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DIATOMS AS BIO-INDICATORS OF NITRATE CONCENTRATIONS IN LOCAL FRESHWATER SPRINGS

Jonathan W. Baker, Charles P. Gilman, Micah F. Morton,
Shannon V. Nayyar and Daryl R. Valley

ABSTRACT

We tested whether diatom abundance and diversity are correlated with differences in nitrate concentration among five freshwater springs in central Pennsylvania. We found no such correlations. We conclude that other environmental factors may be limiting diatom growth and diversity within the study springs.

INTRODUCTION

Ruth Patrick (1966) has shown that the growth rate of diatoms is limited by hydrogen-ion concentration, amount of sunlight, water depth, stream-flow rate, and nitrate, phosphate and iron concentrations. The purpose of this study was to examine whether nitrate concentration affects diatom abundance and diversity in freshwater springs of central Pennsylvania. If so, diatoms may be used as bio-indicators of toxic concentrations of nitrate in spring water. Nitrates and nitrites are nitrogen-oxygen chemical units which combine with various organic and inorganic compounds. Once taken into the body, nitrates are converted into nitrites. The greatest use of nitrates is as fertilizers.

Why are Nitrates/Nitrites being Regulated?

In 1974, the U.S. Congress passed the Safe Drinking Water Act. This law requires the Environmental Protection Agency (EPA) to determine safe levels of chemicals in drinking water which do or may cause health problems. These non-enforceable levels, based solely on possible health risks and exposure, are called Maximum Contaminant Level Goals. The MCLG for nitrates has been set at 10 parts per million (ppm), and for nitrites at 1 ppm, because EPA believes this level of protection would not cause any of the potential health problems described below. Based on this MCLG, EPA has set an enforceable standard called a Maximum Contaminant Level (MCL). MCLs are set as close to the MCLGs as possible, considering the ability of public water systems to detect and remove contaminants using suitable treatment technologies. The MCL for nitrates has been set at 10 ppm, and for nitrites at 1 ppm, because EPA believes, given present technology and resources, this is the lowest level to which water systems can reasonably be required to remove this contaminant should it occur in drinking water. These drinking water standards and the regulations for ensuring these standards are met, are called National Primary Drinking Water Regulations. All public water supplies must abide by these regulations.

What are the Health Effects?

Over the short-term, excessive levels of nitrate in drinking water have caused serious illness and sometimes death. The serious illness in infants is due to the conversion of nitrate to nitrite by the body, which can interfere with the oxygen-carrying capacity of the blood. This can be an acute condition in which health deteriorates rapidly over a period of days. Symptoms include shortness of breath and blueness of the skin. Over the long-term, nitrates and nitrites have the potential to cause the following effects from a lifetime exposure at levels above the MCL: diuresis, increased starchy deposits and hemorrhaging of the spleen.

How much Nitrates/Nitrites are Produced and Released to the Environment?

Most nitrogenous materials in natural waters tend to be converted to nitrate, so all sources of combined nitrogen, particularly organic nitrogen and ammonia should be considered as potential nitrate sources. Primary sources of organic nitrates include human sewage and livestock manure, especially from feeding. The primary inorganic nitrates which may contaminate drinking water are potassium nitrate and ammonium nitrate, both of which are widely used as fertilizers.

FIELD SITES

Kanesatake Spring

Kanesatake spring is located on the grounds of a summer church camp. It runs parallel to Spruce Creek, eventually meeting it at a point three feet beyond the area of sampling. Spruce creek is about 10 feet in width. There are many trees along Spruce Creek, but their shade does not extend to the spring. The spring flows out from the ground and forms a wetland area. The amount of plant coverage exposes the spring water to decaying plant material. Chemical tests showed that the pH was 7.29, and the average nitrate concentration was 7.5 ppm. During the study period, the spring had depths from one to four inches with average depth of 3.2 inches. Two slides used for diatom colonization were attached to a stick placed at a depth of 1.5 inches and where the flow rate was 0.294 m/s.

