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ABSTRACT

In the summer of 2000, diatom communities were sampled from three stream sites in the Upper Mill Creek watershed using three different sampling methods. Diatoms were collected from natural rock substrata, the phytoplankton in the water column and from ceramic tiles placed in the stream. Water chemistry and physical parameters were also measured at each sampling site on each of the three dates. Each sample site was of a different water quality, resulting from various sources of pollution.

Diatom communities were analyzed using similarity and diversity indices and comparisons were also made based on community composition. Differences between sites and differences between sampling methods were tested using analysis of variance (ANOVA). Greater differences in the community were observed between method of sampling rather than between sampling sites. In addition, pollution tolerant taxa were observed at all three sites, indicating that each site was impacted by pollution to an extent.

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INTRODUCTION

Water Quality Assessment

The assessment of water quality has become more important in the last few decades as a result of the environmental movement and laws supporting clean water. It has become especially important to communicate water quality to the general public, who can be adversely affected by polluted water, and to lawmakers, who create and implement laws that manage water resources (Couillard and Lefebvre, 1985). The collection of chemical and biological data is the usual method for water quality assessment under the Water Pollution Control Act of 1972. Together, biological and chemical data explain more about the environment than one parameter alone. The Ohio EPA has done much to codify biological indices (IBI, ICI, QHEI) over the past 15 years using fish and macroinvertebrates. However, determining an ideal and comprehensive method of water quality assessment would best include diverse autotrophic organisms, such as diatoms.

Flowing water is especially difficult to assess as it has the potential to change. Chemical stressors and organisms may enter the system at any point and then move downstream. Water quality in rivers and streams may be expressed by the biological integrity of the system (Hill et al, 2003). Biological integrity is defined as "the capability of supporting and maintaining a balanced, integrated, adaptive community of organisms having a species composition, diversity, and functional organization comparable to that of natural habitat of the region" (Angermeier and Karr, 1994). Measuring the chemical composition of water only provides a "snapshot" in time of the water quality (Pan et al,

1996; Resh et al, 1996; Hill et al, 2000). Instead of this "snapshot" in time, a more time integrative measurement of water quality must be obtained for accurate assessment. By examining the biological integrity of sessile organisms in a water body, one is able to determine the quality of water based on the biodiversity, trophic structure and the indicator organisms that the stream supports. Most organisms have specific habitat requirements and thus are able to only live within a range of environmental conditions. In addition, organisms present in a stream can be a result of the previous two to three weeks of environmental conditions (Pan et al., 1996; Allen et al., 1977). Knowing the habitat specificity of a particular organism can thus provide information about the habitat in which that organism is found. The US Clean Water Act (1977) requires two organismal systems to be monitored, usually fish and macroinvertebrates. Good biomonitoring systems must be biodiverse, have a wide environmental range and have specific growth requirements. Algae, specifically diatoms, have these characteristics as biomonitors. For instance, if a particular diatom can only live in eutrophic conditions, and that particular diatom is found living in a certain section of a stream, one would be able to deduce that that section of the stream is nutrient rich.

By knowing the habitat requirements of a particular organism, we know much about the stream. By sampling the diatoms of Mill Creek, this study sought insight into the factors (water chemistry, flooding and stream habitat quality) which determine the diatom communities.

Background on Diatoms

Diatoms belong to the Kingdom Protista, Division Bacillariophyta. They are unicellular and their cell walls are made of silica. They can be found anywhere there is moisture, land or water, freshwater or saltwater, and there must be enough light for them to carry out photosynthesis. Diatoms usually have a single nucleus with one or more nucleoli inside. They also have cell membranes and a centrosome, which contains chloroplasts, mitochondria and storage bodies. Most diatoms are autotrophic, but some are heterotrophic. When the conditions are right (temperature, light, and nutrients) diatoms undergo cell division. The rate of cell division varies greatly among species. Some species may divide as many as eight times in one day, whereas other species do not divide for weeks (Patrick and Reimer, 1966).

A typical diatom cell or frustule consists of two halves called valves that are connected together by a girdle. The girdle is a circular piece of silica. One valve fits over the other valve much like the top and bottom of a petri dish. Because the valve section of the diatom is generally wider than the girdle, diatoms are usually viewed under a microscope in valve view (as if looking down onto the lid of the petri dish). Thus, most diatoms are described and identified via the appearance of their valve. Diatoms that have radially symmetric valves are centric and diatoms that have more linear shaped valves are pennate. The valve surface of some diatoms may consist of tiny openings in the cell wall called pores. Pores or puncta appear as dots on the valve surface and sometimes look like lines called striae when they are very close together. Thickened lines called ribs can also be present on the valve surface, as well as other types of extensions and spines (Patrick and Reimer, 1966).

Some diatom species also have a raphe. The raphe is a slit in the surface of the valve along which protoplasm moves giving the individual mobility. Certain genera of diatoms, such as *Nitzschia*, *Hantzschia* and *Bacillaria*, have a canal on the surface of the valve and the raphe is located in this canal, called a keel. Other genera have a raphe but do not have a keel, such as *Navicula*, *Gomphonema*, *Amphora* and *Cymbella*. Only diatoms with a raphe are motile. External movement occurs by rotation of the cytoplasm in the raphe and the shape of the raphe determines the path of movement: for example, straight movement or curved movement (Patrick and Reimer, 1966).

All diatoms reproduce asexually via mitosis in a process called vegetative reproduction. Diatoms have the ability to reproduce sexually by the formation of an auxospore, which begins as a zygote. Though, this type of reproduction must occur when the cells of a particular species are of a certain minimum size. If it does not occur when the cells are the correct size for that taxon, auxospore formation will never occur for that cell (Patrick and Reimer, 1966).

On the outside surface of the cell wall, all diatoms are covered with a thin layer of mucilaginous substance that allows diatoms to adhere to substrates. Diatoms are able to attach and glide across substrata by secreting a sticky mucilage made up of extracellular polymeric substances (EPS). The EPS is composed mainly of acidic polysaccharides (Lind et al., 1997). Certain species of diatoms, such as *Gomphonema, Achnanthes, Cocconeis* and *Cymbella*, develop various types of hold-fasts such as gelatinous pads, gelatinous tubes, and gelatinous stalks which assist in adhering to substrata located in swift currents (Patrick and Reimer, 1966).

Diatoms and Water Quality

The impact of pollution and other stresses on an ecosystem has often been assessed from indicator organisms (Washington, 1984). Many states have implemented such protocols using fish and macroinvertebrates in order to meet the US EPA mandate that they establish biologically based water quality criteria (US EPA, 1990; Plafkin et al, 1989). However primary producers, such as algae may be more rapidly responsive to changes in water quality than groups higher up in the food chain. For flowing systems, they may be a more appropriate and sensitive indicator (Dixit et al., 1992).

Like any good indicator organism, diatoms have been shown to respond to numerous factors including conductivity, dissolved oxygen (Stevenson and White, 1995), turbidity (Leland, 1995; Pan et al., 1996), temperature, nitrogen concentration and phosphorus concentration (Hill and Knight, 1988; Keithan et al., 1988; Miller et al., 1992; Pan et al., 1996). In addition to quick response to environmental changes (Triest et al., 2001; Rott et al., 1998), diatoms grow wherever light is present (Allen, 1995; Patrick and Reimer, 1966), they are easy to collect and preserve, and they are distributed all over the world (Patrick and Reimer, 1966). Diatoms are easy to count because they are mostly unicellular (Patrick and Reimer, 1966) and since they reproduce mostly asexually, species identification is based on physical characteristics of the siliceous valves rather than sexual stages (OCC, 1993), as required in many soft algae. The plethora of diatom identification guides makes species identification workable, once mastered (Patrick and Reimer, 1966,1975; Collins and Kalinsky, 1977; Krammer and Lange-Bertalot, 1986, 1988, 1991a,b; Biggs and Kilroy, 2000).

Diatoms as a group have a wide range of environmental tolerances, but as individuals they have strict habitat requirements (Dixit et al., 1992). The genera *Amphora*, *Cocconeis*, *Cymbella*, *Fragillaria*, *Gomphonema*, *Melosira*, *Navicula*, *Nitzschia*, *Rhoicosphenia* and *Synedra* contain many species that are found mostly in eutrophic water (Patrick and Reimer, 1966; Hill et al., 2000). *Achnanthes minutissima*, *Navicula cryptotenella* and *Fragilaria ulna* are also common eutraphenetic species (Rott et al., 1998). Some diatoms are also able to obtain their nitrogen from sources of ammonia (Patrick and Reimer, 1966). Species such as *Cocconeis pediculus*, *Navicula cryptocephala*, *Nitzschia inconspicua* and *Rhoichospenia curvata* are often found living in areas of alkaline, eutrophic waters (Descy, 1979).

Gomphonema parvulum, *Navicula lanceolata*, *Navicula viridula*, *Nitzschia palea* and *Surirella ovata* prefer high levels of nitrogen (Rott et al., 1998; Patrick and Reimer, 1966); whereas *Navicula tripunctata* and *Navicula cryptotenella* prefer more moderate concentrations of nitrate (Rott et al., 1998).

Other environmental conditions are also accounted for by species with known affiliations for such conditions. *Cyclotella meneghiniana*, *Cocconeis pediculus*, *Cocconeis placentula* prefer conditions of high temperatures, high levels of phosphorus and high levels of turbidity (Rott et al., 1998). Pan et al. (1996) found *Nitzschia dissipata*, *Navicula menisculus*, *Achnanthes lanceolata*, *Navicula minima* abundant in stream sites with low turbidity (2-10 NTU), whereas *Navicula trivialis*, *Gomphonema angustatum*, *Surirella minuta* were present in stream sites with high turbidity (13-20 NTU).

There is also a direct relationship between the abundance of motile taxa (*Caloneis*, *Diploneis*, *Frustulia*, *Gyrosigma*, *Navicula*, *Nitzschia*, *Pinnularia*, *Surirella*) (Hill et al., 2000) and the amount of siltation in the stream (Bahls 1993; Mills et al 1993; Stevenson and Pan, 1999). As siltation increases, the percentage of motile diatoms increases (Hill et al., 2000) as they migrate toward light.

A major component of any method of water assessment based on diatoms is the scale of saprobity, or the effects of organic pollution (Descy, 1979). Palmer (1969) developed a system for rating organic pollution based on a compilation of tolerant algae because organic pollution tended to influence algal communities more so than other environmental factors such as light, pH, DO, current flow and temperature. The top diatom genera found to tolerate organic pollution were *Nitzschia* and *Navicula* (Palmer 1969). In particular, *Nitzschia palea, Nitzschia dissipata, Navicula cryptocephala* and *Navicula viridula* were the most pollution tolerant species of these genera (Palmer 1969). *Fragilaria ulna* and *Fragilaria capucina* tolerate more than moderate pollution (Rott et al., 1998).

Another method for diatom based water quality assessment relies on the estimation of community parameters, including diversity, evenness, richness and similarity (Hill et al., 2000). Examining community structure based on similarity and diversity indices has been a common method for quantifying changes in many ecosystems (Washington, 1984). The assumption is that the more diverse the community, the healthier the ecosystem (Hill et al., 2000). Indices based on such measurements have been useful in monitoring the impact of point-source pollution (Freidrich et al., 1992).

Diatoms have long been associated with water quality monitoring, but more recently they have been incorporated into multimetric indices designed for water quality assessment (Kentucky Division of Water, 1993; Bahls, 1993; OCC, 1993; Hill et al., 1998). These indices produce a simplified score based on a multitude of data such as stream chemistry, stream physiology, and stream biota, from which water quality can be determined (Coulliard and Lefebvre, 1985; Barbour et al, 1995).

Diatom communities are also influenced by available habitat, for example, planktonic versus periphytic species. Planktonic species are abundant only in areas of the stream where the current is very slow or absent all together (Patrick and Reimer, 1966). Periphytic species are able to inhabit areas of swifter currents, especially diatoms that produce gelatinous stalks for substrata adherence. Among periphytic species, there are variations among substrata type. Variations in diatom flora have been observed between epilithic (on rocks), epiphytic (on plants), epipelic (on silt) and episammic (on sand) communities (Kelly et al., 1998). Sampling diatoms attached to aquatic plants involves special precautions. Depending on whether macrophytes are submerged or emergent or what taxa the macrophyte is, being careful is key for collecting diatom material off of the macrophyte without dislodging the plant or scraping off part of the plant (Kelly et al., 1998). In addition, sampling epipelic and episammic communities is not recommended because of the nutrients retained in silt which favor eutrophic taxa and the distinct flora that thrives on sand (Kelly et al., 1998). The use of hard surfaces is preferred over aquatic plants and sediments (Kelly et al., 1998). Moreover, most of the currently accepted methods for evaluating or monitoring water quality that involve the use of diatoms focus on the epilithic diatom community (Winter and Duthie 2000).

Diatom Sampling Methods

Obtaining a periphyton sample can be accomplished with various methods that reveal differences in colonization and succession. Harvesting natural substrata by scrubbing rocks or wood or brushing leaves of macrophytes or sampling stream sediments (Kelly et al., 1998; Winter et al., 2000) are not always feasible in large, deep rivers and lakes because natural substrata are sparse (Biggs and Kilroy, 2000) or substrata are too deep for light to reach. In smaller, shallow streams it is possible to use the natural substrata for sampling periphyton. Not only the type of substratum present affects diatom assemblages but also the water quality, water depth, water current, and riparian cover at each sampling site. If the substratum varies from site to site, then the diatom assemblage may be affected by the change in substratum, not just by a change in water quality. Perhaps it is necessary then to sample the "substratum that best reflects the typology of the river" in order to obtain an accurate comparison (Kelly et al., 1998). In attempts to examine the algal population of rivers and lakes, some researchers have turned to artificial substrata as a means of sampling periphyton to avoid the shortcomings natural substrate pose for substrate uniformity and quantitative analysis (Barbiero, 2000; Chessman, 1985; Moschini-Carlos et al., 2000; Triest et al., 2001). Chessman (1985) suggests periphyton responses to water quality variations are best measured via the use of a standardized artificial substratum.

In addition to the lack of attainable natural substrata in large water bodies, it is often difficult to quantify the sample area of natural substrates, such as rocks, wood or plants due to the lack of a smooth flat surface. Moreover, researchers cannot control the

location of natural substrata or the growth time of the algal community present on natural substrata (Barbiero, 2000). As a result, artificial substrata are used because they offer more control in experiments, such as the sample location, the exact age of the algal community, and an exact area of measurement, which is required for quantitative analysis (Weitzel et al., 1979; Barbiero, 2000).

Many different forms of artificial substrata have been employed for algal sampling. Glass slides have been a popular form of artificial sampling (Barbiero, 2000; Triest et al., 2001; Patrick, 1971; Hudon and Legendre, 1987). The standard method using glass slides is known as the Catherwood diatometer (Patrick et al., 1954). This method is useful for collecting benthic and planktonic diatoms (Patrick and Reimer, 1966). Other methods of artificial substrata for diatom sampling include plexiglass discs, polyurethane foam, (Snoeijs and Simenstad, 1995; Goldsmith, 1997), clay tiles (Peterson et al., 1989; McCormick and Stevenson, 1991; Lowe et al., 1996), ceramic panels (Leskinen et al., 1988), and aluminum. Unfortunately, artificial substrata are often disturbed by vandalism or washed out by floods (Biggs et al., 2000). In this study, the first attempt at sampling employed glass slides, but these were all lost during a summer rainstorm. Thus, small ceramic tiles attached to bricks were used as the artificial substrate.

The use of artificial substrata provides researchers with control over where the substrate will be placed, hence where the samples will be collected and the exact growth time allotted for the diatom community (Weitzel et al., 1979). Choosing artificial substrata also allows for greater reproducibility as well as precise measurements of the surface area in which the colony grows (Biggs et al., 2000). However, it is important that

the artificial substrate be comparable to the natural substrate of the water body if the researcher aims to procure an accurate account of the natural population.

Studies have found artificial substrata to be more selective for certain species of diatoms (Barbiero, 2000; Snoejis, 1991) which would result in a false depiction of the natural stream community, whereas overall species diversity of natural and artificial substrata was found to be almost identical (Eulin et al., 1998). In addition, the taxa that were present on natural substrata were also found to be present on artificial substrata though they varied in abundance (Hamilton and Duthie, 1984). Other studies have shown that differences in diatom communities even exist among the various types of natural substrata (Reavie and Smol, 1997). Winter and Duthie (2000) found the strongest relationships between diatom data and environmental variables when all three habitats (epilithic, epipelic and epiphytic) were summed. Nevertheless, there are benefits to using both natural and artificial substrata. Artificial substrata are better aimed at monitoring because of the replicability (Barbiero, 2000) and control they offer. Natural substrata will inevitably reflect the natural community better than artificial substrata because artificial substrata contain newer communities reflective of current conditions or those in the recent past (Tuchman and Stevenson, 1980). In addition, allowing periphyton to grow for a 2-month incubation period will result in a diatom community similar to that of the community on the natural substratum (Cattaneo et al., 1975; Lowe and Gale, 1980; Chessman, 1985). The community will also reflect the water quality and growth conditions for the previous 2 months. Perhaps both artificial and natural substrata should be used in water quality assessment to obtain the best overall analysis.

