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Eukaryal and archaeal diversity in a submerged sinkhole ecosystem influenced by sulfur-rich, hypoxic groundwater

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ABSTRACT

Middle Island sinkhole (23 m depth) is an underwater karst feature in Lake Huron (MI, USA) inundated with hypoxic, high-conductivity groundwater. Here, microbial mats composed of purple-pigmented filamentous cyanobacteria cover carbon-rich sediments. To study the species diversity of *Archaea* and *Eukarya* in this habitat, we constructed clone libraries and sequenced the small subunit ribosomal RNA genes from sediment cores sectioned into five visually distinct layers: the surface cyanobacterial mat (0–0.2 cm), an underlying white crystalline layer (0.2–0.5 cm), and three sub-sections of black organic-rich sediment chosen from distinct layers in the cores (0.5–2.0 cm, 7.5–9.5 cm, and 24.5–28.5 cm). Clone libraries from the cyanobacterial mat were dominated by eukaryal 18S rRNA gene sequences such as nematodes (*Tobrilus gracilis*), ciliates (*Frontonia vernalis*), and tardigrades (*Isohypsibius granulifer*). Shallow organic-rich sediments shared clones with the overlying mat but also included seed shrimp (*Cyprididae* sp.) and copepods (*Leptodiaptomus* spp.). Clone libraries from the deepest sediments were dominated by archaeal sequences similar to known methanogens (*Methanosphaerula* and *Methanosaeta*) and uncultivated *Archaea*, including non-thermophilic *Crenarchaeota*. Phylogenetic trees revealed representation in diverse eukaryotic and archaeal lineages. By chronicling the species composition of freshwater sinkholes, this study expands our knowledge of microbial communities in habitats influenced by hypoxic, sulfur-rich groundwater.

Introduction

Recent reports suggest that intrusion of groundwater into aquatic ecosystems is an important source of nutrients, contaminants, and trace elements that can significantly affect aquatic life (Church, 1996; Moore et al., 2008; Michael et al., 2005; Knauth and Kennedy, 2009). While groundwater can influence all aquatic habitats, most studies to date have focused on the marine environment. To better understand the influence of groundwater intrusion into freshwater habitats, we studied actively venting underwater sinkholes in the Laurentian Great Lakes.

Sinkholes are common geologic features found in areas where near-surface limestone is dissolved by groundwater, forming underground caverns. When caverns collapse, the resulting sinkholes fill with overburden and may contain groundwater (Palmer, 1991). While sinkholes are most often found in terrestrial habitats, submerged sinkholes in Lake Huron are unique underwater karst formations formed by groundwater dissolution of Paleozoic bedrock in the Michigan Basin (Gardner, 1974). Found beneath the lake surface, these submerged sinkholes release groundwater through underwater vents that flood the lake floor. Because the groundwater is heavily modified by the limestone, shale, and evaporite layers through which it flows, it has significantly different physico-chemical properties than the surrounding lake water (Biddanda et al., 2006; Ruberg et al., 2005). The groundwater has relatively low pH (7.0) and high conductivity (1000–1500 µS cm^{-1}) due to high levels of dissolved carbonate, sulfate, and chloride ions, and low levels of dissolved oxygen (0–50 µM) and nitrate (0–0.5 µM) compared to the surrounding lake water (Biddanda et al., 2006). Because of these differences, steep environmental gradients are formed, creating a habitat characterized by high abundance of microorganisms and intense biogeochemical cycling—a “hotspot” of microbial diversity and activity (Biddanda et al., 2006, 2009; McClain et al., 2003).

