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EXTRACTION OF ZYGNEMATACEAN ZYGOSPORES FROM LAKE SEDIMENTS AND THEIR POTENTIAL AS PALEO-INDICATORS OF LAKE ACIDIFICATION

R. A. C. PROJECT NO. 464G

Prepared for Environment Ontario by:

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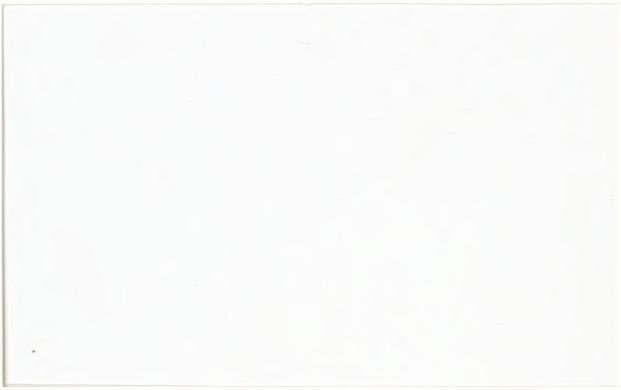

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TABLE OF CONTENTS

List of Figures and Tables

EXECUTIVE SUMMARY

Zygnematacean zygospores can be recovered from lake sediments by a slightly modified palynological technique. In 10 study lakes, zygnematacean zygospores are not practical paleo-indicators of lake acidification because they are diluted (approximately $1:10^5$ to $1:10^6$) by pollen from the surrounding forests. Their potential as a paleo-indicator may be significantly greater in regions with a lower pollen flux.

ABSTRACT

Sediment cores were taken from 10 lakes in the Haliburton-Muskoka region of Ontario. Zygnematacean zygospores were recovered and concentrated from the lake sediments by the progressive removal of inorganic and acid-base soluble organic components. The final residue contains the insoluble zygospores and other sporopollenin microfossils including spores, pollen, dinoflagellate cysts, *Pediastrum*, and fungal spores. In most residues, zygnematacean zygospores are present in concentrations too low for reliable and efficient quantitative analysis. Pollen from the surrounding forest dominates the microfossil assemblage. Because zygnematacean zygospores are less soluble in oxidizing acids than are most other sporopollenin microfossils, controlled oxidation of the residue may enhance their concentration by

removing a portion of the pollen present in the residue. The technique may have potential in regions with a low pollen flux. In contrast, *Peridinium* dinoflagellate cysts and *Pediastrum* coenobia were found in abundance and show promise as paleo-indicators of lake water *p*H.

INTRODUCTION

The purpose of this study is to develop a standard method for the recovery and concentration of zygnematacean zygospores from lake sediments and to evaluate their potential as a paleo-indicator of lake acidification.

Many of the Zygnemataceae are acidophilic and are abundant in naturally acidic habitats (Transeau, 1951; Prescott, 1962; Hoshaw, 1968; Yung et al., 1986). Zygnemataceae are known to proliferate in some lakes and streams that have been experimentally acidified (Hendrey, 1976; Hall et al., 1980; Turner et al., 1987; Detenbeck and Johnson, 1986; Parent et al., 1986). Certain species of the Zygnemataceae increase in abundance as pH drops below 6.0 (Jack, 1985; Stokes and Howell, 1987). The increased growth of certain zygnematacean algae in soft-water lakes during the early stages of lake acidification has prompted an investigation of the potential use of zygospores of this family as paleo-indicators of lake acidification. The main purpose of the investigation was to augment the transfer

functions developed for diatoms and other siliceous scale-bearing Chrysophyceae (Battarbee, 1984; Smol, 1986), in order to provide a more complete overview of past lake dynamics, especially in lakes where a clear relationship between diatoms, Chrysophyceae and pH cannot readily be established.

Lakes by their very nature are sediment traps. A large component of typical lake sediment is organic. This component contains readily preservable representatives of the biota that inhabited the lake in the past. Zygospores of the conjugating filamentous green algae (Zygnemataceae) are preserved as part of the fossil flora in recent sediments and in sedimentary rocks as old as 300 million years (Dickman et al, 1987; Ellis and Van Geel, 1978; Graham, 1971; Head and Zippi, 1991; Jarzen, 1979; Lindgren, 1980; Rich, et al, 1982; Van Geel, and Van der Hammen, 1978; Van Geel, 1976, 1979, 1986; Zippi and Norris, 1991; Zippi et al., 1990).

The zygospores of the Zygnemataceae are thick-walled reproductive bodies which form in conjugating vegetative cells or the conjugation tube as a result of isogamous fertilization. (Transeau, 1951; Randhawa, 1959; Hoshaw, 1968, and Hoshaw and McCourt, 1988). Zygospore formation is a reproductive strategy that seems to enable the Zygnemataceae to survive the extreme environmental fluctuations of seasons (Coleman, 1983). The presence of these zygospores in lake sediments is indicative of environmental conditions that were, at least temporarily, conducive for the growth and

reproduction of the vegetative filaments. Therefore, occurrences of zygnematacean zygospores in lake sediments have potential to be used to reconstruct past environmental conditions. For many species, zygospores have been described and are diagnostic at the species level (Wei et al., 1989; Zippi et al., 1990).

A reliable method for the extraction and concentration of zygospores is necessary before their potential usefulness as paleoecological indicators can be practically assessed. Zygnematacean zygospores are composed of a sporopollenin-like substance (Ashraf and Godward, 1980; Simons et al., 1982; Vries et al, 1983). The term sporopollenin is generally applied to an organic substance found in the wall of pollen, spores, and many algal cysts that is insoluble in acetolysis solution, bases and non-oxidizing acids. Sporopollenin is a biologically produced group of oxidative polymers of carotenoids or carotenoid esters (Shaw, 1971; Brooks and Shaw, 1972), which is resistant to acetolysis (Atkinson et al, 1972). In the laboratory, the sporopollenin of pollen, spores, and dinoflagellate cysts can be degraded to varying degrees by oxidizing acids (chromic or nitric acid). Vries et al (1983) found that zygospores from species of *Spirogyra* and *Mougeotia* survived one hour of oxidation by chromic acid at room temperature, whereas pollen of *Taxus baccata* and *Pinus sylvestris* were dissolved.

Methods exist for the recovery and concentration from sediments and sedimentary rocks of dinoflagellate cysts, pollen, spores and several other types of microfossils of composed of sporopollenin. The established methods of palynological extraction are suitable for zygnematacean zygospores because they are similar in size and composition to other sporopollenin palynomorphs. If present in the original sample, zygnematacean zygospores will be contained in the organic residues that were concentrated by the standard acid-base palynological techniques.

Zygospores may be present in standard palynological preparations from lake sediments, but in samples from forested regions they will almost certainly be diluted several orders of magnitude by the overwhelming abundance of pollen and spores from the surrounding catchment area (Zippi et al., 1990). Although the presence of several types of zygospores may be demonstrated with relative ease, it may be difficult and time consuming to count zygospores in numbers sufficient for statistical significance in a study restricted to zygnematacean species.

Controlled oxidation of the acetolysis residue, however, may remove a sufficient proportion of spores, pollen and dinoflagellates to effectively concentrate the zygnematacean zygospores.

METHODS

Field Methods

Ten lakes in the geologically acid-sensitive region of southern Ontario were cored (Figure 1, Table 1). Target lakes were restricted to small sized, clear water lakes with a pH in the range of 4 to 8. Core sites were selected where morphometric maps indicated a centrally located, deep, flat area of the lake floor (Appendix 2 shows precise core locations on a morphometric map for each lake). A modified KB gravity corer (6.5 cm diameter) and piston corer (5 cm diameter) were used to recover sediment cores. A clear plastic core tube allowed immediate visual inspection of the sediment core to insure that the uppermost sediments had been recovered. A portable extruding device (Glew, 1988) was used to subsample the gravity cores at 0.5 cm intervals for the first 10 cm, then at 1 cm intervals for the remainder of the core. Only the upper 3 cm of core top material was recovered from the piston cores.

