University of Nevada, Reno

Does the diatom record of Lake Vico reflect a recent history of degraded water quality from hazelnut farming in the watershed?

A thesis submitted in partial fulfillment

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Bachelor of Science in Geology and the Honors Program

By

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BACHELOR OF SCIENCE, GEOLOGY

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Abstract

Lake Vico, Italy has experienced water quality issues in modern times due to the advent of hazelnut plantations and increased agriculture around the lake. Anthropogenic activity has affected the lake's trophic state, and this study analyzed whether this was reflected in the diatom record, indicating that the lake's flora are sensitive so such changes in the water quality. Enumeration of diatoms and multivariate were conducted to determine whether taxonomic differences presented themselves within the sediment record of the lake for the past millennium. Cluster analysis and broken stick modeling indicate that there are 4 distinct diatom zones within top 98cm of the lake core, with the uppermost zone representing anthropogenic activity over the last 50 years. Paleochlorophyll data indicate that the trophic state in 2012 may have improved some from the late 1990's. While the recent eutrophication signal is apparent in the diatom flora, considerable down core variation was also noted in the older parts of the core which were analyzed to provide background data. This indicates that the diatom assemblages from the older part of the core could record an interesting paleoenvironmental record.

Acknowledgements

First, I would like to thank my thesis advisor, Dr. Paula Noble. Her guidance and support were invaluable to me in completing this thesis and having the motivation to continue analysis of this lake core. She also provided me with numerous resources that I would have otherwise been want of.

I would also like to thank Drs Noble (Geological Sciences) and Mensing (Geography), Gianluca Piovesan (DAFNE, University of Tuscia, IT), and their collaborators for providing me with the core samples and dating and Dr. Smol and his PEARL lab (Queen's University, Ontario, CA) for paleochlorophyll data necessary for completion of this thesis.

Additionally, I want to thank Marco Aquino López (Queen's University, Belfast, IE) for providing me access to his PLUM model of the core and helping determine sample ages at depth.

Lastly, I want to thank my family and friends for their continued support, love, and optimism; they always helped me see the bright side of any problem I came across along the way.

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Introduction

Lake Vico (Lago di Vico) is a volcanic lake located in central Italy about 50km (31 miles) north of Rome. It is situated in a caldera that was formed after the collapse of the center of the Vico stratovolcano roughly 200-150 thousand years ago (Magri and Sadori, 1999). Its maximum depth is ~50m, and it has an area of around 12 sq. km (Magri and Sadori, 1999). The lake has a small stream inflow, no outflow, and a relatively slow water renewal rate – the rate at which all the lake's water is replaced with 'new' water from another source – of approximately 17 years, which poses a potential problem for water quality, because contaminants brought in through external nutrient loading can remain in the system for a while (Recanatesi, et. al., 2013, Constantini, et. al., 2007).

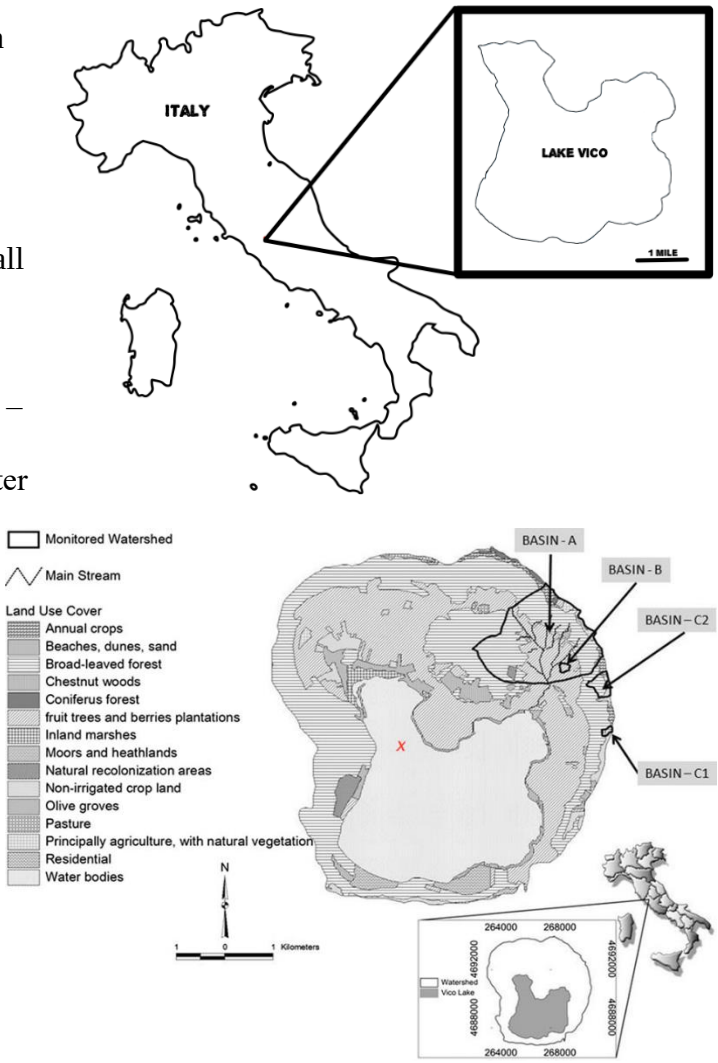

Figure 1 a) Locality map of Italy and inset of Lake Vico. b) Distribution map of land use around the Lago di Vico basin (Recanatesi, et. al., 2013)

Land use within the Lake Vico watershed includes intensive hazel farming and plantations that date to about the last 50 years around the lake (Recanatesi, et. al., 2013).

The increased agricultural activities pose a problem for water quality because increased agricultural runoff has led to a decrease in water quality in the lake from fertilizers and insecticide. Nutrient loading, in the form of orthophosphates and nitrates attributed to fertilizers showed marked increases in Lake Vico in the early 1990's (Dyer, 1995), and the insecticide Lambda-cyhalothrin is commonly used in the region to control hazelnut weevils (Paparatti and Speranza, 2005), causing pollution problems in the lake. Nutrient loading causes eutrophication of the lake, as shown by a shift in the lake from a "state of oligotrophy to one of meso-eutrophy" as a result of erosional runoff which is accelerated by human activity and rainfall in the area (Sabetta et. al., 2010, Recanatesi, 2013). In the early 1990's, sampling of the ambient phytoplankton indicated that increases in both orthophosphate and nitrate in the lake were accompanied by a change in soft-bodied phytoplankton (chiefly green and blue-green algae) that reflect the characteristics of a mesotrophic or sometimes eutrophic lake environment (Dyer, 1995). The work by Dyer was compared to a study some 20 years prior (Gerletti, 1971) following the same sampling protocols, and provide two past "snapshots" that document algal change with water quality degradation.

