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NOTE

Some Fungi and Water Molds in Waters of Lake Michigan with Emphasis on Those Associated with the Benthic Amphipod *Diporeia* spp.

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ABSTRACT. *To determine types of fungi in the water and associated with the benthic amphipod* Diporeia *spp., samples were collected at various depths in Lake Michigan in an area where the* Diporeia *population was in a severe state of decline. No fungi were found associated with living, freshly-dead, or dried* Diporeia *cultured separately from Lake Michigan water. When dead* Diporeia *and other organic substrates (snake skin and hemp seeds) were used to grow fungi in Lake Michigan water, a rich and diverse fungal and water mold community was revealed. A total of 31 species were found, with the most common genera being* Achlya, Aphanomyces, Myzocytium, *and* Pythium. *In general, species were homogeneously distributed in the water; that is, few differences were found in species richness between nearshore (10–15 m) and offshore (60–80 m) waters, and between near-surface (1 m) and near-bottom waters (1 m off bottom). Sampling occurred during the unstratified period (April and October) to maximize the number of species collected, which may have contributed to the uniform spatial pattern observed. While conclusions must be placed in context with our methods of detection, we found no evidence that a fungal infestation was associated with* Diporeia *in this region of the lake.*

INDEX WORDS: *Great Lakes fungi, pathogen, disease, macroinvertebrates.*

INTRODUCTION

Fungi and fungi-like organisms play a vital role in the decomposition of most organic material in aquatic systems. In particular, specific groups actively mineralize insoluble polysaccharides such as chitin, which comprises the exoskeleton and carapaces of aquatic crustaceans (Batko 1975, Söderhäll *et al.* 1991, Czeczuga and Godlewska 2001, Kiziewicz and Kurzatkowska 2004). While most fungal groups are saprobionts, other groups are parasitic on the life stages of both invertebrates and vertebrates, including embryos and eggs (Dick 2001a, b). Most of these pathogenetic fungi occur within the division Oomycota (water molds). The single class Oomycetes within this division have a complex life cycle that includes flagella-bearing zoospores that infect host organisms. While most water molds live freely in the water, they can become associated with host organisms as mostly parasites and predators when environmental conditions change, or when the host is subjected to various stressors (Barron 2003). Various water molds such as *Aphanomyces invadens* can cause massive mortality in fish populations (Noga and Dykstra 1986, Kiryu *et al.* 2002, 2003; Sosa *et al.* 2007), and fungal diseases are widespread in various phytoplankton and crustaceans (Ramaiah 2006).

In this paper we identify some fungal and water molds found in Lake Michigan with an emphasis on species associated with the benthic amphipod *Diporeia* spp. *Diporeia* populations are currently de-

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clining in all the Great Lakes except Lake Superior, and large areas are now completely devoid of this organism (Nalepa *et al.* 2006a). While the disappearance of *Diporeia* has been linked to the introduction and spread of dreissenid mussels (*Dreissena polymorpha* and *Dreissena bugensis*), exact causes for the decline have been difficult to determine (Nalepa *et al.* 2005, Nalepa *et al.* 2006b). By examining fungi associated with *Diporeia* in an area where populations are declining, we explore the possibility that a water-borne fungus may be implicated in observed declines. Pathogens are common in amphipods and are known to reduce or limit populations (Johnson 1985, 1986), or to play a major role in structuring communities (MacNeil *et al.* 2003). A dramatic decline in the amphipod *Corophium violator* in the Baltic Sea was attributed to a yeast pathogen (Segerstrale 1960). In the Great Lakes, Messick *et al.* (2004) used histological techniques to determine the prevalence of parasites and pathogens associated with *Diporeia* from Lakes Michigan and Huron. Incidence levels of yeast-like organisms and fungi were low, only 0.4%, and levels in areas of declining populations were not different from those in areas where populations were stable. To supplement this work, we further examine fungi and water molds associated with *Diporeia* by using the "bait" method. In this method, organic matter is used as substrate for the cultured growth of fungi; hence, the method may detect the presence of fungal taxa not easily observed using histological techniques. In addition to determining the presence of fungi associated with *Diporeia*, we document those taxa present in nearshore and offshore waters of Lake Michigan that readily become associated with organic material.