Laboratory Methods

A slightly modified palynological extraction procedure was employed for the extraction of zygnematacean zygospores from lake sediments. The following section describes the

steps used for the extraction and concentration of sporopollenin microfossils including zygnematacean zygospores from lake sediments in this study. Table 2 summarizes the major processing steps and the effect of each step.

Several excellent technical references for the general palynological processing of sediments and sedimentary rock are Gray (1965), Barss and Williams (1976), and Phipps and Playford (1984) (*Caution:* Phipps and Playford (1984) list solutions as percent of concentrated stock solution rather than molar or weight percent). Evitt (1984) outlines several special techniques for preparing and mounting specimens. These four references give useful advice on dealing with special problems or difficult samples.

A. Sample splitting and measurement

A1. Split sample or remove a homogeneous fraction of uncompacted wet sediment and place approximately 6 to 10 ml into a 15 ml centrifuge tube. If sediment is dried add approximately 5 ml of sediment and fill tube with distilled water.

A2. Centrifuge at speeds sufficient to de-water and compact sediment. (test for differential compaction of sediment at different centrifugation speeds and times and standardize)

A3. Measure the volume of the compacted sediment (3-4 ml is the optimum amount of sediment for easy processing in a 15 ml tube).

B. Addition of marker-species spike

B1. Decant supernatant water.

B2. Add aliquot or tablet of known concentration of marker pollen or spheres (use a species that does not occur in the study area).

B3. Fill tube with distilled water and mix on a vortex mixer.

Adding water at this stage removes water-soluble substances.

C. Removal of carbonates and soluble Fe-oxy-hydroxides

C1. Add 4 ml of cold 10% HCl.

C2. Mix on vortex mixer[1]

C3. Once the reaction has slowed, add an additional 6 ml of HCl.

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[1] – when mixing on a vortex mixer, fill tube to 4 ml with reagent, use mixer, then add remaining quantity of reagent. Appropriate care should be taken not to allow harmful chemicals or valuable residue to splash or leak out of the tube while mixing.

C4. Centrifuge and decant supernatant liquid.

If supernatant liquid is bright yellow-green repeat HCl wash (steps C1-C4). Follow final HCl wash with at least 3 water washes to dilute the dissolved calcium and magnesium and the concentration of acid to approach neutrality.

Caution: samples containing more than 10% calcium carbonate will effervesce and may overflow a 15 ml tube. Use a larger container for calcium carbonate-rich samples. Test reactivity of sample by slowly adding HCl.

At this stage, a homogeneous portion of the sample may be removed for siliceous microfossil analysis (diatoms). The next step will dissolve all siliceous substances.

D. Removal of silicates

D1. Add 4 ml of cold 50% HF.

Caution: This step should be performed in a fume hood.

D2. Mix with a plastic or wooden rod.

D3. Add an additional 6 ml of cold 50% HF.

D4. Place tubes in a hot water bath at 90-100°C for 30 minutes and stir occasionally.

D5. Centrifuge and decant supernatant liquid. Follow with as many water washes as needed to dilute the pH to near neutrality (at least 3 water washes).

E. Removal of fluoride by-products

Depending on the composition of the mineral component of the residue, a fine white gel or crystals may precipitate as a by-product of the reaction with HF. The precipitate may be CaF_2, MgF_2, H_2SiF_6 or H_2SiO_3. These by-products are more commonly produced from samples containing leached, oxidized clays or granitic rock fragments. H_2SiF_6 is soluble in hot water, H_2SiO_3 is soluble in hot HF, and CaF_2, MgF_2 are soluble in hot HCl. Perform the following steps only if necessary.

E1. Add 10 ml of appropriate reagent.

E2. Heat in hot water bath at 90-100°C for 10 minutes.

E3. Centrifuge and wash with water at least 3 times.

E4. Repeat steps using various reagents until by-products are removed.

The composition of the by-products is difficult to identify. A trial and error approach using the reagents listed above may be the most expedient method for the removal of fluoride by-products. Fluorides are the most common by-product, so start with the addition of HCl, heat, and wash.

If the precipitate persists, add water, heat to 100°C, and wash. If precipitate is still present add HF and repeat steps outlined above. The formation of calcium and magnesium fluorides may often be prevented by thorough HCl washes prior to the addition of HF.

F. Removal of humic acids and fine particulate matter

F1. Add 4 ml of 10% NH_4OH and mix thoroughly.

F2. Fill remaining volume with 10% NH_4OH and heat at 90°C for 2 minutes.

F3. Centrifuge and wash with water at least 3 times or until supernatant liquid clears.

G. Removal of cellulose and hemi-cellulose by acetolysis

Steps G1 and G6 are very important! Adding acetolysis solution to a tube containing even a small amount of water results in an explosive reaction. Take extreme care to keep water away from acetolysis solution. Follow steps carefully.

G1. Add 10 ml of glacial acetic acid to dehydrate the sample.

G2. Centrifuge and decant supernatant acid.

G3. Add 4 ml of acetolysis solution and mix thoroughly.

G4. Add an additional 6 ml of acetolysis solution.

G5. Place tube in hot water bath at 90-100°C for 10-15 minutes.

G6. Fill tube to safe level with glacial acetic acid.

G7. Centrifuge and decant supernatant liquid (Balance the tubes with glacial acetic acid).

G8. Wash with glacial acetic acid and centrifuge.

G9. Wash with water and centrifuge.

H. Inspect sample under microscope

H1. Decant as much supernatant water as possible without pouring off any residue.

H2. Mix residue and remaining water with a micro-pipette.

H3. Pipette one drop of residue mixture onto a clean microscope slide.

H4. Evaluate the success of the preceding processing steps by examining the residue under a microscope at low magnification (do not use a coverslip).

H5. Carefully return the residue from the microscope slide to the centrifuge tube.

H6. Repeat any steps (D,E,F,or G) as necessary.

I. Removal of extraneous sporopollenin by oxidation

Several attempts at implementing this step were unsuccessful. See the description of oxidation trials in *Discussion* section. Further investigation of controlled oxidation of organic residue to concentrate the zygnematacean zygospores relative to other sporopollenin microfossils is necessary.

J. Removal of very fine adhering particulate matter

Include this step only if abundant fine particles (<2µm) obscure the sample.

J1. Add 4 ml of 10% NH_4OH and mix thoroughly.

J2. Fill remaining volume with water and centrifuge.

J3. Wash with water at least 3 times.

K. Sieving to remove fine dispersed organic debris

K1. Add 4 ml of 5% (wt/vol) detergent mixture (e.g. Alconox) and mix thoroughly using vortex mixer.

K2. Pour contents of centrifuge tube into sieve tube fitted with a 7 µm screen (see Appendix 1 for sieve tube construction).

K3. Spray with a jet of water until tube is 3/4 full then vibrate sieve tube with a vibrating engraver until it is nearly empty (Zippi, 1986). See Appendix 1.

K4. Repeat step 3 until the water coming through the sieve contains no suspended particles and is not soapy.

L. Slide making

Slide making may take some practice to achieve the proper density of specimens for efficient inspection and analysis later. Slide making should be performed on a slide warming plate at 50°C (See Appendix 1 for details on the preparation of glycerine jelly).

L1. Remove as much water as possible from the centrifuge tube without removing any specimens.

L2. Thoroughly mix residue with remaining water and *immediately* (before any specimens can settle) place a drop onto the center of a clean glass microscope slide.

L3. Add 1 drop of liquified glycerine jelly (Liquify glycerine jelly by placing a glass tube containing a small portion of jelly into a warm [~50°C] water bath).

L4. Mix residue and glycerine jelly with a clean wooden toothpick or dissecting needle. Allow excess water from residue to evaporate.

L5. Place edge of a 22 x 40 mm number 1 coverslip to the glass slide and lower at an angle onto the residue mixture.

