2021

North American Freshwater Snails as Paleoecologic Proxies in Crystal Lake, Medway, Ohio

Jaclyn R. Manker
Wright State University

NORTH AMERICAN FRESHWATER SNAILS AS SUPPLEMNTAL PALEOECOLOGICAL PROXIES IN CRYSTAL LAKE, MEDWAY, OHIO

A thesis submitted in partial
fulfillment of therequirements for the
degree of
Master of Science

by

JACLYN R. MANKER

B.S., Wright State University, 2019

2021
Wright State University

WRIGHT STATE UNIVERSITY
GRADUATE SCHOOL

JULY 19, 2021

I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Jaclyn R. Manker ENTITLED North American Freshwater Snails as Supplemental Paleoecological Proxies in Crystal Lake, Medway, Ohio BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science.

Rebecca Teed, Ph.D.
Thesis Advisor

Rebecca Teed, Ph.D.
Chair, Department of Earth & Environmental Sciences

Committee on Final Examination:

Stacey Hundley, Ph.D. (non-voting)

Chris Barton, Ph.D.

Audrey McGowin, Ph.D.

Barry Milligan, Ph.D.
Vice Provost for Academic Affairs
Dean of the Graduate School

Abstract

Manker, Jaclyn R. M.S., Department of Earth and Environmental Sciences, Wright State University, 2021. North American Freshwater Snails as Supplemental Paleoecological Proxies in Crystal Lake, Medway, Ohio

This study combines various paleoecological proxies found within a sediment core extracted from Crystal Lake, Medway, Ohio in order to assess the lake's sensitivity to past climate changes and how that may have affected lake water levels. Crystal Lake is a natural kettle lake formed at the end of the Wisconsin glaciation. It is now surrounded by approximately 500 residential homes and is privately owned by the HOA of Crystal Lake. A sediment core was extracted from Crystal Lake in 2007 and has been carbon dated to 18000 years before present, indicating that it contains a complete sedimentary history from late Pleistocene until today. Loss on ignition (LOI) and pollen data completed by previous colleagueswill help build a context for this study's research in the contribution of North American freshwater snails, in particular, three species of *Valvata*, as paleoecologic proxies of past aquatic habitats. Pollen data used to reconstruct vegetation changes known as climate zones have shown a progression of vegetation through several stages from postglacial initial colonization to modern vegetative landscape. LOI data shows changes in lithographic composition which can infer periods of eutrophication and correlates with vegetation shifts.

The number and consistency of *Valvata* shells retrieved from the core align nicely with the transition of climate zones. However, because sample size was small, and little is known aboutthe mechanisms of shell transport within the lake, more data is needed and this study remainsinconclusive as to the usefulness of the *Valvata* as climate proxies in Crystal Lake, Ohio.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Acknowledgements

I would like to thank my Thesis Advisor, Dr. Rebecca Teed for all her help and advice as well as my committee members Dr. Stacey Hundley and Dr. Audrey McGowin for theirthoughtful contributions. I would also like to thank all the members of the Teed lab whose workpreceded mine and made it possible, especially Kristen Kopera for her invaluable work in constructing the climate zones referenced in this Thesis. This project was funded in part by the Wright State University Woman in Science Giving Circle.

Introduction

This study focuses on using fossil North American freshwater snails of the *Valvata* genus as supplemental climate proxies for Crystal Lake, Ohio to assess the stability of the lake's perennial waters and its sensitivity to climatic variations. Water level fluctuations of the Great Lakes over a period of a hundred years or more can be linked to historical climate fluctuations such as the Medieval warm period between 1000-700 YBP (Wilcox et al, 2007). Smaller lakes may record a more sensitive record of climate fluctuations on a shorter response cycle (Miller, 1998). This study will be useful in determining the sensitivity of small lakes such as Crystal Lake to past climate fluctuations which will aide in modeling what future changes may look like based on current climate prediction scenarios.

Although the presence of the *Valvata* can indicate certain environmental parameters of Crystal Lake's past, they must be interpreted alongside other data. This study will use Loss on Ignition (LOI) data obtained from past undergraduate researchers of the Teed lab, to enhance the interpretation of *Valvata* data. A study of Newell Lake Basin located in Bellefontaine, Ohio found that high organic content of sediment lithology has been correlated with low lake levels and shoreline shifting, while high carbonate content is associated with deeper water and larger mollusk populations (McDonough, 2001).

Data will be compared to a pollen data analysis of the Crystal Lake sediment core by Kristen Kopera (Kopera, 2019). Pollen data can be used to show shifts in the vegetation surrounding a lake as the pollen dispersion of nearby plants may be preserved within the lake sediments. Because the ecology and environmental requirements of plant species are well known, climatic inferences can be made based on plant assemblages. Data from pollen analyses are organized into climate zones which will aid in *Valvata* data interpretation. When using pollen as a climate proxy, some of the same assumptions must be made as when mollusks are used. Also, some problems occur such as assemblages like the pine and elm assemblage found in Crystal Lakes pollen data for which there are no modern analogues (Kopera, 2019).

The *Valvata* as Supplemental Climate Proxies

The North American freshwater snails are ubiquitous to nearly all freshwater habitats throughout the North American continent (Burch, 1989). They are an important component to many ecosystems and serve fundamental roles within complex food webs as both predator and prey. They exhibit a replacement or nested gradient within families and species that can be strongly correlated with various habitat aspects including hydroperiod, predator biomass (Turner and Montgomery, 2009), macrophyte community composition, trophic level (Costil and Clement, 1996), and abiotic factors influencing water chemistry (Bukowski and Auld, 2014).

Mollusks have long been used as a climate proxy. More than one hundred years ago, scientists observed that fossil mollusks found at what is today a temperate latitude of the Midwest have a modern species range that has shifted to be much farther north from the Great Lakes region into Canada (Baker, 1918). Gastropods are a valuable proxy and can indicate environmental conditions, vegetation, and climate. They have a high-resolution response time to a changing environment of less than 50 years (Moine et al, 2002).

It is for this specialization that these organisms may be quite useful in the reconstruction of past ecosystems and climates. Burch noted in “Freshwater Snails of North America” that while a few species were observed to be generalists of habitat, the restriction of species to specific habitats was the accepted rule (Burch, 1989). Among the generalists noted are several members of genus *Physa,* including *acuta*, *gyrina gyrina*, and *skinneri* (Burch,1989) (Turner and Montgomery, 2009). Genus *Physa* are pulmonates or lunged snails are of little use in the estimation of hydroperiods or water depth as they must remain, at some period, above water. For those reasons, pulmonate snails will not be taken into consideration in this study. This study will focus on only three species of the *Valvata* genus. Figure 1 shows a photo example of each species (from left to right) *V. tricarinata, V. sincera sincera,* and *V. bicarinata.*

Figure 1. *Valvata. V. tricarinata, V. sincera sincera, and V. bicarinata* photographed under a stereoscope. Each snail is approximately 1-5mm in width.

The ecology of *V. bicarinate*, *V. sincera* and *V. tricarinata* are well known and accepted to be specialized. They are extant species of gilled freshwater snails within the *Valva* genus with a wide range that includes a large portion of the northern Great Lakes region of the US and most of eastern Canada (Burch, 1989). They reside on macrophytes within a characteristic environment. While Burch gives a broad generalization of habitat preferences by species in his comprehensive identification book, North American Freshwater Snails (1989), his categorizations are a generalization pieced together from many different malacological authors over many years. For example, Burch lists *V. tricarinata* as a species vaguely described as residing in perineal waters. However, studies suggest that *V. tricarinata* can be found in shallow perennial waters, the species has higher population densities in intermediate to deep waters.

A study by Eggleton in 1952 in Douglas Lake, Michigan found that invertebrate populations, particularly aquatic snails, were correlated to aquatic macrophyte density as well as substrate deposits. Samples were collected using a 1-meter square grid in a grab-style sampling technique. Vegetation density was ranked on a scale of 1-3, with 3 being the densest. Substrate was categorized by sediment type and composition into five categories; brown pulpy peat (later renamed gyttja), sand, muddy sand, black ooze, and sandy mud.

The largest invertebrate populations were found in areas where plant density was ranked 3 and where the substrate was sandy mud. The densest vegetation populations occurred at a depth of 14ft. *V. tricarinata* was present in the snails surveyed in this study. Their peak populations occurring in muddy sand and sandy mud were 4-10 times that of the populations occurring in the other substrates and were associated with aquatic plants of genus *Najas, Chara,* and *Potamogeton* which have an intermediate depth range.

The first underwater study of mollusks was conducted using SCUBA in Long Lake, northwestern Minnesota (Moyle and Bacon, 1968). Before this study, all ecological surveys involving mollusks and aquatic macrophytes has been restricted to above water sampling techniques and therefore only produced partial data in relatively shallow or easily accessible areas. Utilization of SCUBA allowed for a more comprehensive sampling approach.

Six transects of Long Lake were made and samples were taken at each meter of increased depth. A 0.5-meter square grid was placed over aquatic vegetation. The vegetation within the grid was then harvested into a bucket and brought to the surface. Snails were carefully stripped from the vegetation, documented, and preserved. Among the snails of interest in this study was *V. tricarinata.* Though *V. tricarinata* was present at all depths sampled, the highest populations were observed at 9 meters. A chi-square test (P. < 0.05) indicated a significant correlation between *V. tricarinata* populations and deep-water vegetation.

A previous survey of aquatic macrophytes by Schmidt in 1965 in Long Lake established that in shallow waters the plant communities consisted of a sparse population of *Chara contraria, Najas flexis,* and several species of *Potamogeton.* In waters ranging from 1-5 meters, the *Chara* and *Najas* were dominant. In waters ranging from 6-8 meters, *Chara* was still present, however the *Najas* had been replaced by *Nitella opaca.* In the waters deeper than 8 meters only *Nitella* remained, but was not found below 11 meters (Schmidt, 1965). Light was thought to be the limiting factor for the disappearance of *Nitella* as 11 meters was below the thermocline of Long Lake. Moyle and Bacon concluded that a strong association existed between *V. tricarinata* and deep-water vegetation such as *Nitella opaca.*

Burch lists *V. sincera sincera* as a deep lake dwelling species. An excavation of Bristol Fen located in Elkhart County, Indiana found fossil remains of both *V. sincera sincera* and *V. tricarinata* alongside the remains of an unspecified species of *Najas* (Swinehart, 1995). *Najas* as a genus is generally an intermediate aquatic plant, reaching depths up to 4 meters (Schmidt,

1965) (Meriläinen, 1968). This may indicate that the depth preference of *V. sincera sincera* and *V. tricarinata* is negotiable, presenting itself as more of a gradient based on food availability, than a ridged niche. Still, assemblages of *V. sincera sincera* and *V. tricarinata* have been correlated with clear, silt-free, deep water, with continued abundant vegetation and a firm substrate of bottom sediments (Yang et al, 2001).