Emma Spring

Emma spring is surrounded by farm land. It flows out from rocks at the bottom of a steep sloped hill. The stream then runs under and along a road. We placed two sample slides near the spring source at a depth of 5 inches and a flow rate of .3125 m/s. Another two slides were placed about 50 yards downstream at a depth of 3 inches and a flow rate of 0.350 m/s. There were no trees or brush to shade the area where the slides were placed, though many fallen leaves were present. There were no living macrophytes in the stream. We found a frog at the mouth of the spring, and amphipods (*Gammarus minus)* were also abundant in the spring. The average pH of the spring was 6.93, and the average nitrate concentration was 0.733 ppm.

Ell Spring

Ell spring is one of the One-Hundred Springs. It is located in a forested area, but during the study period the winter exfoliated trees provided little shade. Ell Spring starts from the bottom of a hill, and has two main points where water flows from, forming an "L" shape. The two streams join to form one. The average depth was 4.57 inches. We placed slides in each beginning segment of the spring. The first stick was fitted with two slides and placed at a depth of 4 inches. The second, also fitted with two slides, was placed at a depth of 3 inches. Watercress, watercress snails (*Fontigens nickliniana*) and amphipods (*G. minus)* were abundant. The average pH was 7.51, and the average nitrate concentration was 0.44 ppm.

Petersburg Spring

Petersburg spring is a pond fed by a short brook. One slide was placed at a depth of 4.75 inches near the spring source where the flow rate was 0.333 m/s. Another slide was placed in the pond. The area was slightly shaded, but there was no shade where we placed the slides. Amphipods (*G. minus*) were numerous. The average pH was 6.94, and the average nitrate concentration was 0.29 ppm.

Blue Spring

Blue spring's source is located above a farm. A pond is formed directly after the source, then continues as a stream. We placed slides in one spot further down the stream past the bridge. There are a few trees along the stream's banks, but not enough to shade the area where the slides were placed. The dominant macroinvertebrates were isopods (*Lirceus brachyurus*); and amphipods (*G. minus*), snails, pea clams and caddisfly larvae were also numerous. The slides were placed at a depth of 16.5 inches with a flow rate of 0.345 m/s. The average pH was 6.86, and the average nitrate concentration was 0.29 ppm.

METHODS AND MATERIALS

We tested whether diatom growth (colonization rate, species diversity, and relative abundance) is related to a single limiting factor (nitrate concentrations) by attempting to keep other factors as constant as possible. We chose our study springs based on data on nitrate concentrations given by Glazier, Horne aand Lehman (1992) (see Table 5). Using a Hach kit, we estimated nitrate concentrations at these sites on the same day that the diatom samples from the springs were taken. We also measured pH using a Fisher Scientific ® Accumet pH Meter 915.

Using a float, meter stick, and timer we calculated the flow rate at each study site to find a relatively consistent flow rate across the study springs (2.9 seconds per meter, see Fig. 2 and Table 1). We set up the apparatus for sampling in the following manner: we obtained 10 sturdy, stationary sticks, upon which we placed microscope slides. The slides were attached to the sticks through the use of ties and wedging material. The stationary sticks, along with the attached slides were driven into the spring beds by the use of a sledge hammer. The slides were positioned either with or against the current, to evaluate whether there would be a difference in colonization of the diatoms in either case.

At study sites where there was an option between sunlit vs. shaded areas, we placed the slides in the sunlit areas. We predicted that we would find the diatom genera *Nitzschia* and *Pseudonitzschia* within the study springs. These diatoms are recently evolved forms with rapid growth rates of two individuals per day. They are classified as pennate forms which are elongated and bilaterally symmetry. They contain accessory pigments such as chlorophyll and fucoxanthin.

After a period of two weeks, we went back to each study spring and collected the slides. In the laboratory the coverslips were glued with Permount to the slides, making them permanent. A differential interference microscope was used to estimate relative abundance of each genus of diatoms found on each slide. This was difficult because the Permount caused the majority of the diatom colonies to slide ("surf") to the edges of the cover-slip.