MATERIAL AND METHODS

Sampling sites were located in Lake Michigan at various depths off Muskegon, MI (Fig. 1). *Diporeia* were collected with a Ponar grab at 60 m (43° 12.00′ N, 86° 27.37′ W), and at 80 m (43° 12.00′ N, 86° 29.57′ W) in April and October, 2004, respectively. Sampling was conducted in the spring and fall since aquatic fungi grow best at colder temperatures (Srivastava 1967, Khulbe and Bhargava 1977). The *Diporeia* population at the 60-m site was in a severe state of decline at the time of sampling in April, and individuals were no longer found at this site by October. Hence, to collect *Diporeia* in October, we sampled progressively offshore until individuals were found, and that was not

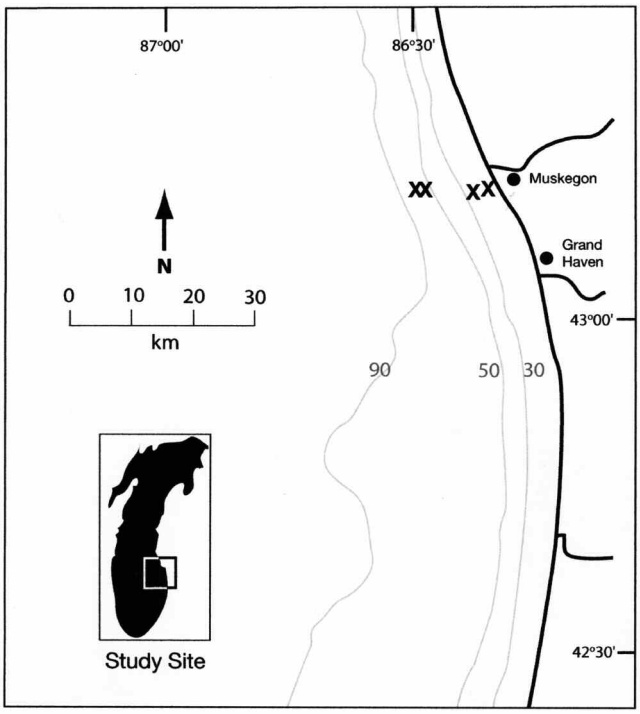

FIG. 1. Location of sampling sites (X) in southeastern Lake Michigan off Muskegon, MI. Sites were located at 10–15 m, 25 m, 60 m, and 80 m. Depth contours are 30, 50, and 90 m.

until we reached the 80 m depth. Intact Ponar grab samples (organisms, sediment, and overlying water) were placed into plastic bags, then placed into a cooler, iced, and brought back to the laboratory and kept at 4°C. Water samples were also collected at each site with a rinsed, 1-L Niskin bottle from three vertical depths: 1 m, 4 m, and 1 m off the bottom. In addition to collecting water samples at sites where we collected *Diporeia*, we also collected water at two additional sites in both April and October—one site was located at 10–15 m (43 11.49 N, 86 20.64 W) and the other site was located at 25 m (43° 12.00′ N, 86° 22.67′ W). *Diporeia* had already disappeared from these two shallower sites several years earlier (Nalepa unpublished data).

Within 24 h of collection, 10–20 *Diporeia* of various sizes (~ 4–7 mm) were removed from the sediment samples, dried in a dessicator at room temperature for 48 h, and then placed in clean, sealed vials. These dried specimens were sent via overnight express to Medical University, Bialystok, Poland for analysis of attached fungi. Also sent were the water samples collected from each site and vertical depth, as well as 10–20 live specimens

from each collection location. The latter individuals were shipped in rinsed, insulated thermos bottles filled with Lake Michigan water collected from the hypolimnion.