V. bicarinata is thought to be the shallowest dwelling of the three *Valvata* of interest to this study and the most elusive from literature. Burch mentions *V. bicarinata* as residing in riverine habitats (Burch, 1989). It has a discontinuous distribution scattered among eastern and midwestern North American States including New Jersey, Pennsylvania, Iowa, Illinois, Tennessee, Alabama, Georgia, and North Carolina (Burch, 1989; Dillon et al., accessed 06/2021). Ohio is not listed as an area of documented distribution by Freshwater Gastropods of North America Association (it is also interesting to note that Dillon et al. do not list Ohio as having a documented population of *V. tricarinata* as well). Indiana was also not included as part of the distribution range for *V. bicarinata*, however a study of past and present gastropods of Indiana compared museum collections to current sample sites and determined that while *V. bicarinata* was once widespread in riverine environments, it is currently extinct from the state (Pyron et al, 2008).

Another issue with both *V. bicarinata* and *V. tricarinata* is that both species can produce polymorphs which closely resemble each other (Burch, 1989). The polymorph *V. bicarinata normalis* (Walker, 1902) has a third ridge in addition to the two characteristic ridges from which its name is derived. Burch lists eight polymorphs for *V. tricarinata*, however does not give a physical description of them and no scientific literature was found.

There are other potential biases and assumptions which must be made in the modeling of such paleo ecological proxies such as snails. Because a sediment core represents a thanatocoenosis and not a biocenosis, no assumptions about population sizes can be made due to small sample size and a lack of understanding of shell transport within the lake. Only the presence or absence of a species will be used to infer any ecological conditions. Also, although sedimentological conditions affecting shell preservation will be explored in this study, it should be noted that the absence of a species from a sample site may not mean that the species is absent from the lake as a whole.

Another assumption which must be made in the use of snails is that the environmental conditions in which they thrive today are the same as those in which they thrived in the past. This must be approached with some flexibility as snails have shown that they can be adaptable to some extent in their tolerance for some environmental parameters (Yang et al, 2001).

The question this study seeks to answer is can the presence or absence of *Valvata* snail species be used to infer past lake water levels and can that information be used to predict future conditions for Crystal Lake. If the water levels of Crystal Lake have changed in response to climate variations in the past, then that should be reflected in the consistency of *Valvata* shells throughout the core.

SITE DESCRIPTION

Crystal Lake is a series of four interconnected kettle lakes located in Medway, Clark County, Ohio at GPS coordinates 39.8888 N, -84.0239 E (Figure 2.). The largest, Main Lake, is connected to North Lake, South Lake, and Hidden Lake via a natural channel system. The lake system is fed on its northern side by tributary streams but may also be groundwater fed as wetlands exist to the east of the lake complex (Clemens, 2001).

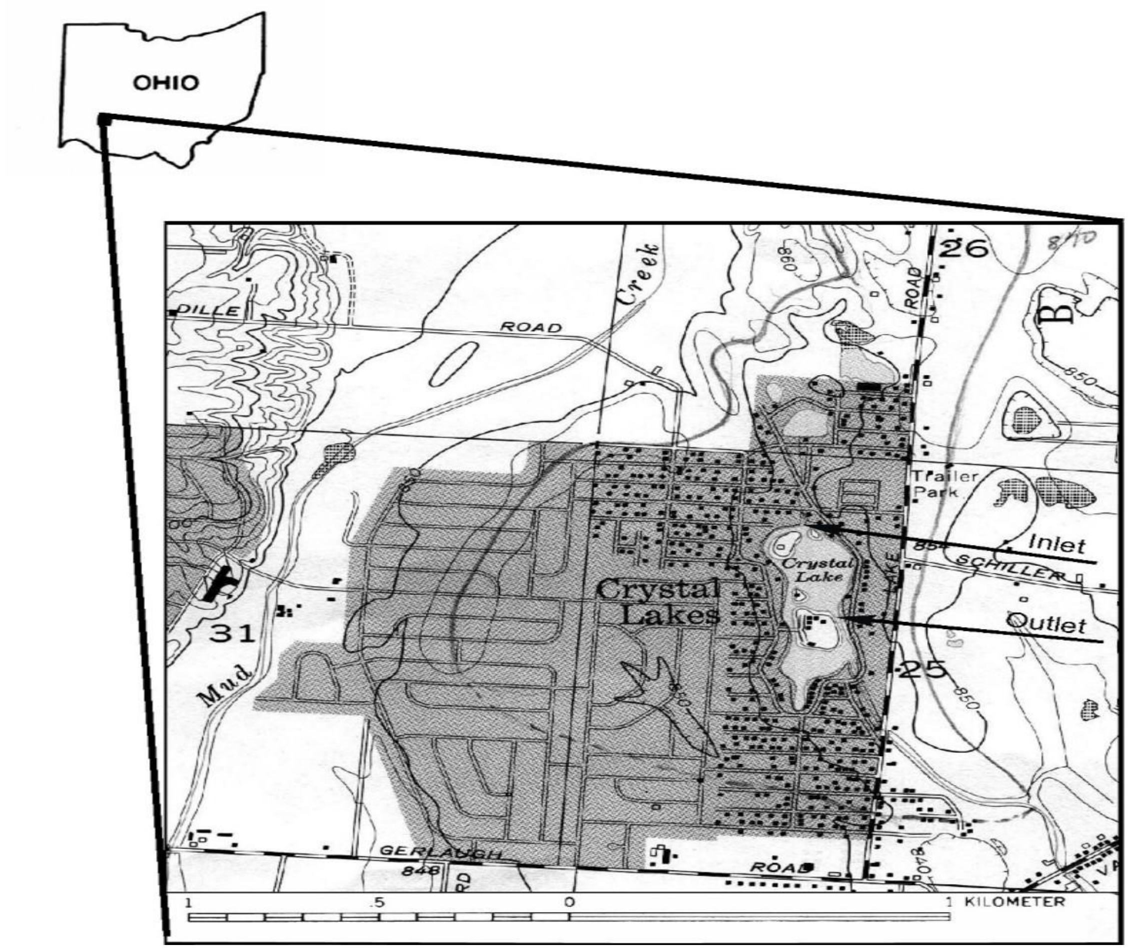

Figure 2. Map of Crystal Lake. From Teed et al, 2008. Crystal Lakes located in Medway, Clark County, Ohio, a northeast suburb of Dayton. The small black squares represent residential structure

Crystal Lake is surrounded by a residential community of around 500 small, originally intended vacation residences, which are now occupied year-round (Figure 2.). It is owned and maintained by the homeowner's association who has graciously permitted the lake to be used for research purposes although the primary use of the lake is for the aesthetic and recreational enjoyment of the association's residents. Activities enjoyed by the residents of Crystal Lake include boating, fishing, and swimming (within a designated area).

The surface area of Main Lake is approximately 7.3 hectares. A carbonate shelf makes most of the Main Lake shallow with depths ranging from 1.2 – 3.4 meters, the exception being a single deep area which is approximately 11 meters in depth (Figure 4.). The edges of Main Lake as well as the entirety of the three surrounding smaller lakes are populated by lilies as well as various other aquatic macrophytes (Figure 3.).

Figure 3. Main Lake photographed in August of 2018. Many aquatic macrophytes including lilies are abundant along lake edges. The deepest portion of the lake is visible in the upper right-hand side of this photo. The blue arrow points to the approximate location where the core was extracted.

Crystal Lake is a natural lake (Black, 1991; Bowell, 1980) and like the vast majority of Ohio's natural lakes, is post-glacial in origin, a feature of the Wisconsin Glaciation which began receding from the area around 20,000 YBP (Black, 1999).

A feature that may result from glaciation is a kettle lake. Kettle lakes are formed when a retreating ice sheet calves a large piece of ice. The ice block is then gradually buried by till and glacial out wash. Eventually the ice block melts and the sediment and till encasing it and collapsing to form the basin of the lake (Maizels, 1977). Crystal Lake was thought to have formed at the end of the Wisconsin glaciation in this manner. This origin was confirmed when a sediment core extracted in 2007 by T. Lowell and colleagues, revealed gravel underlay the lakes sediments (T. Lowell, unpublished, 2008). Lowell's team was able to recover a carbon datable sample of charcoal from drive CL-T12. Testing of the sample returned a date of 14,750 ± 80 ^{14}CBP and was calibrated to 18,089 ± 320 calendar years BP (Kopera, 2019).

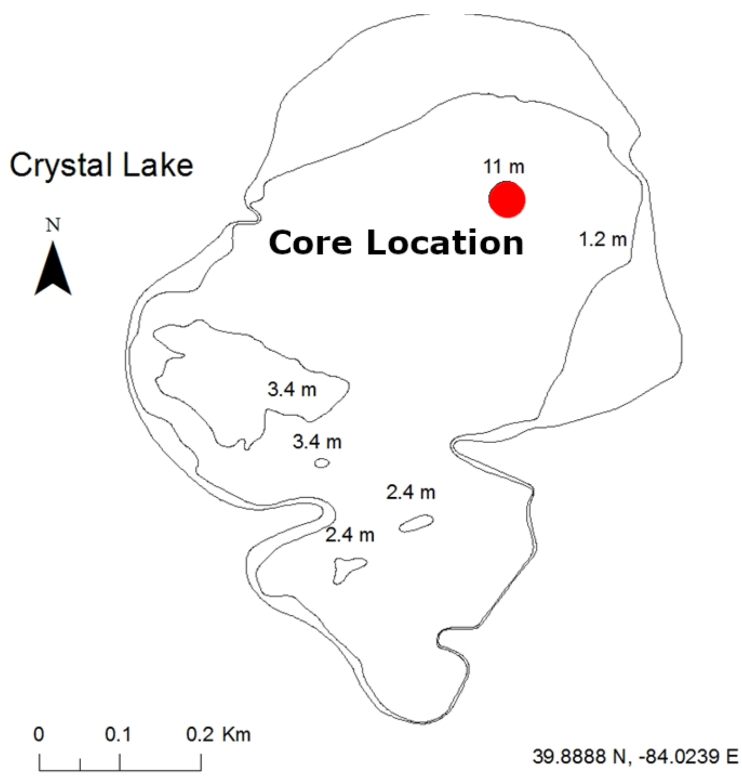

Figure 4. Bathymetric map of Crystal Lakes, Ohio from Kopera (2019), modified from Agather (2018). The deepest area of Main Lake is indicated by the red dot pictured above.