L6. Allow slide to remain on the warming plate until mixture has spread to reach all edges of the coverslip.

L7. Invert the slide, so that the coverslip is down (but raised up off the slide warmer with toothpicks under the edges) to allow specimens to settle through the layer of glycerine jelly closer to the coverslip for 5 minutes.

L8. Remove from warming plate and allow slides to cool in an inverted position for at least 1 hour. Slides may be inspected but should be handled gently (avoid oil immersion and cleaning) for at least 2 days. After 2-3 days excess glycerine jelly may be cut away from the edges of the coverslip with a razor blade. Gently clean with alcohol, then seal edges with a clear plastic or varnish.

L9. Store in a cool place (not overly hot or humid for prolonged times) with slides positioned horizontally. If the slides are stored on edge, over time, the residue will flow.

L10. Store remainder of residue. Add a few drops of glycerine and a drop of 5% copper sulphate solution to a vial containing the residue and tightly cap.

Processing strategy

The overall processing strategy must be: less is better. The number of steps used in the processing of samples should be kept to a minimum. Specimens are lost by repeated centrifugation and decanting or pipetting of supernatant liquid. The loss is insignificant during the early stages of processing before the zygospores are concentrated and as long as enough residue is settling to form a distinct pellet. The risk of specimen loss increases as the zygospores are increasingly concentrated. Although the number of processing steps should be kept to a minimum, all samples should be processed in a similar manner in order to reduce statistical bias resulting from different processing techniques.

MICROSCOPY

Strew mounts were examined using light microscopy. Often only one layer of the zygospore wall is preserved after paylonological treatment. Although they are tough and durable as fossils, these cell membranes may be thin, transparent and of similar optical contrast to most of the common mounting

media. Many specimens may be difficult or impossible to view under bright field illumination. Phase contrast or interference contrast illumination is essential for the successful identification and enumeration of the fossil zygospores.

ENUMERATION

Fossil categories were tallied until the count reached a minimum of 350 planktonic specimens exclusive of *Botryococcus braunii* and the *Lycopodium* marker species, or until 2 entire strew mounts were completely counted.

RESULTS OF ANALYSIS

Observations of identifiable zygnematacean zygospores were extremely rare in the organic residues that were concentrated from the 10 study lakes and the Plastic Lake core. After thorough reconnaissance and quantitative enumeration of the residues, identifiable zygnematacean zygospores were found only in 3 of the 10 study lakes. Only one zygospore of *Zygogonium tunetanum* was observed in the surface sediment sample from Plastic Lake. Two other specimens of questionable identification were observed in

surface sediment samples from Tock Lake and Four Mile Lake. Identifications were made by comparison to reference slides of zygospores extracted from field collections of zygnematacean filaments recovered from the study area and and by consulting existing taxonomic literature (Transeau, 1951; Randhawa, 1959; Wei et al., 1989).

Zygospores from one species of *Debarya* were found with greater regularity in samples from the lower portion of the Plastic Lake core (Head and Zippi, 1991). However, the zygospores of *Debarya* sp. were not found in any surface sediment samples and do not occur in abundance in samples above 17 cm in the core (Table 3).

In contrast, dinoflagellate cysts and *Pediastrum* coenobia were found in abundance in the surface sediments of the 10 study lakes and in the Plastic Lake core samples and show potential as paleo-indicators of lake water *p*H (Zippi et al., 1990; 1991a; 1991b).

DISCUSSION

Effects of HCL, HF, NH_4OH and acetolysis

Processing with HCL, HF, NH_4OH and acetolysis solution effectively concentrates all sporopollenin microfossils and

other acid-base insoluble particles including zygnematacean zygospores. The concentration occurs by the progressive dissolution of mineral and organic components. The sample is demineralized with HCl and HF, humic acids are dissolved with NH_4OH, and cellulose is removed by acetolysis. Some of the sporopollenin microfossils present at this stage are pollen, spores, dinoflagellate cysts, various algal cysts such as *Pediastrum*, fungal spores and hyphae, and *Botryococcus*. If zygnematacean zygospores are present in significant numbers processing can stop without resorting to harsh oxidation.

Concentration of zygnematacean zygospores by oxidation

In Ontario lakes, a problem of concentration arises because the zygnematacean zygospores are diluted by the extremely rich pollen flora. The total pollen, spores, and dinocyst abundance outnumbers the zygnematacean zygospores by several orders of magnitude. The ratio of zygnematacean zygospores to pollen and other sporopollenin microfossils is estimated to be less than 1:100,000. This concentration is unworkable for practical paleoecological analysis.

A possible solution to this problem is the further concentration of zygnematacean zygospores from pollen-rich organic residues by controlled oxidation. In several experimental trials with zygospores recovered from acid-acetylation residues of mature zygnematacean filaments,

zygnematacean zygospores proved to be more resistant to oxidation by chromic acid than spores, pollen and dinocysts.

Oxidation trials

In order to test the relative resistance of zygnematacean zygospores to oxidation, field samples from the Haliburton-Muskoka area of *Mougeotia laetevirens*, *Spirogyra jatobae*, *S. jaoii*, *S. corrugata*, *Zygogonium tunetanum*, plus pollen, spores and dinoflagellate cysts, were acetolyzed, treated with hydrofluoric acid (HF), and subsequently oxidized with chromic acid for 90 minutes at 50°C. The residue was periodically examined (at 10 minute intervals) under the microscope to check the progress of the oxidation.

After 90 minutes at 50°C in chromic acid, the pollen, spores, and dinoflagellate cysts are mostly dissolved. Zygospores remain, but they are thinned and lightened to a point that makes identification difficult. Various factors such as wall thickness, sculpturing and possibly composition causes differential susceptibility to dissolution among species of zygospores. Consequently, not all species are dissolved at the same rate. Thick, solid-walled species survive, while thinner and more deeply sculptured wall types are less resistant (sculpture such as deep pitting increases surface area for faster reaction).

If oxidation is to be carried out on a sample, the marker grains must be insoluble in oxidizing acids. Spores are among the very first sporopollenin microfossils to be dissolved by oxidizing acids and are therefore inappropriate as marker grains.

CONCLUSIONS

The rarity of zygnematacean zygospores in the study lakes precludes further analysis of their relationship to lake water pH. The concentration of zygnematacean zygospores is diluted by the overwhelming supply of pollen from the surrounding forests. Because the single zygospore species (*Debarya* sp.) that occurs in abundance in some samples of the the Plastic Lake core was not recovered from any surface sediments, precise environmental interpretations cannot be related to its occurrence.

The use of zygnematacean zygospores as a paleo-indicator for lake acidification in the recent past may prove useful in acid-sensitive regions where the pollen supply is much lower. One possible area is The Netherlands, where algae occurs in abundance in shallow pools and ponds.

The method of concentration outlined in this paper can be used to concentrate zygnematacean zygospores from lake sediments.