History of Mill Creek

Mill Creek is a 4th order stream located in Cincinnati, Ohio. The creek is 28.1 miles long and flows through southeastern Butler County and central Hamilton County where it empties into the Ohio River. The drainage area of the Mill Creek watershed is 166.2 square miles and the stream flows over sand and gravel from glacial deposits and outwash. There are five major tributary streams of the Mill Creek (Ohio EPA, 1994). My research focuses on the upper Mill Creek and the northernmost tributary stream, East Fork Mill Creek.

Beginning in the late 1700's and continuing to the present day, the Mill Creek was used primarily for the removal of wastes: industrial and urban. In fact, the Mill Creek was named to attract water-driven mills that might have been established throughout the outlying area. Today, major industries are settled along the creek in the watershed and continue to discharge storm-water and NPDES-permitted effluents into the stream. Additionally, 158 combined sewers overflow into the Mill Creek or its tributary streams during rain events when the sewer system is unable to contain the large volume of combined domestic sewage, storm water and industrial wastes (Ohio EPA, 1994). This results in a creek that is unsuitable for human contact during the rainy season and compromises the habitat for fish, shellfish and wildlife.

As agricultural land is replaced with residential and commercial sprawl development throughout the upper Mill Creek watershed, the amount of urban run-off and storm water continues to increase. Resulting from this urbanization is erosion of the natural stream and flooding. To contain the flooding and erosion, from 1981 until 1992

the Army Corps of Engineers re-channelized and over-widened lower portions of Mill Creek hence removing the natural riparian vegetation (Ohio EPA, 1994).

The Mill Creek continues to be examined by various groups in Cincinnati (Mill Creek Restoration Project Inc., Mill Creek Watershed Council) with the hopes of some day regaining the aquatic ecosystem that it was before it was degraded by human impacts. Past studies of the Mill Creek have been based on fish and macroinvertebrate communities. Therefore, Mill Creek is a good candidate to observe differences in water quality based on diatom communities through a variety of sampling methods.

Research Objectives

The objectives of this study were to describe the diatom communities of the upper Mill Creek using different three sampling methods; and to assess the relationship between these communities and water quality over three dates in summer of 2000. There is a lack of standardization with regard to diatom sampling. Thus, it is important to evaluate the methods used for community analyses. Other studies have only made comparisons between various natural and artificial hard substrata, while the suspended algal community has been generally overlooked. This study compares all three sampling methods (rock substrata, artificial hard substrata and phytoplankton) to determine which provide similar results, and which offer novel information. Furthermore, the impact of environmental pollutants on the Mill Creek, such as urban development and wastewater effluent, has never been examined via diatoms. I will compare three sites that differ in disturbance to ascertain the different effects each disturbance has on the diatom community.

Hypotheses

Water Quality Impact On Diatoms

1) Stream sites, which are affected by different environmental disturbances, will display a different diatom community as a result of the varying water quality. Therefore, the diatom community at the site that receives wastewater effluent will be different than the community at the site that receives construction run-off. Both of these sites will be different than the undisturbed site.
2) Genus and species level data will provide more resolution in how the diatom community responds to environmental disturbances than indices at the community level.

Sampling Methodology

3) The composition of the diatom community from water column and substrate sampling will differ. If this hypothesis is supported, it would suggest that different sampling procedures would need to be employed to gain a complete picture of the diatom community.
4) Sampling from natural substrates may reflect both old and new diatom growth. I predict that growth on the tiles will be more representative of recent settling species. Over time, the tile community will become similar to that of the natural substrate.

METHODS

Sampling Area

The 28.1 mile Mill Creek (Figure 1) flows south from its headwaters in Butler County, through the middle of Hamilton County, to where it flows into the Ohio River. The upper Mill Creek watershed in Butler County is being developed for commercial and residential uses. The lower Mill Creek watershed in Hamilton County is heavily impacted by industrial wastes, urban runoff, storm-water and domestic sewage. Sample site A is located on East Fork Mill Creek upstream from the Butler County/Hamilton County line. At the time of sampling, there were not any known direct sources of pollution into this site. Sample site B (WWTP – designates main source of impact) is located on East Fork Mill Creek just at the Butler County/Hamilton County line. This site is located just downstream from the Butler County Wastewater Treatment Plant (WWTP), which discharges treated effluent into the creek. Sample site C (C/D – designates main source of impact) is located on the main stem of Mill Creek just at the Butler County/Hamilton County line. This site received run-off from upstream construction and development (C/D).

Sample Collection

Diatom samples were collected from each study site using three sampling methods: periphyton rock scrubs, periphyton on ceramic tiles, and suspended phytoplankton. Sites were sampled on three dates: 03AUG00, 30AUG00, and 10OCT00.

At each sample site, field measurements of water temperature (YSI Model 51 meter) and dissolved oxygen (YSI Model 51 meter) were taken. Canopy cover and

current velocity were also noted. Stream discharge was determined by multiplying the cross sectional area (m^2) of the channel by the current velocity (m/sec).

Two one-liter water samples were also collected at each site on 03AUG00, 30AUG00, and 10OCT00 and saved in a brown bottle and a clear bottle respectively. Back at the lab, water from the brown bottle was used to obtain measurements of chlorophyll *a*, phosphate, nitrate and ammonia from each sample site. A 500mL portion of the sample was filtered through a Gelman A/E type glass-fiber filter using suction filtration (<10psi) and then 50mL of the filtrate were used to measure each nutrient: phosphate, nitrate and ammonia, according to Standard Methods (Clesceri et al., 1998) using an external standard regression. The filter was placed in a small centrifuge tube with 20mL 90% acetone for chlorophyll measurements. The samples were stored in the refrigerator until the solution was later read on a spectrophotometer at 630nm, 645nm, 665nm, and 750nm. Not all samples were read within the recommended 24-48 hour period so 0.01mL of 4N HCl was added to the samples and they were re-read at the same settings after sitting 5 minutes. The water from the clear bottle was used to measure conductivity with a Coleman portable conductivity meter and turbidity with a HACH (model 5300) turbidimeter.

Periphyton samples were collected from the natural substrata by scrubbing rocks from the stream. Rocks, with evidence of algal growth, were collected from the streambed to cover the bottom of a washbasin with an area of 75,000 mm^2. The rocks were scrubbed using a small stiff brush (Kelly et al., 1998) and then rinsed with creek water to fill a 500mL-graduated cylinder. A 50mL sample was taken out of this mix and saved in a centrifuge tube and brought back to the lab. A 2mL sample was set aside for

measurement of chlorophyll *a*. After the rest of the mixture had settled overnight, the liquid was decanted and replaced with 95% ethanol for preservation and later species identification. The 2mL sample was placed in 20mL 90% acetone and refrigerated in the dark. The samples were stored in the refrigerator until the solution was read on a spectrophotometer at 630nm, 645nm, 665nm, and 750nm. Not all samples were read within the recommended 24-48 hour period so 0.01mL of 4N HCl was added to the samples and they were re-read at the same settings after sitting 5 minutes.

Algal samples were also collected from artificial substrata. Glass slides were arranged in hollow slide boxes and glued to metal rods. These had been placed into the stream at each sample site on 08JUN00, but were washed away during a rain event. Because of the flash flooding that occurs on Mill Creek during summer rain events, an alternative method of artificial substrate was used instead. Nine white glazed ceramic tiles (35mm x 35mm each) were super-glued onto six house bricks for a total of three tiles on each brick. Two bricks were placed in the stream at each sample site on 20JUL00. Two tiles evident of diatom colonization were retrieved from each sample site on 03AUG00 (14 days), 30AUG00 (41 days), and 10OCT00 (82 days). One tile was stored in a labeled petri dish for later species identification. The other tile was placed in 20mL of 90% acetone for chlorophyll *a* measurements. The chlorophyll samples were stored in the refrigerator until the solution was read on a spectrophotometer at 630nm, 645nm, 665nm, and 750nm. Not all samples were read within the recommended 24-48 hour period so 0.01mL of 4N HCl was added to each sample and they were re-read at the same settings after sitting 5 minutes.

Algal samples were also collected from the phytoplankton community. A 4L sample of creek water was filtered, using a 10-micron mesh phytoplankton net, and the filtrate was concentrated into a 22-mL glass tube (Patrick and Reimer, 1966). The sample was preserved at the lab with 3-4 drops of Lugol's Iodine until tea colored, for later species identification.

Diatom Sample Processing

Tile Samples

Each tile was placed in an 800mL beaker and was boiled in 25-50mL of concentrated HNO_3 until the tile was clean of all organic material. After the beaker cooled, 500-600mL of distilled water was added to rinse, a cover was placed on the beaker and the mixture was allowed to settle overnight in the hood. The supernatant was carefully aspirated down to 100mL so as not to disturb the settled materials and then 500-600mL of distilled water was added again. This was repeated until the pH was neutral. The remaining 100mL were stored in a bottle. Of the 100mL, 20mL were carefully poured into a coverslip lid onto three 22-mm coverslips. A lamp was directed at the coverslip lid and the solution was left to air dry overnight. Slides were mounted according to Patrick and Reimer, 1966 and Biggs and Kilroy, 2000. Three microscope slides were placed on a hotplate. Using a glass rod, one drop of Naphrax® mounting media was placed on each slide. One dried coverslip, diatoms facing down, was mounted onto the Naphrax on each slide. The slides were heated until the mountant began to bubble and then they were removed from the hot plate. This was repeated until very few bubbles appeared. Slides were examined at 1000X under oil immersion using an

Olympus CH-2 microscope. Diatoms were identified to species using (Patrick and Reimer, 1966; Patrick and Reimer, 1975; Collins and Kalinsky, 1977; Krammer and Lange-Bertalot, 1986, 1988, 1991a, 1991b). A minimum of 350 valves were identified and counted.

Rock Samples

From each 50mL sample of rock periphyton, 20mL were boiled in concentrated nitric acid. The solution was rinsed with distilled water and allowed to settle overnight. The supernatant was aspirated down to 100mL and the solution was rinsed with distilled water again. This was repeated until the solution had a neutral pH. The final 100mL sample was stored in a bottle. From this 100mL, 5-10mL was carefully pipetted onto three 22-mm coverslips in a coverslip lid and the samples were left to air dry overnight under a lamp. Three microscope slides were placed on a hotplate. Using a glass rod, one drop of Naphrax mounting media was placed on each slide. One dried coverslip, diatoms downward, was mounted onto the Naphrax® on each slide. The slides were heated until the mountant began to bubble and then they were removed from the hot plate. This was repeated until very few bubbles appeared. The slides were examined at 1000X under oil immersion using an Olympus CH-2 microscope. A minimum of 450 diatom valves were identified to species (Patrick and Reimer, 1966; Patrick and Reimer, 1975; Collins and Kalinsky, 1977; Krammer and Lange-Bertalot, 1986, 1988, 1991a, 1991b) and counted.

Phytoplankton Samples

Each 22mL phytoplankton sample, concentrated from 4 liters, was boiled in concentrated nitric acid to remove all organic materials. Each sample was then rinsed to a neutral pH using distilled water. 100mL of each sample were stored in a bottle. From each 100mL sample, 20mL was pipetted onto three 22-mm coverslips in a coverslip lid and allowed to air dry overnight under a lamp. Three microscope slides were placed on a hotplate. Using a glass rod, one drop of Naphrax mounting media was placed on each slide. One dried coverslip, diatoms downward, was mounted onto the Naphrax® on each slide. The slides were heated until the mountant began to bubble and then they were removed from the hot plate. This was repeated until very few bubbles appeared. The slides were examined at 1000X under oil immersion using an Olympus CH-2 microscope. All 3 slides were examined and as many diatoms as possible were counted and identified to species (Patrick and Reimer, 1966; Patrick and Reimer, 1975; Collins and Kalinsky, 1977; Krammer and Lange-Bertalot, 1986, 1988, 1991a, 1991b) for each site, though at most sites planktonic taxa were sparse.

Data Analysis

All data were analyzed in Microsoft Excel, Systat 10.0 for Windows, and Ecostat Statistical Program.

Cell density was calculated for each sample as either #cells/mm^2 or #cells/mL. Algal communities were analyzed for each sample by calculating species richness, species diversity (using Shannon-Weiner function), species dominance (using Simpson's dominance), and community evenness (using Shannon-Weiner log base 10 function)

(Washington, 1984). Density and dominance values were Log10 transformed for normality.

The aforementioned dependent variables (Log Density, Richness, Diversity, Evenness and Log Dominance) were used to determine differences between sites and differences between methods using one-way analysis of variance (ANOVA) and interactions between sites, dates and methods were tested using two-way analysis of variance (ANOVA) (Zar, 1999). Pearson correlations between environmental variables (NO3, PO4, NH3, Turbidity, Conductivity, Dissolved Oxygen, Temperature) and diatom community indices (Density, Diversity, Dominance, Evenness, Richness) were explored to determine relationships between variables. Species comparisons between samples were made using percent similarity. Relationships between diatom species and all variables were analyzed using cluster analysis plotted in multi-dimensional scaling scatter plot using Euclidean distances (Wilkinson et al., 1996). The distribution of the relative abundance of species was examined by ranked-abundance curves on a logarithmic x-axis to analyze the impact of pollution on the community (Lobo, 1997).

RESULTS

Diatom Community Analysis

A total of 21 genera and 98 species of diatoms (Table 1) were collected from the three study sites (Figure 1). Each sample was analyzed by calculating cell density (#cells/area), species richness (# species present), Shannon diversity (log base 10), Shannon evenness, and Simpson's dominance for each site on each date and for each method. Cell density was greatest on natural substrata (rock) and least in the phytoplankton community (Table 2, Figures 2.a., 2.b., and 2.c.). *Nitzschia* was the most abundant taxa followed by *Amphora*, *Rhoicosphenia*, *Cocconeis*, *Achnanthes*, *Navicula*, *Fragilaria* and *Gomphonema* (Table 3). Eight genera were present across all sites and by way of all three sampling methods (Table 4). Twenty species in eleven genera comprised greater than 4% of the Mill Creek diatom community that was sampled (Table 5). Thirteen species comprised greater than 4% of the tile community, while only nine species made up greater than 4% of the rock and phytoplankton community (Table 5).

The cell density of the phytoplankton community never surpassed 39 cells/ml (Table 6). On the other hand, the cell density gradually increased on the artificial substrate (tiles), but only once exceeded the community growing on rocks (site C on 8/30/00) (Table 6). The cell density of the tile community at sites B and C on 8/30/00 was very similar to the rock community on the same date (Table 6). But overall, the average cell density on rocks was 14,482 cells/mm^2 compared to the average cell density on tiles 2,455 cells/mm^2 and the average cell density in the phytoplankton community 24 cells/ml (Table 7, Figure 2.a.). On average, site A had the highest cell density for rock and tile communities (Figure 3.c., 4.b.). Site C had the highest cell density for

phytoplankton communities, but the density was not much greater at site C than site A (Figure 5.b.). Average genera density was highest at site A for tile and rock periphyton communities, but was highest at site C for phytoplankton communities (Figure 6.a., b., c.).

Taxa richness was greatest on natural rock substrata at 22.3 species compared with 18.8 species present on tiles (Table 7). The phytoplankton community was comparable to the rock community with 21 taxa (Table 7, Figure 7.a.). Overall, natural communities (rock and plankton) contained more species than the community on the artificial substrata (tile).

Overall species diversity and community evenness was greatest in the phytoplankton community and least in the algal community growing on tiles (Table 7). All three sampling methods had high species diversity, ranging between 0.52 to 1.22 (Table 7, Figure 8.a.). Site A, above the wastewater treatment plant (WWTP) effluent was the most diverse for all three sampling methods (Table 7). In addition, evenness was always above 0.60 in rock and phytoplankton communities, but dropped down to between 0.41 and 0.67 for tile communities (Table 6). Hence, dominance was low for all sites on all dates in rock and phytoplankton communities, but went up to 0.66 in the tile communities. The dominance in tile communities decreased over time, as the community matured and diversified. Site A of tile, rock and phytoplankton communities had the lowest dominance and thus the greatest diversity (Table 6, Figures 8.b., 9.b.).

Tile communities at all three sites were dominated by *Cocconeis placentula* (Figure 10.a., b., c.). Rock communities at all three sites were dominated by *Nitzschia inconspicua* (Figure 11.a., b., c.). The phytoplankton community at each site was

dominated by different species: *Nitzschia inconspicua* and *Rhoicosphenia curvata* at site A, *Cocconeis placentula* at site B, and *Aulacoseira alpigena* and *Nitzschia palea* at site C (Figure 12.a., b., c.).