Crystal Lake has been described as eutrophic (Wisebaker, 2008) with the presence of a thermocline. In her 2007 study of Crystal Lake, Wisebaker found the nutrient content to fall within the parameters described by the Trophic Scale index as ranking a 60-70, placing the lake into a lower eutrophic category (Carlson, 1977).

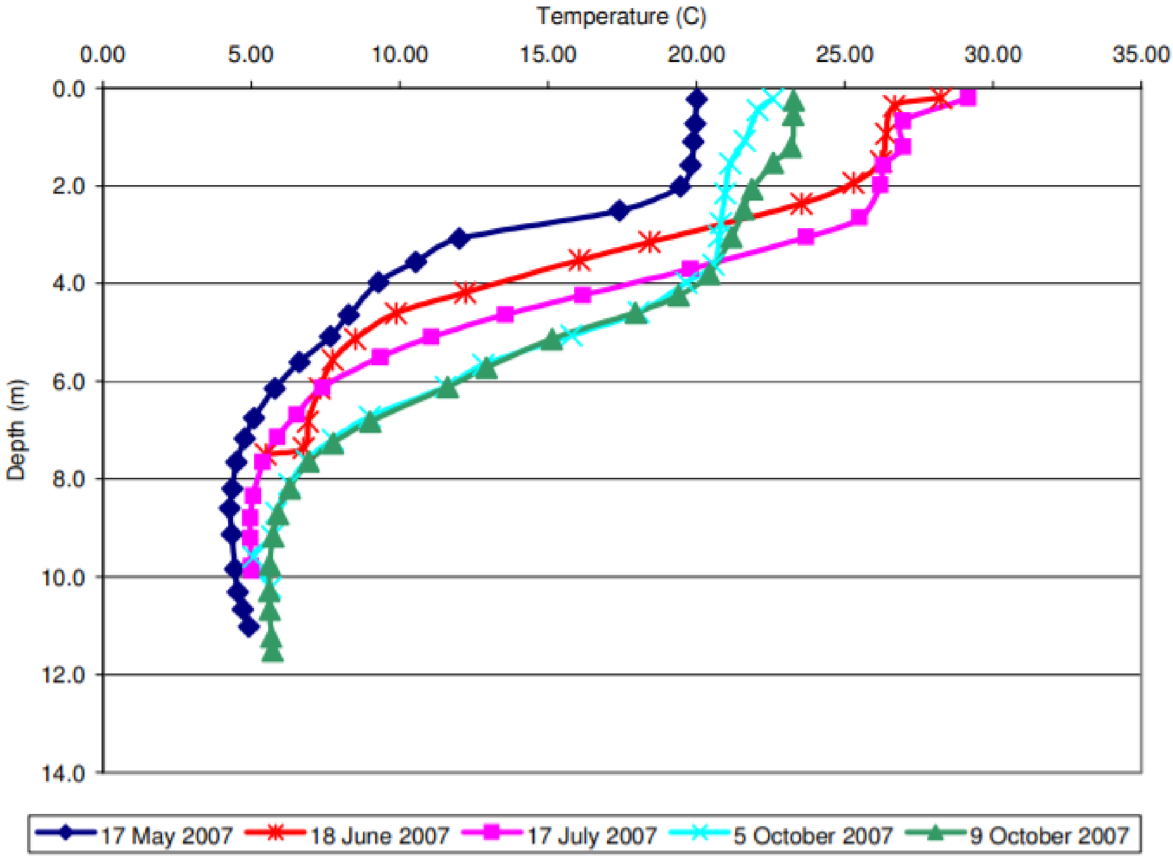

Figure 5. Temperature profile of Crystal Lake, Medway, Ohio (Wisebaker, 2008).

The lake experiences turnover twice a year in the spring and the fall (Collins, 1999). Figure 5 is a temperature profile of Crystal Lake from spring to fall in 2007 (Wisebaker, 2008).

The thermocline of the lake is visible where the greatest temperature change occurs with the least change in depth.

The lake experiences an increase of algae and photosynthesis seasonally, but even at peak bloom times dose not experience anoxic conditions at its mean depth of 6 meters, the exception being the deepest portion of the lake (Figure 6.). Based on data from Wisebaker, 2008, total chlorophyll, biomass, and nutrient count place June as the lakes most productive month.

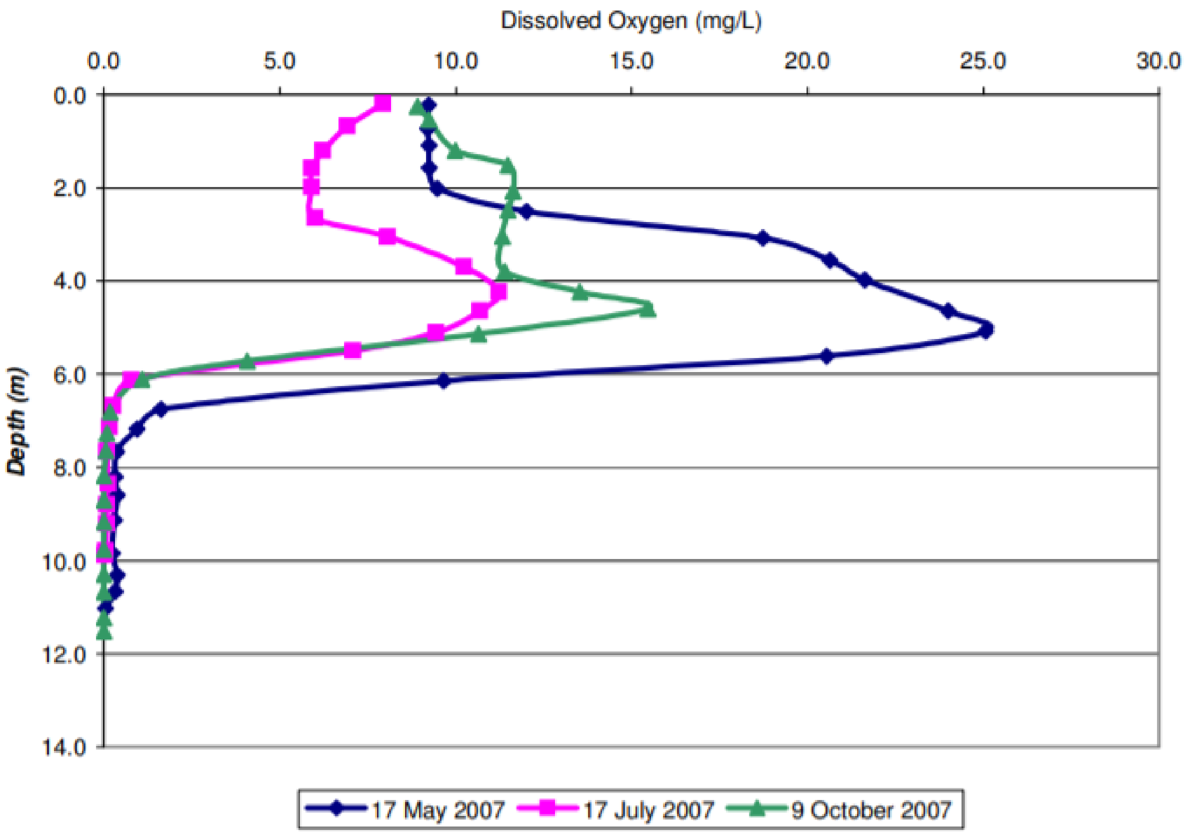

Figure 6. Dissolved oxygen profile of Crystal Lake (Wisebaker, 2008).

Methods

Sediment core collection

In October of 2007, T. Lowell and colleagues extracted a sediment core from Crystal Lake, located in Medway, Ohio for the purpose of research. The core was removed from the deepest portion of Main Lake which has a depth of approximately 11 meters. The collection of the sediment core from Crystal Lake was a joint research collaboration between the University of Cincinnati and Wright State University. Dr. Thomas Lowell, from the University of Cincinnati, and his research team, were joined by Dr. Songlin Cheng and Dr. Rebecca Teed from Wright State University. A Livingston-Wright piston corer provided by the University of Cincinnati was used to extract the sediment core (Figure 7.).

Figure 7. Preparing to core. Joint effort between colleagues of Wright State and UC to core Crystal Lake, 2007. Picture complements of Dr. Songlin Cheng.

The core was composed of 15 individual but somewhat overlapping drives which total 8.4 meters in length (Figure 8.). After extraction, the core was left in the care of Dr. Rebecca Teed who split it into halves. Each portion was wrapped in aluminum foil and plastic film, and then placed the core in cold storage at 42 degrees Fahrenheit. The fifteen drives were later correlated by loss on ignition to confirm their exact depths.