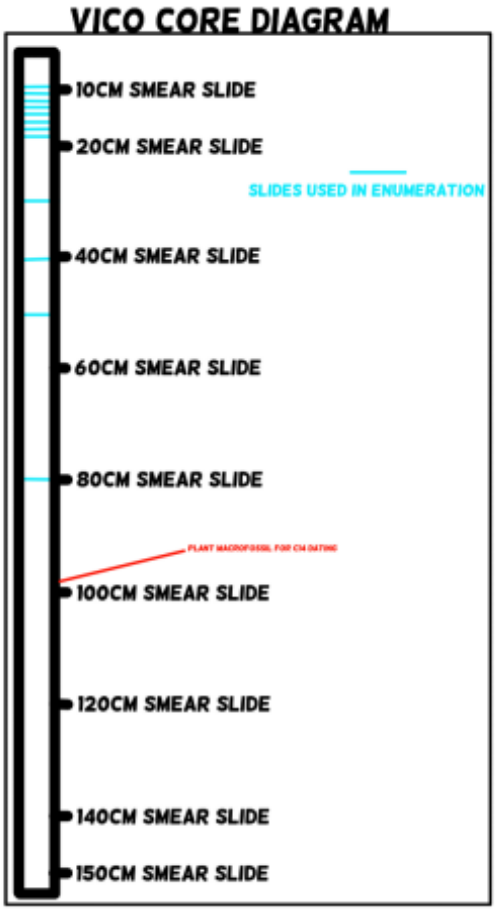

Figure 2 Schematic diagram of Lake Vico core showing depths of samples for smear slide and diatom enumeration. Additionally labeled is the depth of the plant microfossil used for C-14 dating.

While the lake was formerly used as a source of water for some 10,000 people, current conditions in the lake show that water quality is not sufficient to be distributed as potable. Not only have higher nutrient levels led to eutrophication, but these changes in water quality have allowed for the presence of parasites such as *T. franki* in the water that cause cercarial dermatitis (swimmer's itch) with prolonged contact (Cirpiana, et. al., 2011).

This study aims to analyze the diatom taxa at the lowest taxonomic level possible (genus or species level) in a sediment core taken from Lake Vico dating back over the past half-century as a means to determine a more detailed representation of change in the algal community and relate it to the described anthropogenic activities. It is hoped that the documented shift in water quality will be reflected in the diatom record. In addition to high resolution sampling over the last 70 years, as a comparison, diatoms were also counted and analyzed from lower down in the core, spanning the past millennia. These older samples provide a baseline of the range of variation in the diatom population in response to environmental change before widescale human activity around the lake, particularly hazelnut farming accompanied by chemical pesticides and fertilizers. I hypothesize that the last 50 years of hazelnut farming have sufficiently altered the water quality to affect the diatom populations. The null hypothesis is that the diatoms are not particularly sensitive to nutrient loading over the last 50 years and will not be statistically different from previous diatom population seen in the older part of the core. In addition to enumeration of diatoms within the core, paleochlorophyll from the upper 4cm of the core will be used as a cross check of eutrophication and compared to the diatom results.

Methods and Materials

In 2013, Dr. Mensing along with his team, including Dr. Paula Noble, cored Lake Vico, recovering a sediment core of 152cm in length from the lake floor to determine whether this lake could produce a usable paleolimnological record. The core was dated using Pb-210 and C-14 taken from a plant macrofossil found at 98cm within the core as well as Cs-137 found in the sediments. Using Pb-210 and C-14 data, an age model was produced using the program Plum developed by Marco Aquino López (Lopez et. al., 2017), including maximum, minimum, and mean ages for each sample based on the Pb-210 and C-14 data available.

This study will focus on the uppermost 7.5cm of the core because results from Pb-210 dating provide a detailed age model from this part of the core and indicate that the mean age at that depth would be roughly 50 years ago, placing the samples at an optimal position for determining anthropogenic effects from hazelnut farming and other agriculture in the area. Analyses of paleochlorophyll from the first 4.5cm of core were also performed in the lab of Dr. Smol that will be compared to the diatom record as a cross check for changes in algal productivity levels.

Samples of lake mud were processed for diatoms using a modified method of Battarbee et. al. (2001). This method removes organic material and produced a siliceous slurry composed mostly of diatoms. From this slurry, strewn slides of diatoms were produced at half centimeter intervals for the first 10cm of the core, with subsequent slides made at 1cm intervals up to 30cm intra-core depth. Further slides were produced at 40cm and 70cm below lake floor as well. Slides from the uppermost 7.5cm were the main focus of the study, being counted at 1cm intervals from 0.5-7.5cm depth for a total of 8 slides

while additional slides at a coarser interval were examined from the lower part of the core down to 71cm to get a general baseline for the last roughly 940 years to determine variation over the past millennium. The age model has the greatest uncertainty below the base of the interval dated by Pb-210 and therefore high resolution sampling cannot be easily reconciled to specific historical events and dates prior to the 20th century. For this reason, only a few baseline samples from the older part of the core were taken.

Live diatom samplings from plankton tows – as well as periphyton sampling along shore – were taken in 2013 by Dr. Noble, and slides of these samples were made available for comparison.

Prior to enumeration, diatom slides were examined under 100x oil immersion transmitted light microscopy in order to identify all diatom taxa present to the lowest taxonomic level possible. This work was done with the help of Dr. Noble and using Hoffman et. al. (2013) as the principal identification reference for the periphyton. Most identification was done to the genus level, however, some of the more common diatoms were identified to the species level, and these are the most important species to the analysis of the enumeration. An identification guide was made for easy reference while enumerating and contains photos of the species or species groups, categorized taxonomically. Enumeration was conducted by counting 500 diatoms per slide to determine relative abundance of diatom taxa.

Count data were then analyzed in time series graphs and with multivariate analysis techniques (ordination and constrained cluster analysis) to determine whether there were any statistically significant changes in the flora down core. The quantitative data analysis was conducted in the R environment using the packages vegan, analogue,

and rioja to produce various graphical representations including principal component analysis (PCA) ordinations, multivariate transformations, and broken stick models (see Appendix A.). PCA and broken stick models were conducted to determine statistically significant trends in the data in order to determine the most effective transformation to be performed for final data analysis. Principal component analysis is a data reduction technique that allows for identifying axes (called principal components) representing the maximum amount of variance in the sample set and is useful in identifying patterns in species distribution amongst samples in the dataset.

Using a stratigraphically constrained cluster analysis, adjacent samples were grouped by similarity to one another. This technique allows for the recognition of breaks between stratigraphic intervals of samples, producing a zonation. These zones were used in conjunction with cluster analysis and PCA ordination to determine the most significant species for distinguishing potential anthropogenic effects.

Furthermore, the results of the ordinations and stratiplot analyses were visually compared to a time series plot of the paleochlorophyll data acquired from samples representing the top 4.5cm of the core and the past 50 years.

Results

Core Chronology:

The Plum age model is for the top of the core and is based on Pb-210 data. It shows that sample age resolution decreases down core (Figure 3) based on larger uncertainties (Table 1).

