Owasco*		*Onondaga*		*Maumee Bay*	
	Bottom	*Cluster*	*Bottom*	*Cluster*	*Bottom*	*Cluster*	*Bottom*	*Cluster*	*Bottom*	*Cluster*
Ontario bottom	88	62	44	45	9	7	14	8	29	39
Ontario cluster		92	37	30	33	39	0	0	0	1
Oneida bottom			92	87	29	13	0	0	20	20
Oneida cluster				95	18	3	1	0	33	31
Owasco bottom					93	70	0	0	0	1
Owasco cluster						95	0	0	0	0
Onondaga bottom							97	86	57	55
Onondaga cluster								72	47	42
Maumee Bay bottom									98	86
Maumee Bay cluster										98

ity, where each band represents a unique sequence type (phylotype). The decreased number of bands in the fingerprints of cluster communities can be further explained in conjunction with the plate count and CLPP results that revealed site-specific alterations in density, activity, and metabolic diversity resulting from *Dreissena* colonization. Specifically, cluster communities exhibited higher bacterial density and activity, as expressed by the AWCD and *S*, which were higher in cluster communities than in bottom sediment communities at four of the six sites (Owasco, Oneida, Ontario, and Onondaga). It is possible that although the presence of *Dreissena* selected for a bacterial community that exhibited dominant members (this study, [14]), the variety of carbon and nitrogen sources in the *Dreissena* feces/pseudofeces [41] might select for increased metabolic potential in the community.

Whereas future manipulative, experimental studies should aim to determine the cause of the observed phenomenon, we hypothesized that *Dreissena* impact their immediate environment and subsequently alter the bacterial community structure and activity by increasing resources through the deposition of organic material and associated nutrients in the form of pseudofeces [10]. The observed increases in cluster community bacterial density (at five of the six sites) and AWCD and *S* (at four of the six sites) supported this idea. This is likely caused by local nutrient alterations that occur in the vicinity of the cluster, as much of the particulate matter filtered by *Dreissena* is concentrated, excreted as pseudofeces, and made available to benthic organisms [10, 21]. Consequently, a substantial community of detritivores and decomposers can be supported within the *Dreissena* clusters [44]. Microbes in this environment can further mineralize a significant portion of the nutrients contained in the *Dreissena* wastes, thereby increasing bioavailable nutrients and altering nutrient cycling and nutrient concentrations in and near *Dreissena* clusters [21].

It has been shown that macroinvertebrate densities are elevated in *Dreissena* clusters [6, 36, 40], and many of these may consume particulate matter and simultaneously consume bacteria, thereby negatively affecting the benthic bacterial community. In some terrestrial ecosystems, grazing increases primary productivity [33], although this may be most likely to occur under conditions in which losses of a limiting nutrient are high or when grazers import nutrients from outside the system [9]. Therefore, it is possible that high macroinvertebrate density in the *Dreissena* clusters affects bacterial productivity differently, depending on rates of loss and importation of nutrients or carbon at the different sites. Bottom communities from two of the six sites (Maumee Bay and Erie, PA) exhibited slightly higher AWCD and *S* than did *Dreissena* cluster communities. We do not have measurements of the macroinvertebrate grazers at these sites, but the observed trend suggests that grazing in the *Dreissena* clusters is not stimulating production. Oneida Lake was the location of the highest AWCD (Fig. 4) and *S* (Fig. 5). *Dreissena* clusters in Oneida Lake harbor high invertebrate densities relative to background counts [32], and our observations of macroinvertebrate density within *Dreissena* clusters of the six lakes sampled indicate that Oneida Lake macroinvertebrate densities were higher than those in the other lakes (Lohner and Mayer, personal observation). Therefore, although invertebrate grazing may play a role in the observed pattern of AWCD, manipulative studies of the effects of grazing on the bacterial community in the *Dreissena* cluster environment will be needed to definitively provide a mechanism for the observed field patterns.