The ranked abundance curve for diatom assemblages averaged across all sites and dates determined tile communities to be most dominated by one species. Rock communities followed that trend to a lesser degree, while the curve for phytoplankton communities showed a more even distribution (Figure 13.a.). The steep ranked abundance curve for tile communities at sites B and C showed dominance (comprising greater than 70% of the population) by one main species, *Cocconeis placentula* (Figures 10.b., 10.c.). While on the contrary, the curve for site A showed 5 species each comprising greater than 10% of the population (Figure 13.b.). The curves for rock periphyton communities displayed greater evenness than the tile periphyton communities, but once again, the curve for site A was less steep showing a more even distribution with five species each comprising 10% or more of the community (Figure 13.d.). The abundance curves for phytoplankton communities were smoother than the steep curves for tile and rock communities. But like the tile and rock periphyton communities, site B and C had the steeper curves indicating dominance by a few species while site A had less dominance by a few species (Figure 13.c.).

The percent community similarity matrix (Table 8) determined diatom communities on tiles and rocks to have 41% similarity, tile and phytoplankton communities had 37% similarity and rock and phytoplankton diatom communities had 44% similarity. For rock periphyton communities, site B (WWTP) and C (C/D) were most similar at 61%. Sites B and C were also the most similar for tile periphyton

communities at 83%. Phytoplankton communities were all less than 43% similar. Tile communities were most similar with rock communities at sites A and B having 51% and 45% similarity respectively. Tile communities were also most similar with phytoplankton communities at sites A (47%) and B (49%). Overall, species composition was more similar between rock and phytoplankton communities, both natural communities.

Water Chemistry Analysis and Physical Parameters

Measurements of physical parameters of the stream water and water chemistry were obtained for each site on each date to obtain information about the water quality for each sample (Table 9, Table 10, Figures 14 – 22). Site B (WWTP), which was downstream from a wastewater treatment plant and received treated effluent, had high levels of ammonia on 8/3/00 and 8/30/00 (1500-2023 ug/L) (Figure 14). Site B also had the highest levels of nitrate (1500-2600 ug/L) (Figure 15) and phosphate (1500-2000 ug/L) (Figure 16).

Measurements of dissolved oxygen and water temperature could not be obtained at site A on 8/30/00 or 10/10/00 due to equipment difficulties. Though, the measurements of dissolved oxygen and temperature did not vary much from site B or C for those same dates. As the summer ended water temperature decreased (Figure 17) and dissolved oxygen increased (Figure 18.). Water temperature ranged from 10.6 °C and 23.9 °C and declined seasonally. Dissolved oxygen remained between 4-10 ug/L at all sites and dates (Figure 18).

Site A and C consistently had lower levels of ammonia, nitrate, phosphate and conductivity. However, site C (C/D) which was downstream from a construction site and commercial development upstream received high peak run-off and silt, and therefore had the highest levels of turbidity (between 20-70 NTU) while water turbidity at site A and B remained less than 10 NTU (Figure 19). Levels of conductivity generally did not fluctuate or vary much between sites and dates, except at site B (WWTP) on 10OCT00 (Figure 20).

Site A had the lowest stream discharge, while site B (WWTP) and C (C/D) had more discharge resulting from added WWTP effluent and the combination of construction runoff and the draining of retention ponds upstream respectively (Figure 21). Chlorophyll biomass varied sharply between site and sampling method (Figure 22 a – c). Chlorophyll a was greatest in tile communities at site B (WWTP) on 10OCT00, which coincides with the nitrate, phosphate, and conductivity peaks that were present then at that site. However, chlorophyll a was highest in rock communities at site A, the undisturbed site. The algal communities on natural substrata at site A were much more matured and developed, consisting of macroalgae and other algal groups in addition to diatoms. Whereas, rock substrata at site B had did not have the same all-inclusive algal communities and consisted mainly of diatoms. Chlorophyll a of the phytoplankton community was greatest at site C (C/D) where turbidity was also highest.

Statistical Analysis

Pearson correlation (Table 11) showed strong positive relationships between NO_3^{+1}, conductivity, NH_4^+ and PO_4^{-3}. There was a strong negative relationship between

conductivity, NO_3^{+1}, PO_4^{-3} and NH_4^+ with turbidity. There was also a negative relationship between taxa richness and nutrients (PO_4^{-3}, NH_4^+, NO_3^{+1}) and conductivity.

A one-way ANOVA with sampling method as a factor showed significant differences ($p<0.05$) between measurements of density, diversity, evenness and dominance for sites A and B (Table 12). Cell density between methods was the only significant difference ($p=0.001$) at site C (Table 12).

One-way analysis of variance was also used to examine any difference in density between sites (Table 13), as well as differences in richness, diversity, evenness and dominance. There were significant differences ($p<0.05$) in diversity, evenness, and dominance between sites using the tile sampling method. There was a marginally significant difference ($p=0.052$) in evenness among sites for rock communities as well as a marginally significant difference ($p=0.056$) in density among sites for phytoplankton communities.

Two-way analysis of variance was used to analyze community differences among site and method and to determine whether there was any interaction between site and method (Table 14). Results showed no interactions between site and method. No significant differences in taxa richness were found between sites or methods. Species diversity ($p=0.055$), community evenness ($p<0.05$) and dominance were significantly different ($p<0.05$) between sites and methods. Cell density was significantly different ($p=0.000$) between methods only.

Cluster Analysis

Water chemistry data, species data and similarity/diversity indices were analyzed by cluster analysis based on Euclidean distances and the clusters were plotted in a scatter plot using multi-dimensional scaling. The cluster plot (Figure 23.a.) of the water chemistry data and similarity/diversity indices by sampling method, site, and date, showed a definite separation of site B from sites A and C. Site A and C data points clustered closely together, but with little overlap.

The cluster plot (Figure 23.b.) of standardized species data displayed rock samples as a somewhat separate group from tile and phytoplankton samples. Tile samples did not cluster together much, while phytoplankton samples formed a cluster in the middle right portion of the graph. Clustering water chemistry data with species data did not provide much more information (Figure 23.c.). Rock samples separated the most from tile and phytoplankton samples. Among all sampling methods, sites A and C are more spread out; while data points from site B clustered along the middle to lower portion of the graph.

DISCUSSION

Water Quality

Diatoms reflect ecological conditions based on physical and chemical factors (Pan et al., 1996). Hence, continued efforts for improving water quality assessment protocols have incorporated diatoms in recent years. Diatom based monitoring has become more common for water quality assessment because diatoms are found living in all aquatic habitats, they can be identified to species, and they react quickly to nutrient enrichment (Rott et al., 1998). This research found some relationships between diatoms and environmental factors as expressed by the community variation among sites of different water quality.

The water quality varied between site A, B and C in regards to the levels of nutrients, conductivity and turbidity while the water temperature and dissolved oxygen were fairly similar for each site. As expected, levels of nutrients and conductivity were highest at site B, which received treated sewage effluent. While site C had much higher levels of turbidity due to the excess run-off from the construction site upstream. In terms of negative environmental impacts, site A remained undisturbed with low levels of nitrate, phosphate, ammonia, conductivity and turbidity (Tables 9 & 10).

It was expected that diatom communities would be different at each site as a result of different water quality. Although this hypothesis was accepted, its counterpart was not accepted. Examining the genera and species present in the stream provided information about the water quality, but because all three sites were of substandard water quality, detecting differences based on species and genera alone was not as informative.

However, basing comparisons on indices at the community level did provide differences between sampling sites.

Community indices were utilized to determine differences between each site and which communities were impacted by environmental pollution. This relies on the concept that environmental stability allows for high diversity in a stable community (Washington, 1984). The diatom community reacted to the change in water quality at each site, with the community on average showing the most diversity and evenness at the undisturbed site, A. Site A had the greatest density of diatoms for tile and rock samples and also had the lowest dominance (Table 7), while phytoplankton density was greater at site C (Table 7). Statistically, taxa richness and cell density were not significantly different between each site and species diversity was only marginally significantly different with the degrees of freedom available ($p = 0.055$) (Table 14). However, evenness and dominance were significantly different between each site ($p < 0.05$) (Table 14) with the communities at site A being the most even and the communities at site C being the most dominant (Figures 10 - 12 a, b, c). Communities at site C were dominated by a different species depending on the substrata (Figures 10 c, 11 c, 12 c). The phytoplankton density was higher in the more turbid site (C) than it was at the less turbid sites (A) and (B). Phytoplankton generally are more abundant in conditions of low turbidity which means improved underwater light conditions and less suspended matter (Marker et al., 1997). However, this main channel of Mill Creek is longer than the East Fork of Mill Creek (Sites A & B), receiving water from retention/detention ponds, constructed wetlands and farm ponds. Moreover, habitat heterogeneity was higher with

pools, riffles, and runs, allowing more sources for suspended algal development and more habitats for diatom sloughing.

Site C had the highest levels of turbidity and generally had high species richness, which was supported by a Pearson correlation showing a strong positive relationship between turbidity and species richness (Table 11). The turbidity was not causal, but not stressful to diatom development in the pooled section with low nutrient concentrations. Low nutrient waters are routinely more biodiverse than enriched waters (Tilman, 1982). In addition, Pearson correlation showed a strong negative relationship between richness and phosphate illustrated in Figure 7.c. with site B generally having the lowest richness since it was the most eutrophic site except in the rock community. Furthermore, in contrast with Horner and Welch (1981), the colonization rate of diatoms on tiles was lower in the nutrient rich water of site B (Figure 3.a.), which they found to be higher in eutrophic conditions. The environmental impacts of treated wastewater and construction run-off affected the diversity, evenness and dominance of the community but did not statistically affect the cell density or richness of the community. The natural diatom community growing on rocks was negatively impacted more by wastewater effluent than construction run-off in terms of density, diversity and evenness.

The variation in species composition of the community at each site can be attributed to the different water quality at each site, however, the sites (A & B) on the East Fork of Mill Creek were already impacted by urban runoff. In various studies (Rott et al., 1998; Pan et al., 1996; Palmer, 1969), diatoms have been categorized and rated based on their tolerance or affinity to pollution. Each species of diatom has a range of tolerance to different environmental conditions. Monitoring changes in diatom

communities can reveal changes in water quality, if based on characteristic responses to those specific changes in water quality (Lange-Bertalot, 1979). Site B contained *Achnanthes lanceolata*, *Nitzschia amphibia* and a greater abundance of *Cocconeis placentula* than was present at site A or site C. Not only did site B (WWTP) contain more pollution tolerant species, but it also had less diatom cell growth than sites A or C, except in tile communities where cell density at sites B and C was similar (Figure 6.a – b). Pan et al (1996) found *Achnanthes lanceolata* in areas of phosphorus enrichment. In addition, communities at site C contained *Navicula viridula* (Pan et al, 1996) and an abundance of *Aulacoseira alpigena*, which are associated with turbid conditions (Figures 6.c and 12.c). Diatom communities at both sites B and C were more dominated by *Cocconeis placentula* and *Nitzschia inconspicua*, both pollution tolerant species (Rott et al., 1998), while site A contained a more diverse and evenly distributed community composed of species that were not abundant at site B or C. Species that are less tolerant of pollution, such as *Achnanthes minutissima* (Rott et al., 1998) and *Rhoicosphenia curvata* (Lange-Bertalot, 1979) were more abundant at site A (Table 5). Overall, the diatom communities at all three sites were composed mostly of taxa associated with eutrophication.

The diatom assemblages of site B and C had a higher percentage of similarity than did either with site A, except in the phytoplankton sample (Table 8). Diatom communities of tiles had 83% similarity between site B and C, both disturbed sites (Table 8); while site A was only 32% and 33% similar, respectively. Likewise, rock communities at site B and C were 61% similar, and only 40% and 49% similar to site A, the undisturbed site (Table 8). Overall, all three sites contained more pollution tolerant

taxa than pollution sensitive taxa. But site A contained a more diverse and natural community than was observed at sites B or C, which both received a different source of pollution. The effect of the stressors was clearer in looking at the decrease in density of the community and the lower scores of the community indices of diversity and evenness; while water quality assessment based on dominant taxa was less useful.

The cluster plot based on community indices and environmental variables grouped site A and C together (Figure 23.a.) and grouped site B apart. Whereas the cluster plot based on species data and environmental variables did not separate the three sites as much. Instead data were loosely clustered according to sampling method, with tile and phytoplankton samples closer than rock samples. This supports previous conclusions that differences in sites were better detected via diversity and similarity indices as opposed to the specific composition of the community. Perhaps in a stream that on average is of poor water quality, such as Mill Creek, species data alone will not provide as much resolution because the environment directs most of the community to be pollution tolerant.

Sampling Methodology

Sampling diatoms by way of a natural substrate is often difficult to obtain and quantify. The phytoplankton community of lotic systems is unstable because of its nature and so isn't always the best method for water quality analysis. Thus, many researchers have resorted to the use of artificial substrata. But communities on artificial substrata are often different than those on the natural substrata of the same stream section (Tuchman and Stevenson, 1980; Blinn et al., 1980). Thus, variations in the diatom community

cannot be completely attributed to water quality when they could also be attributed to substrata.

In this study, the species distribution of the diatom community varied between substrata as predicted. Each sampling method procured a different composition of dominant species (Figures 10 – 12, a - c), but there were common species found across all substrata, such as *Cocconeis placentula* and *Nitzschia inconspicua* (Table 5). The horizontal genera, *Cocconeis* and *Achnanthes* are common primary colonizers, followed by *Gomphonema* and *Nitzschia*, upright genera (Korte and Blinn, 1983). Overall, phytoplankton samples produced the greatest number of species across all dates with 66 species, while tile and rock samples had 55 and 56 species respectively (Table 1). Not much difference was detected in the number of genera present for each sampling method as it ranged from 14 - 16 genera per method, with rock samples having the most genera and phytoplankton samples having the fewest genera, but most species across sampling dates (Table 1). Suspended algae can move some distance and integrate more habitats and microhabitats than any stationary sample from a pitted surface.

Though the periphyton communities differed between tiles and rocks, most genera present on the rocks were also present on the tiles (Hamilton and Dutie, 1984). Likewise, most of the periphyton community on both rocks and tiles was composed of pennate diatoms such as *Achnanthes*, *Navicula*, *Nitzschia*, *Gomphonema* and *Rhoicosphenia* and only a few centric diatoms such as *Cyclotella* and *Melosira* (Table 4, Figures 10, 11, 12) (Korte and Blinn, 1983). Other centric diatoms, such as *Aulacoseira* and *Thalassiosira* were found only in the phytoplankton community. The phytoplankton community varied much more between sites (Table 8, Figures 12.a., b., c.) than the tile or rock communities,

which is expected given the fact that the water column contains sloughed periphytic species in addition to true planktonic species. Furthermore, the phytoplankton community is more susceptible to rapid changes as the current transports diatoms downstream from other upstream habitats.

The bar graphs of genera density displayed a similar trend between all three sites for each sampling method (Figures 6.a., b., c.). Of the three sampling methods, tiles and rocks had similar trends, while phytoplankton samples seemed to vary among sites the most. The periphytic communities of the tile and rock substrata along with phytoplankton communities were similar in richness (Table 7) representing the most conservative changes needed for water quality monitoring. Of the 21 genera that were observed in Mill Creek, 12 genera were present in all three sampling methods (Table 4).

But in terms of overall similarity, rock and tile communities were only 41% similar while rock and phytoplankton communities were 44% similar (Table 8). Tile communities were only 37% similar to phytoplankton communities (Table 8). Hence, the artificial substrate was more similar to the hard natural substrate. But the two natural substrata were more similar to each other than with the artificial substrata. The use of natural substrata for sampling diatoms provided a better representation of the natural community than that of artificial substrata. But utilizing different sampling regimes provided more information about the flora of Mill Creek than one method alone provided.

In addition, the community similarity and diversity indices varied between the three sampling methods. The average cell density of the phytoplankton community was sparse in contrast with the abundant rock periphyton community (Table 2), but the diversity was higher (Table 7) since planktonic samples were composed of all suspended

diatoms including scoured periphyton species plus true planktonic species. Density was significantly different between all sampling methods (Table 14). Diatom communities on rocks, consisting of old and new diatom growth, were much more matured than the communities on the introduced artificial substrata, which better demonstrated the early stages of diatom colonization. Due to fluctuations in current velocity and physical parameters, such as nutrient enrichment and siltation, the phytoplankton community did not have a stable environment in which to proliferate.

Community variables (density, diversity, evenness, and dominance) were significantly different ($p>0.05$) between methods at site A and B; while only density was significantly different ($p=0.01$) between methods at site C (Table 12). Tile samples displayed significant ($p>0.05$) community differences (diversity, evenness, and dominance) between sites; while rock and phytoplankton communities only picked up marginal differences between evenness and density respectively (Table 13).