Table 1. Sample Ages, Minimum, Mean, Maximum

Sample number	Minimum age (years ago from 2012)	Mean age (years ago from 2012)	Maximum Age (years ago from 2012)	Minimum Uncertainty (yrs)	Maximum Uncertainty (yrs)
1	1.952273	3.248378	4.478172	-1.296105	+1.229794
2	5.586819	9.745134	13.434516	-4.158315	+3.689382
3	9.761364	16.241889	22.39086	-6.480525	+6.148971
4	13.141115	23.264369	33.248443	-10.12325	+9.984074
5	16.211952	30.812571	46.859082	-14.60062	+16.04651
6	19.207184	38.360774	60.1863	-19.15359	+21.82553
7	21.219594	44.166253	71.803032	-22.94666	+27.63678
8	22.114772	48.229009	81.366042	-26.11424	+33.13703
9	97.029368	195.985903	334.877642	-98.95654	+138.8917
10	185.842085	328.028737	496.851226	-142.1867	+168.8225
11	313.596392	492.083847	671.631636	-178.4875	+179.5478
12	750.416461	940.107555	1119.58826	-189.6911	+179.4807

This model was used to determine mean ages of each sample at the start of the project in order to first choose a subset of samples for diatom analysis that covered the interval of interest. The age model extends further down the core by adding a C-14 date from a depth of 98cm.The resolution below the Pb-210 age model is significantly poorer, however the C-14 data does allow for extrapolating a maximum age, including uncertainties for the C-14 date for the lowermost samples.

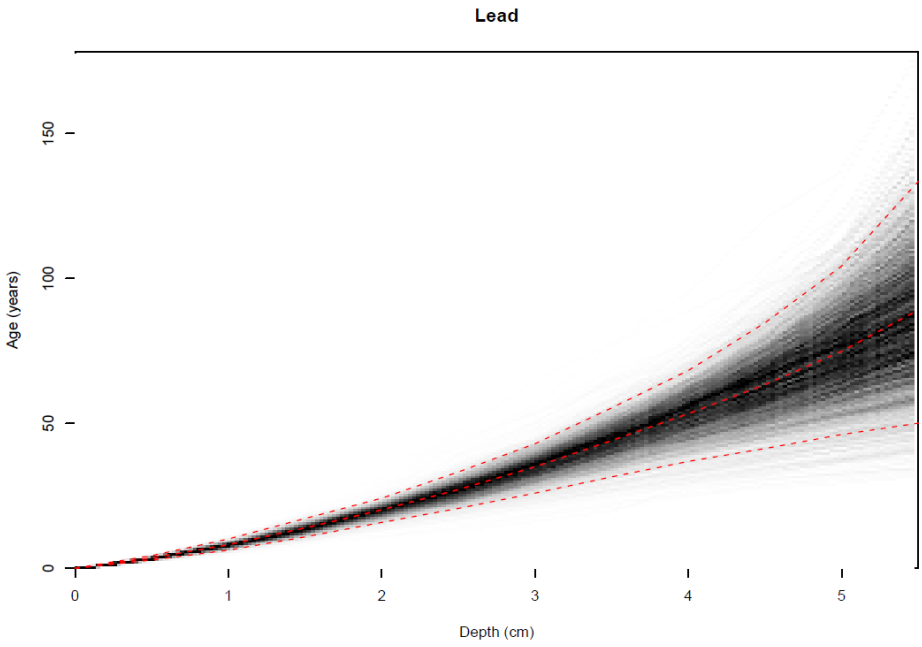

Figure 3 PLUM age model of samples from Lake Vico, Italy using Pb-210 and C-14 data. Expressed are minimum and maximum ages as well as mean age according to standard deviations of the age model (courtesy of Marco Aquino Lopez).

Table 2. Diatom Counts

Age (years ago from 2012)	3	10	16	23	31	38	44	48	196	328	492	940
depth (cm)	0-0.5	1-1.5	2-2.5	3-3.5	4-4.5	5-5.5	6-6.5	7-7.5	20-21	29-30	40-41	70-71
CA	1	0	2	1	0	0	1	0	0	0	0	0
CY	3	8	1	6	7	4	1	2	5	6	4	1
CO	38	44	47	38	18	23	23	13	25	9	6	17
GE	9	5	6	18	23	20	19	20	16	31	22	21
EN	30	31	32	23	27	25	23	41	24	41	16	19
EP	4	6	6	6	2	3	2	1	7	6	2	4
LO	166	152	176	182	145	145	142	159	198	184	266	254
LS1	34	19	16	30	57	48	30	40	45	18	43	2
SU	0	0	0	0	0	1	0	0	0	0	0	1
NA	29	20	23	34	18	13	4	3	8	14	10	15
PL	1	0	0	6	1	3	3	5	0	0	0	4
RH	0	0	2	0	0	1	0	0	1	0	0	0
STR	0	0	0	0	0	0	0	1	0	0	0	0
UC	3	10	2	13	5	47	15	1	0	1	8	14
STP	41	38	48	34	29	27	33	26	64	52	56	19
SM	6	2	6	5	18	9	15	15	24	14	9	14
SB	71	87	68	33	70	70	60	96	42	56	10	33
SC	13	16	15	17	15	5	4	14	11	1	3	7
SV	26	49	29	18	36	6	35	12	11	10	9	8
AS	7	1	3	9	2	1	3	3	0	1	0	1
NI	10	3	6	16	2	9	13	6	4	11	10	10
AU	0	0	0	0	6	9	19	20	0	15	1	31
KA	1	0	0	2	2	1	4	4	3	3	2	1
KO	0	0	0	0	0	1	0	6	4	11	15	13
CYM	1	0	0	0	0	0	0	0	1	0	0	2
GO	2	0	1	0	0	0	0	0	2	0	0	0
CA	1	0	1	0	0	0	0	0	1	0	0	0
UB	1	1	0	0	1	0	6	0	0	0	0	2
UA	0	0	5	8	2	8	2	0	0	3	3	5
LS2	0	0	0	0	8	12	0	0	0	0	0	0
PLAC	0	6	1	0	1	1	13	8	0	5	2	1
GYRO	0	1	0	1	0	0	0	0	0	0	0	0
UE	0	0	1	0	0	0	0	0	0	0	0	0

Table 2. Diatom Counts cont.

Age (years ago from 2012)	3	10	16	23	31	38	44	48	196	328	492	940
depth (cm)	0-0.5	1-1.5	2-2.5	3-3.5	4-4.5	5-5.5	6-6.5	7-7.5	20-21	29-30	40-41	70-71
DIP	1	1	1	0	0	0	4	1	0	3	1	0
U	0	0	2	0	2	6	14	3	1	0	2	0
CYS1	0	0	0	0	0	0	0	0	1	2	0	1
PS	1	0	0	0	0	0	0	0	0	0	0	0
CO1	0	0	0	0	3	2	0	0	0	0	0	0
HAL	0	0	0	0	0	0	0	0	3	2	0	0
CT	0	0	0	0	0	0	12	0	0	1	0	0

Diatom analysis:

A total of 40 taxa were counted from 12 samples with an emphasis on the uppermost ~7.5cm of the core which was counted at a higher resolution. Ages of each sample indicate that up to 7.5cm intra-core depth, would place the uppermost samples within the 50-year timeframe since the advent of hazelnut plantations in the area. Resolution of subsequent samples becomes less significant as these samples are to be used as a baseline for taxa diversity and abundance over the course of the past millennium. Diatom counts show that the most prominent taxa throughout all the samples are *Lindavia oscellata*, *Cocconeis placentula*, *Encyonema*, *Staurosira brevistriata*, *Staurosira venter*, and *Stephanodiscus*.