Simpson's Diversity and Equitability Indexes were calculated for each spring, as shown in Tables 2 and 3. This was done to obtain some relative information as to how diverse the genera of diatoms were within each spring and how evenly distributed each genus was within that particular biome (study spring). We also constructed rank-abundance graphs for each of the study springs to gain insight into what might have been occurring within each of the diatom communities.

RESULTS

Nitrate concentrations in the study springs decreased in the order of Kanasatake, Emma, Ell, Petersburg, and Blue (Fig. 1). As intended, pH and flow rate were similar among all of the sampling sites (Fig. 2, Table 1).

Figure 1.

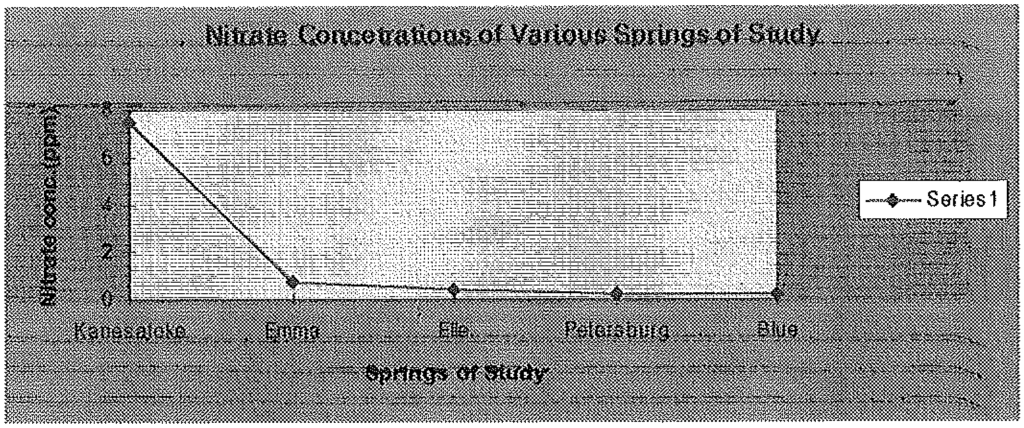


Figure 2.

