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Research paper

Resting cysts of freshwater dinoflagellates in southeastern Georgian Bay (Lake Huron) as proxies of cultural eutrophication

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ABSTRACT

Resting cysts attributed to the freshwater dinoflagellate genus *Peridinium* were found in surface sediments from Severn Sound, southeastern Georgian Bay (Lake Huron, Laurentian Great Lakes of North America). Two distinct cyst morphotypes were present and they were assigned to *Peridinium wisconsinense* Eddy, 1930 and *Peridinium willei* Huitfeldt-Kaas, 1900 by establishing cyst–theca relationships through germinations and single-cell LSU rDNA analysis on an excysted cell of *Peridinium willei*. Sediments recovered from deep, sheltered portions of Severn Sound and restricted basins like Honey Harbour contained between ~750 and 8500 cysts/cm^3. However, winnowing by bottom currents and high concentrations of dissolved oxygen adversely impact the dinoflagellate cyst record on the lakebed, and cyst concentrations in easily remobilized muds on bathymetric highs were <100 cysts/cm^3.

Down-core changes in the relative abundances of these two cyst morphotypes were attributed primarily to cultural eutrophication related to land-use changes around Severn Sound over the last six centuries. Cysts of *Peridinium willei*, a cosmopolitan dinoflagellate species that occurs in a broad range of temperature, pH and nutrient conditions, comprise 60–74% of the cysts identified in *Ambrosia* (ragweed)-rich sediments in the upper 20 cm of a gravity core taken from Honey Harbour. Euro-Canadian settlement and land-clearing that began in the Midland-Penetanguishene region around A.D. 1840 are evident in the increase in *Ambrosia* (ragweed), Gramineae (grasses) and other herbs (non-arboreal pollen) that mark the base of the *Ambrosia* zone (pollen zone 4) as well as an overall increase in terrigenous flux. In addition to siltation, this terrigenous flux increased the availability of limiting nutrients to the previously oligotrophic waters of Severn Sound, leading to increased cyst flux in Honey Harbour peaking at nearly 3000 cysts/cm^2/y in A.D. 1966, an order of magnitude higher than cyst fluxes prior to the Euro-Canadian *Ambrosia* zone.

Peridinium wisconsinense was the more common dinoflagellate cyst species in Honey Harbour prior to Euro-Canadian settlement, when cyst flux was an order of magnitude lower. This is consistent with the restriction of this species to relatively warm, oligotrophic to mesotrophic lakes in North America. An earlier increase in *P. willei* at the expense of *P. wisconsinense* in the core from Honey Harbour within pollen zone 3 d (~700 to ~150 yBP) is attributed to earlier land-clearing by the Wendat ("Huron"), who practiced agriculture in the Penetanguishene peninsula between ~A.D. 1450–1650. The cysts of these freshwater dinoflagellates thus appear to be sensitive to cultural eutrophication.

1. Introduction

Despite a general increase in awareness of non-pollen palynomorphs (e.g. van Geel, 2006) freshwater dinoflagellate cysts remain underutilized in paleolimnological studies. The occurrence of fossilizable cysts belonging to recent freshwater dinoflagellates was documented several decades ago by Wall and Dale (1968) and Norris and McAndrews (1970), but subsequent reports of their occurrence in freshwater environments have been surprisingly rare (Miller et al., 1982; Burden et al., 1986; Zippi et al., 1990). While most palynologists ignore the presence of cysts of freshwater dinoflagellates in their slides, as suggested decades ago by Norris and McAndrews (1970),

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those who do recognize cysts with probable dinoflagellate affinity may be dissuaded from giving them further consideration due to their often low concentrations, the poorly resolved taxonomy and poorly documented biogeographic and stratigraphic record. Despite this, renewed interest in these cysts and their (paleo)ecological significance (e.g. Kouli et al., 2001; Alster et al., 2006; Tardio et al., 2006a,b; Chu et al., 2008, 2009; Leroy et al., 2009), recent advances in DNA research on the vegetative stages (e.g. Kim et al., 2004; Logares et al., 2007) and laboratory life-cycle studies (e.g. Rengefors and Anderson, 1998; Tardio et al., 2008) are encouraging for paleoenvironmental application of freshwater dinoflagellate cysts.