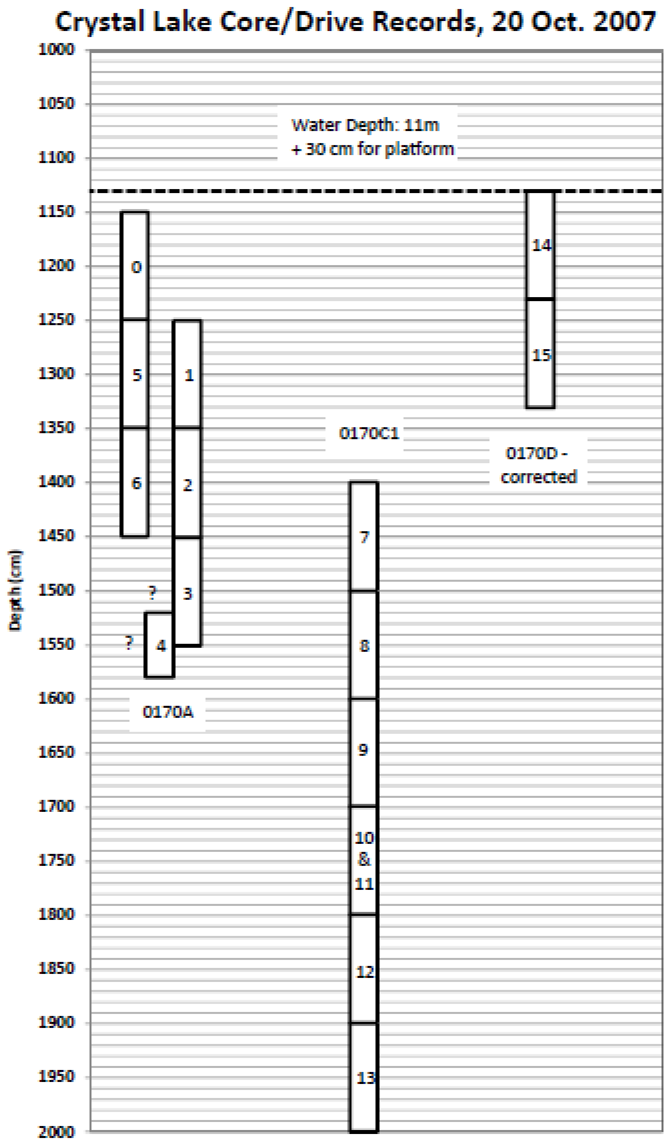

Figure 8. Diagram of sediment drives. From Kopera, 2019, a diagram of the 15 drives removed by T.Lowell and colleagues in 2007 from Crystal Lake, Ohio. The dotted line represents the sediment surface.

Sieving

Samples were removed from each drive in approximately 2-cm blocks with a volume of approximately 10 cubic cm. Their depth from the surface recorded using a meter stick. Each sample was placed on a No. 80 sieve and flushed via quirt bottle with high quality, Type 1 water obtained from a Milli-Q filtration device or if unavailable, distilled water was used. The mesh opening of the sieve was 180 µm. Small paint brushes were used to assist the breakup of sediments and the retrieval of any objects from the sediments. Samples were stored in lens jars until documented then placed into specimen vials for storage.

Sample Identification

Snails were identified to genus and species using J.B. Burch's North American Freshwater Snails (1989).

Carbon Dating

Organic material from charcoal weighing greater than 10 mg or terrestrial plant remains weighing greater than 20 mg were suitable for carbon dating and were processed by Direct AMS Labs. The near basal date obtained by T. Lowell in a previous study was processed by NOSAMS facility.

Loss on Ignition

Loss on ignition (LOI) is a method used for estimating the content of organic matter and calcium carbonate in lake sediments. It is commonly used because it is a relatively cheap, fast, and accurate means of obtaining data (Heiri et al., 1999). The process involves the collection of uniform sample sizes from a sediment core that are then dried in a muffle furnace at 105˚C for a period of 12-24 hours. The samples are then weighed to obtain a dry weight (DW_{105}). The samples are then heated to 500-550˚C during the first reaction during which organic matter is oxidized to produce CO_2 and ash (Heiri et al., 1999). Samples are again weighed to obtain DW_{550}.

The second reaction occurs when samples are again heated to 950˚C, removing CO_2 from $CaCO_3$ resulting in oxide. Samples are again weighed to obtain DW_{950}. The remaining portion of the sample may be referred to a mineral. The calculations to achieve percent of organic matter, $CaCO_3$, and mineral are expressed in equations 1 and 2 as given in Heiri et al. (1999).

$$LOI_{550} = ((DW_{105} - DW_{550})/ DW_{105}) * 100 \qquad \text{Equation (1)}$$

$$LOI_{950} = ((DW_{550} - DW_{950})/ DW_{105}) * 100 \qquad \text{Equation (2)}$$

LOI heating times may vary depending upon sample size, muffle furnace model, and crucible placement. Exact times and temperatures for the reproducibility of results are lab and substrate dependent (Heiri et al., 1999).

As a paleoenvironmental proxy, LOI data can be useful in assessing past water quality parameters such as eutrophication. Sediments of mesotrophic and eutrophic lakes can be classified as such if their sediments contain greater than 20% organic matter by LOI_{550} (Dean, 1999). Organic carbon content of sediments can be assumed as approximately ½ of the percent organic matter, therefore if a sediment sample contains 20% organic matter, it is 10% organic carbon, sometimes referred to as gyttja (Dean, 1999).

If mineral content is not highly variable, organic carbon and $CaCO_3$ are inversely related, although not linearly. The relationship is closer to a logarithmic relationship. A single percent increase in organic carbon may cause a several percent decrease in $CaCO_3$. This is because for every organic molecule of carbon decomposed by microorganisms, six O_2 molecules are consumed, and six CO_2 molecules formed (the reverse of photosynthesis). As CO_2 concentrations rise, calcite dissolves. Particulate organic carbon is usually the dominant source of carbon in sediments, however a nominal amount of dissolved carbon may sorb to clay particles (Dean, 1999).

In lakes with underlying glacial deposits, carbonate rocks may be a source of $CaCO_3$. However, when organic carbon becomes greater than 12%, the amount of CO_2 produced during the decomposition of that matter can lower pH to a point where dissolution of $CaCO_3$ occurs before it can deposit on the sediment floor (Dean, 1999).

The LOI data used in this study was obtained from samples taken from the Crystal Lake sediment core and processed by undergraduate researchers of the Teed lab. The data set contains 120 observations which span the length of the sediment core. These observations will serve as supplemental data in the reconstruction of Crystal Lake's past.

Statistical Modeling of LOI Data

The LOI data set was modeled using an Excel file imported into R Studio and figures were created using the gg plot add in. The plots were constructed using geom_smooth feature in gg plot which converts a scatter plot into a smooth line with darker surrounding shading representing standard deviation.

A binary regression was done using the statistical programming package R to explore the relationship between snail preservation in the sediment core and the % organic material and % $CaCO_3$ from the LOI data. For the binary regression, samples containing either shells or shell fragments were assigned a "1" to indicate presence, or "0" to indicate absence. The results are tabulated in Table 3.

Hester-Dendy Samplers

Palaeoecological proxies' usefulness in ecosystem reconstruction are much enhanced by establishing modern parameters where past ecosystems currently exist. To fully understand how sensitive to climatic changes the *Valvata* genus is, conducting a survey for modern populations is important.

Hester-Dendy samplers are a relatively inexpensive and easily deployed option for use in ecological surveying of benthic invertebrates (Valenty and Fisher, 2012). Their general construction consists of a stacked series of wooden or tile squares which, once deployed, sit in contact with the sediments. This provides and inviting shelter for many benthic invertebrates. Samplers are left for several weeks to allow for colonization, then retrieved, and examined for organisms. Placed in differing habitats, a Hester-Dendy sampler may give insight into the environmental preferences of invertebrate populations.

Construction of Hester-Dendy Sampler

Materials for construction of each sampler:

- Four 3” X 3” X 1/8” balsa wood plates
- Two 2” X 2” X 1/8” balsa wood plates
- Seven 1” X 1” X 1/8” balsa wood plates
- One eye bolt 6” in length
- Thirteen ¼” washers
- Two ¼” nuts
- Nylon rope (1 spool, 1cm thick)

Construction of Sampler

A hole was drilled through the center of each plate. Larger plates were placed at the bottom of the eye bolt with smaller plates above. Plates are spaced using washers at various intervals to create different sized habitats for colonization (Figure 9). Samplers were anchored to buoys with bailing wire of appropriate length for deployment depth.

Samplers were modified from their original construction by adhering flat rocks to their bottom to enable proper deployment. Total cost of samplers was around $35.00.

Figure 9. Photo of Hester-Dendy sampler. Hester-Dendy sampler constructed as per instructions listed in the methods section. Styrofoam buoy labeled as a research device and listing contact info for the curious (left). Modified Hester-Dendy (right).

Ecological Sampling of Crystal Lake

Three Hester-Dendy devices were deployed at three sites accessible by land into the main lake (Figure 10). Sample site #1 was a shady shallow area with a depth of 20cm. Upon deployment at site #1, the device did not sink which is necessary for contact with the benthos. The device was left in place wedged into tree roots. All other devices were taken back and modified by adhering flat rocks to the bottom using a waterproof adhesive.

Modified devices were deployed 08/05/2020. Sample site #1 was replaced with modified device and original was dislodged from the root system and returned to the lab for examination. Water level at site # 1 was noted to be 23 cm. Sample site #2 was sun exposed and heavily occupied by lily pads. The depth of site #2 was 39 cm. Site #3 was 1+ meter in depth and located at the end of a small fishing pier. Environmental parameters for each site are listed in Table 1. Devices were marked with buoys and left *in situ* for six weeks to allow for proper colonization by macroinvertebrate populations. Hester-Dendy devices were wrapped in cheese-cloth during removal to protect against the loss of sample organisms, placed in plastic containers with a tight-fitting lid and transported to Wright State lab. Organisms were identified and counted.

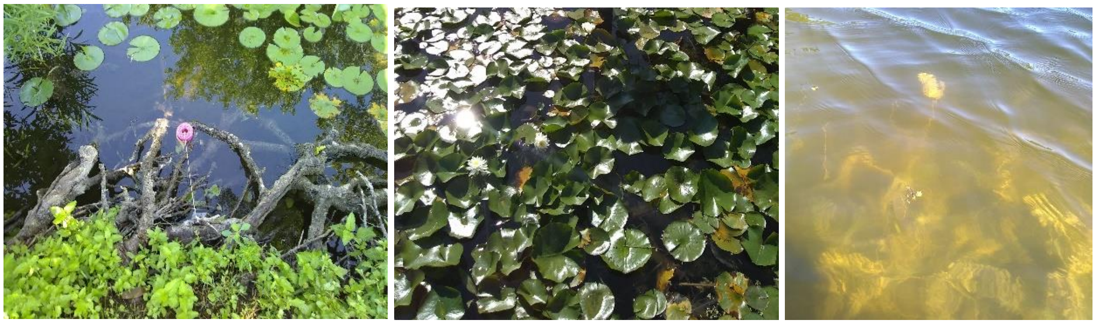

Figure 10. Hester-Dendy sample sites (from left); site#1, site#2, site#3.

Table 1. Temperature, depth, pH, and total suspended solids (TSS) for sample sites.

site	Temperature (C)	Depth (cm)	pH	TSS
1	25.3	23	8.7	211
2	29.4	39	8.35	195
3	25	100+	8.25	199

Results

Radiocarbon Dating

In addition to the initial carbon date obtained from a previous study by Dr. T Lowell's team, four dates were obtained (Table 2.).