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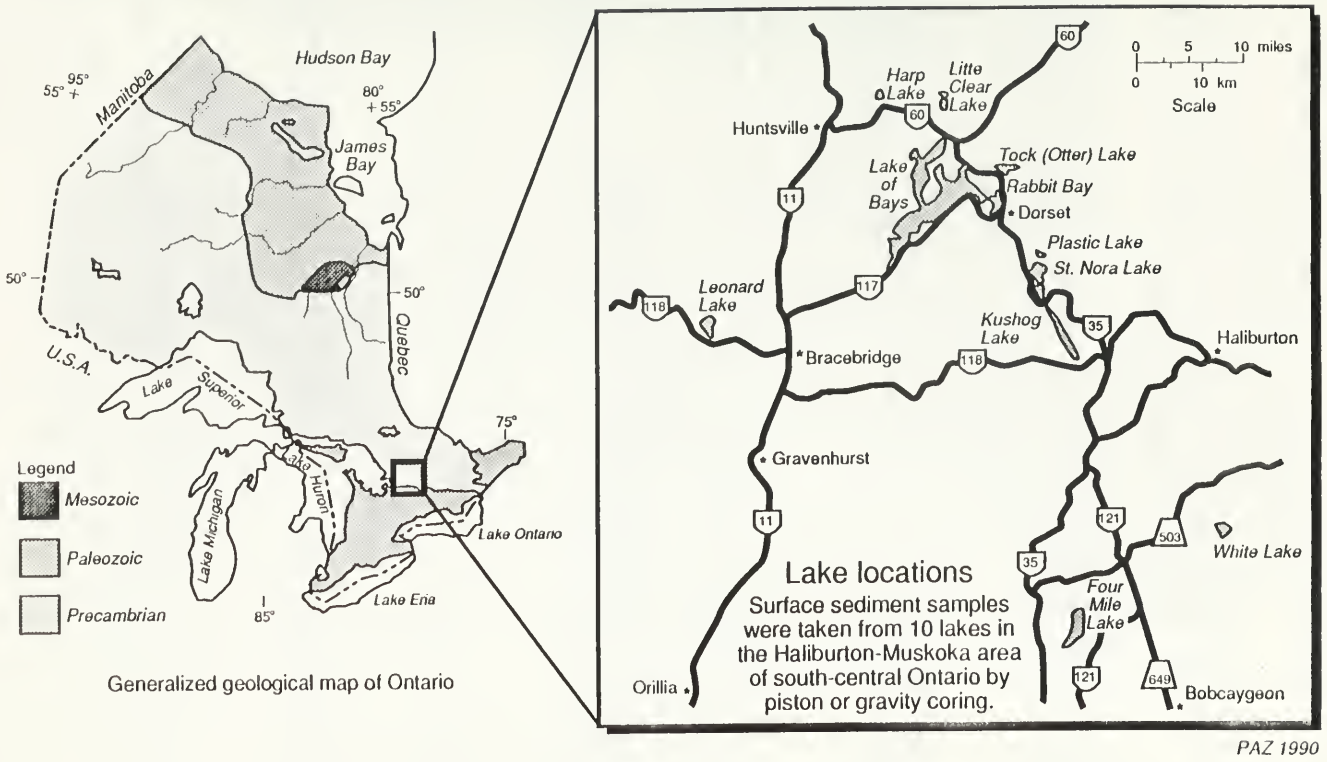

Figure 1 - Locations of 10 lakes in the Haliburton-Muskoka area of south-central Ontario.

Lake	*pH*	*ALK* (µeq/l)	*area* (h)	*depth* (m)	*core type*	*location (Twp., lat./lon.)*
Leonard	5.5	0.3	467	13	grav.	Monck, 45°04'/79°27'
Plastic	5.8	14.7	32.1	15.5	grav.	Sherborne, 45°11'/78°50'
Tock	6.2	2.2	288	14.5	pist.	McClintock, 45°16'/78°53'
St. Nora	6.2	2.1	647	14	grav.	Sherborne, 45°09'/78°50'
Harp	6.3	61.1	71.4	13	pist.	Chaffey, 45°23'/79°07'
Little Clear	6.3	7	136	12	pist.	Sinclair, 45°24'/79°00'
Rabbit Bay	6.8	3.5	17428	18	pist.	Muskoka, 45°15'/78°54'
Kushog	6.8	3.1	1580	15.5	grav.	Stanhope, 45°04'/78°47'
White	7.4	67.7	432	9	pist.	Galway Township, 44°50'/78°29'
4 Mile	7.8	89.4	1942	20	pist.	Summerville, 44°41'/78°31'

Table 1 - Lake locations and morphometric data for 10 lakes in the Haliburton-Muskoka region of south-central Ontario. *p*H and ALK data from the Ontario Ministry of the Environment, Dorset, Ontario.

Step 1

Measure de-watered sediment volume

add known quantity of marker grains
yields density: specimens/ml

Step 2

Carbonate removal

HCL

removes calcareous algae and carbonate rock fragments

Step 3

Humic acid removal

NH_4OH

removes humic acid, oxidized cellulose,
amorphous organic particles and some clay-sized minerals

Step 4

Silica and silicate removal

HF

removes silica and silicate microfossils and mineral grains

Step 5

Acetolysis

$H_2SO_4 : (CH_3CO)_2O$ (1:9)

removes cellulose and hemi-cellulose

Step 6

Sieving (7 µm)

removes fine particles

Step 7

Sporopollenin residue

pollen, spores, dinoflagellate cysts, *Pediastrum* coenobia, and
zygnematacean zygospores

Step 8

Acid-oxidation

HNO_3 or CrO_3

limited success concentrating zygnematacean zygospores
from pollen-rich organic residues

Table 2 - Summary flow chart of the steps for concentrating zygnematacean zygospores from lake sediments.

Depth (cm)	*Debarya* sp.	Other Zygnemataceae (number of specimens)
0	0	*Zygogonium tunetanum* (1)
1	0	
2	0	
3	0	
4	0	
5	1	
6	2	
7	1	
8	4	
9	0	
10	0	
11	0	
12	0	*Mougeotia* sp. (1)
13	1	
14	0	
15	0	*Mougeotia laetevirens* (1)
16	0	
17	11	
18	14	
19	20	
24	0	
29	0	
34	0	*Spirogyra*? sp (1)
39	26	

Table 3 - Occurrences of Zygnemataceae in the Plastic Lake core. Zygnematacean zygospores are rare in the surface sediments of the 10 study lakes. The only commonly occuring species in the Plastic Lake core was *Debarya* sp., but it was not recovered from any of the surface samples.

Appendix 1 - Preparation of solutions and apparatuses.

Acids and bases

Acid solutions are specified in volume percent. Mixtures should be prepared with distilled water. Where concentrations are not specified, use a concentrated stock solution.

Acetolysis solution

Mix 9 parts acetic anhydride [$(CH_3CO)_2O$]:1 part sulfuric acid (H_2SO_4). *Caution:* Keep away from water. Tubes and beakers should be dry or de-watered by rinsing with glacial acetic acid before use.

Glycerine jelly mounting medium

Mix 10 g of gelatine powder (e.g. Knox gelatine) into 60 ml of warm (50-80°C), distilled water until dissolved. Add 60 ml of glycerine and 0.25 g of copper sulfate (fungal growth inhibitor) and continue to mix gently for approximately 10 minutes. Avoid creating air bubbles while mixing. Filter mixture while still warm through a fine mesh (20-25 µm). Allow any bubbles to rise while still warm, then cap and store in a cool place.

Repeated reheating of mountant may result in mountant instability. For slide making, remove only a small portion of glycerine jelly sufficient for the number of slides about to be mounted. Keep the remainder refrigerated.

Sieve tube construction

The sieve tube is constructed by mounting fine mesh Nitex* screening between an outer sleeve and an inner tube that have been cut from polypropylene centrifuge tubes. The outer sleeve is cut from a 100 ml round-bottom centrifuge tube that has an inner diameter equal to the outer diameter of the inner tube. The inner tube is cut from a 50 ml centrifuge tube. Several outer sleeves may be cut from a single 100 ml tube. The sleeves should be cut about 1 cm (1/2") long. Sleeves that are cut too thin may slip off during sieving and sleeves that are too long are difficult to remove once screen is in place. The inner tube should be cut 5-8 cm (2-3") long from a smooth sided (non-graduated) 50 ml tube. Raised graduated markings will form a gap between the outer sleeve and the inner tube allowing larger particles to leak through. Tubes cut too short will allow residue to easily splash over the side when sieving is enhanced with vibration, and will limit the volume of residue and water.

Tube cutting: Centrifuge tubes are placed in a vice or suitable holder (tighten only enough to immobilize) and cut

to length with a fine-tooth hack saw blade. Cutting with a razor yields poor results and is very dangerous. The ragged edges can be neatly shaved using a razor knife. The ragged edges of the inner tube further smoothed by passing the shaved cut end lightly over an open flame. (Do not heat treat the outer sleeve. The melted plastic rolls inward making the inner diameter too small.)