Freshwater dinoflagellates, particularly the genus *Peridinium*, are reasonably common in the Great Lakes region (Eddy, 1930; Prescott, 1962; Schindler and Nighswander, 1970; Nicholls, 1973; Carty, 2002). Two species of *Peridinium*, *P. wisconsinense* and "*Peridinium* large" have been identified in phycological analyses from Severn Sound (Severn Sound Environmental Association, pers. comm.). Dinoflagellate cysts have been reported from small lakes in the Great Lakes basin (Burden et al., 1986; Zippi et al., 1990, 1991), and attributed to the genus *Peridinium*, but assignment to species was not rigorously determined, but only inferred on the basis of gross morphological similarity. This paper brings together the phycological and palynological approaches by confirming the identity of the two dominant cyst morphotypes present in Severn Sound (Fig. 1) through germinations and DNA analysis.

The Severn Sound region is especially suitable for studies of cultural eutrophication, defined by Ekdahl et al. (2004) as "the process by which human activities increase nutrient input rates to aquatic ecosystems and thereby cause undesirable changes in surface-water quality", because two distinct episodes of human settlement are separated by two centuries of little anthropogenic impact. The Wendat ("Huron") people farmed the land around Severn Sound between ~A. D. 1450 and 1650, when they were massacred by the Iroquois (Heidenreich, 1971 in O'Brien, 1976), and it was only ~A.D. 1840 that Euro-Canadian settlers extensively cleared land and established communities in the Midland-Penetanguishene region (Chittenden, 1990). A previous study by Burden et al. (1986) related down-core variations in the pollen, dinoflagellate cyst, and *Pediastrum* record from two small lakes in or near Awenda Provincial Park near the mouth of Severn Sound to the changes in land-use over the last six centuries (Fig. 2).

This paper evaluates the potential of the freshwater dinoflagellate cysts as proxies of cultural eutrophication, since dinoflagellate cysts have proven useful as eutrophication indicators in marine settings (e.g. Saetre et al., 1997; Dale et al., 1999; Matsuoka, 1999; Pospelova et al., 2002; Dale, 2009; Shin et al., 2010). Eutrophication has previously been documented in several lakes in southern Ontario using various proxies, including diatoms (Ekdahl et al., 2007), thecamoebians (Reinhardt et al., 2005; McCarthy et al., 2010), and a variety of non-pollen palynomorphs (e.g. Turton and McAndrews, 2006), but dinoflagellate cysts remain underexploited, despite the documented occurrence of freshwater dinoflagellates in phycological studies.

We examine the spatial distribution of dinoflagellate cysts in surface sediments in the Severn Sound region of southeastern Georgian Bay, whose occurrence was first noted by McCarthy et al. (2006) and studied further by Gregg (2006), and compare this with the occurrence of cysts identified by Zippi et al. (1990, 1991) in surface sediments from small lakes in the surrounding region (Fig. 1). We also examine the relative abundances of these freshwater dinoflagellate cysts over the last six centuries in core SV5-C from Honey Harbour, Ontario, and compare the down-core distribution with that documented by Burden et al. (1986) for nearby lakes. We also demonstrate the affinity of the two commonly fossilized cyst morphotypes, and examine the distribution and ecology of these dinoflagellate species.