The diatom community changed in response to different types of water quality at each site depending on which sampling method was employed. Overall, density was greatest in rock samples; richness was greatest in rock samples; diversity was greatest in phytoplankton samples; evenness was greatest in phytoplankton samples and dominance was highest in tile samples. More than likely, phytoplankton samples provide the most diverse samples because they can contain both planktonic and periphytic species. This also supports the hypothesis that multiple sampling regimes provide the most information about the community. A downfall of sampling by way of artificial substrata is its selectivity for particular diatom species. As was also found in other studies, *Cocconeis placentula* was dominant on tiles (Figure 10 a, b, c) (Lowe et al., 1996; Barbiero, 2000).

Because it is a small monoraphid diatom that tightly attaches horizontally to substrata, it is commonly a primary colonizer (Brown and Austin, 1973; Eulin and LeCohu, 1998; Korte and Blinn, 1983). But over time, the diversity and evenness of the tile community increased and dominance decreased.

As predicted, the tile community became more similar to that of the natural rock periphyton community over time. On average, the periphyton on the ceramic tiles continued to grow throughout the study, eventually covering the tile (Hamilton and Duthie, 1984) but never completely equating the mature community on the rock substrata. This usually can take anywhere from three weeks (Blinn et al., 1980) to two months (Cattaneo et al., 1975; Lowe et al., 1980; Chessman, 1985). Growth was the highest at site A, which had moderate levels of nutrients and turbidity in contrast with the two disturbed sites in with the high nutrient levels of site B and the high turbidity of site C. There was no significant difference in diatom cell density between sampling dates, except for the tile community. This indicates that artificial substrata are useful in colonization studies (Peterson and Stevenson, 1989). Thus the use of artificial substrata may complement the natural substrata by being a more sensitive indicator of more recent changes in water quality.

The diatom assemblage of tiles was not the best representation of the community growing on the natural rock substrata with only 41% similarity (Barbiero, 2000). The fact that tile periphyton communities were consistently different than rock and phytoplankton communities would suggest that the differences are not just a result of random colonization, but are a result of type of substrate (Barbiero, 2000). But despite some species assemblage differences, the communities of both tile and rock substrata

appear to similarly adjust to the different water quality at each site (Figures 10.a., b., c., 11.a., b., c.). The diatom community on tiles may have needed more time to grow and mature in a stressed environment, such as Mill Creek.

Cluster analysis of species data showed a separation of rock communities from tile and phytoplankton communities (Figure 23.b – c.). Rock, tile and phytoplankton communities at site B clustered together when analyzed by community indices and water chemistry (Figure 23.a.). Sites A and C slightly clustered when analyzed by the community analyses and water chemistry, indicating more similarity than site B (Figures 23.a.). But when analysis was done using species data, the data clustered based more on substrate type than sampling site (Figure 23.b. – c.). This indicates that the variation in community composition is explained more by the sampling methods than by the sampling site.

Conclusions

Overall, site differences were detected within the algal community, by analyzing community structure via diversity, evenness, dominance and richness and by water chemistry analysis. Comparisons of species and genera composition alone did not provide enough information about specific site differences. Whereas community level analyses provided insight into water quality differences based on physical and chemical parameters. Both approaches together, species/genus level data and community level indices, provided the most comprehensive information about site differences and differences between sampling methods.

In addition, there were community differences depending on which method of sampling was utilized. Each sampling method provided different perspectives into the community structure. Similar trends would have been discovered had just one sampling method been used; however each method offered a unique quality. Artificial substrata expressed new growth that might not have been detected on the natural substrata that have been around for many years. Artificial substrata also provided information into colonization rates of diatom communities. On the other hand, the community growing on the natural substrate is, by definition, the community that best characterizes the stream and there is no need to wait for the community to mature, as is necessary on artificial substrata to avoid dominance by early colonizers. Including phytoplankton sampling provided information about a completely different type of community and is the easiest to sample and quantify. Despite that there were significant differences between the three sampling methods – tile, rock, phytoplankton, they all provided useful information in exploring the diatom community structure and how the community is affected by environmental changes.

Figure 1. Map of Mill Creek, located in Hamilton County and Butler County (southwest Cincinnati, Ohio). Sampling sites indicated by letters A, B, C.

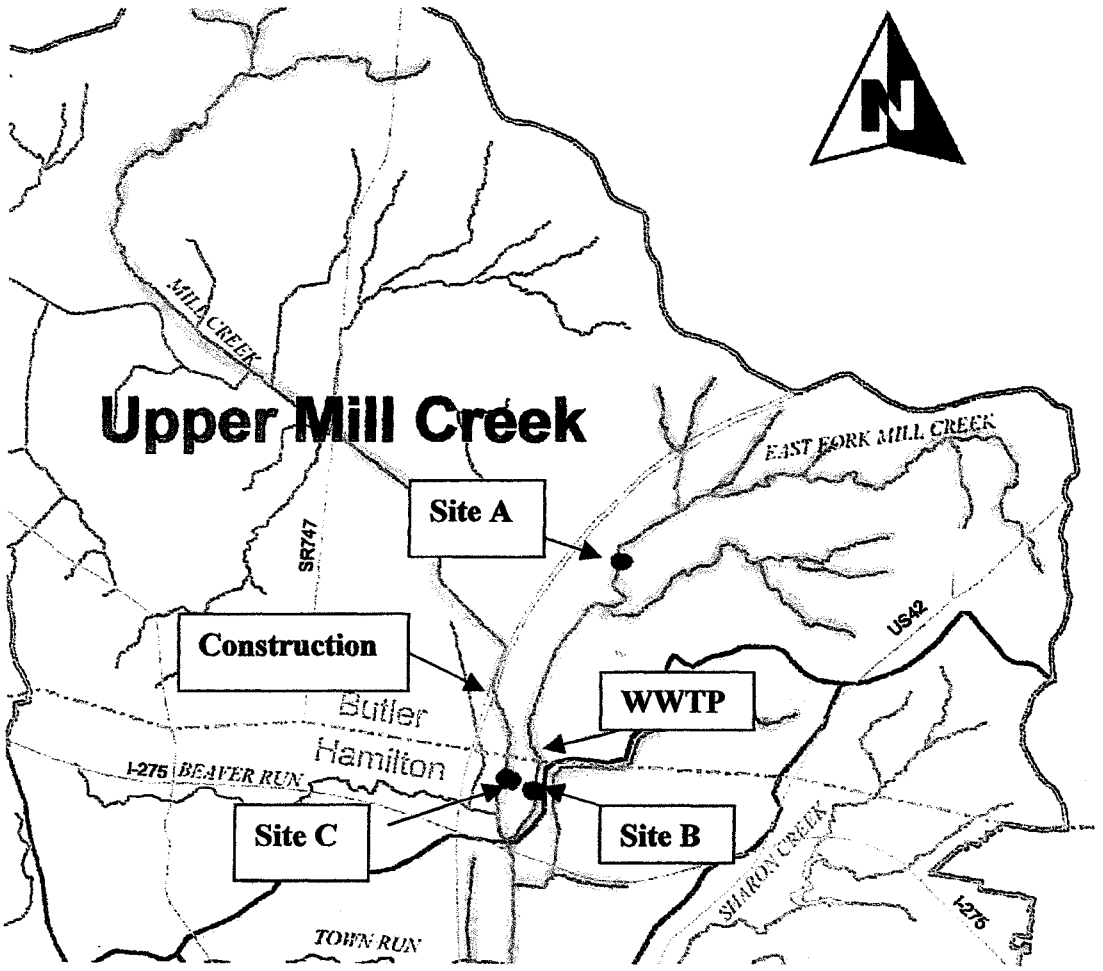

Figure 2.a. Cell Density (#cells/mm2) or (#cells/ml) averaged across all sites and all dates by sampling method

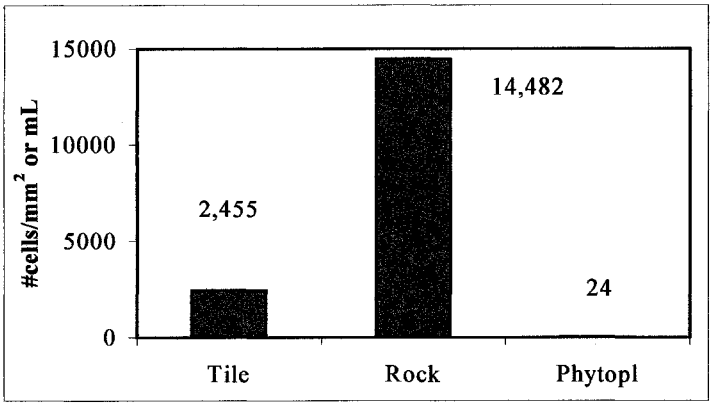

Figure 2.b. Cell Density (#cells/mm2) or (#cells/ml) averaged across dates by sampling method. Post Hoc Tukey tests determined that there were no significant differences between sampling methods (tile, rock, phytoplankton).

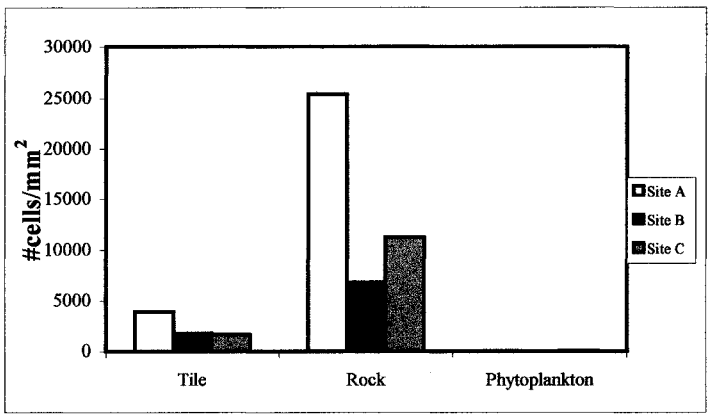

Figure 2.c. Cell Density (#cells/mm2) or (#cells/mL) averaged across dates by sampling site. Post Hoc Tukey tests determined that site A was significantly different from site B and site C.