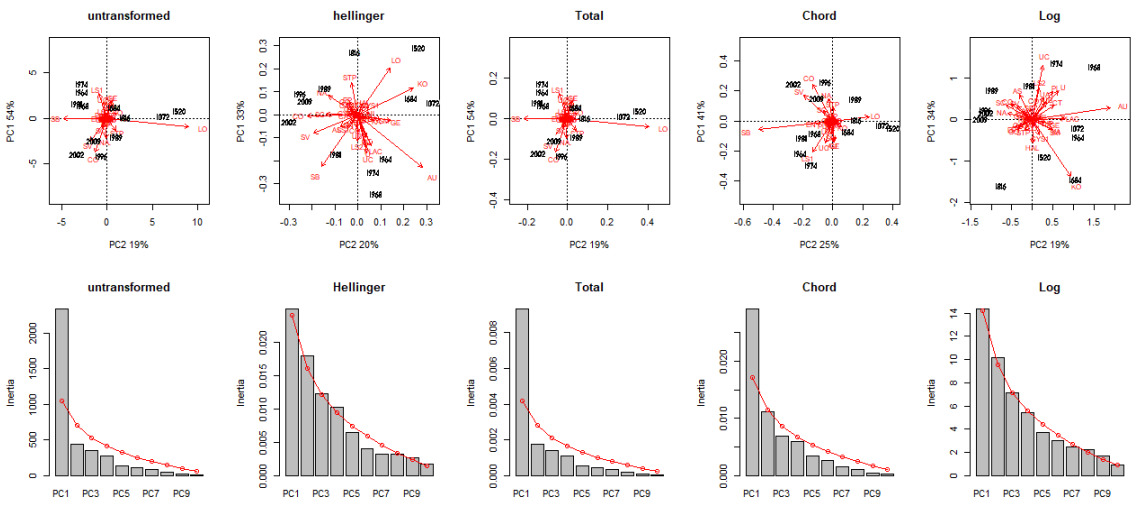

Figure 4 Multiple transformation of data along with analyses to determine significance of each principal component axis. Hellinger, Chord, and Log transformations show that both PC1 and PC2 are significant in data interpretation.

As a first step in data reduction, a Principal Component Analysis was conducted and the results of the first 2 axes were plotted (Figure 5). This multivariate technique is a data reduction tool that helps look for patterns. It was seen that the uppermost samples plotted apart from those lower down, although there were also large differences between the older pre-1950 samples, indicating a fair amount of variability in the past millennium unrelated to the recent eutrophication problems with hazelnut farming. Since diatom data typically show unimodal responses, not linear responses, these data are commonly transformed before conducting a PCA (Legendre and Legendre, 2012). As a trial and error, plots of the first and second axes of the PCA along with a broken stick model were run to determine the best transformation to have statistically significant 1st and 2nd axes. In Figure 4, broken stick diagrams for each transformation are shown, indicating that for the Hellinger, Chord, and Log transformations, both PC1 and PC2 are significant while in

the untransformed and Total transformation ordinations, only the first principal component axis is significant in data interpretation.

Of the transformations tried, the Hellinger transformation was chosen for interpretation and used because both PC1 and PC2 axes were significant. The Hellinger transformation has been used in paleoecologic and ecologic studies for quite some time (Legendre and Legendre, 2012) and thus provides a good basis for use in this study. An enlarged plot of the ordination, using the Hellinger transformation, shows the diatom data as well as the sample ages along 2 principal component axes. Eigenvectors for each taxon present within the samples represent directionality along the principal component axes and which taxa are drawing each sample in a given direction along one or both principal component axes. Samples may be grouped according to where they fall on either PC1 or PC2, meaning that the uppermost samples from 0.5-3.5cm may be grouped together while 5.5-7.5cm samples can be grouped according to their positions along both axes. The oldest samples can also be grouped together as they fall largely within the first quadrant together. This means that the uppermost samples are more similar to each other than to older samples, however there is more variance between some of the older samples than between them and the modern grouping (last 50 years), indicating a large degree of variability in the past.

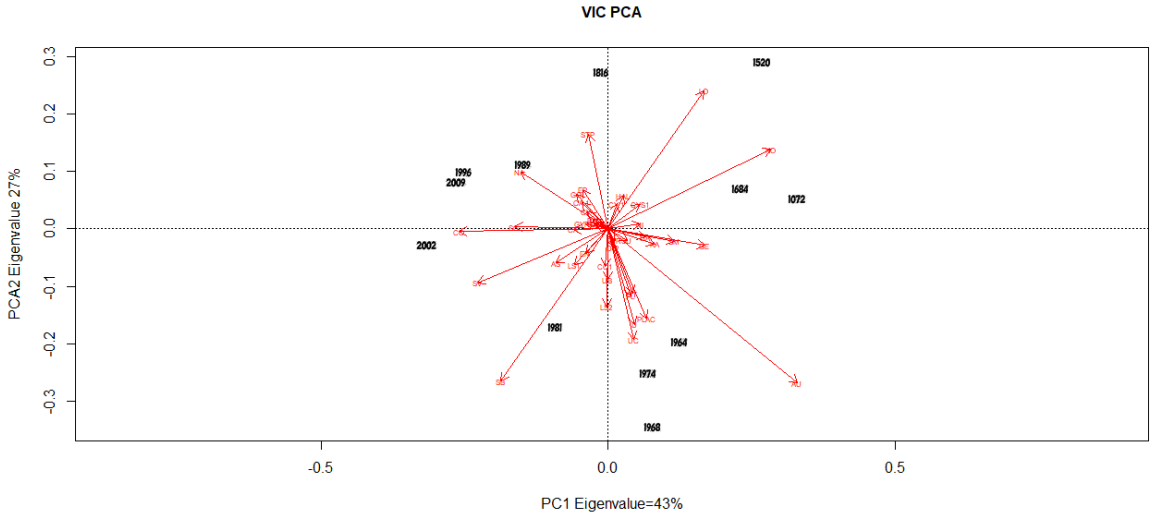

Figure 5 Principal Component Analysis using the Hellinger transformation, showing samples and taxa representations along each principal component axis. Numbers in black are sample ages, and arrows and letter labels represent distributions of taxa.