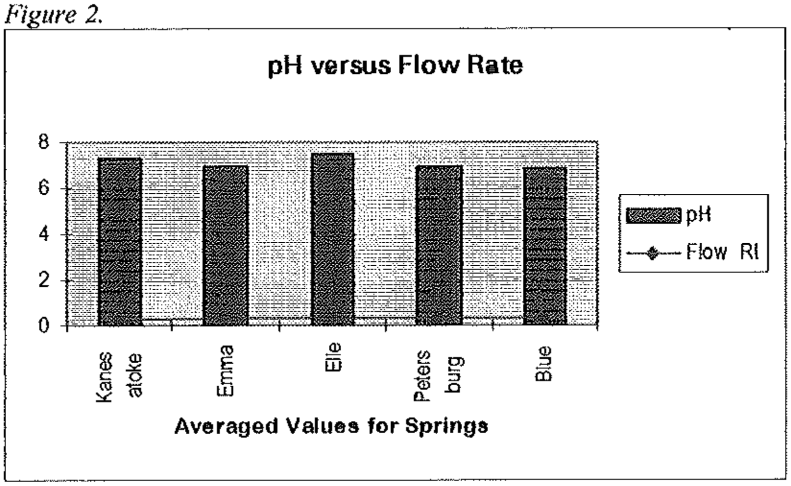


Table 1

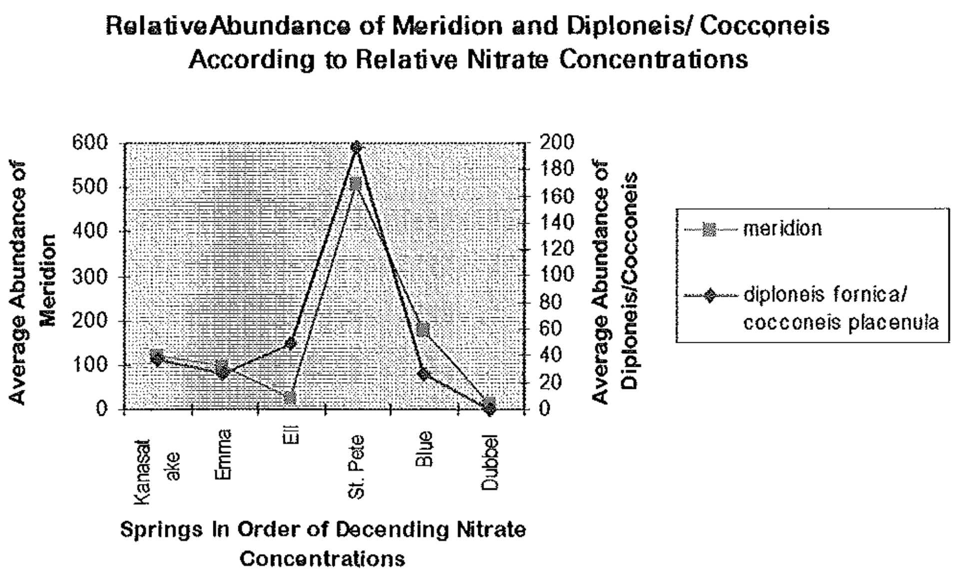
	Kanesatoke	Emma	Elle	Petersburg	Blue
pH	7.3	6.93	7.51	6.94	6.86
Flow rate	0.294	0.3125	0.333	0.345	0.345

Diatom genera and their abundance in the five study springs are listed in Table 2, in order of decreasing nitrate concentrations. Dubbel spring is also included, though no data on nitrate concentration were available. Only the genera *Meridion* and *Cocconeis/Diploneis* occurred in all springs. Their relative abundance is shown in Fig. 3.

Table 2

	Kanasatake	Emma	Ell	Petersburg	Blue	Dubbel
Pop Var	1872.389	1024.785	255.375	30891.8	2966.606	124.9219
Average nitrate concentrations	7.5	0.733333	0.44	0.29	0.29	
	Kanasatake	Emma	Ell	Petersbu rg	Blue	Dubbel
Meridion	123	97	28.5	509	180.5	13
Diploneis fornica/ Cocconeis placenula	37.5	27.5	50	197.3	27.5	
Navicula	26	49	5	63	32.75	
Eunotia					1	1
Diatoma		42.5	24.5	79.7	8	32
Climacosphia moniligera				9.3	7.5	
Stephanodiscus					1	
Gomphonema geminatum		5			100.3	
Nitzschia					2.25	
Synedra		1.5		1	1	
Stauroneis					1	
Desmid-*Cosmarium*						11.5

Figure 3.



Simpson' Diversity Index (D), equitability (E), and the sums of each spring's P_i^2 are given in Table 3. Fig. 4 graphs the E values for each spring in order of descending nitrate concentrations. It shows that the equitability of the diatom community was highest in Kanesatake, Emma and Ell springs, which had the the highest nitrate concentrations. In contrast, The diatom community in Kanasatake spring had the lowest Simpson's Diversity Index (Fig. 5). Figs. 6-11 depict are rank-abundance curves for each study spring.

Table 3

	D	E	∑Pi^2
Kanasatake	2.021	0.6737	0.4948
Emma	3.438	0.573	0.29087
Ell	2.9623	0.74058	0.33757
Pete	2.3941	0.39902	0.41769
Blue	2.9526	0.2684	0.3387
Dubbel	2.51	0.6275	0.3984

Figure 4.