Vibration sieving

The use of a vibrating engraver can enhance and speed the sieving of organic microfossils (Zippi, 1986). The necessary hardware is inexpensive and readily available. A standard vibrating engraver* and a short length of narrow-diameter rubber tubing are all that is required. Cut a short piece of tubing so that it fits tightly over, and extends slightly past, the engraving tip. Holding the top of the sieve tube between thumb and forefinger and the engraver in the opposite hand, simply touch the rubber tip to the side of the sieve tube. While water is in the tube, this vibrating action disaggregates and suspends the residue, greatly speeding the sieving process.

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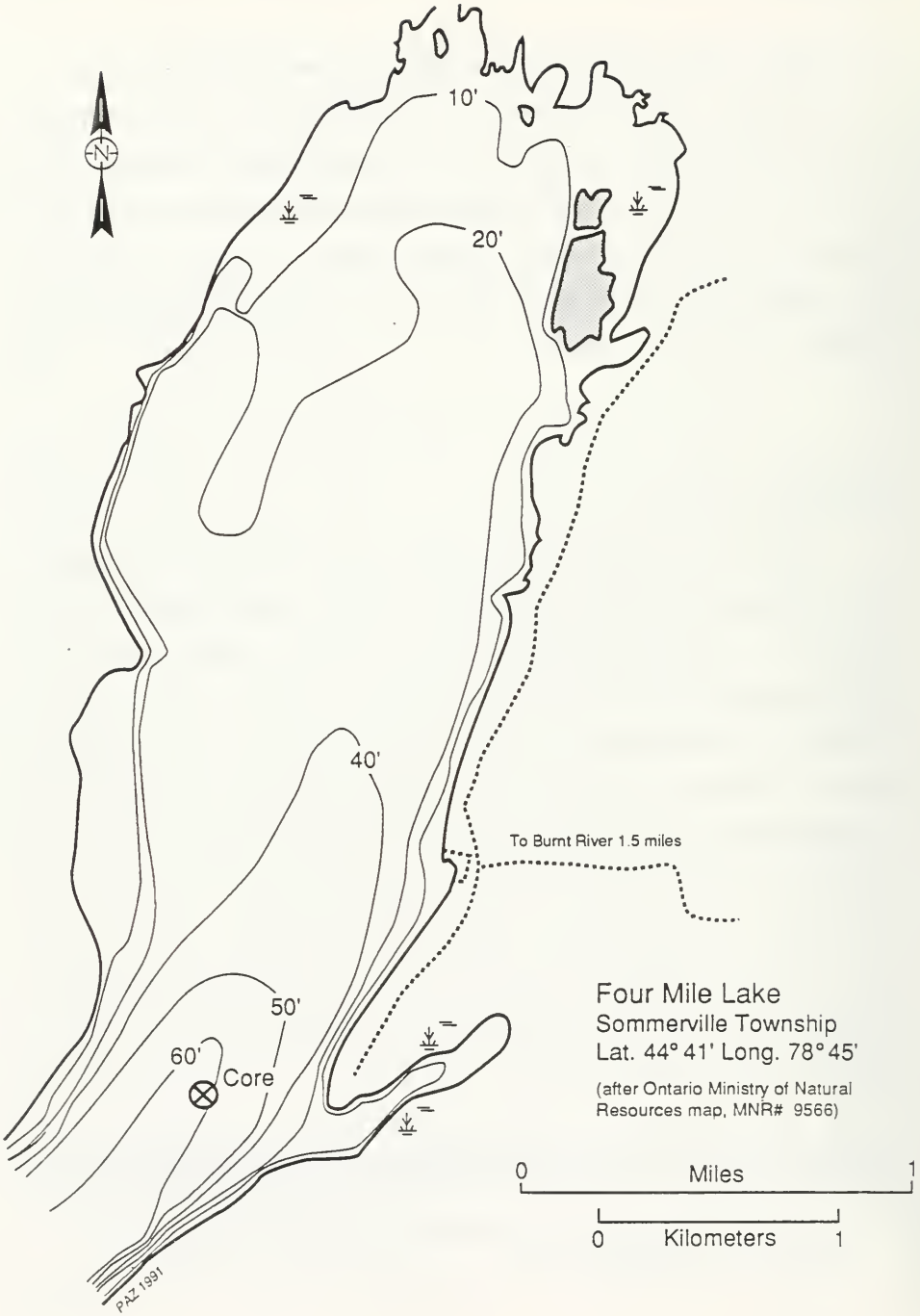
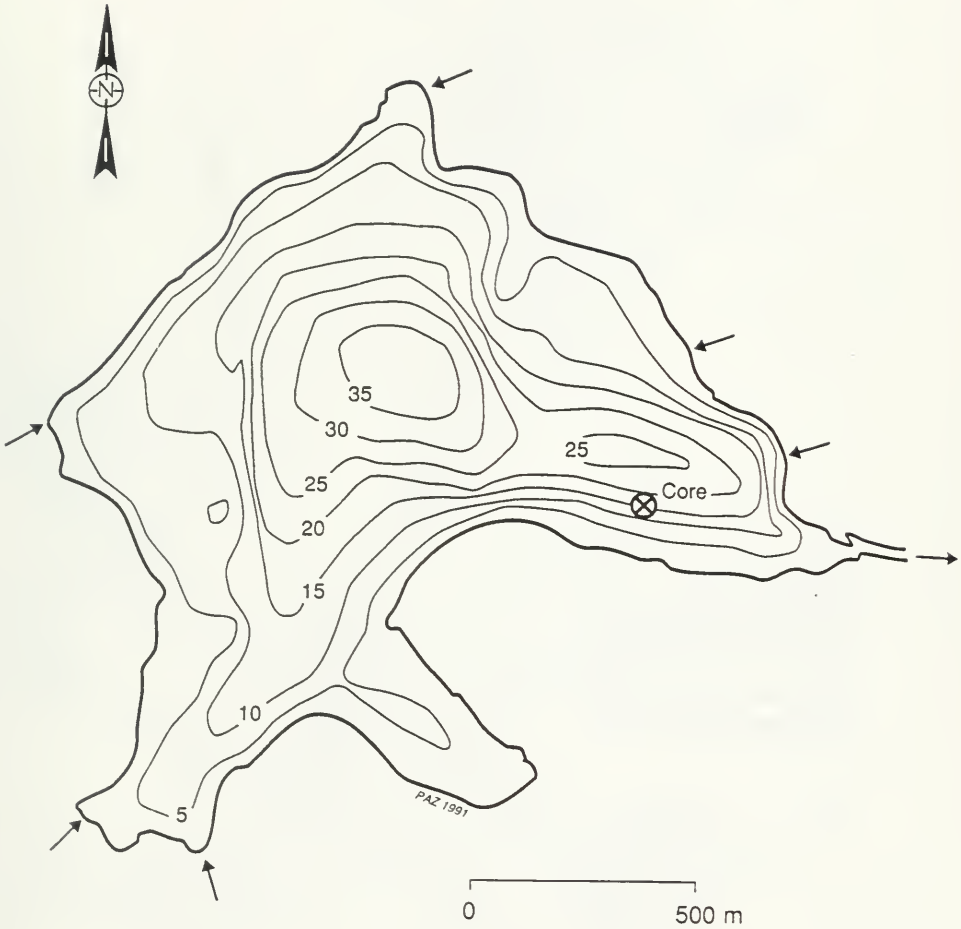
*- Nitex screens - Tetko, 420 Sawmill River Rd., Amelsford, NY 10523, or B & SH Thompson, 140 Midwest Rd., Unit 11, Scarborough, Ont., M1P 3B3