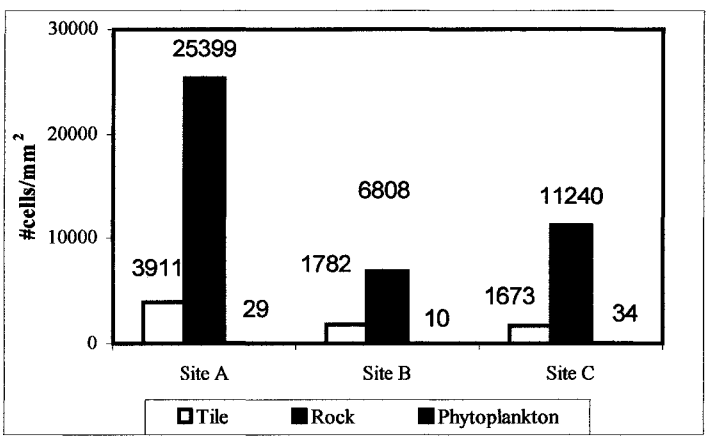

Figure 3.a. Cell Density (#cells/mm^2) on tiles by site and date

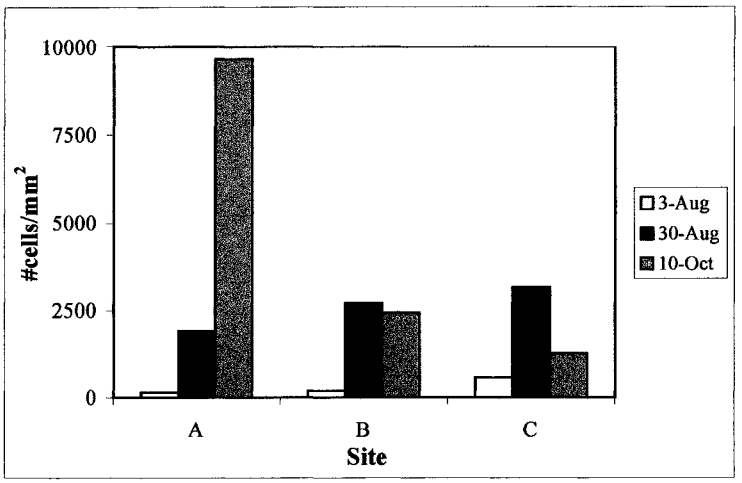

Figure 3.b. Cell Density (#cells/mm^2) on tiles by date averaged across sites

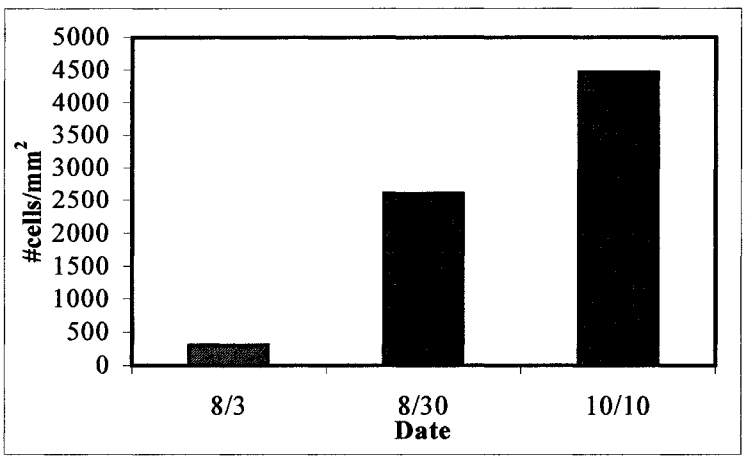

Figure 3.c. Cell Density (#cells/mm^2) on tiles by site averaged across dates

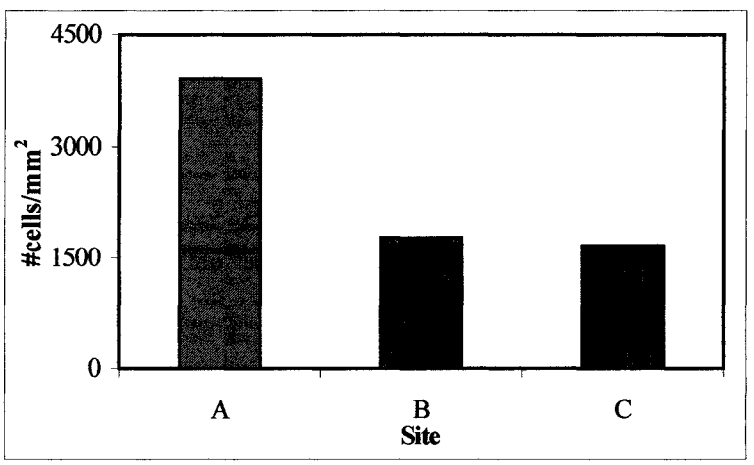

Figure 4.a. Cell Density (#cells/mm^2) on rocks by site and date

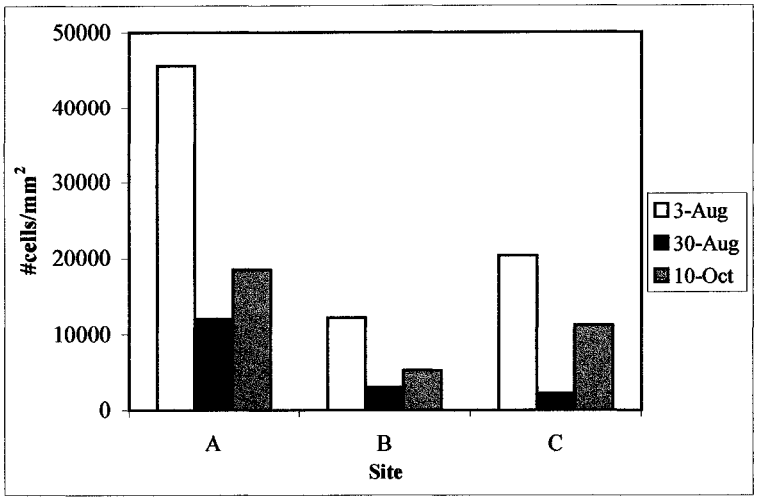

Figure 4.b. Cell Density (#cells/mm^2) on rocks by site averaged across dates

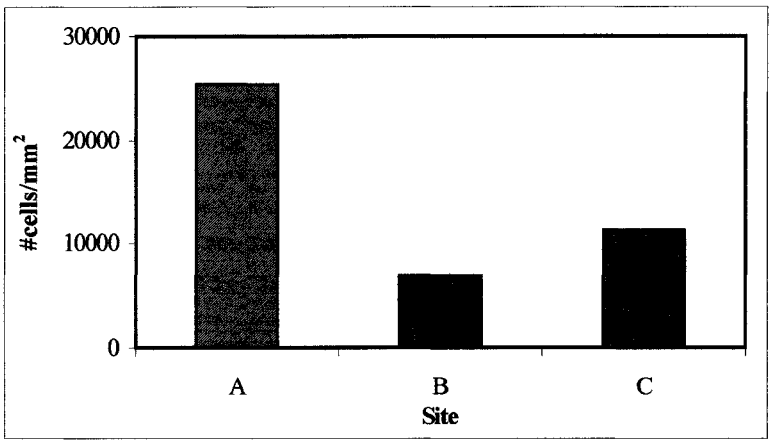

Figure 4.c. Cell Density (#cells/mm^2) on rocks by date averaged across sites

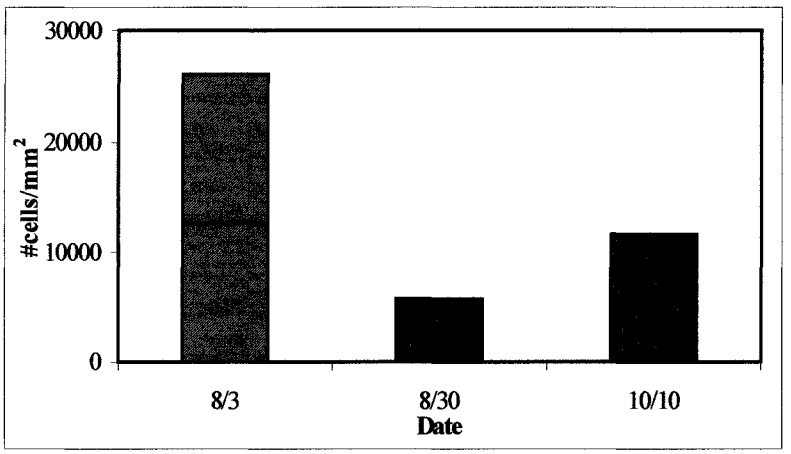

Figure 5.a. Cell Density (#cells/mL) of phytoplankton by site and date

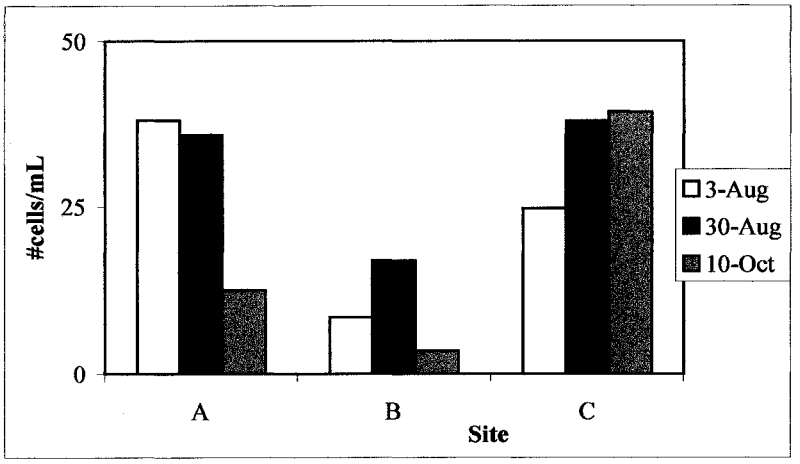

Figure 5.b. Cell Density (#cells/mL) of phytoplankton by site averaged across dates

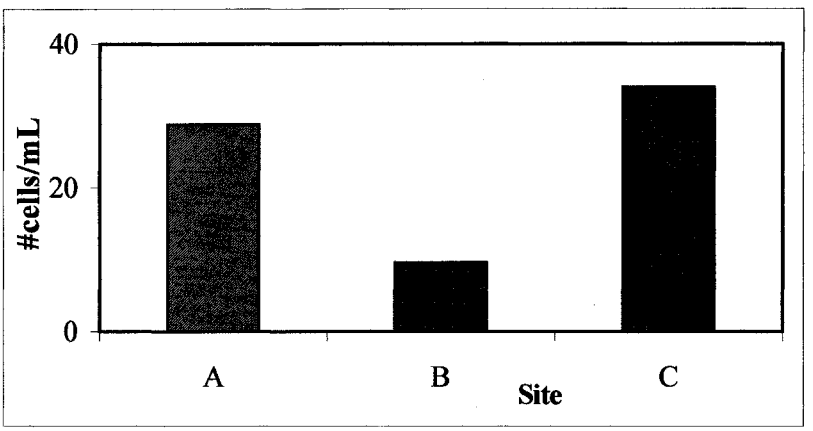

Figure 5.c. Cell Density (#cells/mL) of phytoplankton by date averaged across sites

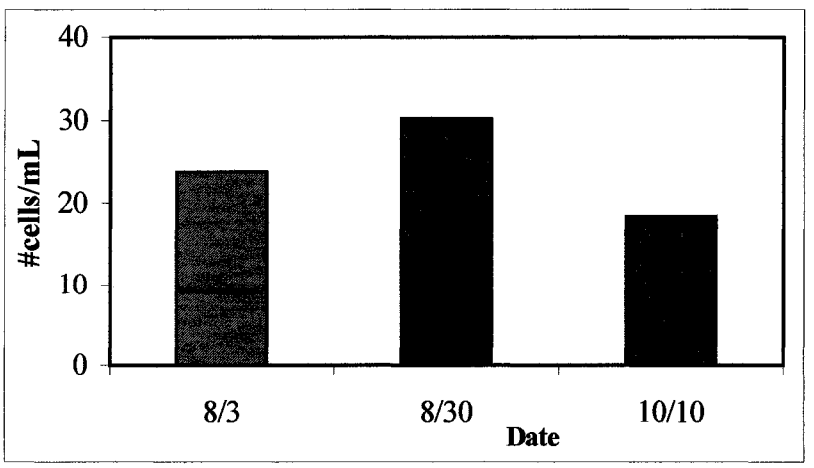

Figure 6.a. Cell Density (#cells/mm^2) of dominant genera on tiles by site averaged across dates

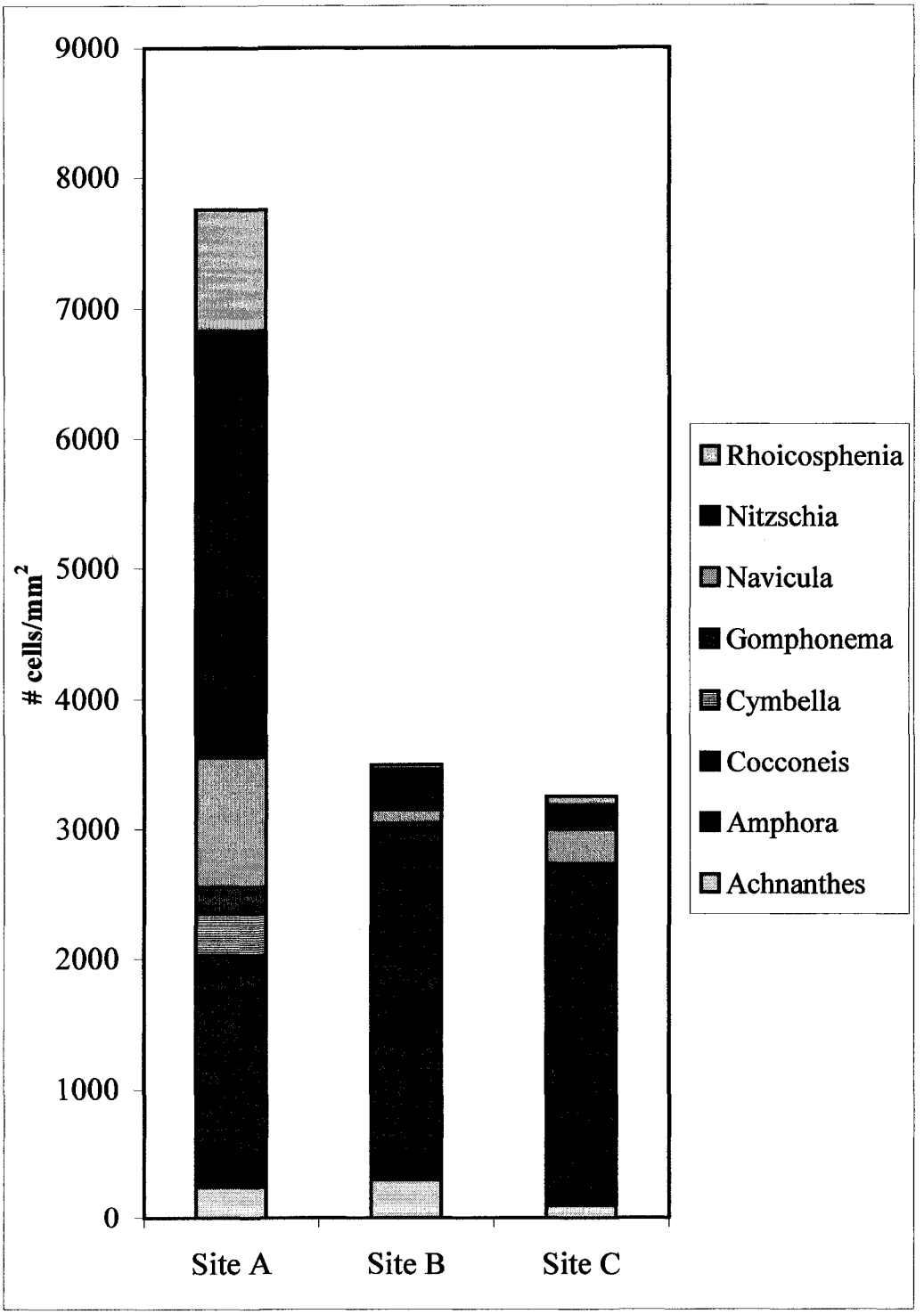

Figure 6.b. Cell Density (#cells/mm^2) of dominant genera on rocks by site averaged across dates

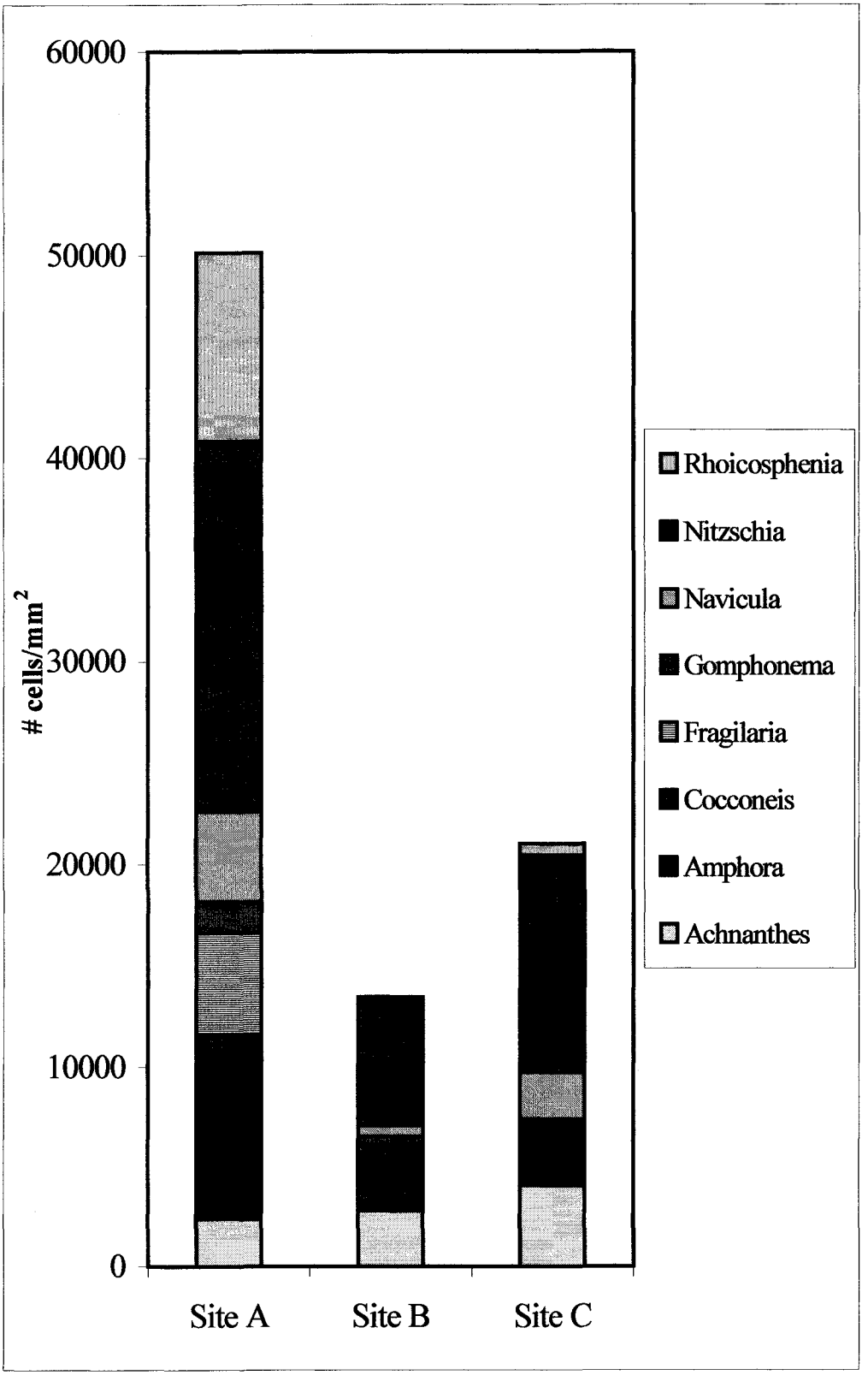

Figure 6.c. Cell Density (#cells/mL) of dominant genera in phytoplankton by site averaged across dates