Fungi were isolated using the bait method in which organic material is used as substrate for fungal culture (Seymour and Fuller 1987). In addition to *Diporeia*, hemp seeds (*Cannabis sativum*) and snake skin (*Natrix natrix*) were also used as substrate for fungal growth. Both hemp seeds and snake skin are standard substrates used in culturing fungi for identification. Each of the three bait items was placed separately into 1-L containers with Lake Michigan water. Living and freshly-dead *Diporeia* from the thermos bottles, and previously dried *Diporeia*, were placed into petri dishes containing distilled water. All containers were covered with glass plates to partly protect the water from outside bacteria and then incubated for approximately 1 month at room temperatures. During this period, microscopically-determined mycelia were removed from each of the substrates and transferred to sterilized petri dishes containing distilled water. Several preparations were made for each sample, and aquatic fungi were identified using the vegetative organs (shape and size of the hyphae), the asexual organs (shape of sporangium and spores), and the generative organs (structure of the oognium, oosporangium, and antheridium). Observations and measurements were made on colonies developed on these baits. Incubation at room temperature proved to be adequate for vigorous growth of every representative we isolated. Keys used for fungi identification are given in Sparrow (1960), Bedenek (1972), Batko (1975), Fassatiova (1983), Seymour and Fuller (1987), Dick (1969, 1990), and Pystina (1998).

Fungi found in Lake Michigan water exclusive of the bait method were determined using solid media culture techniques (agar-medium method). Water (1 mL) from each of the sites and vertical depths was sowed onto culture plates containing Sabouraud glucose agar with Gentamicin and chloramphenicol 2 (SGCs; BioMerieux, France) and then incubated for 3–5 days at 28°C. Observed fungi were further transferred to plates with Sabouraud agar and incubated at room temperature for 5 days, during which developing colonies were counted, examined, and identified (Zaremba and Borowski 2001). In this way, pure fungi developed with the structures needed for identification. Species identification was based on morphological and biometrical characters of the mycelium and conidia (Fassatiova 1983, Zaremba and Borowski 2001).

For purposes of analysis, the sites were divided into three depth categories: 10–15 m, 25 m, and 60–80 m. Independence in the number of species collected between the 2 months (April, October), three sites, and three vertical depths (1 m, 4 m, 1 m off bottom) were tested using a G-test (log-likelihood) within a 2 × 3 × 3 contingency table (Sokal and Rohlf 1969).

RESULTS

No fungi (mycelia) were found on living, freshly-dead, or dried *Diporeia* placed in distilled water indicating that, at the time of collection, no fungi able to grow under the described circumstances were associated with these individuals. When using organic bait (snake skin, hemp seeds, and dried *Diporeia*) and the agar-medium method to culture fungi from Lake Michigan water, we identified 18 genera and 31 fungal species (Table 1). Of these, 18 were found on snake skin, 12 on *Diporeia*, 5 on hemp seeds, and 3 on Sabourand glucose agar. The most common genera were fungi within the class Oomycetes: *Achlya* with seven species found, *Pythium* with four species, and *Aphanomyces* and *Myzocytium* and with three species each. Of these major genera, some preferences were noted in the substrate selected. For instance, the four *Pythium* and three *Aphanomyces* species grew exclusively on snake skin, and all seven of the *Achlya* species grew on *Diporeia*, with three of these growing on *Diporeia* exclusively.

Although sampling date, site, and water-column depth jointly lacked independence ($0.05 > P > 0.01$), consistent patterns in the distribution of total species were not apparent across these factors (Table 1). The lack of independence was likely a result of the greater number of species (12) found at 1-m water depth at the 60-m site in April as compared to the other date × site × water depth combinations (maximum of six species). Five of the seven species of *Achlya* were only found on that date and location. The total number of species was generally similar at the three sites (15, 11, and 15 species at 10–15 m, 25 m, and 60–80 m sites), and vertically within the water column (16, 14, and 11 species at 1m, 4 m, and 1 m off the bottom).

DISCUSSION

A number of studies have examined the occurrence of fungal communities in Great Lakes waters (Paterson 1967, Qureshi and Dutka 1974, El-

TABLE 1. List of fungal and water mold taxa found in waters of Lake Michigan in 2004. Given are the types of organic substrate or "bait" upon which each taxa grew, and the month, site, and vertical depth at which each taxa was found. Organic substrate: D = Diporeia spp., H = hemp seed, S = snake skin, Sa = Sabourand glucose agar medium. Given in parenthesis after each taxa: r = rare in large European lakes, c = common in large European lakes.