Table 2. Radiocarbon date results obtained from sieving the Crystal Lake sediment core.

Radiocarbon Dates from Crystal Lake Sediment Core	
Depth (cm)	Date (Years Before Present)
1130	Modern
1200	175 ±106
1300	3000 ±46
1688	13506 ± 130

The date listed as "Modern" could not be given by radiocarbon dating because it was deemed to be post-1950. This was supported by the retrieval of a small piece of plastic from the same sample (Figure 11.).

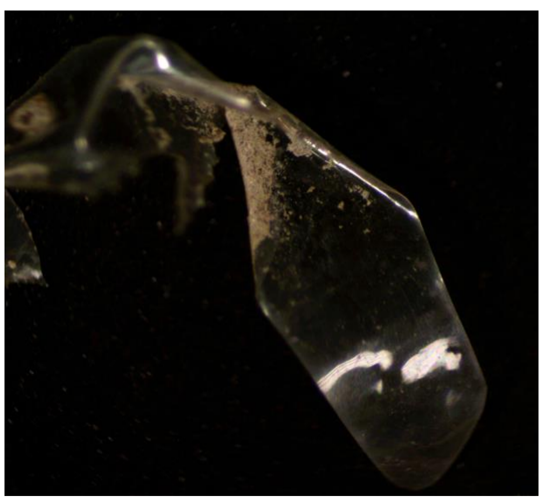

Figure 11. Photo of plastic film. Small piece of plastic film (~ 10mm) retrieved from sample depth of 1130cm. That samples organic matter returned a modern radiocarbon date (~1950 +).

Sieving Results for *Valvata* snails

A total of 73 samples were processed by sieving. Only the three species of *Valvata*; *V. bicarinata, V. tricarinata,* and *V. sincera sincera* were counted for this study. Figure 12 shows the number and depth as well as radiocarbon dates corresponding to depth. Climate zone depths as determined by Kopera (2019) from pollen analysis of the core are also given to enhance context. The frequency and quantity of *Valvata* are most prevalent in climate zones 3 and 4.

The number of *Valvata* shells greatly declined at the end of zone 4 and disappeared from the sediment core until the end of zone 6, where small numbers *V. bicarinata* shells appeared in two samples. *V. sincera sincera* was only observed once in a sample from zone 7 alongside *v. bicarinata.*

V. tricarinata was the species with the most shells at the end of zone 3 but experienced a steady decline. *V. tricarinata* was not observed in any samples from zones 5, 6, or 7.

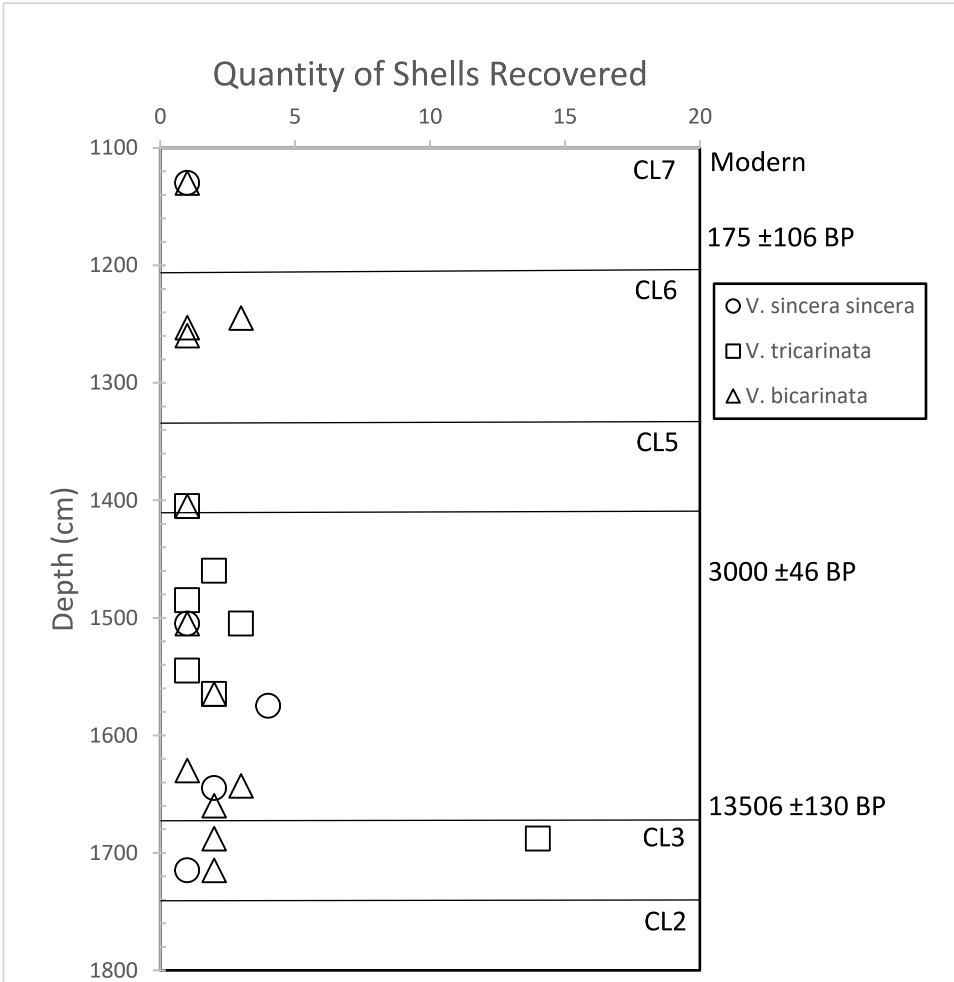

Figure 12. Quantity and depth at which each species of *Valvata* were retrieved via sieving. Climate zones (CL)as determined by Kopera 2019 are abbreviated at right and radiocarbon dates listed.

LOI Sediment analysis results (Lithology)

Figure 13 is a plot of percent mineral versus depth. Percent mineral declines from a depth of ~1890cm to 1300cm where it then begins to increase again.

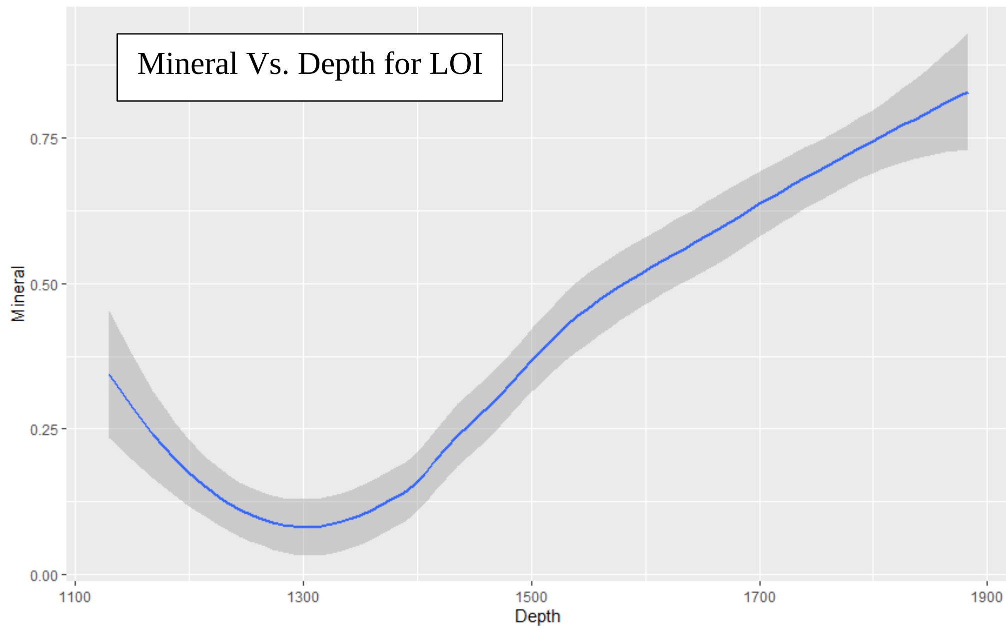

Figure 13. Percent mineral versus depth for the Crystal Lake sediment core. The blue line represents the mean for data while the shaded areas are standard deviation.

Figure 14 is a plot of $CaCO_3$ versus depth for the Crystal Lake sediment core. $CaCO_3$ increases from ~1890cm until it peaks at 1300cm, then begins to decline. The plots of percent mineral versus depth and $CaCO_3$ verse depth appear to be inversely related while percent organics appears to fluctuate independently (Figure 15.).

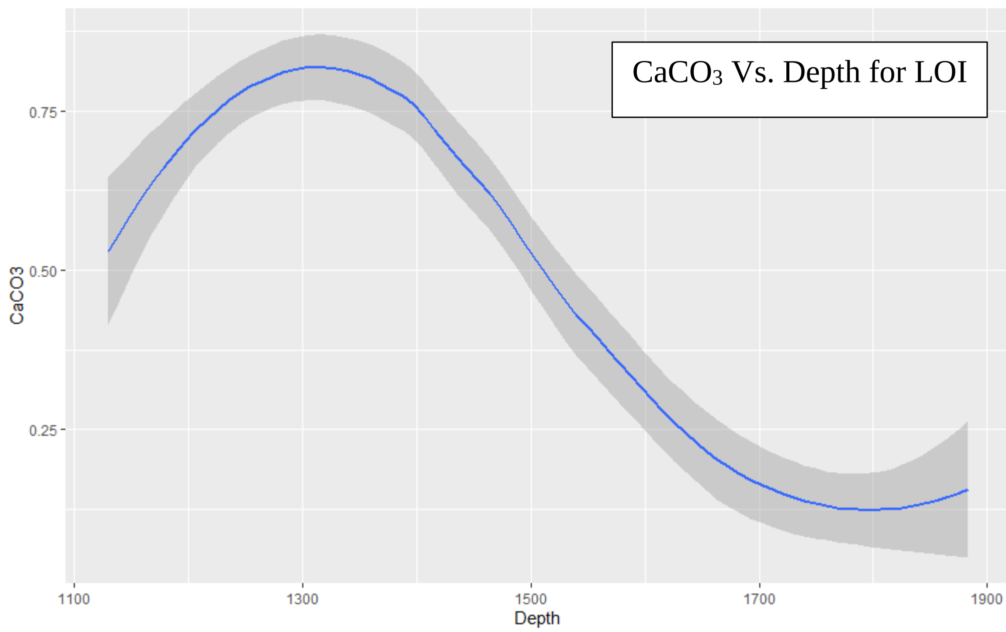

Figure 14. Percent $CaCO_3$ versus depth for the Crystal Lake sediment core.