Middle Island, near Alpena, MI (USA) (Fig. 1) is the location of a model sinkhole ecosystem we study to understand microbial diversity and activity in hypoxic groundwater-influenced environments. The physico-chemical environment of Middle Island sinkhole has been described previously (Ruberg et al., 2008). Briefly, this 23 m deep feature is affected by dense, hypoxic groundwater flooding the lake floor to a depth of 1 m. Elsewhere in Lake Huron, sediments are sand, bedrock, or cobble where oxygenated lake water reaches the sediment surface. In Middle Island sinkhole, the lack of oxygen significantly affects the sediment environment. Here, purple-pigmented mats formed by filamentous cyanobacteria (*Phormidium* spp.) cover the sediment surface (Nold et al., 2010). The cyanobacteria experience 5–

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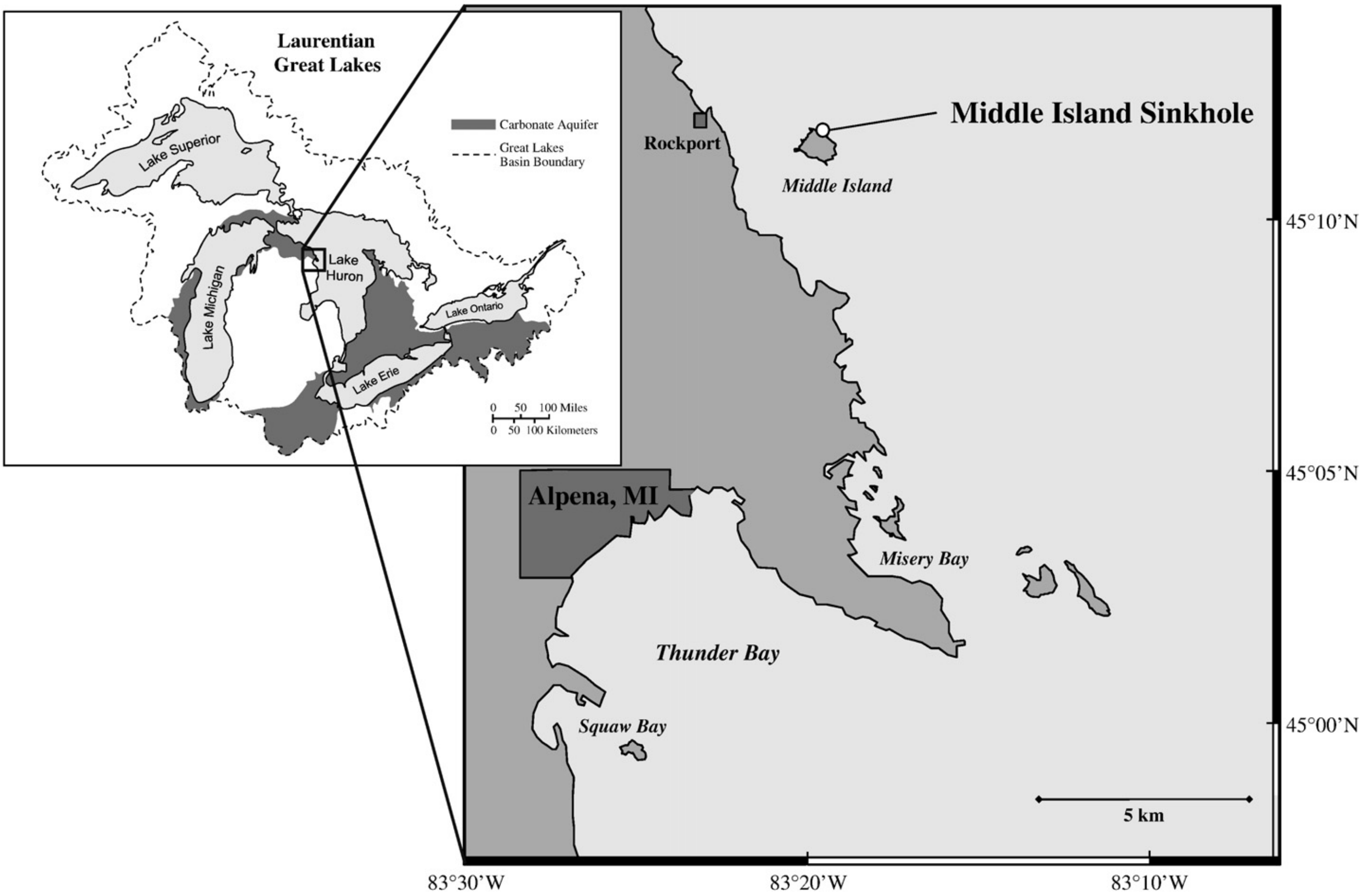

Fig. 1. Map depicting the location of Middle Island sinkhole in Lake Huron.