2. Methods

2.1. Germination experiments and single-cell LSU rDNA analysis

Fresh surface sediments from nearshore environments off docks at 44.86°N, 79.82°W in Honey Harbour were sampled using an Ekman grab sampler on October 13, 2009 at water depths between 1 and 2.2 m. Lake water pH was 7.3 and surface-water temperature between 9.6 and 10.1 °C. Dissolved oxygen (DO) concentrations at the sediment-water interface measured using a Hydrolab ranged from <1 to 8.7 mg/L, with bedrock hollows characterized by anoxic conditions bordered by bedrock highs with well-oxygenated bottom water. Fig. 3 illustrates seasonal variations in concentrations of dissolved oxygen in the Honey Harbour water column, confirming the very low DO measurements during sampling for phycological studies.

About 0.5–1 cm^3 of wet sediment was rinsed through a 25 μm metallic-meshed calibrated sieve (Retsch, Haan, Germany). From this residue, the cyst fraction was separated using the density method (sodiumpolytungstate at density 1.3 g cm^{-1} (Bolch, 1997)). Subsequently, single cysts of both morphotypes were transferred to Nunclon 0.5 ml microwells with four different freshwater media (MWC and MWC-Se;Guillard and Lorenzen, 1972), AF6 and DY-V (Andersen et al., 1997) at 15 °C, light intensity of ~35 μEm^{-2} s^{-1} and a 16-h:8-h light–dark cycle. The first incubation was initiated on 20 October 2009 and followed up until 18 December 2009, the second from 10 November 2009 to 11 January 2010 and a third incubation took place from 7 March 2010 until 18 March 2010. Only during this last incubation, the pH of the medium was explicitly adjusted to the in situ pH of the lake (ca. 7.5).

Cysts were regularly checked for germination and observations of the cells were performed under an Olympus CKx41 inverted light microscope (Olympus, Tokyo, Japan). Encysted and excysted cysts and vegetative stages were photographed using the latter and an Olympus BX51 light microscope. Cysts were also coated with platinum–palladium to a thickness of ca. 15 nm and studied using a Jeol JSM-6335 F field emission scanning electron microscope. Vegetative stages were identified using Popovský and Pfiester (1990). Confocal microscopy was performed using a C1 confocal microscope (Nikon Belux, Brussels, Belgium). No staining was necessary since the cysts were sufficiently autofluorescent. Cyst autofluorescence was excited with the 488 nm wavelength of an Ar-ion laser. Z-stacks were taken with a step size of 0.35 μs with a Pixel dwell time of 7.92 μs. The objective used was a 60×/1.40/0.13 Plan-Apochromat lens with oil immersion. After correcting the z-axis for differences in refractive index between the immersion oil and glycerine jelly (here a factor of 78% of correction was used), images were rendered with Volume Graphics VGStudioMax© software.

The identity of *Peridinium willei* was also confirmed by single-cell PCR amplification and sequencing with the objective of blasting the obtained genetic sequence with other(s) available at GenBank. This approach was only applicable to *Peridinium willei*, as no genetic data on *Peridinium wisconsinense* was available for comparison.

An excysted cell of *Peridinium willei* was transferred with a micropipette to a 200 μL PCR tube, frozen and vortexed after adding a sterile glass bead of 0.7–1 mm to the tube to enhance rupture of the theca and release of cellular contents. The PCR amplification was performed in two steps. For the first PCR run, the reaction mixture contained 0.5 units of the DNA polymerase, 1 μL 1×PCR buffer, 8 μL 2 mM dNTPs, 2 μL 10×BSA, 1.5 μL (10 μM) of primers SR1 (Yamaguchi and Horiguchi, 2005) and 25 F1 (Kogame et al., 1999), and 2 μL (10 μM) of primer LSUR2 (Takano and Horiguchi, 2004). Thermal cycling followed an activation step of 5 min, 35 cycles of 94 °C for 45 s, 56 °C for 1 min and 72 °C for 2.5 min, and a final elongation step of 72 °C for 6 min. For the nested PCR reaction, 1 μL of PCR product from the first reaction was used as template. The 50 μL reaction mixture contained 0.75 units of DNA polymerase, 5 μL 1×PCR Buffer, 20 μL 2 mM dNTPs, 2.5 μL (10 μM) of primers D3A (Nunn et al., 1996) and