The taxa that have the largest eigenvectors in the direction of the oldest samples are *Lindavia oscellata* and *Kobayasiella*. The most dominant taxa affecting the samples from 5.5-7.5 appear to be *Placoneis*, *Aulacoseira,* and *Cyclotella sp.2,* while *Staurosira brevistriata* appears to have the largest contribution to the sample at 4.5cm depth. The uppermost 4 samples are placed along PC1 and PC2 along with *Staurosira venter, Cocconeis placentula, Navicula,* and *Staurosira construens.*

Cluster groups were identified using a stratigraphically constrained cluster analysis on the diatom enumeration data (Figure 6a). The broken stick model run on the cluster analysis shows the number of statistically significant groups to be 6 (Figure 6b); essentially there are 5 statistically significant breaks between sample groups. This is illustrated in figure 6b by the point where the two lines intersect in the graph. The red line

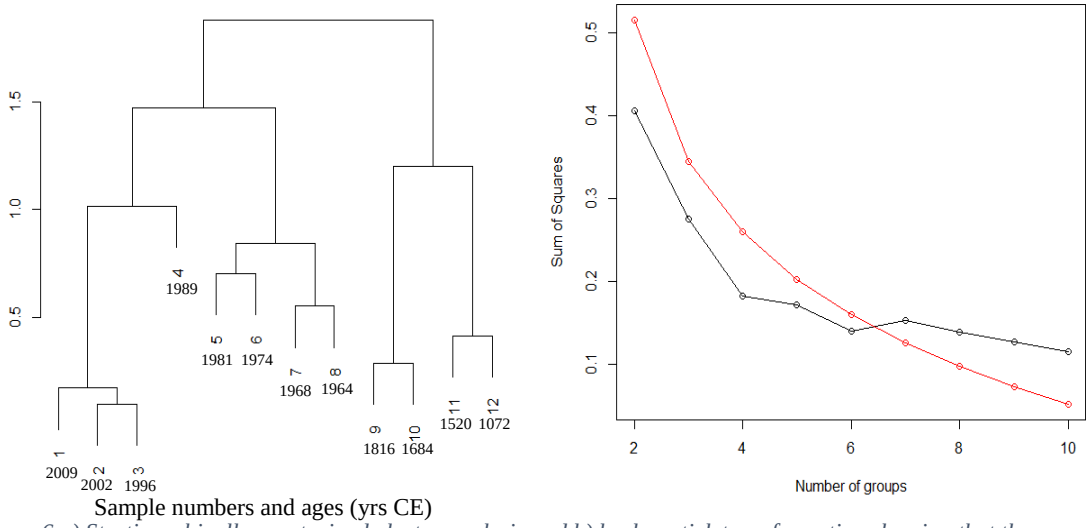

Figure 6 a) Stratigraphically constrained cluster analysis and b) broken stick transformation showing that there are 5 significant zonations possible for the data collected. Groups can be broken up on the cluster diagram based on the greatest variability between samples.

represents a randomly generated value of the sum of squares for the data set analyzed. Six groups of the actual data exceed the values of the random (broken stick) model, indicating that they are not generated just by random chance and are significant. These groups can then be broken out into zones based on the most significant differences between them by separating the groups from the top of the diagram downward. However, it is difficult to interpret all 6 statistically significant groups in the data, so only the 3 most prominent breaks (4 groups, or zones) were then used in Figure 7.

Figure 7 shows the zones from the cluster analysis superimposed on a time series graph of the most abundant taxa present in each of the samples – those that represent greater than 1% of at least one sample. The number of taxa that exceed this 1% cutoff is 22 compared to the original taxa list of 40 taxonomic groups. These taxa are the ones used to distinguish zonations between samples. The species *Asterionella formosa* is only found in the upper 2 zones (zones 3 and 4) whereas the genera *Planothidium*,

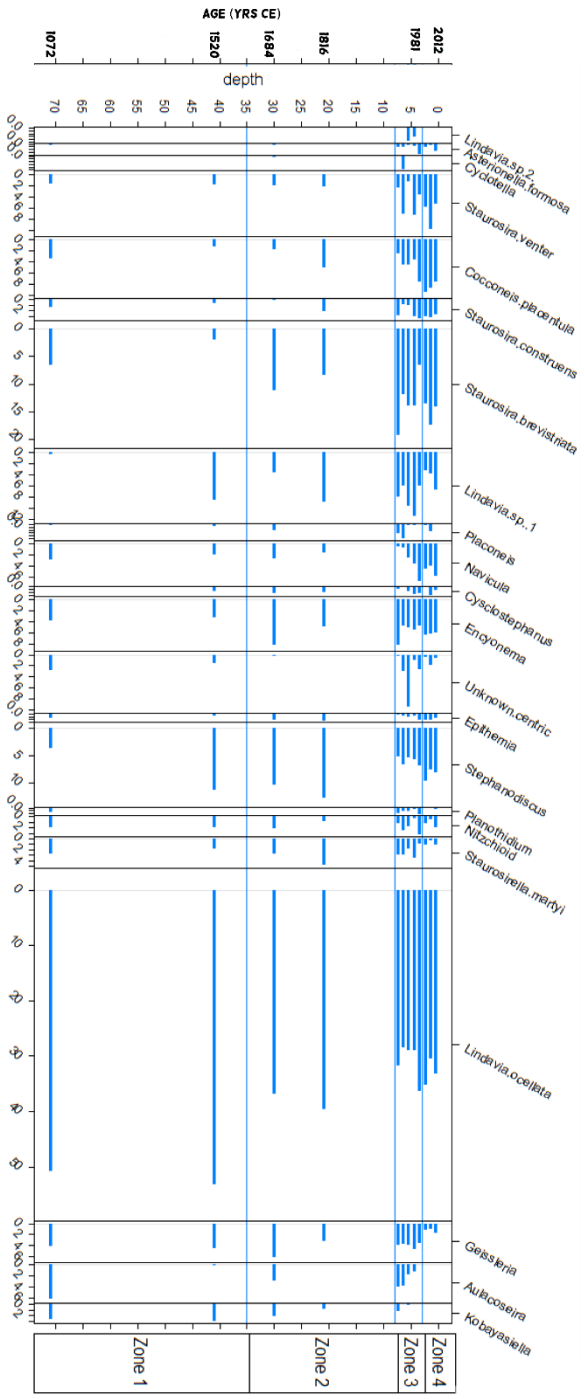

Figure 7 Zonation of most dominant taxa within selected samples. Relative abundance in percent. Blue lines across entire graph represent zonal boundaries as indicated by legend of zones.

Kobayasiella, and *Aulacoseira* only appear above zone 1. There are also greater percentages of *Staurosira venter*, *Cocconeis placentula*, *Staurosira construens*, *Staurosira brevistriata*, *Lindavia sp. 1*, and *Navicula* in the uppermost sections of the core, zones 3 and 4. In Zone 1, *Lindavia oscellata* shows a larger signal than in subsequent zones.