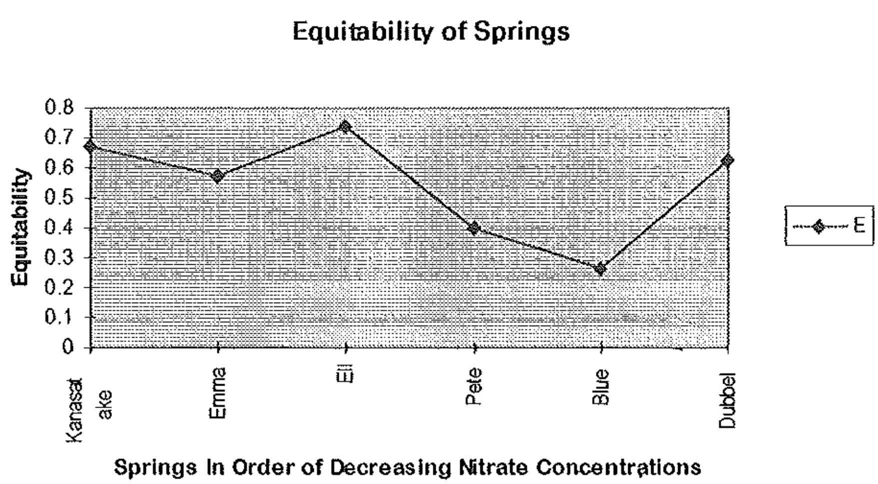
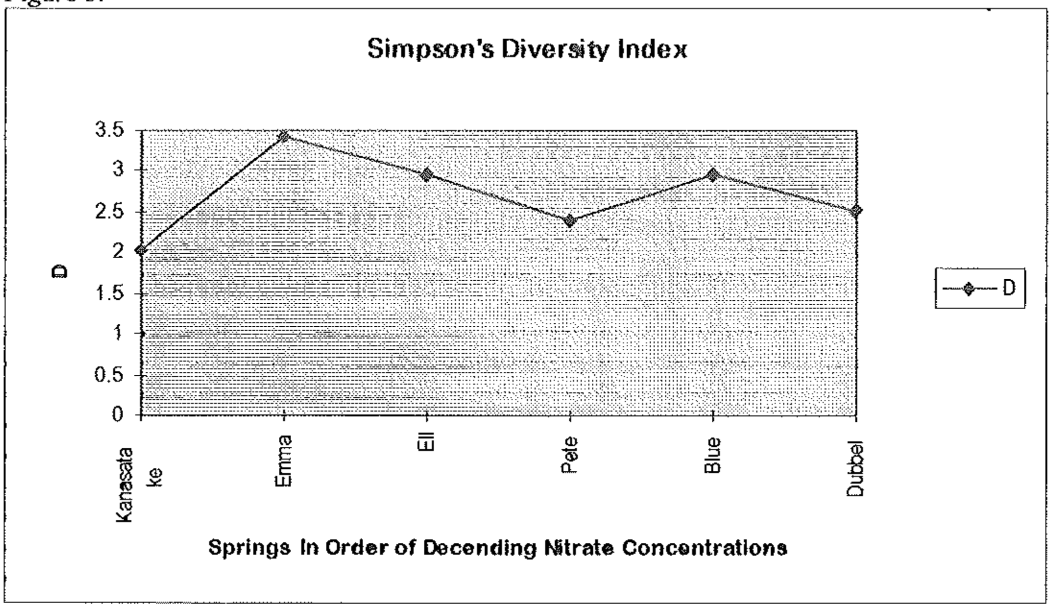


Figure 5.



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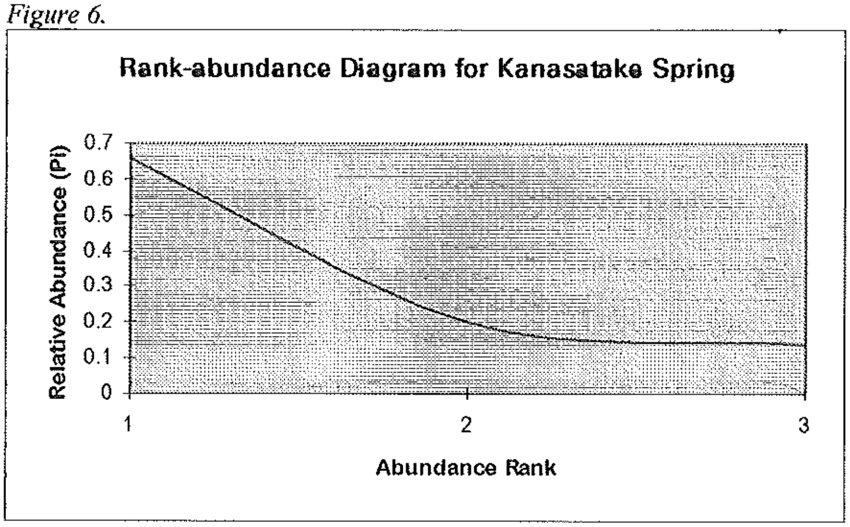