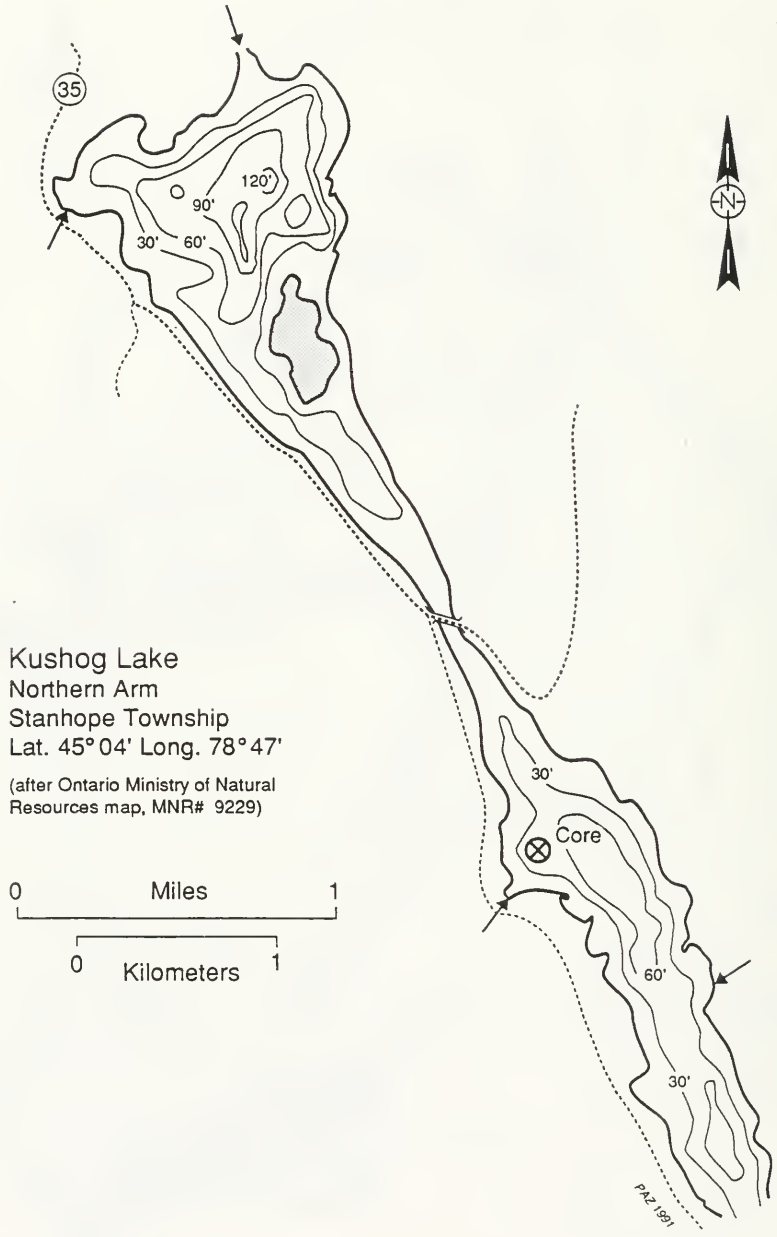
- Vibro-graver- Burgess Vibrocrafters Inc., Grayslake, Illinois (available at hardware stores).


10'
20'
40'
50'
60'
Core
To Burnt River 1.5 miles
Four Mile Lake
Sommerville Township
Lat. 44° 41' Long. 78° 45'
(after Ontario Ministry of Natural Resources map, MNR# 9566)
0 Miles 1
0 Kilometers 1
PAZ 1991



Harp Lake
Chaffey Township
Lat. 45° 23' Long. 79°07'

(source: Ontario Ministry of the Environment, Dorset, Ontario)

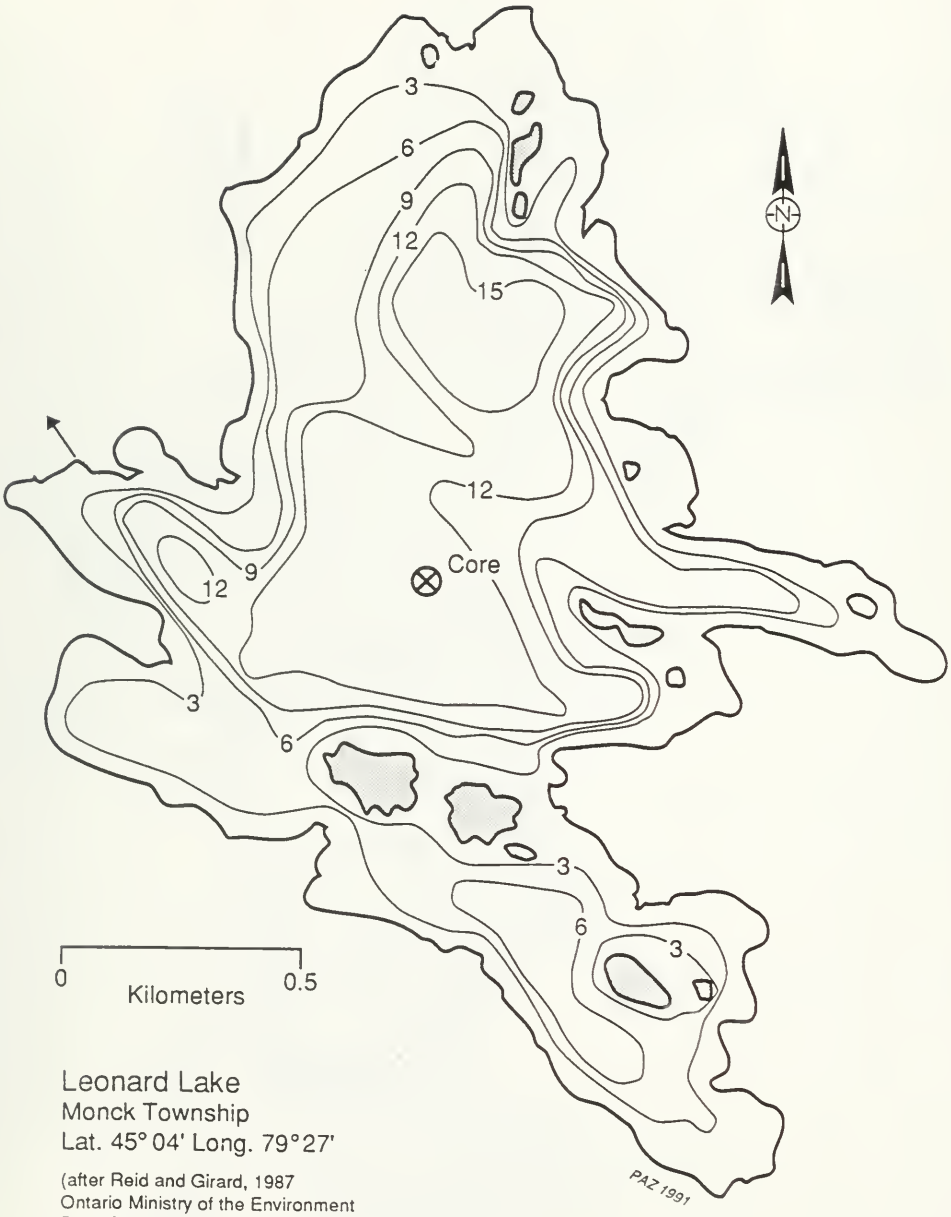


Kushog Lake
Northern Arm
Stanhope Township
Lat. 45° 04' Long. 78° 47'

(after Ontario Ministry of Natural Resources map, MNR# 9229)