The independent fluctuation of percent organic material in figure 15 is due to the small amount of material it represents in comparison the mineral and calcium carbonate content.

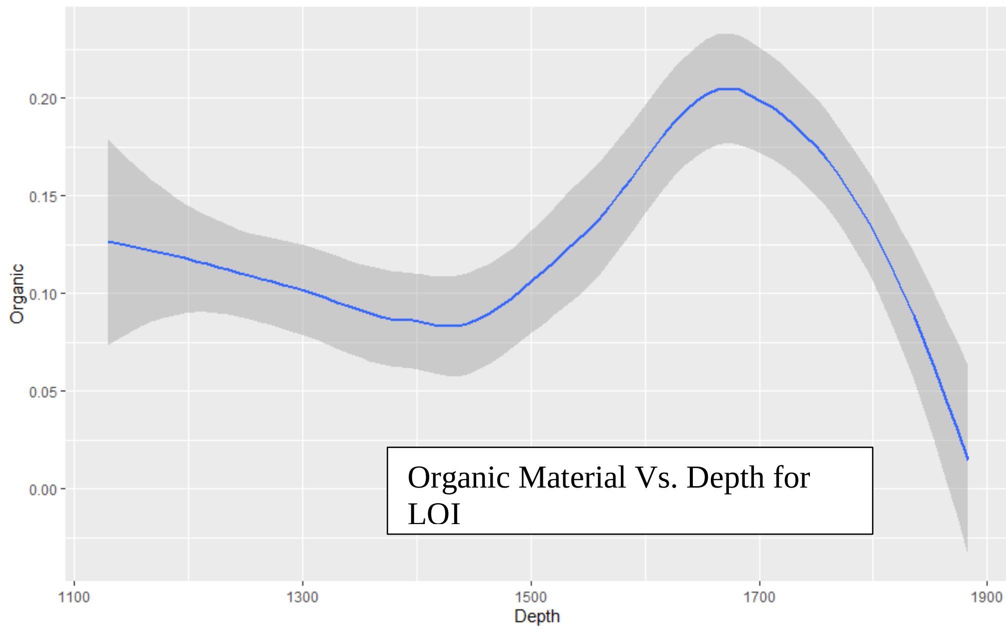

Figure 15. Percent organic mineral versus depth for Crystal Lake sediment core.

Although percentages of mineral $CaCO_3$ and organic material are all parts of a whole, the response between fluctuations in organic material and $CaCO_3$ is not 1:1. For every percent increase in organic material the decrease in $CaCo_3$ is much greater. Mineral percentages also fluctuate as these three components are part of a whole. Figure 16 shows changes in organic material with depth and the response of $CaCO_3$ fluctuations.

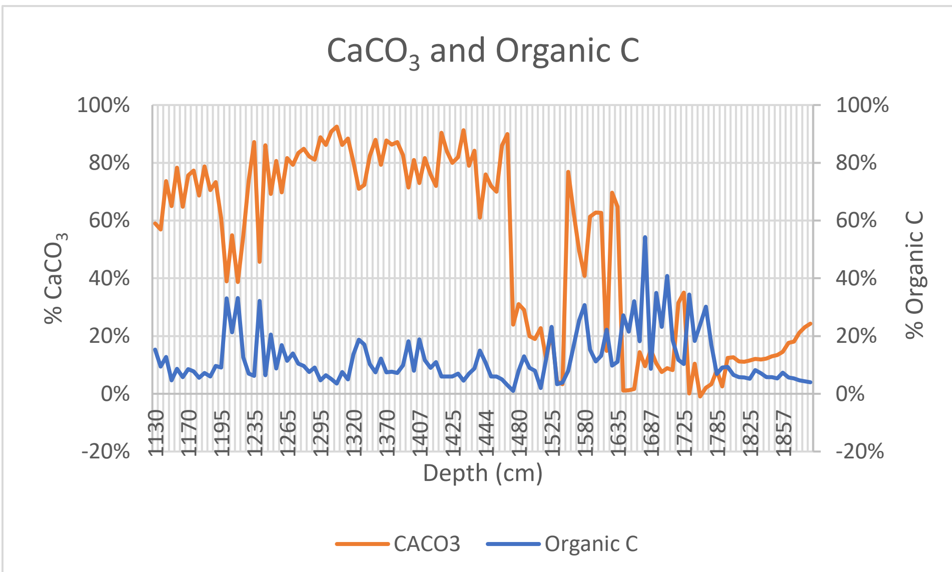

Figure 16. Fluctuations in percent organic matter as well as $CaCO_3$ by depth in the Crystal Lake sediment core.

Nine samples from the 120 observations in the LOI data set contained organic matter greater than 24% indicating periods of eutrophication. Table 3 lists the depths and percentage of organic matter for these samples.

Table 3. Percent organics above 24%. Occurrences where percent organics exceeded 24% and are associated with eutrophication.

Organic Matter Exceeding 24%	
Depth (cm)	%Organic
1202	33
1210	33
1235	32
1575	25
1580	31
1685	54
1695	41
1755	24
1765	30

These eutrophic events are marked with lines overlying the pollen diagram from Crystal Lake (Kopera, 2019). The blue lines in Figure 17 represent these nine observations which can be correlated with six events.

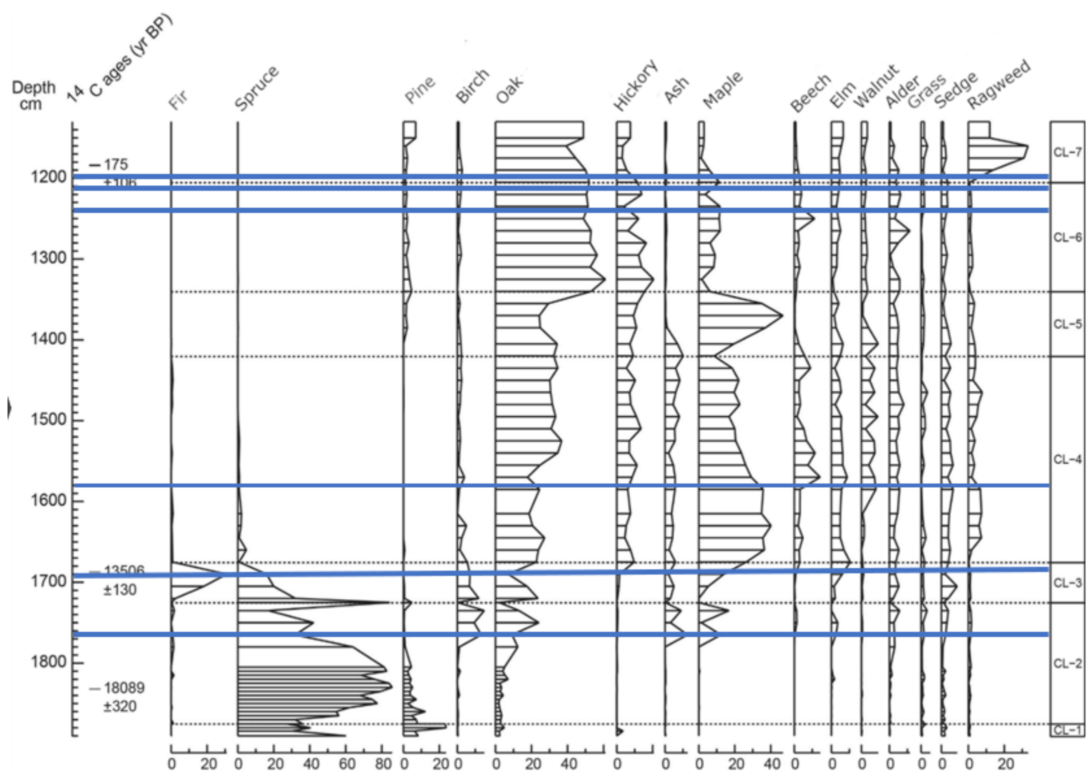

Figure 17. Eutrophic events combined with pollen data. Kopera 2019 pollen data with orange markers indicating eutrophic events. Modified from Kopera 2019.

At each event corresponding to organic matter >24%, there is a slight vegetation shift. At 1765-1755cm there is a decline in spruce and oak, and a spike in birch, hickory, and maple. At 1695-1685cm where the largest % organic matter occurs (> 40%), there is a peak in fir and a sharp decline in oak. Maple and walnut begin to increase. However, in between these depths at 1688cm, the % organics drops to 9% and coincides with the dominance of *V. tricarinata*. The vegetation change at 1580-1575cm consists of a decline in oaks, maple, sedges, and grass, and an increase in beech and walnut. Near the transition from zone 6 to zone 7, there are three eutrophic occurrences. At 1235 cm, there is a drop in hickory and beech. At 1210 cm, hickory recovers and hits a peak. At 1202 cm there begins general decline of all pollen taxa except ragweed, which rises sharply.

Table 4 lists the results for a binary regression performed to determine if either variable organic matter or calcium carbonate was of any significance to shell preservation in the sediment core. A binary regression is a statistical test used to determine the likelihood that a variable, which cannot be enumerated (such as genetic trait, sex, or medical condition), is random. The results in Table 4 list Pr (>|Z|) for both variables. This is the P value corrected for standard error. The smaller the P value, the less likely a variables relationship is random. To be considered significant, a P value should be > 0.05.

Table 4. Binary regression results. Results for a significant correlation between percent calcium carbonate and shell preservation.

Significance of Organic Matter and CaCO3 to Shell Preservation

	Estimate Std.	Error z value		Pr(>|z|)
Organic	1.9636	2.8164	0.697	0.4857
CaCO3	1.3872	0.8349	1.662	0.0966

Neither factor returned a significant P value. However, the likelihood that shell preservation and percent $CaCO_3$ was random was considerably lower than the likelihood that percent organic matter and shell preservation is random.

Figure 14 shows the percent $CaCO_3$ by each of the drives comprising the core for which there is LOI data using boxplots. The minimum percent for each drive is represented by the bottom line or "whisker" extending from the box. The bottom of the box is the 1st quartile, the line represents the median or mean, and the top of the box is the 3rd quartile. The line extending from the top of the box represents the highest percentage. Dots represent outliers.