		April, 2004									October, 2004								
		15 m site			25 m site			60 m site			10 m site			25 m site			80 m site		
Taxa	Organic substrate	1 m	4 m	14 m	1 m	4 m	24 m	1 m	4 m	59 m	1 m	4 m	9 m	1 m	4 m	24 m	1 m	4 m	79 m
Chromista																			
Hyphochytriomycetes																			
Hyphochytriales																			
Rhizidiomyces apophysatus Zopf (r)	H							X											
Oomycetes																			
Pythiales																			
Myzocytium microsporum (Karling) Sparrow (r)	D															X			
Myzocytium proliferum Schenk (r)	S																		X
Myzocytium zoophthorum Sparrow (r)	D,S						X		X			X	X						
Pythium aquatile Höhnk (c)	S							X									X		
Pythium debaryanum R. Hesse (c)	S												X						
Pythium epigynum Höhnk (r)	S							X											
Pythium rostratum E.J. Butler (c)	S		X					X				X							
Saprolegniales																			
Achlya hypogyna Coker and Pemberton (r)	D							X						X					
Achyla diffusa J.V. Harv. (r)	D,H							X											
Achyla oblongata de Bary (r)	D,H							X											
Achyla polyandra Hildebr. (c)	D							X											
Achyla proliferoides Coker (r)	D							X											
Achyla prolifera Nees (c)	D							X											
Achyla treleaseana Humphrey Kaufman (c)	D,S						X	X							X				
Aphanomyces irregularis W.W. Scott (c)	S					X													
Aphanomyces laevis de Bary (c)	S											X	X						
Aphanomyces stellatus de Bary (c)	S												X						
Fungi																			
Chytridiomycetes																			
Blastocladiales																			
Catenophlyctis variabilis (Karling) (c)	S					X													
Chytridiales																			
Achlyogeton entophytum Schenk (r)	S											X							
Nowakowskiella elegans Nowak. (Schröt) (c)	H	X						X											
Phlyctorhiza endogena Hanson (r)	S			X															
Rhizophlyctis chitinophila (Karling) Sparrow (r)	D						X												
Rhizidium ramosum Sparrow (r)	D				X														
Zygomycetes																			
Zoopagales																			
Syncephalis cordata Tiegh. & G. Le Monn. (r)	S		X																
Ascomycetes																			
Hypocreales																			
Fusarium moniliforme Sheld. (r)	S																X		
Eurotiales																			
Aspergillus niger Tiegh. (c)	S,Sa													X					
Penicillium funiculosum Thom (r)	S		X																
Pleosporales																			
Alternaria alternata (Fr.) Keissl. (c)	S											X			X		X		
Saccharomycetes																			
Saccharomycetales																			
Candida albicans (C.P. Robin) Berkhout (r)	Sa																		X
Geotrichum candidum Link (r)	Sa											X							

Shaarawi *et al.* 1977, Sherry 1986, Kwasniewska 1988), but it is difficult to compare our results to those of these previous studies because of different techniques of collection, culturing mediums, and levels of identification. Kwasniewska (1988) used a select agar medium and identified seven dominant genera of yeasts and filamentous fungi in Lake St. Clair, of which only one was found in our Lake Michigan samples (*Fusarium*). Likewise, Qureshi and Dutka (1974) used various agar mediums and found 25 genera in Lake Ontario waters off the Niagara River mouth, of which only five were found in Lake Michigan (*Alernaria*, *Aspergillus*, *Fusarium, Geotrichum*, *and Penicillium*). We identified 18 genera when using both the bait and the agar-medium methods, but only three were identified using the latter method. Hence, when just considering the agar-culture method, it appears that fewer genera were found in the Muskegon area of Lake Michigan relative to these previous studies in Lake St. Clair and the Niagara River area of Lake Ontario. It should be noted, however, that these earlier studies were mostly conducted in the 1970s when, because of greater nutrient loads and absence of *Dreissena*, more organic material was likely present in the water column. Fungi tend to be more abundant and diverse in areas subjected to organic enrichment (Quershi and Dutka 1974).