10% of surface sunlight irradiance, and are photosynthetically active. Underlying these mats lay thick (>2 m) organic-rich sediments whose carbon source is supplied by phytoplankton in the overlying lake water (Nold, unpublished data). Sediment porewaters are saturated with methane below 5 cm depth, and methane is oxidized near the sediment surface (Nold et al., 2010).

For nearly 20 years, molecular biological techniques have successfully been employed to understand earth's microbial diversity (Kane, 2004; Madsen, 2008). Using culture-independent approaches, bacterial species from diverse and possibly novel lineages were recently detected in Middle Island sinkhole (Nold et al., 2010). The potential for the discovery of additional unique microbial lineages is high. *Archaea* are now known to be highly diverse (Hershberger et al., 1996; Robertson et al., 2005) and potentially abundant in aquatic habitats (DeLong et al., 1994; Fuhrman and Steele, 2008). *Archaea* play important roles in nutrient cycling, especially the carbon and nitrogen cycles. *Archaea* belonging to the *Euryarchaeota* are responsible for methanogenesis (Nedwell, 1996) and working in consortium with a sulfate-reducing bacterial partner, are capable of methane oxidation in anaerobic environments (Orphan et al., 2001; Michaelis et al., 2002). *Archaea* are also involved in global nitrogen cycling. Aquatic *Crenarchaeota* were recently implicated in the aerobic oxidation of ammonium (Prosser and Nicol, 2008; Jetten, 2008; Leininger et al., 2006) and may act as general heterotrophs in the open ocean (Fuhrman and Steele, 2008). We are not aware of any published reports of archaeal diversity in submerged sinkhole ecosystems.

Microbial eukaryotes also play important roles in freshwater ecosystems. Recent molecular surveys are uncovering the enormous diversity of microbial eukaryotic species. We are finding that in aquatic habitats, these organisms are ubiquitous and widely distributed (Finlay, 2002; Moreira and Lopez-Garcia, 2002; Countway et al., 2005). Marine picoeukaryotes (organisms <5 µm) are distributed throughout the world's oceans, although coastal regions may harbor indigenous species (Massana et al., 2004). In contrast to marine environments, the microbial eukaryotes in freshwater ecosystems are not as well studied. We have learned that freshwater habitats also contain cosmopolitan microeukaryotes, but these species are different than marine organisms (Richards et al., 2005) and vary depending on physico-chemical factors such as trophic status of the water body (Lefranc et al., 2005) and biological factors such as grazing (Robertson et al., 2005). Microeukaryotes play a large role in carbon cycling and can remove up to 100% of bacterial production in freshwater environments (Zingel et al., 2007; Caron et al., 2008). Some species of stramenopile flagellates and anaerobic ciliates are active in hypoxic environments, and can be major consumers of bacterial biomass (Stoeck et al., 2007). For all their importance, there is only one reported effort looking at the eukaryotic diversity in groundwater, a study of deep subsurface yeasts (Ekendahl et al., 2003). To our knowledge, there are no published accounts of microbial eukaryotic diversity in sinkhole sediments.

While the Laurentian Great Lakes are important freshwater resources, there are relatively few molecular surveys of their microbial communities (Mueller-Spitz et al., 2009; Hicks et al., 2004; Wilhelm et al., 2006; Pascoe and Hicks, 2004; Keough et al., 2003; MacGregor et al., 1997, 2001) and none from submerged sinkhole habitats. At the center of this study is the desire to answer the fundamental ecological question: How many and what types of microbial taxa are present? Here, we report results from an initial survey to describe the diversity of *Archaea* and *Eukarya* inhabiting Middle Island sinkhole sediments. We used one primer pair to PCR amplify archaeal and eukaryal small subunit ribosomal RNA (SSU rRNA) genes from different sediment