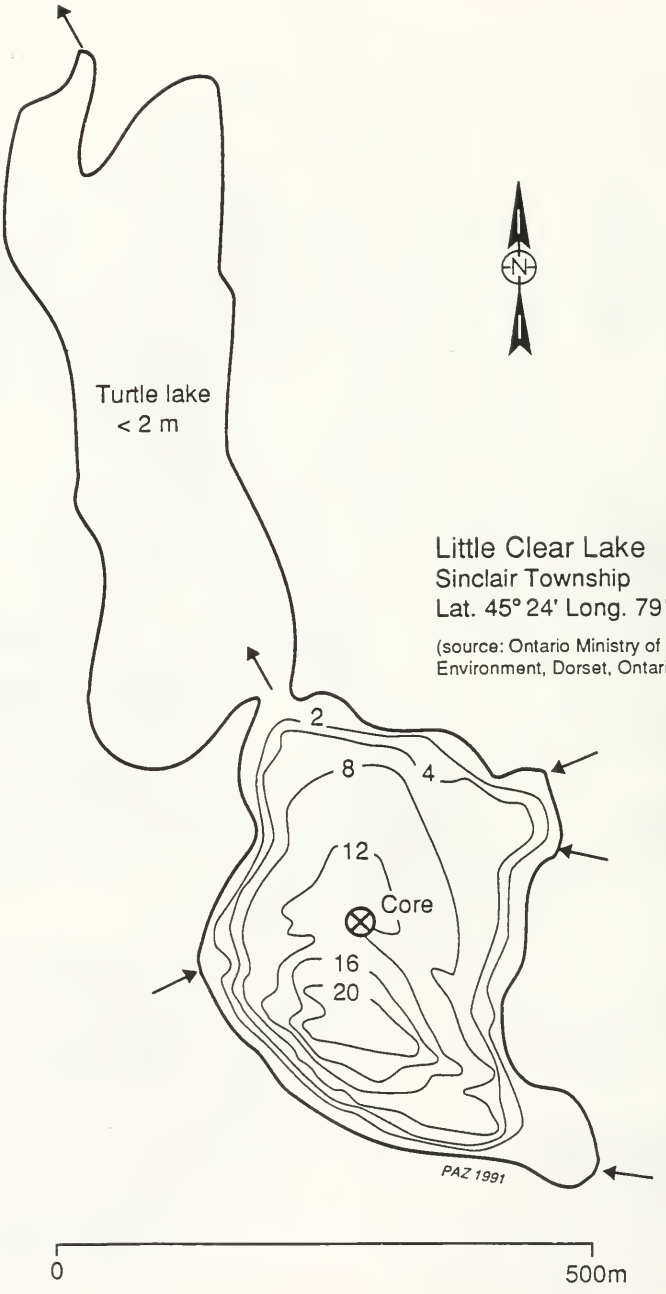
0 Miles 1

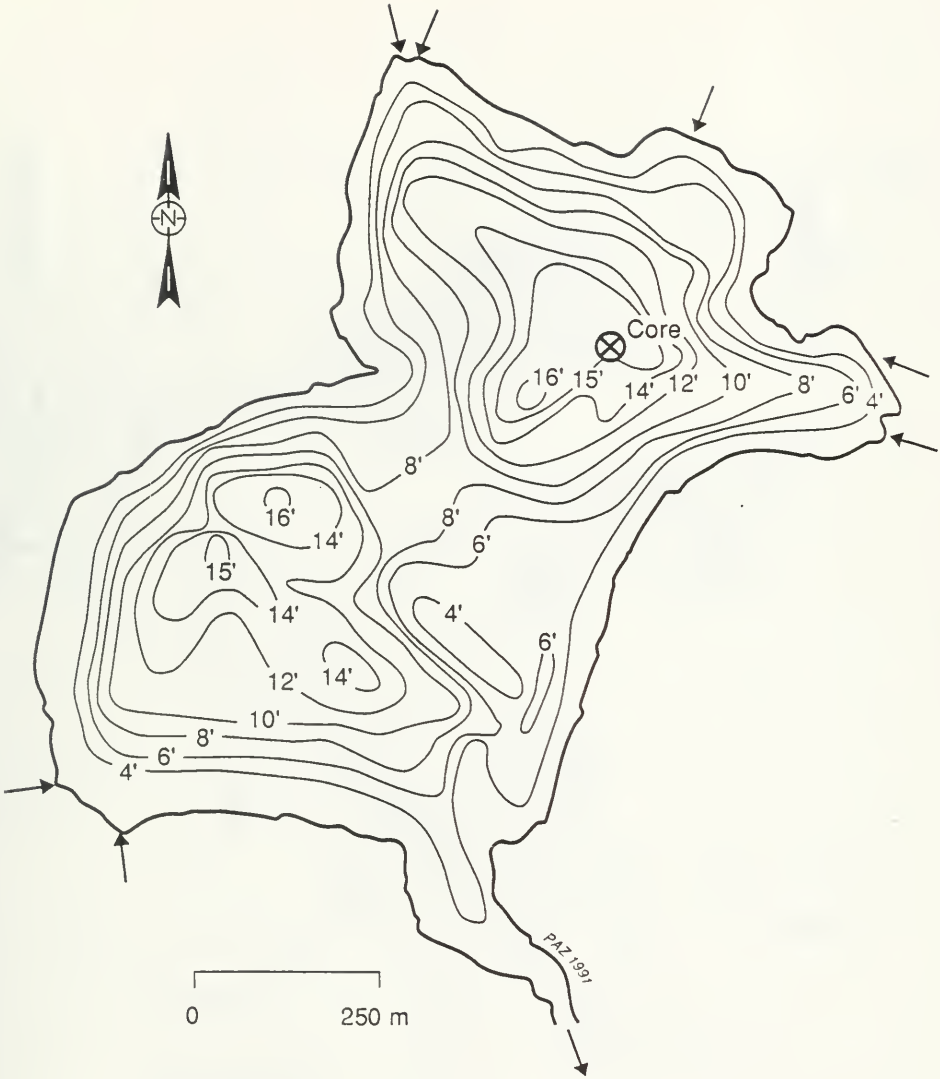
0 Kilometers 1



Leonard Lake
Monck Township
Lat. 45° 04' Long. 79°27'

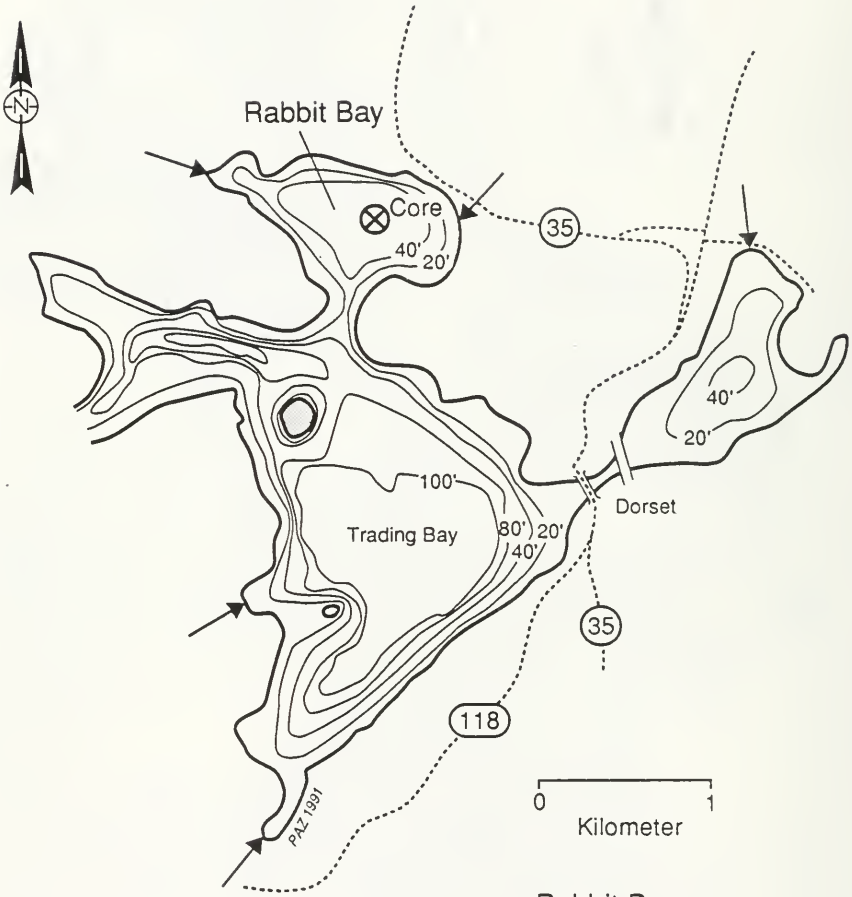
(after Reid and Girard, 1987
Ontario Ministry of the Environment
Data Report DR 87/2)