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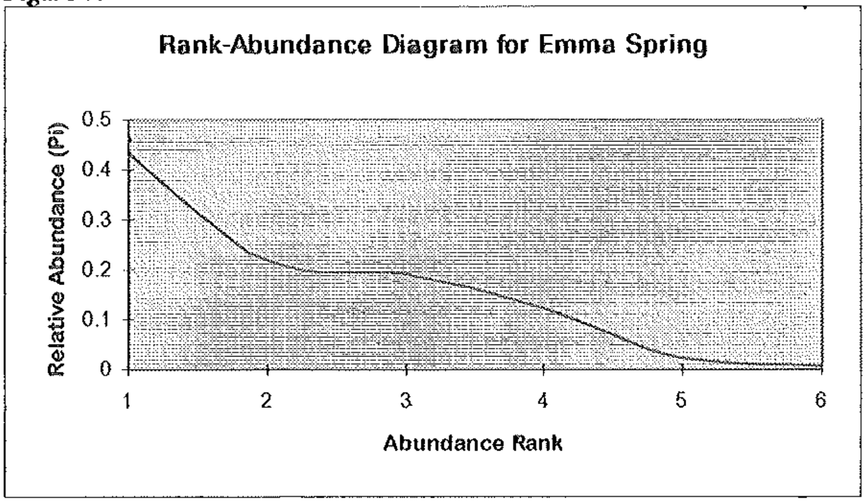

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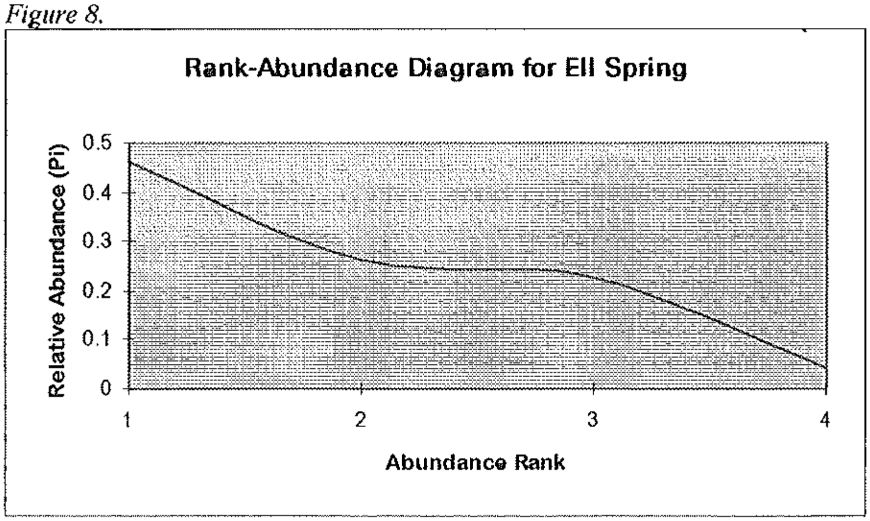


Figure 9.