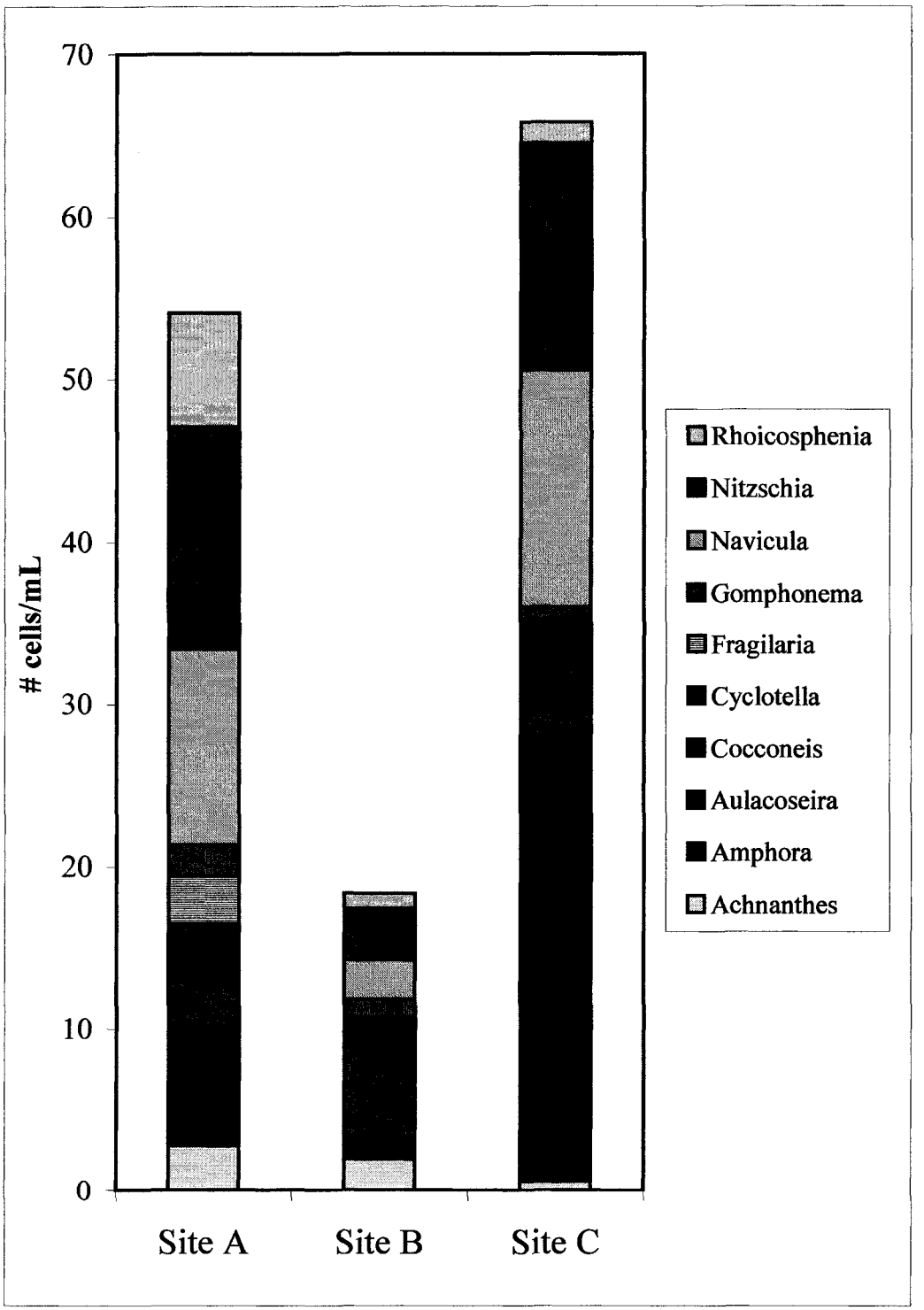

Figure 7.a. Species Richness by sampling method averaged across sites and dates

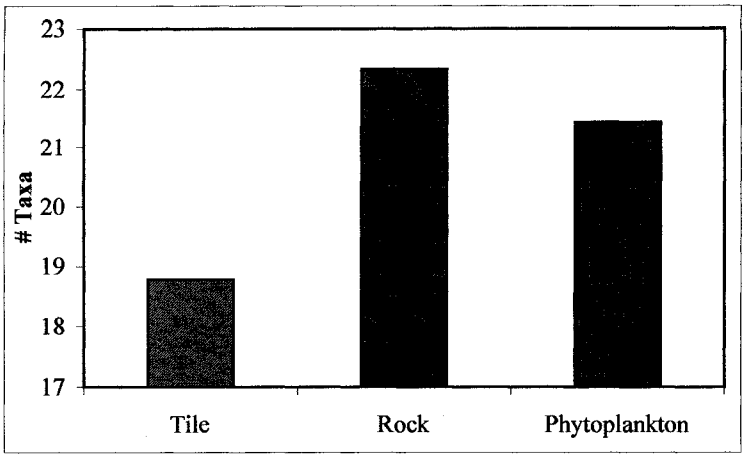

Figure 7.b. Species Richness by sampling method and date averaged across sites

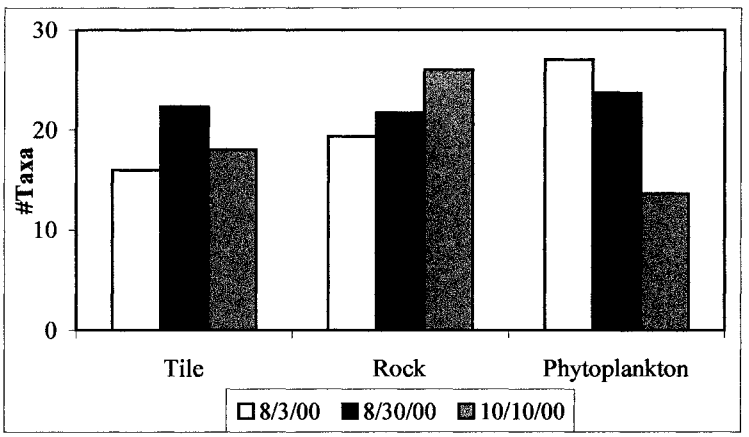

Figure 7.c. Species Richness by sampling method and site averaged across dates. Post Hoc Tukey tests determined that there were no significant differences between site; and there were no significant differences between method.

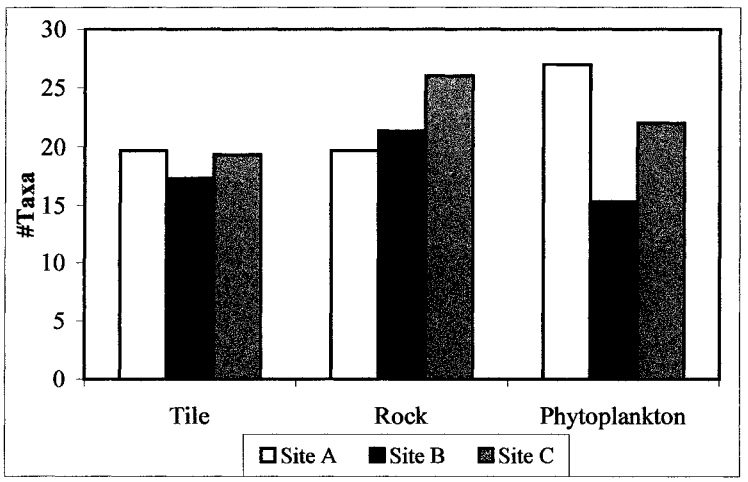

Figure 8.a. Shannon (H') Diversity by sampling method averaged across sites and dates

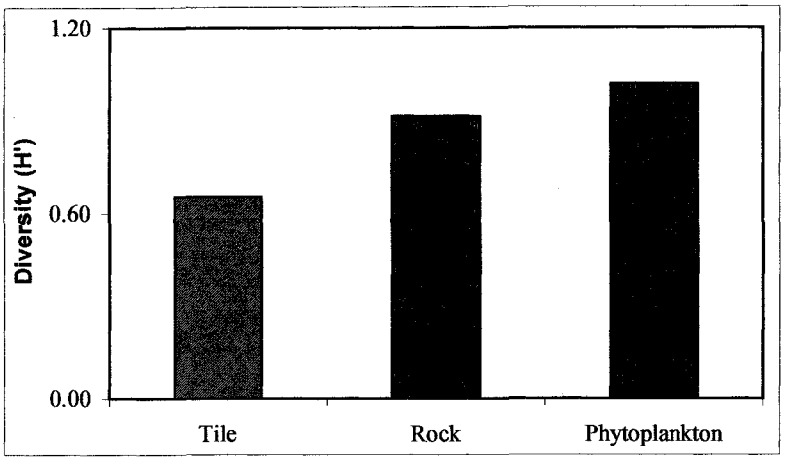

Figure 8.b. Shannon (H') Diversity by sampling method and site averaged across dates. Post Hoc Tukey tests determined that there were no significant differences between method; and there were no significant differences between site.

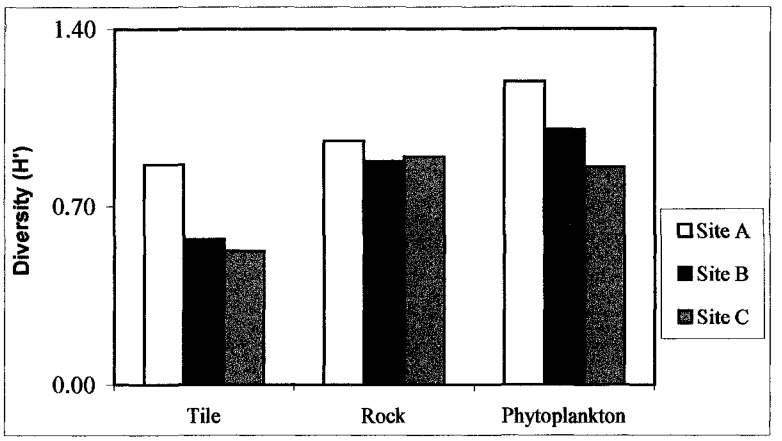

Figure 8.c. Shannon (H') Diversity by sampling method and date averaged across sites

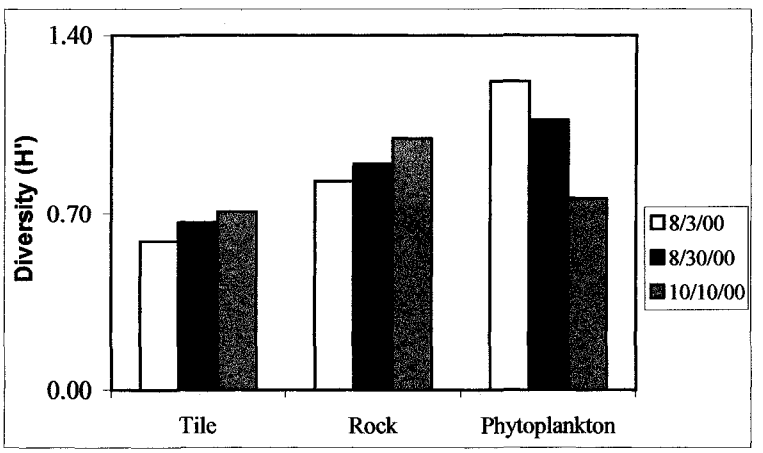

Figure 9.a. Simpson's Dominance by sampling method averaged across sites and dates

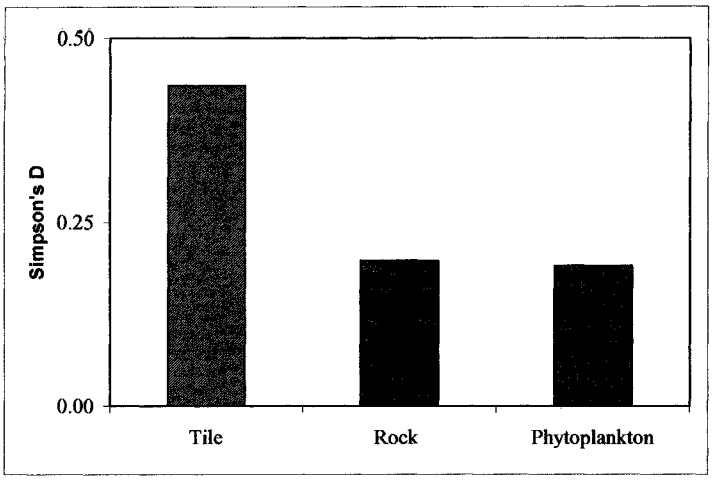

Figure 9.b. Simpson's Dominance by sampling method and site averaged across dates. Post Hoc Tukey tests determined that there were no significant differences between sites; and there were no significant differences between methods.

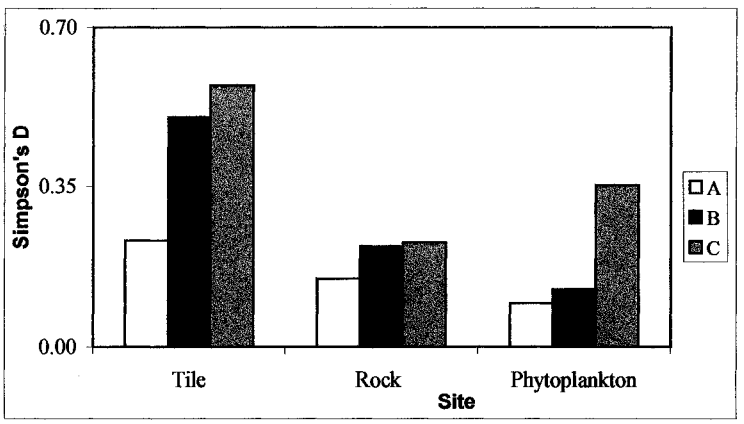

Figure 9.c. Simpson's Dominance by sampling method and date averaged across sites

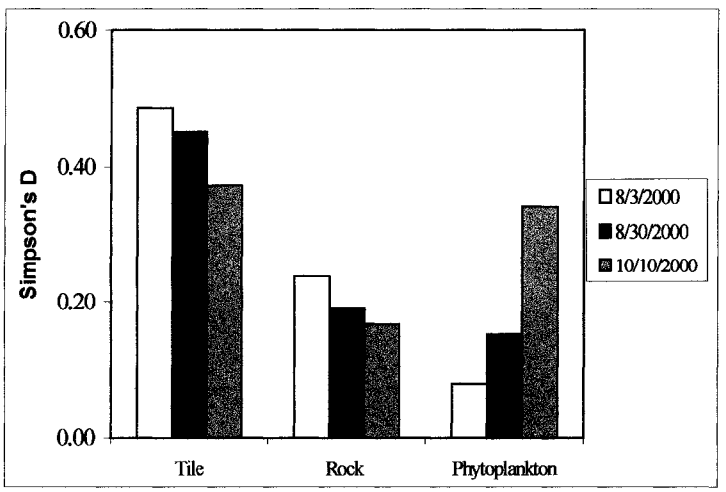

Figure 10.a. Species comprising >4% of the periphyton community on tiles at site A

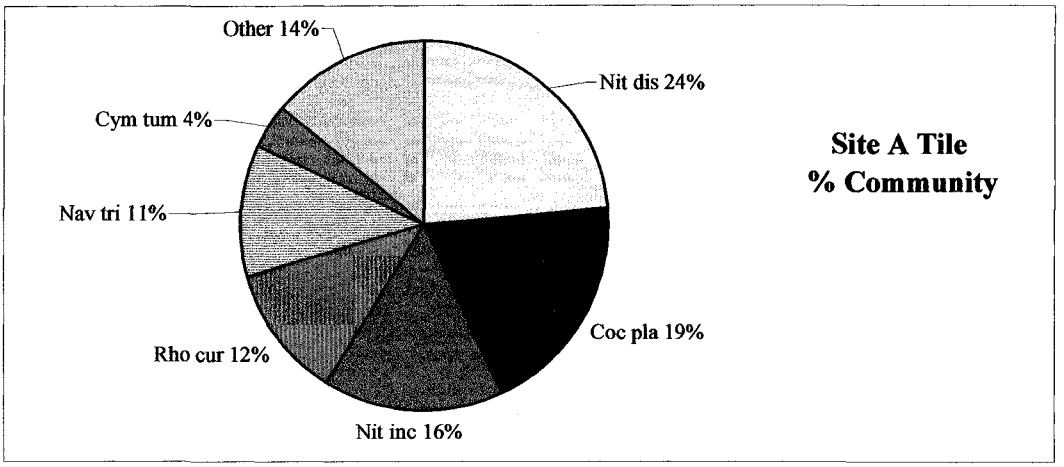

Figure 10.b. Species comprising >4% of the periphyton community on tiles at site B

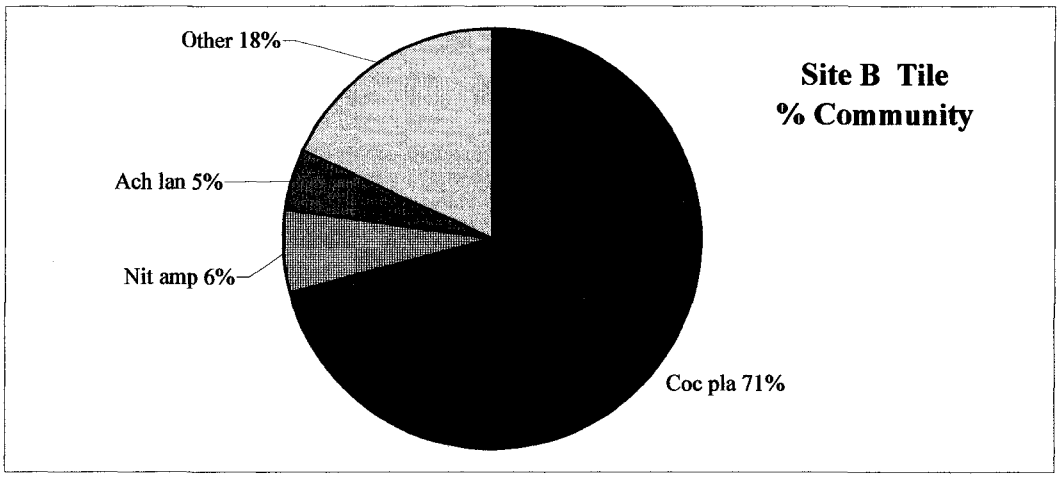

Figure 10.c. Species comprising >4% of the periphyton community on tiles at site C

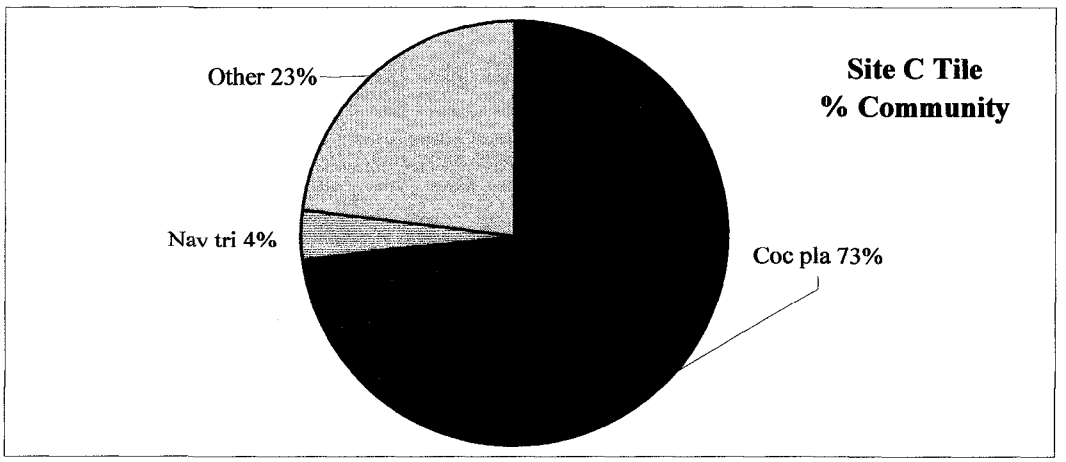

Figure 11.a. Species comprising >4% of the periphyton community on rocks at site A