Turtle lake
< 2 m
N
Little Clear Lake
Sinclair Township
Lat. 45° 24' Long. 79° 00'
(source: Ontario Ministry of the Environment, Dorset, Ontario)
2
8
4
12
Core
16
20
PAZ 1991
0
500m



Plastic Lake
Haliburton County
Sherbourne Township
Lat. 45° 11' Long. 78° 50'

(after Girard et al., 1985
Ontario Ministry of the Environment
Data Report DR 85/1)

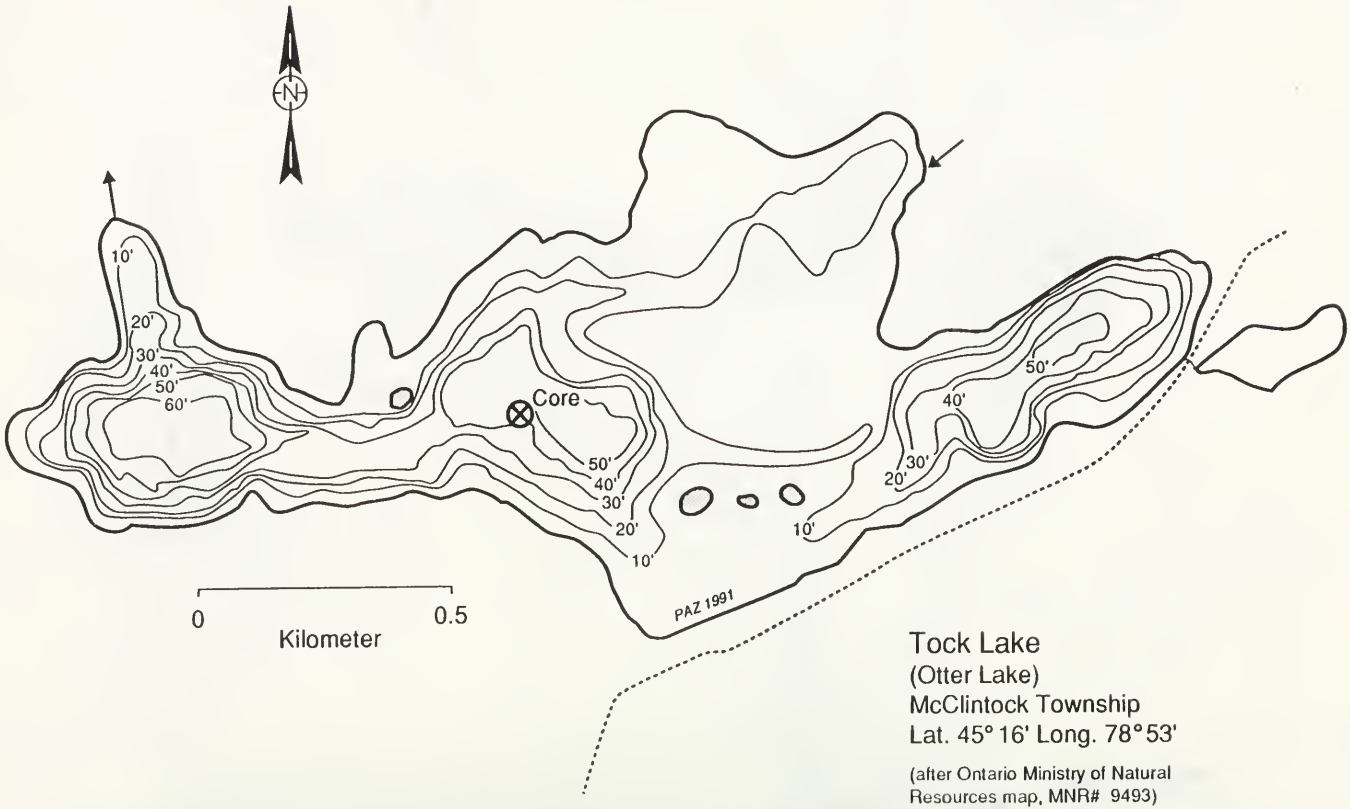


Rabbit Bay
(Lake of Bays)
Muskoka Township
Lat. 45° 15' Long. 78° 54'

(after Ontario Ministry of Natural Resources map, MNR# 9239)

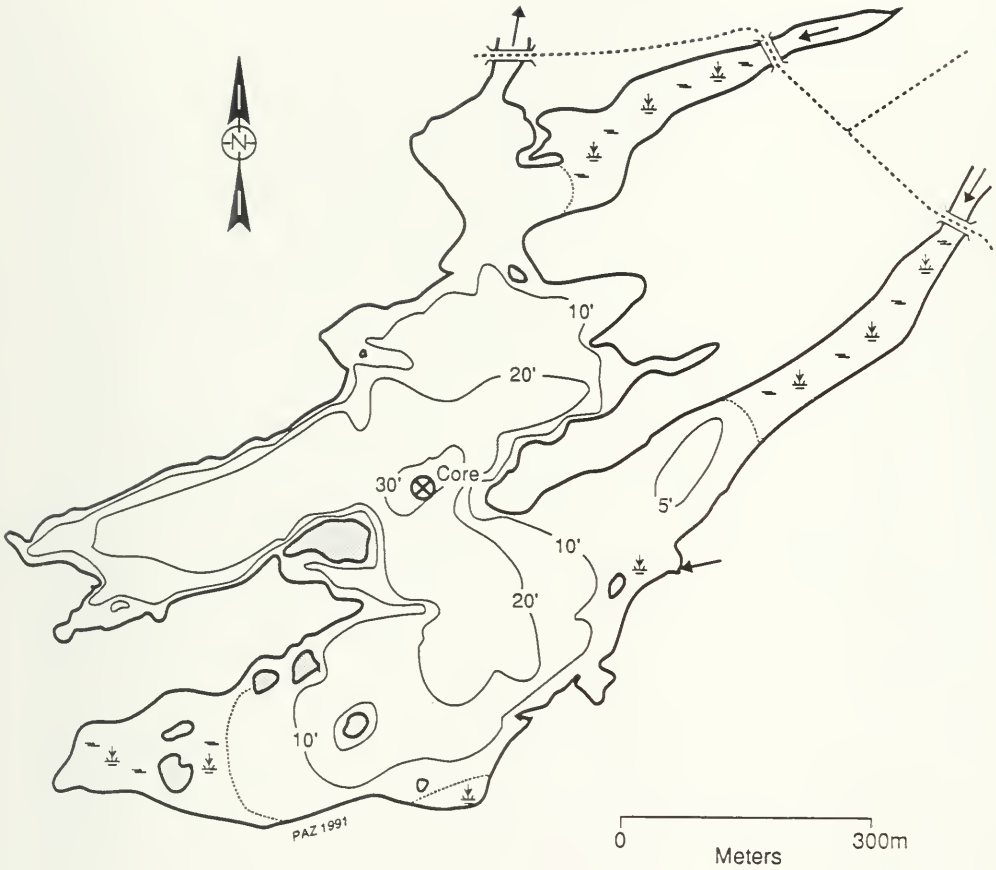
St. Nora Lake
Haliburton County
Sherbourne and Stanhope Townships
Lat. 45° 09' Long. 78° 50'

(after Ontario Ministry of Natural Resources map, MNR# 9614)



Tock Lake
(Otter Lake)
McClintock Township
Lat. 45° 16' Long. 78° 53'

(after Ontario Ministry of Natural Resources map, MNR# 9493)



White Lake
Galway Township
Lat. 44° 50' Long. 78° 29'

(after Ontario Ministry of Natural Resources map, MNR# 9593)