Based on occurrences in other water bodies that have used the "bait" method, mostly lakes in Europe, 18 of the 31 species found in Lake Michigan might be considered rare, while the other 13 might be considered common (see Table 1; B. Kiziewicz, unpublished data). The genera with the most species in Lake Michigan, all within the class Oomycetes, were *Achyla*, *Aphanomyces*, *Myzocytium*, and *Pythium*. All seven species of *Achlya* grew on dead *Diporeia* when it was used as a substrate. These species typically colonize chitin-and-keratin containing substrates such as insect exuviae, and dead fish and amphipods (Czeczuga and Godlewska 1994, Czeczuga *et al.* 2004). Studies have also shown that some of these species (*A. diffusa*, *A. polyandra*, *A. prolifera,* and *A. proliferoides*) can be parasitic on fish eggs and adults (Srivastava and Srivastava 1977, Czeczuga *et al.* 2002). The species of *Aphanomyces* and *Pythium* found in Lake Michigan are mostly saprobiotic, living on decayed animal and plant remains. The exception is *Aphanomyces laevis*, which is a widely-occurring species that is parasitic on fish (Dykstra *et al.* 1986). *Myzocytium zoophthorum* was the most frequently encountered species; this species along with other species of this genus are described as parasitic on small invertebrates, mostly rotifers (Batko 1975). Another species found in Lake Michigan, *Fusarium moniliformae*, has been described as an opportunistic pathogen of crustaceans (Ramaiah 2006).

The main purpose of this study was to determine fungi and water molds associated with *Diporeia.* Population densities of *Diporeia* in the Lake Michigan study area off Muskegon have been in a steady state of decline since the late 1990s. The decline of *Diporeia* occurred first in shallow, nearshore waters and then proceeded offshore, which is consistent with the offshore expansion of dreissenid mussels (Nalepa *et al.* 2006a). The population density of *Diporeia* at a 45-m site off Muskegon was 10,000/m^2 in early 1997, but declined to near 0/m^2 by the end of 2002 (Nalepa *et al.* 2006b). At our 60-m site, *Diporeia* were present in spring 2004, but had disappeared by fall 2004. Densities at our 80-m site have not been regularly monitored, but the population was likely in the state of decline at the time samples were taken. For example, densities at a nearby 100-m site were 4,400/m^2 in 2002, 1,040/m^2 in 2004, and 4/m^2 in 2005 (Nalepa unpublished data). Given these population declines, it is noteworthy therefore that no fungi were found associated with living, freshly-dead, or dried *Diporeia* when cultured apart from Lake Michigan water. Instead, all the fungal species found on *Diporeia* developed after individuals were cultured in Lake Michigan water, indicating fungi were present in the water, but not associated with *Diporeia*.

While relative abundances of fungi were not determined, some insights into distributions can be derived from the relative number of species found. Unexpectedly, gradients related to sampling depth (distance offshore) and vertically in the water-column were not apparent. That is, more species were expected at shallow sites compared to deeper ones, and more near bottom than near surface in the water column. Aquatic fungi utilize a variety of organic substrates for growth, and likely enter lakes through watershed inputs (Dick 1971). Thus, more fungal species might be expected in nearshore areas and near the bottom sediments where organic matter content would be highest. Since, as noted, aquatic fungi grow best at colder temperatures (Srivastava 1967, Khulbe and Bhargava 1977), we collected during the unstratified period in April and October to maximize the number of species found. Sampling during this period of uniform mixing may have contributed to the lack of observed gradients both hori-

zontally and vertically within the water column. The exception to this pattern of homogenous distributions was the relatively high number of *Achlya* species found near the surface at the 60-m site in April. Species of this genus are chitinohilic and readily colonize dead amphipods. The decreasing *Diporeia* population at this site may have contributed to the occurrence of these species, but *Achlya* were not found throughout the water column and were not found in October at the 80-m site where the *Diporeia* population was also likely decreasing.

In summary, this study seems to confirm the finding of Messick *et al.* (2004) that fungi associated with living or freshly-dead *Diporeia* in Lake Michigan were absent or rare. While a rich and diverse community of fungi and water mold species was found in Lake Michigan waters when using various substrates as organic "bait" (including freshly-dead *Diporeia*), no fungi could be cultured using *Diporeia* as a substrate separate from Lake Michigan water. Within the context of our detection methods, therefore, there was no indication that a fungal infection was primarily the cause of the population decline. While some results suggest that a pathogen/disease may be a potential reason for the decline of *Diporeia* in the Great Lakes (Dermott *et al.* 2005), further, more-detailed studies are needed to explore this possibility.

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