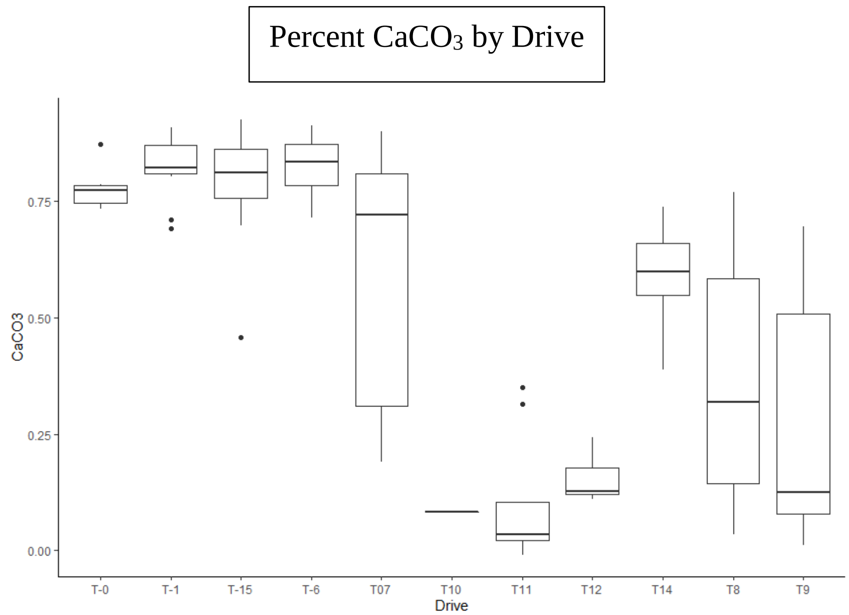

Figure 18. Box plot of percent $CaCO_3$ by drive. Created from LOI data.

Hester-Dendy Sampler

Of the four samplers deployed, three were recovered. The first sampler deployed at sample site #1, was retrieved at the time of the modified Hester-Dendy samples deployment. That sampler contained a single *Physa acuta* snail. *Physa acuta* is not of interest to this study, it is a lunged generalist who appears in numerous habitats across many parts of North America. The second sampler deployed at site#1 was never recovered and was thought to have been removed from the site by a curious person.

The sampler at site#2 also produced a single *Physa acuta*. Sampler at site#3 contained a small leech, but no other benthic organisms. Figure 15 shows an example of how the samplers appeared after removal from their respective sample sites and disassembled in the lab.

Figure 19. Photo of retrieved Hester-Dendy sampler. Hester-Dendy sampler from site#3. The small leech is visible on the bottom left plate.

Discussion

Valvata and Lake Water Levels

The data contained in the pollen, sediment, and snail records from the Crystal Lake core support each other and indicate that Crystal Lake has responded to climatic changes over time.

No samples were sieved from climate zone 1. This zone comprises only a few centimeters of the core and did not appear to have snails, therefore no samples were sieved from this zone.

Climate zone 2 samples (1870-1725 cm), characterized by the dominance of spruce, are Pleistocene deposits. LOI data shows a gradual decline in mineral matter alongside a steep increase in organic matter content. This is the first zone where percent organic reaches above 24%, indicating a period of eutrophication (Dean, 1999). This deposit of sediment containing > 24% organic matter occurs at a depth ranging from 1765-1755 cm and coincides with a brief decline in spruce and oak, and a peak of birch, ash, and maple. This could indicate a brief dry, warm period. No snail shells were present in the portion of the core representing zone 2.

V. tricarinata first appeared in climate zone 3 (1720-1675), thought to be the lower boundary of the Pleistocene based on pollen data from Kopera (2019). *V. sincera sincera* and *V. bicarinata* were also present in the sediment core for the first time but appeared in far fewer numbers. Their first appearance was documented at a depth of 1715 cm and contained one *V. sincera sincera* and one *V. bicarinata. V. tricarinata* first appeared at 1688 cm in a sample containing 14. This climate zone was characterized by a sharp spike in fir pollen (Kopera, 2019). This indicates a cold to cool, moist environment, comparable to modern boreal woodlands where fir thrives today.

The large number of *V. tricarinata* shells at the end of climate zone 3 and the presence of *V. sincera sincera* suggest that the lake depths were adequate to support intermediate to deep dwelling benthic macrophytes (Eggleton, 1952) (Schmidt, 1965). The presence of riverine dwelling *V. bicarinata* supports an extremely moist environment, possibly with shallow streams feeding into the lake.

Two deposits containing organic matter > 24% occur in climate zone 3. The first at 1695-1690 cm and the second at 1685 cm. These two deposits are separated by mineral rich sediment where organic content dropped abruptly from 35% to 9% and then rebounded to 54%, the highest percentage of organic content found in the LOI data. The sample containing the greatest number of *V. tricarinata* shells was in the mineral rich sediment between these organic rich deposits. Overlying the mineral rich sediment was a thick layer of charcoal ladened sediment which yielded a radiocarbon date of 13506 ±130 calendar years. Shortly after the organic rich deposit containing 54% organic matter, fir disappeared from the pollen record and the transition into climate zone 4 and the Holocene warming begins (Kopera, 2019). The mineral rich sediment deposit could represent a final, short-lived cooling which was not reflected in the pollen record due to the longer response time of trees supporting the response of smaller lakes to short climate variations (Miller et al, 1998).

Climate zone 4 (1660-1420 cm) is the zone where deciduous taxa begin to dominate over conifers representing 88% of the pollen data (Kopera, 2019). Throughout this zone *V. tricarinata, V. bicarinata,* and *V. sincera sincera* are consistently present. This indicates a period of stability within the lake. Outside the lake, the plant taxa also appear relatively stable (Figure 13.) and include oak, hickory, maple, alder, and sedges which are indicative of a warming, but humid climate (Kopera, 2019).

Organic material in the sediments begins to decline during this period (Figure 15.) and $CaCO_3$ content increases (Figure 14.). The conditions inferred by the pollen and LOI data appear to be the most favorable conditions for the *Valvata* in this study as this is a period in which their shells are consistently present throughout samples from this zone. A study conducted in Twiss Marl Pond, southern Ontario, Canada, found that peak populations of *V. sincera sincera* dominated that lake around 10,920 BP (calender years) (Yu, 2000). This date probably occurred within climate zone 4 (this is an assumption based on the placement of the other carbon dates) and the time period for which *Valvata* were consistently present in Crystal Lake. However, pollen data from the Twiss Marl study revealed that *Pica* was still the dominant pollen for that area. This indicates that the *Valvata* may be more flexible to climate and that other factors may be more important to their success in an environment. Only deposit of organic matter >24% occurs during this climate zone and coincides with a brief decline in beech. Shortly after the end of climate zone 4, the *Valvata* disappear from the sediment core and do not reappear until midway through zone 6. They are replaced by lunged snail shells. This indicates that their disappearance is not an issue of preservation, otherwise no shells would be present.

Climate zones 5 and 6 represent a period of increased warming and dryness (Kopera, 2019). The hottest, driest period of the Holocene, the hypsithermal, is thought to have occurred between 8,000-4,000 BP for Ohio (Shane, 1987).

A carbon date from White Fish Dune Pond in Wisconsin, established the transition from pond to bog occurred around 3620 BP and coincided with lowered water levels within Lake Michigan. A radiocarbon date from Crystal Lake at 1300 cm returned a date of 3000 ±46 BP. A drastic change in trends occurs in the LOI data at this depth. $CaCO_3$ levels peaked and began to decline (Figure 14.). Organic material increases (Figure 15.), and for the first time in the LOI data, percent mineral increases (Figure 13.). This may represent a time of low water levels and a lowered pH due to increased decomposition of organic matter.

In the mid to upper portion of climate zone 6, beech makes a recovery and *V. bicarinata* reappears in the sediment core. This indicates a return to moister conditions. Though *V. bicarinata* has the shallowest depth presence of the *Valvata* in this study, and *V. sincera sincera* only appears once in climate zone 7, *V. tricarinata* never reappears in the core. Its disappearance from the sediment core could be associated with lake levels which never totally recovered from the Holocene thermal maximum. Lake levels at or below todays 6-meter average would not have been able to support the intermediate to deep dwelling benthic macrophytes which have been associated with optimum population densities for *V. tricarinata*.

In summary, *Valvata* presence and consistency does correlate with distinct climate zones. There does seem to be a connection between *Valvata* presence, LOI data, and pollen data.

LOI data and Shell Preservation

Although the binary regression did not produce a significant result $P > 0.0966$, it may become significant if a greater sample size was used. The box plot of $CaCO_3$ content by drive did not accurately predict where the most shells could be found (Figure 14.). The drive with the highest mean % $CaCO_3$ with the lowest deviation was drive 6. However, the most shells were recovered from drives 9,8, and 7. All of which had a lower mean %, but higher deviation.

Hester-Dendy Samplers

The Hester-Dendy samplers produced disappointing results. There were several reasons for this. Being constrained to sampling sites reachable only by land limited the sample sites to depths which would be unlikely to target peak population densities of *V. tricarinata* and *V. sincera sincera*, should those species still reside in Crystal Lake.

Samplers were used only once. A study comparing the efficiency of Hester-Dendy samplers which had previously been deployed to new samplers found that the efficiency of attracting benthic invertebrates increased with sampler deployment. It was thought that the roughening of the pressed wood after use may have made colonization easier. It was also hypothesized that treatments used in manufacturing of the wood, may have been detectable to organisms on new samplers, but worn away on older ones (Valenty and Fisher, 2012).

The failure of the Hester-Dendy devices may also have been due to exposure times and dates. A more comprehensive study exploring peak exposure times and dates would be needed to optimize placement of devices within Crystal Lake.

Conclusion

Sensitivity of Crystal Lake to Climate Change

The presence and number of *Valvata* shells appear to align nicely with the climate zones established by Kopera (2019). The disappearance of the *Valvata* from the sediment core at the end of zone 4, their absence in zone 5, and the reappearance of *V. sincera sincera* and *V. bicarinata* midway through zone 6, suggest that the lake was growing shallower during that time as the basin filled with sediment. This is supported by the presence of lunged snails such as *Physa* and *Planorbidae*. However, some aspects of this study remain unresolved. The mechanism of shell transport within Crystal Lake is unknown. It is also unknown if any modern populations of *Valvata* are currently residing within the lake due to the failure of the Hester-Dendy sampling. More carbon dates are needed to establish a stronger timeline that would allow for resolution of the climate zones. Until these unresolved questions can be addressed the hypothesis posed in this study cannot either be accepted or refuted and must remain unresolved.