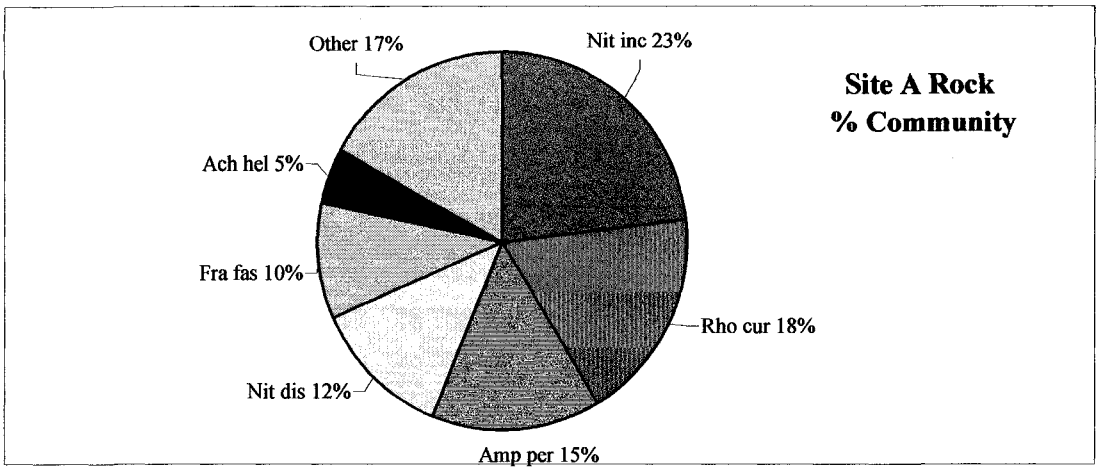

Figure 11.b. Species comprising >4% of the periphyton community on rocks at site B

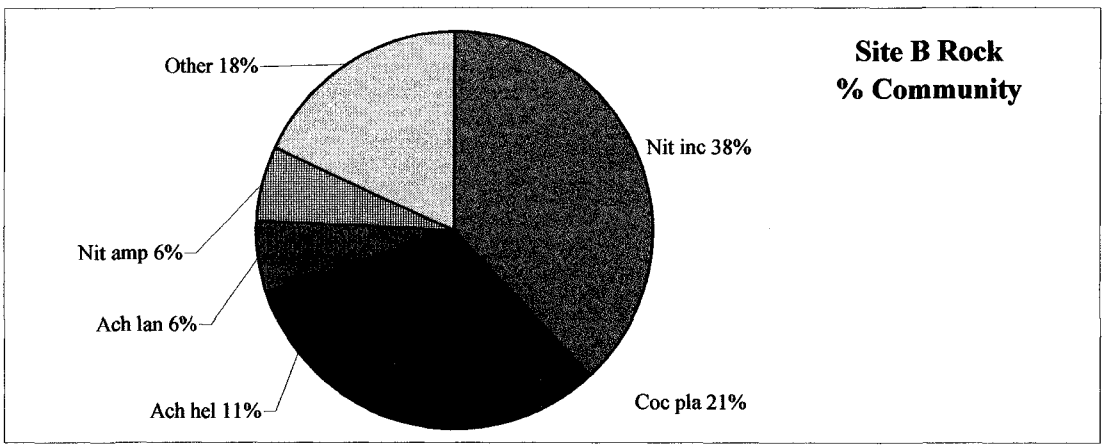

Figure 11.c. Species comprising >4% of the periphyton community on rocks at site C

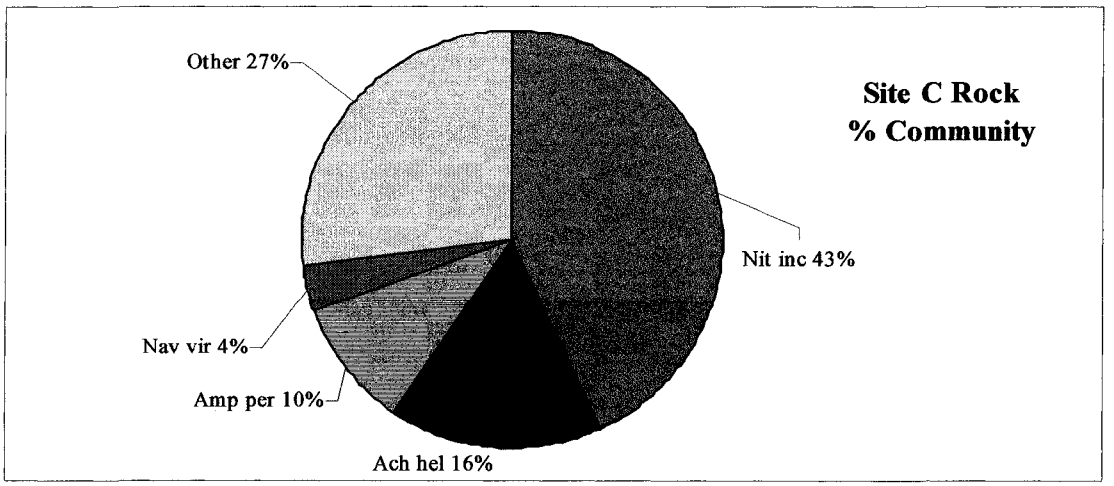

Figure 12.a. Species comprising >4% of the phytoplankton community at site A

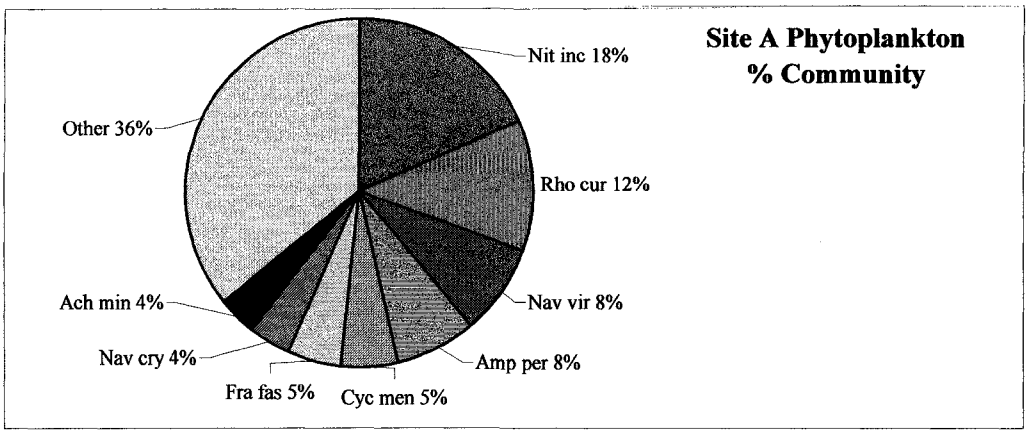

Figure 12.b. Species comprising >4% of the phytoplankton community at site B

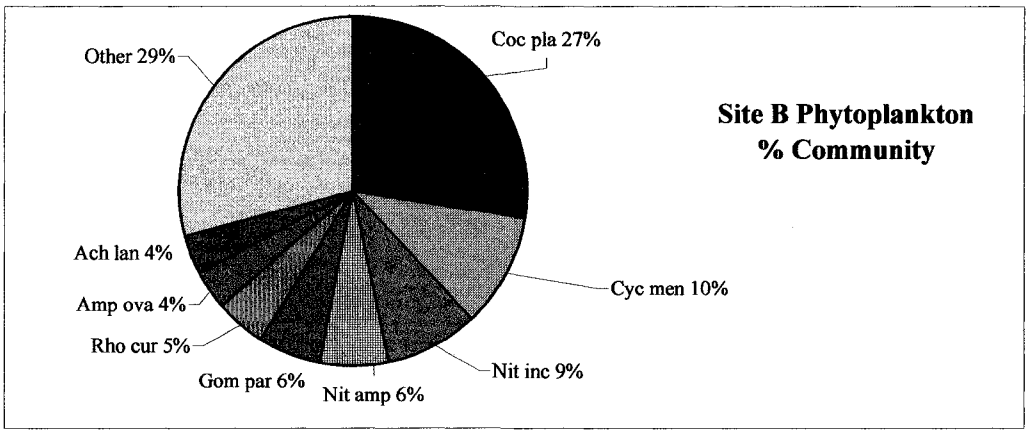

Figure 12.c. Species comprising >4% of the phytoplankton community at site C

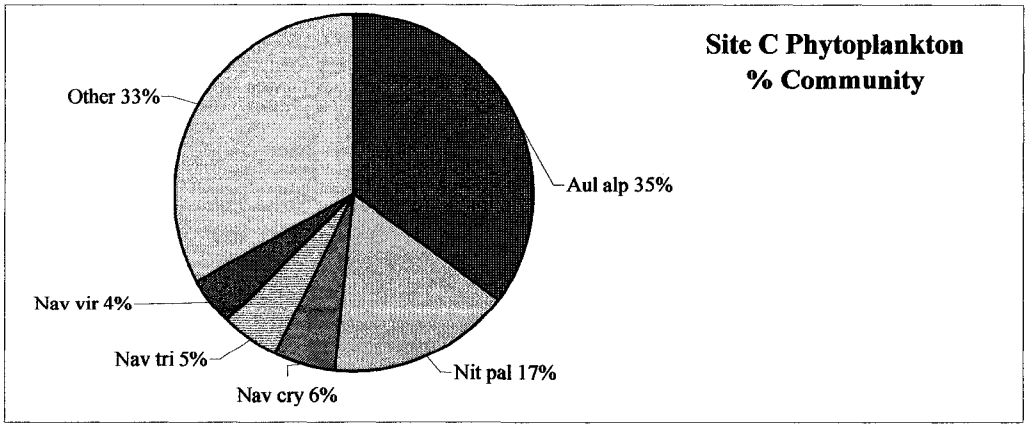

Figure 13.a. Ranked % abundance of diatom species by sampling method, averaged across all sites and all dates

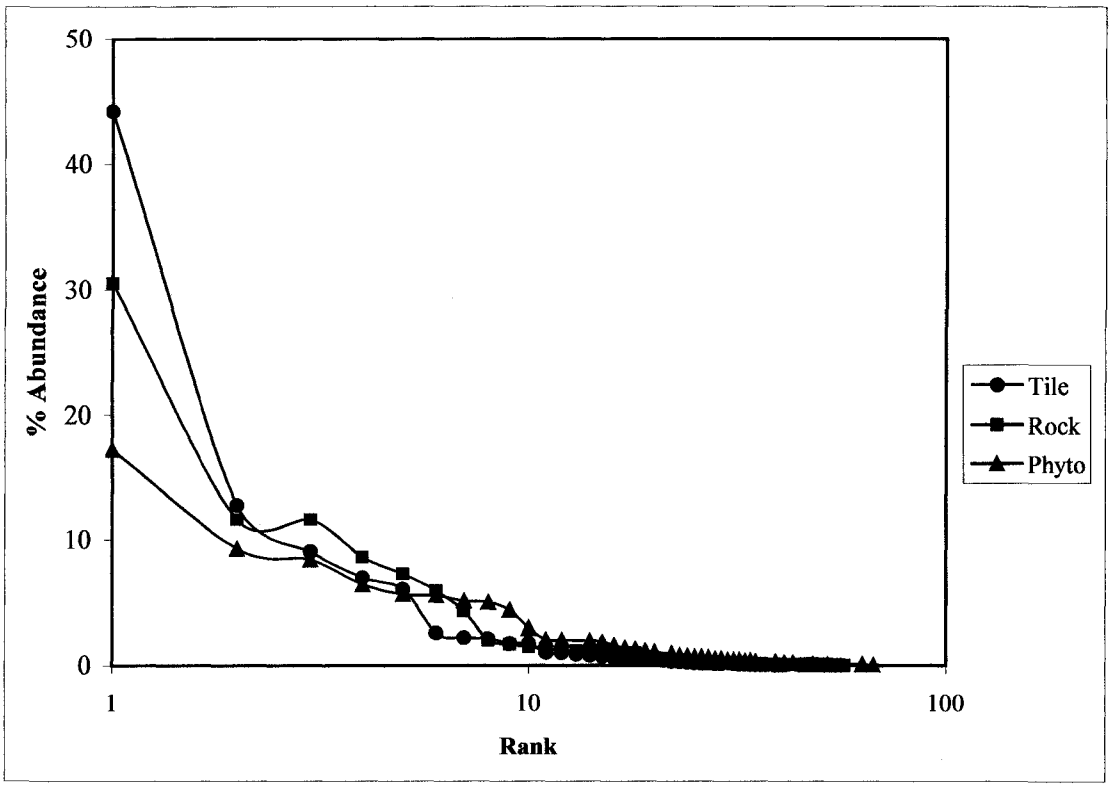

Figure 13.b. Ranked % abundance of tile periphyton species by site, averaged across dates

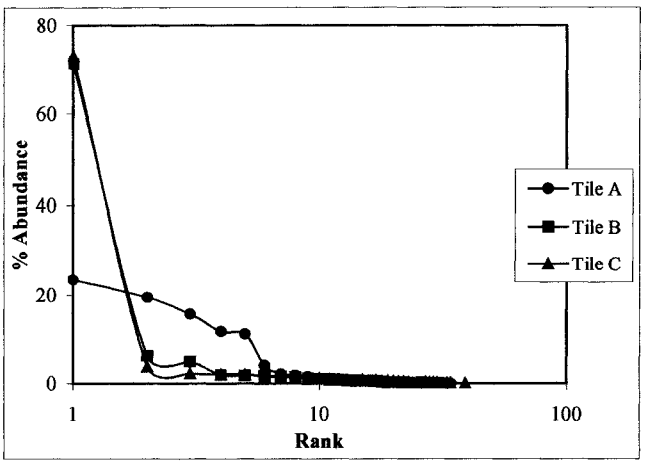

Figure 13.c. Ranked % abundance of phytoplankton species by site, averaged across dates

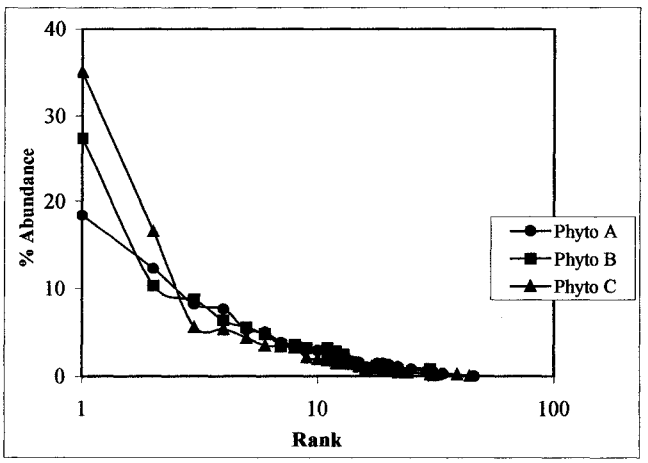

Figure 13.d. Ranked % abundance of rock periphyton species by site, averaged across dates