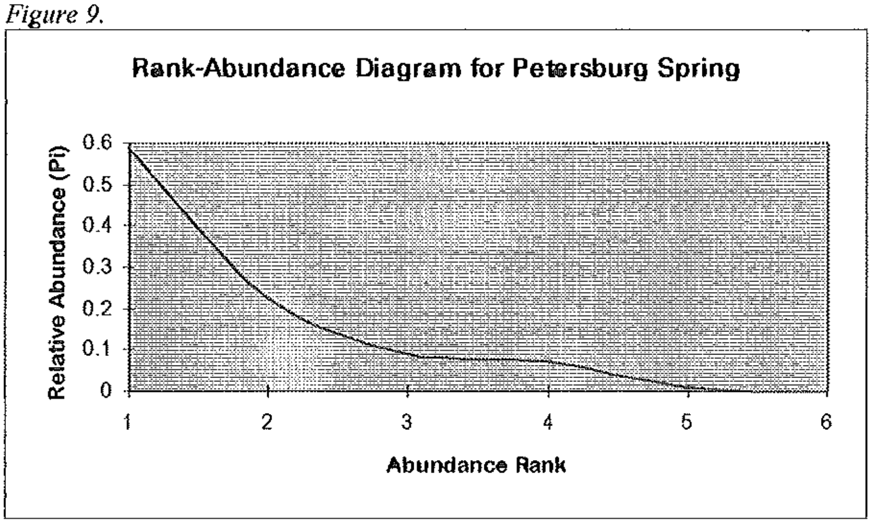


Figure 10.

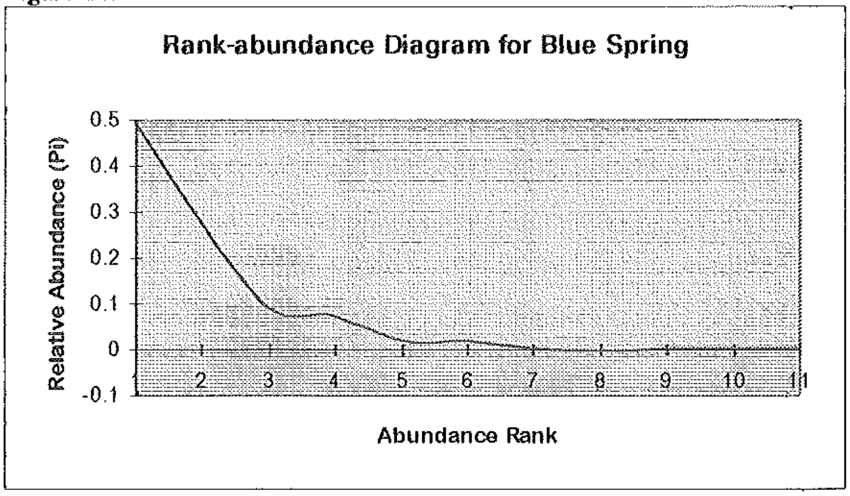
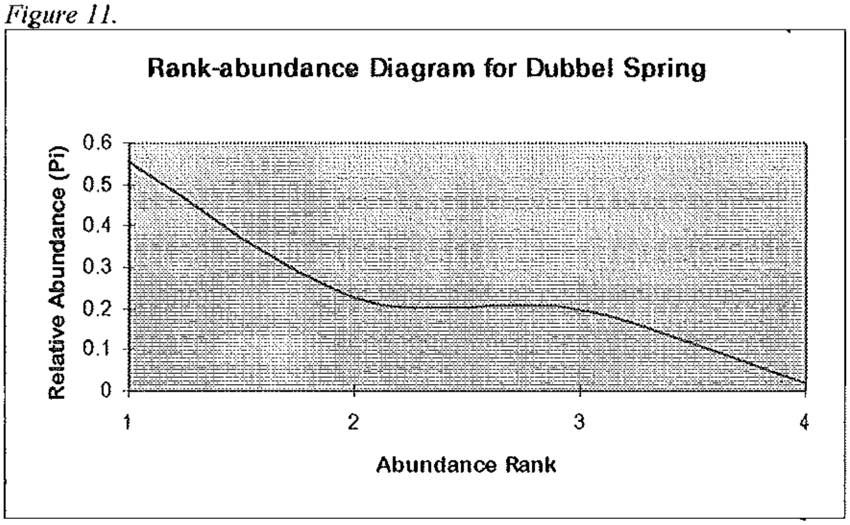


Figure 11.