Another argument explaining the higher AWCD and *S* in bottom sediment communities in the Maumee Bay and Erie, PA sites could concern trophic similarity, as both sites are located in Lake Erie. However, Maumee Bay is situated in the hypereutrophic western basin of Lake Erie, whereas the Erie, PA site is situated in the mesotrophic eastern basin. The western basin receives large inputs of water, sediment, and nutrients via the Detroit and Maumee Rivers [39]. For example, the Maumee River watershed is the largest single watershed in the Great Lakes basin (approximately 17,000 km^2) and contributes more than half the input of suspended solids to Lake Erie [23, 39], as well as an estimated 2,240 tons of phosphorus annually [1]. In contrast, the eastern basin of Lake Erie is small compared to the western basin, and the region lacks the phosphorus and sediment inputs found in the western basin as a result of smaller tributary rivers and a narrow drainage basin [4]. Therefore, trophic similarity, or simply the fact that both sites occur in Lake Erie is an unlikely explanation for the observed pattern. On the other hand, the physical characteristics of the two sites might provide a more plausible explanation for the observed pattern. Despite the trophic differences described above, the two sites are similar in that they were the only sites located on exposed Great Lakes shoreline; the other four sites were in small inland lakes (Fig. 1, sites D, E, and F) or in a protected embayment of Lake Ontario (Fig. 1, site C). Shoreline wave action in Lake Erie can move materials as large as cobble and boulders [22]; therefore, the Maumee Bay and Erie, PA sites are likely exposed to higher wave activity than any of the protected sites. It is possible that in this physical environment, macro- and microinvertebrates graze heavily in the protected cluster environment or that organic material is removed from the clusters by turbulence. Consequently, we suggest that *Dreissena* clusters are likely to increase bacterial activity and diversity except in areas where other conditions, such as high physical disturbances, counteract these increases.

Analysis of carbon substrate guild usage revealed that neither cluster nor bottom communities preferentially

metabolized a particular guild of carbon. A significant correlation existed between guild usage by cluster and bottom communities, suggesting that although the colonization of *Dreissena* altered the structure, activity, and metabolic diversity of sediment communities, substrate preference remained unchanged. Therefore, because proportional guild usage was largely invariant between cluster and bottom sediment communities, the overall *S* appeared to provide a good reflection of functional diversity in the communities.

The two methods employed in this study describe separate aspects of the bacterial community and demonstrate the importance of adopting a multiphasic approach to produce a more comprehensive picture of bacterial community structure and function. In our experimental setup, we used heterotrophic plate counts, DGGE, and CLPP, to detect differences in bacterial density, community structure, and metabolism, respectively. Like all culture-dependent procedures, heterotrophic plate counting and CLPP analysis are selective, as they utilize specific carbon sources in high concentrations, relative to the *in situ* environment [28]. Therefore, the contribution of oligotrophic bacteria and obligate anaerobes might not be well represented. Nonetheless, these analyses do provide indices of relative community-level bacterial density and metabolic potential and an effective means to compare the culturable subsets of different communities [18]. DGGE can also present limitations when analyzing complex samples after 16S rRNA gene amplification. Multiple *rrn* operons and/or comigration of bands of different sequence, but similar melting behavior can lead to misinterpretations concerning community structure [8, 36]. Despite these limitations, in this study DGGE served as a valuable tool to monitor community alterations in response to *Dreissena* colonization. The analysis of DGGE fingerprints is not standardized. Despite the popular use of band-based similarity measures (e.g., Dice and Jaccard), we used the Pearson's product moment correlation coefficient, which uses the entire data set by correlating points along the densitometric curve in each fingerprint. This was preferred with respect to band-based similarity methods because it is insensitive to relative differences in intensity and background, features common to DGGE fingerprints. Evidence suggests that because more of the data in the fingerprint are used than in band-based algorithms, the Pearson-UPGMA clustering method is superior to band-based methods for analyzing DNA fingerprints [19]. Furthermore, when Dice similarity analysis was performed on these fingerprints (data not shown), it was often necessary to manually correct for bands incorrectly called or missed by the software, which introduced an unwanted degree of bias to the analysis.

Few previous studies have addressed the response of the benthic bacterial community to *Dreissena*, a pervasive, introduced species. Our data suggest that the structure and metabolic function of the benthic bacterial community of lakes is greatly impacted by *Dreissena* colonization. Therefore, mechanistic studies that explore whole-system impacts by investigating how *Dreissena* colonization impacts specific bacterial processes and the impacts of the processes across trophic levels need to be preformed.

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