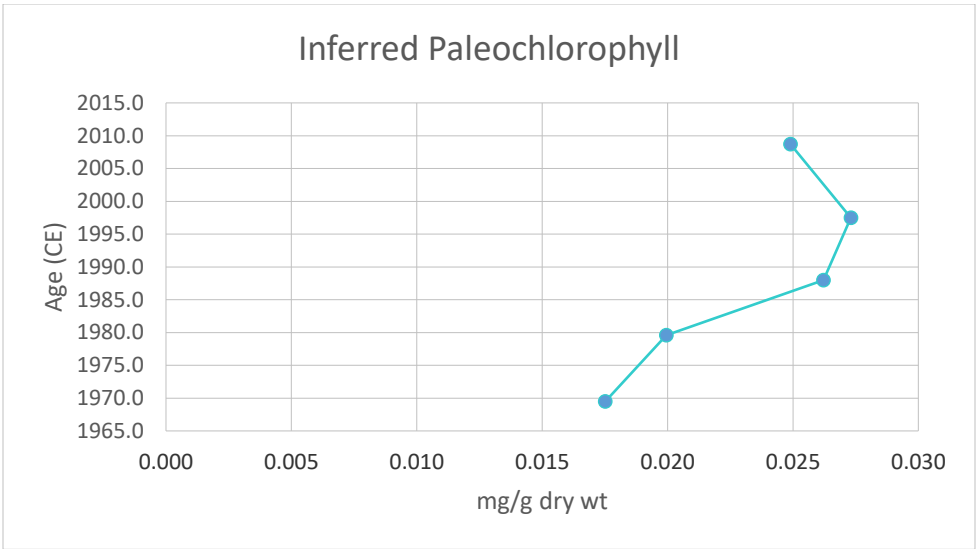

Figure 8 Graph of paleochlorophyll data for the first 4.5cm of core (courtesy of John Smol).

In addition to analyses of taxa abundance, paleochlorophyll data for the uppermost 4.5cm, consisting of samples that represent ages from ~1969 to 2012, show a shift in the amount of paleochlorophyll within the lake over time. Since 1969, the amount of paleochlorophyll by dry weight of sample has increased until approximately 1998 or 1999 after which time the amount of paleochlorophyll dropped in the topmost core sample to 0.025mg/g dry weight.

Discussion

Analyses of the diatom counts show that there appears to be a trend differentiating the uppermost samples of the core from the lower samples, indicating that the diatom record is sensitive to picking up the degradation in water quality due to anthropogenic activity. Cluster analysis in Figure 6 shows that the most significant break between data points occurs between samples 8 and 9, (=7.5cm and 20cm respectively; 48 and 196 years ago from 2012). The second most significant break occurs between samples 4 and 5, (=3.5cm and 4.5cm respectively; 23 and 31 years ago from 2012). This indicates that there is a significant variability between both the uppermost section of the core as well as between the upper 3cm of the core and subsequent samples. This distinction between the uppermost 4.5cm of the core and subsequent samples is also illustrated by the stratiplot zonations found using cluster analysis and shown in Figure 7.

As shown by the ordination plot (Figure 5), PC1 shows the most significant separation between samples, potentially indicating differences between anthropogenic activity and its influence on the sample sets. The PC2 axis also shows a significant grouping of the top 4 samples compared to the samples taken from 5.5-7.5cm. Between these samples is the sample from 4.5cm which can indicate that this is a sort of transitional phase.

Figure 7 shows a difference in the presence of such taxa as *Planothidium, Kobayasiella*, and *Aulacoseira* between the first and second zones, indicating that these taxa are the most likely influence on the separation of these two zones. Because *Kobayasiella* is a typically low-nutrient genus that is negatively affected by anthropogenic activity (Spaulding and Edlund, 2009), zone 4 is likely the most influenced

by human activities and thus can be labeled as the anthropogenic zone due to the loss of *Kobayasiella* in that zone. Zones 1-3 all show the presence of this taxa and thus have had less pollution in comparison. *Planothidium* species are tolerant of higher nutrient concentrations, further supporting the hypothesis that the uppermost 7.5cm of core, representing the lake's history since the advent of hazelnut plantations and increased human activity, shows sensitivities to anthropogenic pollution in the lake. *Aulacoseira granulata* has been found in eutrophic waters in Italy, however it is commonly considered a mesotrophic phase, thus it may represent the transitional phase between the anthropogenic zone and lower zones within the core (Atlante et. al., 2009).

The presence of *Asterionella formosa* in the zones 3 and 4 at the top of the core also indicates that there has been some kind of anthropogenic influence on the uppermost part of the core. *Asterionella formosa* is known to be a species that increases with anthropogenic lake eutrophication and is typically introduced into systems by humans (Spaulding and Edlund, 2009). Because of this, its presence in the uppermost section of the diatom data from the core indicates that there has been both human influence on the lake as well as eutrophication of Lake Vico over the past 50 years.

Additionally, there is an increase in periphyton taxa from the bottom to the top of the core and a turnover in the species of euplankton up core. Shallow periphyton species such as *Staurosira venter* and *Cocconeis placentula* associated with the top sections of the core can indicate that there are more algae at the edges of the lake because both of these species are commonly found growing entangled in aquatic algae along the shallow areas of lakes. An increase in these species suggests that perhaps a significant response to the eutrophication is the increased growth of shallow water aquatic algae. These

increases, combined with the decrease in *Kobayasiella* in the uppermost zone (zone 4) collectively reflect eutrophication during the period of increased hazelnut farming.

Supporting the hypothesis that the lake's diatoms reflect eutrophication, the paleochlorophyll record shows an increase in the levels of chlorophyll in samples from around 1970 to 1997 or 1998. Interestingly, in the most recent sample, however, it appears that paleochlorophyll levels are decreasing, indicating that the lake is potentially becoming less eutrophic and that any remediation efforts underway since the late 1990's may be having positive effects.

Conclusion

The lake core record from Lake Vico records the degraded water quality over the past 50 years due to increased anthropogenic activity in the area, particularly in the form of agricultural runoff. Analyses of the diatom record with a focus on the past 50 years shows that the lake's flora have in fact shown a significant change in diversity and abundance of specific genera and species that record the effects of eutrophication.

Such taxa as *Aulacoseira* and increases in species such as *Staurosira venter, Staurosira brevistriata,* and *Cocconeis placentula* in the samples representing the most recent sediments in the lake show that eutrophication has been taking place and is evident in the diatom record. Furthermore, the loss of *Kobayasiella* and the overall increase in periphyton up-core indicate that anthropogenic affects have significantly altered the species diversity and their distribution within the lake as well. These data are supported by the paleochlorophyll data which show increased values from the early 1970's through the late 1990's. Paleochlorophyll from the core top is lower and may indicate a recent improvement since the late 1990's and are encouraging in terms of lake management practices. The lower part of the core additionally shows significant variation, indicating that additional past factors, either anthropogenic or climatic affected the diatom population over the last millennium. However, these earlier variations were not the focus of this study, and the limitations of the age model on the core prevent any specific interpretations to be put forward.