DISCUSSION

Our original hypothesis was that we would find a correlation between the relative abundance of diatoms and the nitrate concentrations among our study springs. Errors made in making permanent slides of the diatom samples led to a change in plans. We decided to look at the diversity of diatom genera on the individual slides. By looking at the slides, we obtained a relative ratio of the different types of diatom genera found within each spring (note: species classification was not undertaken because of lack of suitable keys).

The nitrate concentrations determined in this study during the winter were compared with those carried out by Glazier et al. (1992) during the summer (see Table 5). Kanasatake and Emma springs appeared to have somewhat higher nitrate levels in the summer than in the winter, though not enough data were available to statistically test this result. Perhaps nitrate inputs to these springs were higher in the summer because of enhanced microbial activity in the marsh surrounding Kanasatake spring, and because of increased use of fertilizers on farmland surrounding Emma spring. However, the nitrate levels in the other three springs did not appear to differ much seasonally. In any case, Kanasatake spring clearly had the highest nitrate levels of all of the springs studied.

Table 5. Nitrate concentrations of study springs during the summer of 1992 (Glazier et al., 1992) and the winter of 1998 (this study).

Spring of Study	Summer '92, $[NO_3]$(ppm)	Winter '98, $[NO_3]$(ppm)
Kanasatake	*8.4*	*7.5*
Emma	*3.7*	*0.73*
Ell	*0.1-0.2*	*0.44*
Petersburg	*less than 0.1*	*0.29*
Blue	*0.3*	*0.29*

It may be no coincidence that the fewest diatom genera were sampled in Kanasatake spring. The sample slides from this spring also exhibited the lowest Simpson's diversity index (Tables 3 and 6, Fig. 5). Three genera of diatoms were identified in Kanasatake. All of the other springs, with relatively low nitrate concentrations, contained at least four or more different genera of diatoms. In addition, the equitability of the diatom community in Kanasatake spring was exceeded only by that in Ell spring (Tables 3 and 6, Fig. 4). This value is a measure of how evenly distributed the diversity of the community is among all the involved genera. Although no significant correlations were found between these community desriptors and nitrate concentration, these data suggest that such descriptors may prove to be useful as indicators of nitrate pollution. Perhaps high nitrate levels, as found in Kanasatake spring, suppress the species diversity of diatom communities.

Table 4. Comparisons of diatom-community patterns in Kanasatake spring with those in other springs with lower nitrate concentrations.

	Kana s	vs	Emma	Kanas	vs	Peters	Kanas	vs	Blue	Kanas	vs	Ell
D	2.021	<	3.438	2.021	<	2.394	2.021	<	2.953	2.021	<	2.962
E	.6737	>	.573	.6737	>	.3990	.6737	>	.2684	.6737	<	.7406
$[NO_3]$	7.5	>	.733	7.5	>	0.29	7.5	>	0.29	7.5	>	0.44

D: Simpsons Diversity Index, E: Equitability Index, $[NO_3]$: nitrate concentration in ppm.

A different picture is revealed if the abundance of specific genera is examined. For example, *Meridion* and *Diploneis/Cocconeis*, the only genera that occurred in all of the springs, were most abundant in Blue spring, which has an intermediate nitrate concentration (Fig. 3). Perhaps Shelford's Law of Tolerance can help explain these results.

In conclusion, no significant effects of nitrate concentration were observed on diatom communities in our study springs. Perhaps other nutrients such as silicon and phosphorus, which are known to be essential for diatom growth, are more important. Future work should consider these and other factors as potential determinants of diatom abundance and diversity in freshwater springs. It is unlikely that a single-factor analysis, as done in this report, will be sufficient.

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P-funk and the heavenly father who has given us all this wondrous beauty and inspired us to explore the natural world in which we live our daily lives. THANKYOU for making this possible.

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