Future Work

While the *Valvata* shell data aligns with pollen and LOI data, it would be enhanced by the study of another snail genus sieved from the core. Due to time constraints, positive identifications were not made on all snails removed from the sediment core. *Valvata* were chosen for this study because of ease of identification and readily studied ecologies.

A modern ecological study of snail population in Crystal Lake would also be useful in establishing a modern analogue and to determine whether any species of *Valvata* currently reside in Crystal Lake. This should be done using improved sampling techniques such as collection of benthic macrophytes by biomass or by collection grids. Improvements on the Hester-Dendy samplers is also a viable option.

Oxygen isotope 18 data could help correlate events with dates when compared to other studies. Some research suggests that a sample containing as few as four *V. tricarinata* is sufficient to return accurate results (Miller et al, 1998). This may be possible to obtain from some sampled depths.

Although much research has been done among many contributors, the full history of Crystal Lake cannot yet be fully understood. Future research will aide in filling in the missing pieces and enabling a model of the future for Crystal Lake.

References:

Baker, F. C. (1918). Post-glacial mollusca from the marls of central Illinois. *The Journal of Geology*, *26*(7), 659-671.

Black, L. P. Water Planning Unit, Ohio Dept. of Natural Resources, Div. of Water. (1991). *Natural lakes in Ohio (Larger than five acres).* (Open File Report #5) Columbus: Ohio.

Bowell, D.F. Dept. of Natural Resources, Div. of Water (1980). Inventory of Ohio's lakes. (Ohio water inventory report No. 26). Columbus: Ohio.

Bukowski, S. J., & Auld, J. R. (2014). The effects of calcium in mediating the inducible morphological defenses of a freshwater snail, Physa acuta. *Aquatic ecology*, *48*(1), 85-90.

Burch, J. B. (1989). *North American freshwater snails* (No. 3). Malacological Publications.

Carlson, R. E. (1977). A trophic state index for lakes 1. *Limnology and oceanography*, *22*(2), 361-369.

Carolina, S., Gallery, M. A., Gallery, V., Gallery, G., & Gallery, T. FWGNA> Species Accounts>atidae> Valvata bicarinata.

Clemens, T. C. (2001). *The Depositional Components and the Origin of Crystal Lakes, Medway, Clark County, Ohio* (Master's Thesis). Wright State University, Dayton, OH.

Costil, K., & Clement, B. (1996). Relationship between freshwater gastropods and plant communities reflecting various trophic levels. *Hydrobiologia*, *321*(1), 7-16.

Dean, W. E. (1999). The carbon cycle and biogeochemical dynamics in lake sediments. *Journal of paleolimnology*, *21*(4), 375-393.

Eggleton, F. E. (1952). Dynamics of interdepression benthic communities. *Transactions of the American Microscopical Society*, *71*(3), 189-228.

Fischer, F. W. (1974). *Early and middle Woodland settlement, subsistence and population in the central Ohio Valley*. Washington University in St. Louis.

Franzen, D. S., & Leonard, A. B. (1942). A preliminary survey of the Mollusca of Kingman County, Kansas. *Transactions of the Kansas Academy of Science (1903-)*, *45*, 334-343.

Fritz, P., Morgan, A. V., Eicher, U., & McAndrews, J. H. (1987). Stable isotope, fossil Coleoptera and pollen stratigraphy in late Quaternary sediments from Ontario and New York State. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *58*(3-4), 183-202.

Dillon, R.T., Jr. and colleagues. (2019). The Freshwater Gastropods of North America. World-wide electronic publication, https://www.fwgna.org, accessed 07/2021.

Goodfriend, G. A. (1992). The use of land snail shells in paleoenvironmental reconstruction. *Quaternary Science Reviews*, *11*(6), 665-685.

Heiri, O., Lotter, A. F., & Lemcke, G. (2001). Loss on ignition as a method for estimating organic and carbonate content in sediments: reproducibility and comparability of results. *Journal of paleolimnology*, *25*(1), 101-110.

Hubendick, B. (1958). Factors conditioning the habitat of freshwater snails. *Bulletin of the World Health Organization*, *18*(5-6), 1072.

Kopera, K. (2019). *Vegetation Sensitivity During the Mid-Holocene Warming in Western Ohio* (Master's Thesis). Wright State University, Dayton, OH.

Kornijów, R., Measey, G. J., & Moss, B. (2016). The structure of the littoral: effects of waterlily density and perch predation on sediment and plant-associated macroinvertebrate communities. *Freshwater biology*, *61*(1), 32-50.

M.D. Guiry in Guiry, M.D. & Guiry, G.M. 2018. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org

Maizels, J. K. (1977). Experiments on the origin of kettle-holes. *Journal of Glaciology*, *18* (79), 291–303.

MCDONOUGH, J. (2001). The marl lake sediments of Newell Lake Basin, Bellefontaine, Ohio: A record of Holocene environmental change. In *Keck Geology Consortium 2001 Symposium Proceedings, Wooster, Ohio*.

Megard, R. O. (1967). Limnology, primary productivity, and carbonate sedimentation of Minnesota lakes.

Meriläinen, J. (1968). Najas minor All. in North America. *Rhodora*, *70*(782), 161-175.

Miller, B. B., Tevesz, J. S., & Carney, J. S. (1998). Holocene environmental changes in the Whitefish Dunes area, Door Peninsula, northern Lake Michigan basin, USA. *Journal of Paleolimnology*, *19*(4), 473-479

Moine, O., Rousseau, D. D., Jolly, D., & Vianey-Liaud, M. (2002). Paleoclimatic reconstruction using mutual climatic range on terrestrial mollusks. *Quaternary Research*, *57*(1), 162-172.

Moyle, P., & Bacon, J. (1968). Distribution and abundance of molluscs in a fresh water environment. *Journal of the Minnesota Academy of Science*, *35*(2), 82-85.

Pyron, M., Beugly, J., Martin, E., & Spielman, M. (2008). Conservation of the freshwater gastropods of Indiana: historic and current distributions. *American Malacological Bulletin*, *26*(1/2), 137-151.

Schmid, W. D. (1965). Distribution of aquatic vegetation as measured by line intercept with SCUBA. *Ecology*, *46*(6), 816-823.

Swinehart, A. L. (1995, January). Paleoecology of an alkaline peatland in Elkhart County, Indiana. In *Proceedings of the Indiana Academy of Science* (Vol. 104, No. 1-2, pp. 43-46).

Turner, A. M., & Montgomery, S. L. (2009). Hydroperiod, predators and the distribution of physid snails across the freshwater habitat gradient. *Freshwater biology*, *54*(6), 1189-1201

Valenty, J., & Fisher, S. J. (2012). Effect of previous use and processing technique on performance of multiplate Hester–Dendy samplers. *Freshwater Science*, *31*(1), 78-82.

Van den Berg, M. S., Coops, H., Noordhuis, R., van Schie, J., & Simons, J. (1997). Macroinvertebrate communities in relation to submerged vegetation in two Chara-dominated lakes. *Hydrobiologia*, *342*, 143-150.

Wilcox, D. A., Thompson, T. A., Booth, R. K., & Nicholas, J. R. (2007). Lake-level variability and water availability in the Great Lakes. *US Geological Survey Circular 1311*.

Wisebaker, A. R. (2008). The Impact of Nutrient Availability and Algal Community on Carbon Isotope Fractionaion in Crystal Lake, Clark County, Ohio.

Yang, J., Karrow, P. F., & Mackie, G. L. (2001). Paleoecological analysis of molluscan assemblages in two marl deposits in Waterloo Region, southwestern Ontario, Canada. *Journal of Paleolimnology*, *25*(3), 313-328.

Yu, Z. (2000). Ecosystem response to Lateglacial and early Holocene climate oscillations in the Great Lakes region of North America. *Quaternary Science Reviews*, *19*(17-18), 1723-1747.

Appendix:

Table 1a. The *Valvata* data

Depth Range	Average Depth	*V. sincera*	*V. tricarinata*	*V. bicarinata*	Dup	*Date*	*plus/min*
CL-7							
1130-1146	1130	1		1		C14Modern	
1149-1151	1150						
1164-1167	1165						
1174-1176	1175						
1186-1188	1187						
1199-1201	1200					C14=175	106
1204-1206	1205						
1209-1211	1210						
CL-6							
1224-1226	1225						
1234-1236	1235						
1244-1246	1245			3	Y		
1252-1254	1253			1	Y		
1259-1261	1260			1	Y		
1264-1266	1265						
1269-1271	1270				Y		
1279-1281	1280						
1284-1286	1285						
1294-1296	1295				Y	C14= 3000	46
1299-1301	1300				Y		
1301-1303	1302				Y		
1309-1311	1310						
1324-1326	1325						
1334-1336	1335				Y		
CL-5							
1339-1341	1340						
1364-1366	1365						
1374-1376	1375						
1389-1391	1390						
1404-1406	1405		1	1			
1414-1416	1415						
1419-1421	1420						
CL-4							
1434-1436	1435						

1449-1451	1450						
1459-1461	1460		2		Y		
1474-1476	1475						
1484-1486	1485		1				
1504-1506	1505	1	3	1	Y		
1524-1526	1525				Y		
1544-1546	1545		1		Y		
1564-1566	1565		2	2	Y		
1574-1576	1575	4			Y		
1584-1586	1585				Y		
1604-1606	1605				Y		
1617-1619	1618				Y		
1629-1631	1630			1	Y		
1642-1644	1643			3	Y		
1644-1646	1645	2					
1659-1661	1660			2			
CL-3							
1685-1687	1686						
1687-1688.5	1688		14	2	Y	C14=13506	130
1688-1690	1689				Y		
1700-1702	1701						
1702-1704	1703						
1704-1706	1705						
1706-1708	1707						
1708-1709	1708						
1715-1717	1715	1		2	Y		
1725-1727	1725				Y		
CL-2							
1739-1741	1740						
1755-1757	1756						
1769-1771	1770						
1784-1786	1785						
1794-1796	1795						
1814-1815	1815						
1829-1831	1830						
1844-1846	1845						
CL-1							
1859-1861	1860						
1873-1875	1874						