Appendix A. R Codes for Analyses

PCA R code	Library(vegan) diatvic<- read.csv("VICDA2.csv",row.names=1) dvicrh<- decostand(diatvic, method= "total") dvicr.pca<- rda(dvicrh,scaling=3) diatvic diatvicsub <- subset(diatvic, select=(-UC)) diatvicsub2 <-diatvicsub[1:8,] diatvics <-decostand(diatvicsub, method="total") diatvic2<- decostand (diatvicsub2, method="total") diatvics.pca<- rda(diatvics, scaling=3) diatvic2.pca<- rda(diatvic2,scaling=3) par(mfrow=c(1,2)) plot(diatvics.pca,type="none",scaling=3, main="Minus UC") dvicr.sp<- scores(diatvics.pca, scaling=3, display="species") arrows(0,0, dvicr.sp[,1], dvicr.sp[,2], length=0.1, col=2) text(diatvics.pca, scaling=3, display="species", cex=.5) points(plot(diatvic2.pca,type="none",scaling=3, main="Minus 940") dvicr.sp<- scores(diatvic2.pca, scaling=3, display="species") arrows(0,0, dvicr.sp[,1], dvicr.sp[,2], length=0.1, col=2) text(diatvic2.pca, scaling=3, display="species", cex=.5)

PCA biplot and stratplot R code	#PCA biplot and stratplot VIC diatom data
	library(vegan)
	library(rioja)
	
	diatvic<- read.csv("VICD4.csv", row.names=1)
	
	#transform file with "total" transformation in decostand
	dvicrh<- decostand(diatvic, method="total")

	str(dvicrh)
	
	#run PCA
	dvicr.pca<- rda(dvicrh, scaling=3)
	dvicr.pca
	
	summary(dvicr.pca)
	#draw plot
	plot(dvicr.pca)
	
	#for a better plot with arrows
	plot(dvicr.pca, type="none", scaling=3, xlab="PC1 Eigenvalue=43%", cex.lab=1, ylab="PCA2 Eigenvalue 27%", main="VIC PCA", cex.main=1)
	dvicr.sp<- scores(dvicr.pca, scaling=3, display="species")
	arrows(0,0, dvicr.sp[,1], dvicr.sp[,2], length=0.1, col=2)
	text(dvicr.pca, scaling=3, display="species", cex=.3)
	points(dvicr.pca, scaling=3, display="sites", cex=.5, col=4)
	text(dvicr.pca, display="sites", scaling=0, cex=.5, col=1)
	#cex in points function, size of circle function of value of variable

PCA and Multiple Transfomations R code	#RUN PCA on VICO diatom counts. Several transformations are experimented with, following Legendre & Legendre, 2012, section7.7, in order to get a broken stick model test that shows significance through axis 2.
	
	library (vegan)
	diatvicr <- read.csv("VICD4.csv", row.names=1)
	diatvicr
	
	#run PCA on untransformed data
	dvicr.pca <- rda(diatvicr, scaling=3)
	dvicr.pca
	
	#transform file with Hellinger transformation in decostand
	dvicrh <- decostand(diatvicr, method="hellinger")
	str(dvicrh)

	#run PCA on hellinger transformed data
	dvicrh.pca <- rda(dvicrh, scaling=3)
	dvicrh.pca
	
	#transform file with transformation in decostand, method=total
	dvicrt <- decostand(diatvicr, method="total")
	str(dvicrt)
	
	#run PCA on transformed data, method=total
	dvicrt.pca <- rda(dvicrt, scaling=3)
	dvicrt.pca
	
	#transform file with normalized transformation in decostand
	dvicrn <- decostand(diatvicr, method="normalize")
	str(dvicrt)
	
	#run PCA on transformed data
	dvicrn.pca <- rda(dvicrn, scaling=3)
	dvicrn.pca
	
	#transform file with log transformation in decostand
	dvicrl <- decostand(diatvicr, method="log")
	str(dvicrl)
	
	#run PCA on transformed data
	dvicrl.pca <- rda(dvicrl, scaling=3)
	dvicrl.pca
	
	#summary numbers of PCAs
	summary(dvicr.pca) #PC1 54%, PC2 19%, PC3 11%
	summary(dvicrh.pca) #PC1 33%, PC2 20%, PC3 15%
	summary(dvicrt.pca) #PC1 54%, PC2 19%, PC3 11%
	summary(dvicrn.pca) #PC1 41%, PC2 25%, PC3 13%
	summary(dvicrl.pca) #PC1 34$, PC2 19%, PC3 15%
	
	par(mfrow=c(2,5))
	biplot(dvicr.pca, main="untransformed", scaling=3, ylab="PC1 54%", xlab="PC2 19%")

	biplot(dvicrh.pca, main="hellinger", scaling=3, ylab="PC1 33%", xlab="PC2 20%") biplot(dvicrt.pca, main="Total", scaling=3, ylab="PC1 54%", xlab="PC2 19%") biplot(dvicrn.pca, main="Chord", scaling=3, ylab="PC1 41%", xlab="PC2 25%") biplot(dvicrl.pca, main="Log", scaling=3, ylab="PC1 34%", xlab="PC2 19%") screeplot(dvicr.pca, bstick = TRUE, npcs = min(10, if (is.null(dvicr.pca$PCA)) dvicr.pca$PCA$rank else dvicr.pca$PCA$rank), ptype = "o", bst.lty = "solid", main="untransformed") screeplot(dvicrh.pca, bstick = TRUE, npcs = min(10, if (is.null(dvicrh.pca$PCA)) dvicrh.pca$PCA$rank else dvicrh.pca$PCA$rank), ptype = "o", bst.lty = "solid",main="Hellinger") screeplot(dvicrt.pca, bstick = TRUE, npcs = min(10, if (is.null(dvicrt.pca$PCA)) dvicrt.pca$PCA$rank else dvicrt.pca$PCA$rank), ptype = "o", bst.lty = "solid", main="Total") screeplot(dvicrn.pca, bstick = TRUE, npcs = min(10, if (is.null(dvicrn.pca$PCA)) dvicrn.pca$PCA$rank else dvicrn.pca$PCA$rank), ptype = "o", bst.lty = "solid",main="Chord") screeplot(dvicrl.pca, bstick = TRUE, npcs = min(10, if (is.null(dvicrl.pca$PCA)) dvicrl.pca$PCA$rank else dvicrl.pca$PCA$rank), ptype = "o", bst.lty = "solid",main="Log") #CONCLUSION, as described in Legendre & Legendre (2012),section 7.7, the best general transformation for these data is the Hellinger transformation, method="hellinger", because there are zeros, a short gradient, and PCA was used. The Broken stick test for the Hellinger transformation shows that both PC1 and PC2 are likely significant.
Analogue and Stratiplot R code	#1coding for the analogue package for making a graph of the data. #2. bring in data VICDA4<-read.csv("VICDA4.csv", header=TRUE, sep=,) str(VICDA4)

	age<-VICDA4[,41] age VICDA<- VICDA4[,-41] VICD<- VICDA[,-41] VICD # file without age and depth data #3. stratigraphic diagram with depth as the y axis Stratiplot(depth~.-age,chooseTaxa(VICDA4, max.abund=5), sort="wa", length=1, yticks=c(0,5,10,15,20,25,30,35,40,45,50,55,60,65,70), varTypes="relative", type=c("h", "l"))

Analogue Plot	#1 Download and then load the package "analogue" by Simpson & Oksanen (2016). You can load using the library function (as shown below), the require function, or with the package manager.
	library(analogue)
	
	#2 bring in data, make objects for x and y in Stratiplot
	VICDP <- read.csv("VICDP.csv", header=TRUE, sep=,)
	str(VICDP)
	VICD<-VICDP[,1:40]
	
	#Take out the unknowns
	VICforplot<-VICD[,-24]
	VICforplot<-VICforplot[,-21]
	VICforplot<-VICforplot[,-20]
	
	samples <-c(1,2,3,4,5,6,7,8,9,10,11,12)
	age<-VICDP[,41]
	depth<-VICDP[,42]
	
	#chooseTaxa() function in analogue allows you to subset data removing rare taxa, and minimum occurrences in samples
	VICD <- chooseTaxa(VIC, max.abun=1)
	str(VICD)
	
	#Make a stratigraphic diagram with Depth, as the y axis
	
	Stratiplot(y=depth, x=VICforplot, sort="wa", yticks=c(0,5,10,15,20,25,30,35,40,45,50,55,60,65,70), varTypes="relative", type=c("h"))

	#c1uster analyses. Perform a stratigraphically constrained cluster analysis to see how samples group over time into zones. Transform the data using a Chord transformation before running the Cluster analysis. Finally, run a broken stick on the cluster analysis to see how many clusters are significant.
	
	library(vegan)
	
	VICchord <-decostand(VICD, method="normalize")
	
	library(rioja)
	
	VIC.dist <- dist(VICchord)
	VIC.clust <-chclust(VIC.dist, method="coniss")
	par(mfrow=c(1,2))
	plot(VIC.clust)
	bstick(VIC.clust)
	
	#Remake the Stratigraphic plot adding the zones based on the cluster and broken stick results
	Zone.bound<- c(3,8,35)
	Stratiplot(y=depth, x=VICforplot, sort="wa", yticks=c(0,5,10,15,20,25,30,35,40,45,50,55,60,65,70), varTypes="relative", type=c("h"), zones=Zone.bound, drawLegend=TRUE)

Appendix B. Abbreviations for Diatom Taxa

CA	*Campylodiscus*
CY	*Cyclostephanus*
CO	*Cocconeis placentula*
GE	*Geissleria*
EN	*Encyonema*
EP	*Epithemia*
LO	*Lindavia ocellata*
LS1	*Lindavia sp.1*
SU	*Surirella*
NA	*Navicula*
PL	*Planothidium*
RH	*Rhopalodia*
UC	*Unknown centric*
STP	*Stephanodiscus*
SM	*Staurosirella martyi*
SB	*Staurosira brevistriata*
SC	*Staurosira construens*
SV	*Staurosira venter*
AS	*Asterionella Formosa*
NI	*Nitzchioid*
AU	*Aulacoseira*
KA	*Karayevia*
KO	*Kobayasiella*
CYM	*Cymbella*
GO	*Gomphonema*
CA	*Caloneis*
UB	*Unknown biraphid*
UA	*Unknown araphid*
LS2	*Lindavia sp.2*
PLAC	*Placoneis*
GYRO	*Gyrosigma*
UE	*Unknown epithemioid*
DIP	*Diploneis elliptica*
U	*Unknown*
CYS1	*Cymbella sp.1*
PS	*Psammothidium Group*
CO1	*Cocconeis sp.1*
HAL	*Halamphora*
CT	*Cyclotella*

Appendix C. Select Diatom Taxa of Lake Vico

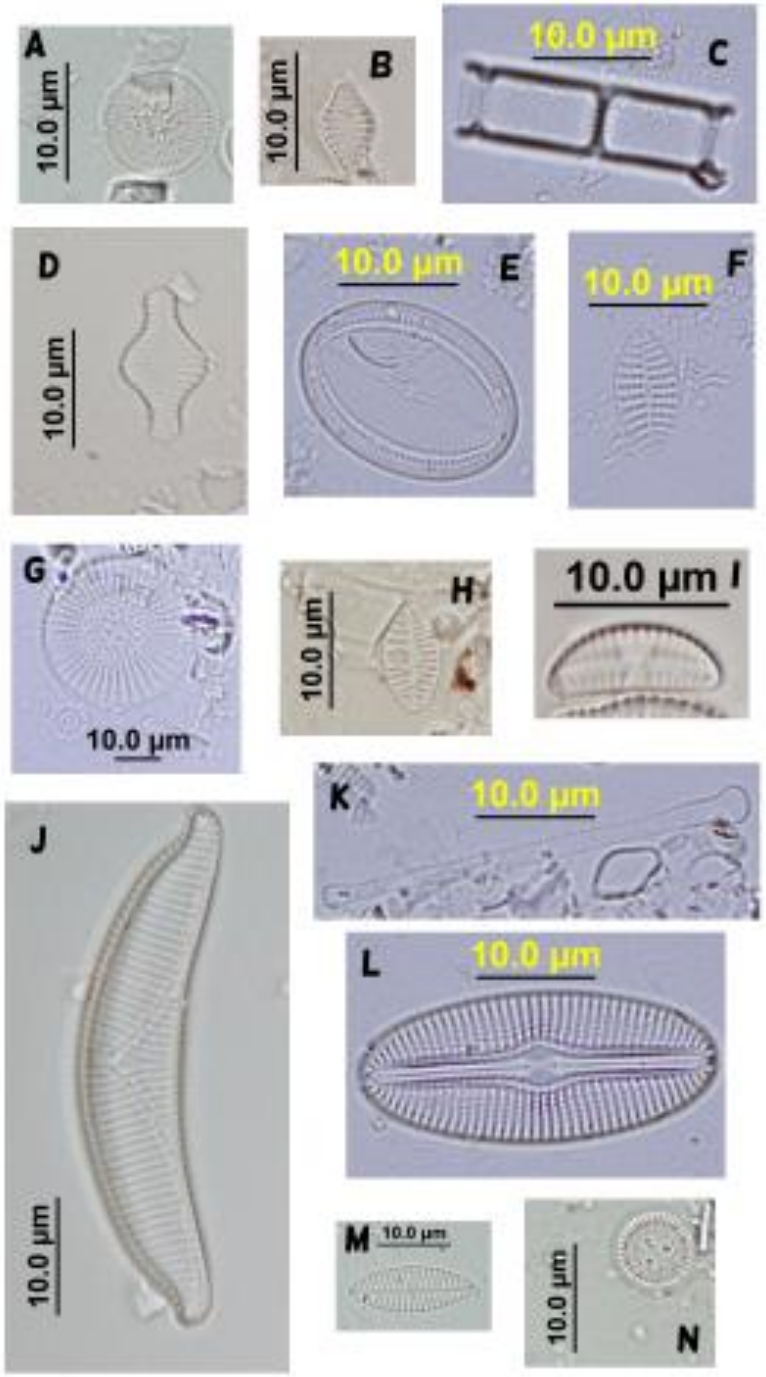

Figure 9 Select diatoms of Lake Vico. A) Stephanodiscus. B) Staurosira venter. C) Aulacoseira granulata. D) Staurosira construens. E) Cocconeis placentula. F) Karayevia. G) Cyclostephanus. H) Planothidium. I) Encyonema. J) Epithemia. K) Asterionella formosa. L) Diploneis elliptica. M) Navicula. N) Lindavia oscellata

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