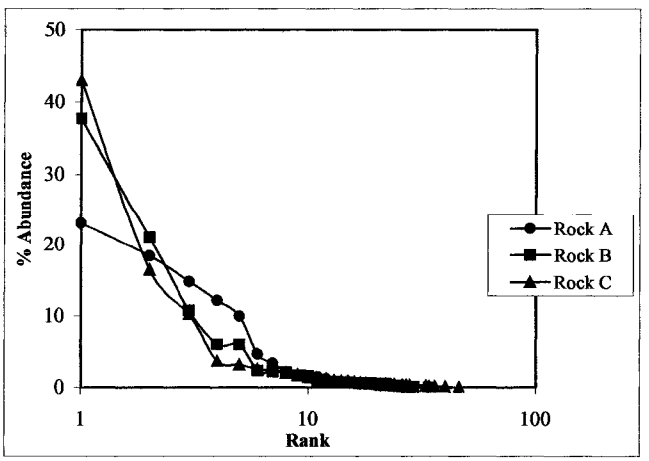

Figure 14. Ammonia (ug/L) in Mill Creek by site and date

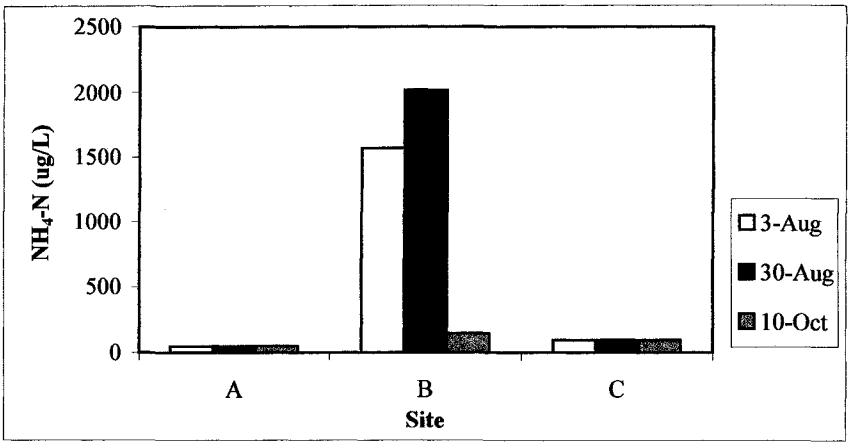

Figure 15. Nitrate (ug/L) in Mill Creek by site and date

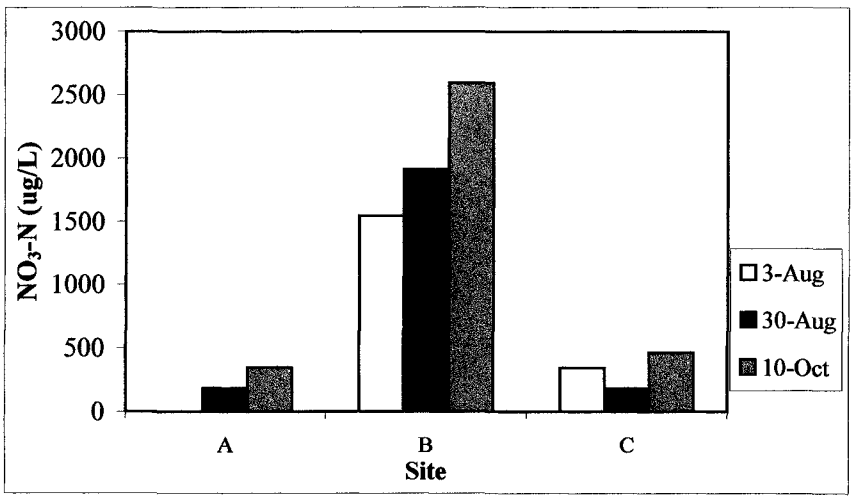

Figure 16. Phosphate (ug/L) in Mill Creek by site and date

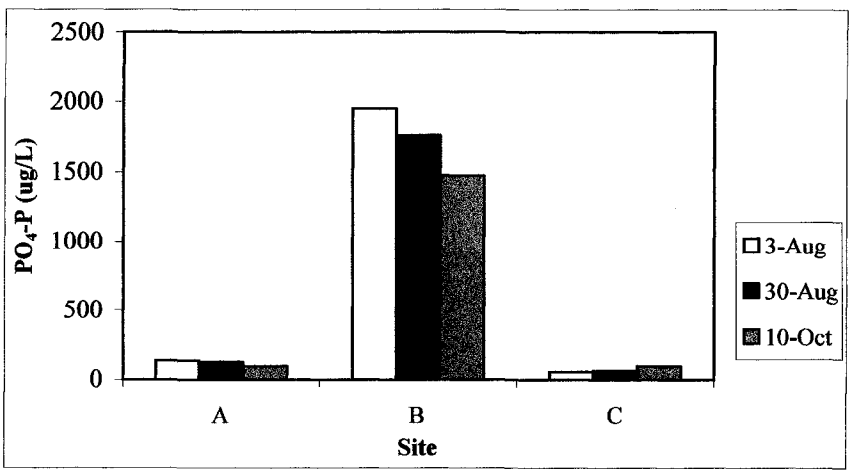

Figure 17. Water Temperature (°C) in Mill Creek by site and date

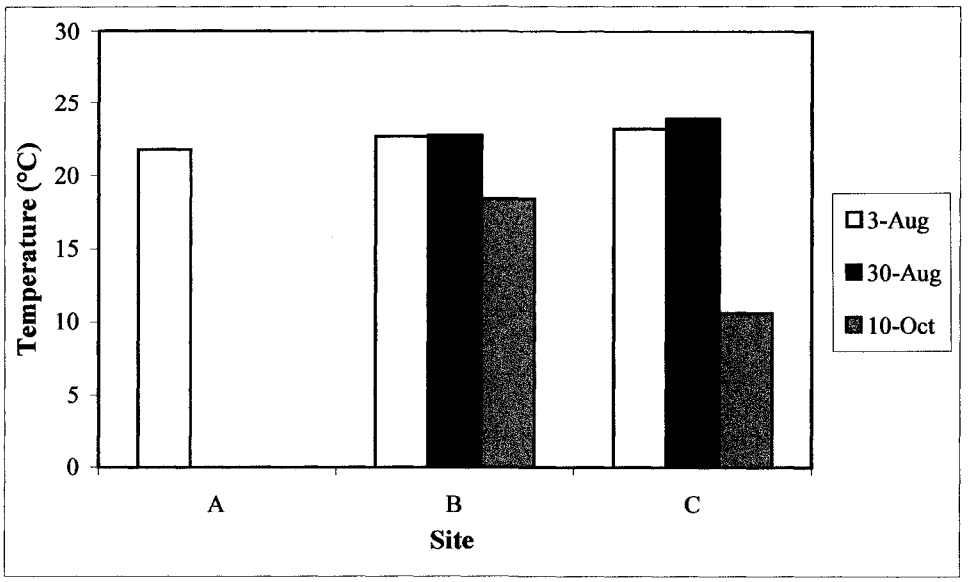

Figure 18. Dissolved Oxygen (ug/L) in Mill Creek by site and date

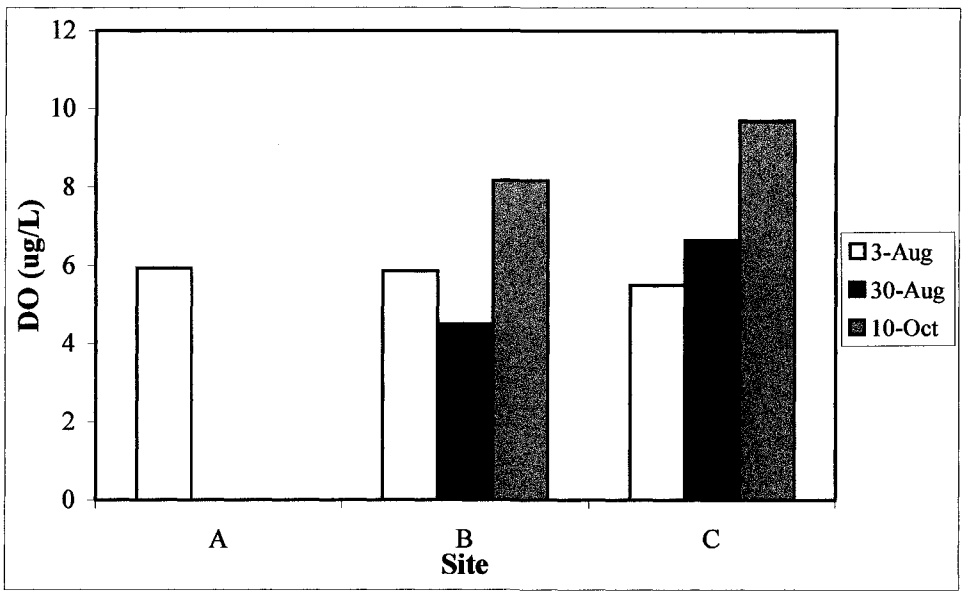

Figure 19. Turbidity (NTU) in Mill Creek by site and date

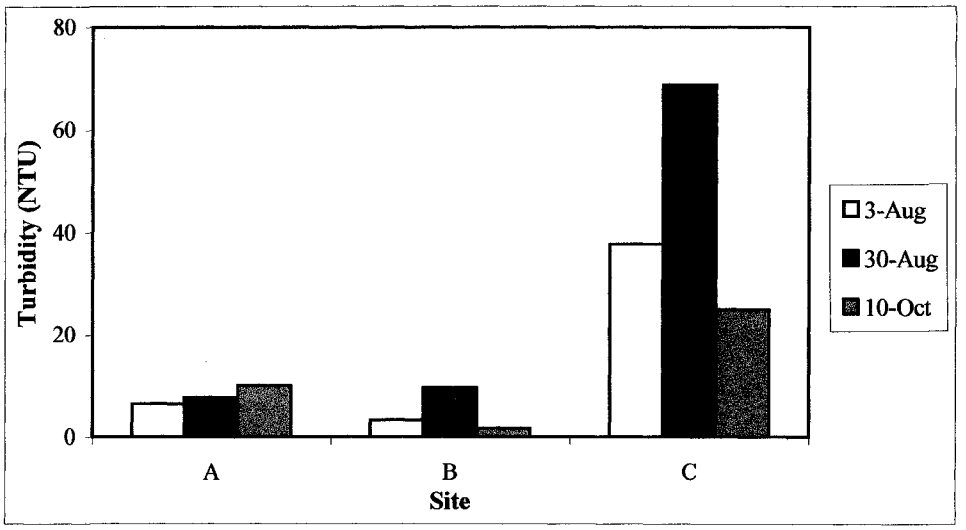

Figure 20. Conductivity (uS) in Mill Creek by site and date

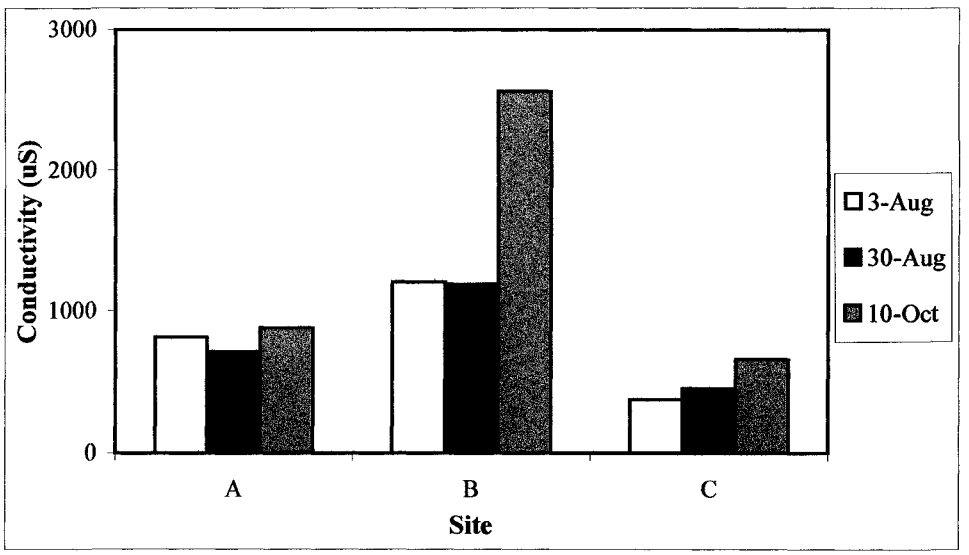

Figure 21. Discharge (m^3/sec) of Mill Creek by site and date

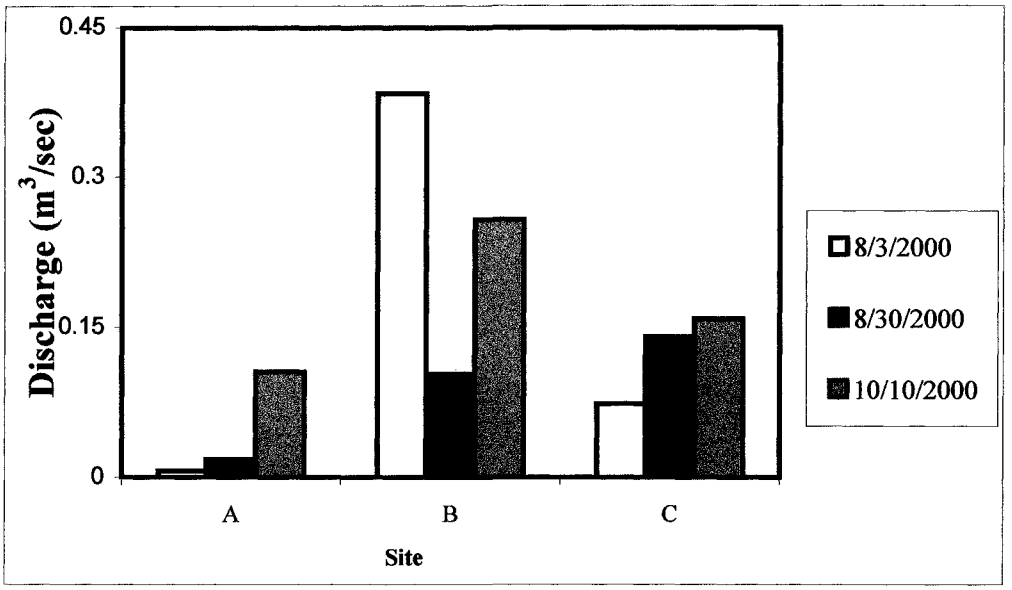

Figure 22.a. Chlorophyll *a* (ug/cm^2) on tiles in Mill Creek by site and date

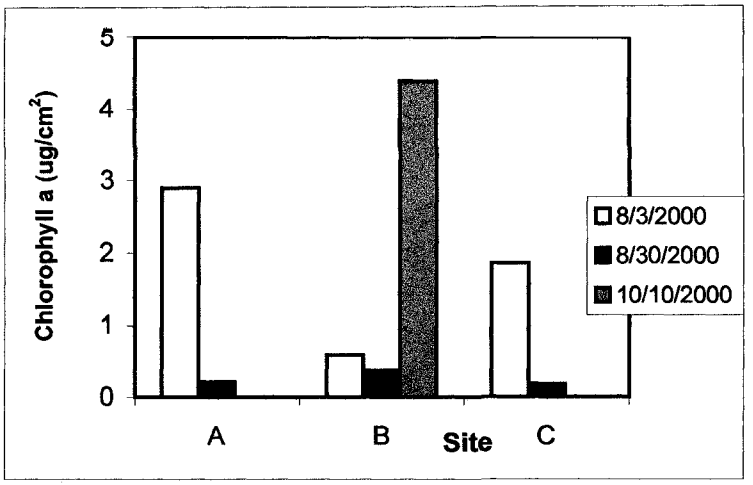

Figure 22.b. Chlorophyll *a* (ug/cm^2) on rocks in Mill Creek by site and date

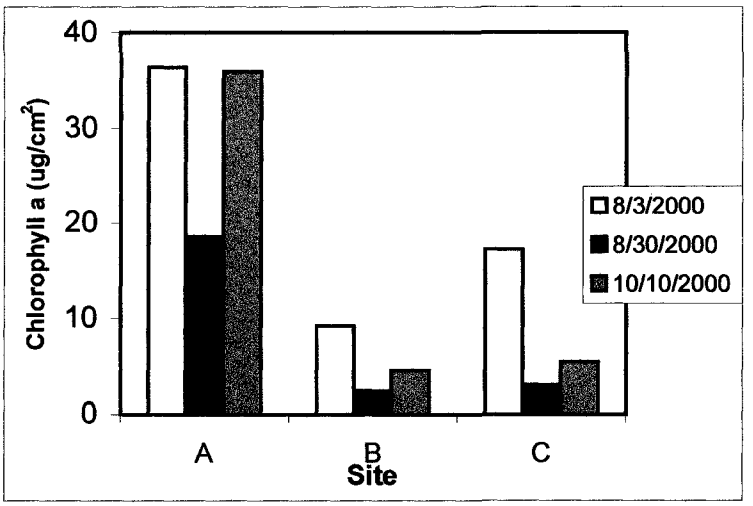

Figure 22.c. Chlorophyll *a* (ug/L) of phytoplankton in Mill Creek by site and date

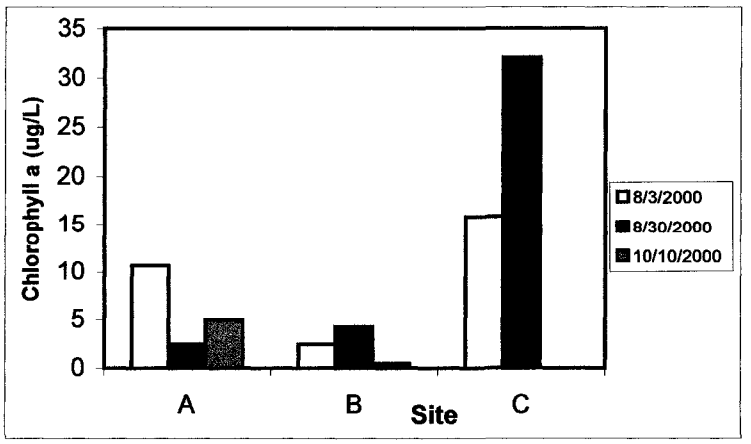

Figure 23.a. Cluster Analysis Grouped by Sample Type (Tile, Rock, Phytoplankton), Site (A, B, C) and Date (8/3, 8/30, 10/10) Using Log Density, Diversity, Evenness, Richness, Log Dominance and Standardized Water Chemistry Variables: PO_4, NO_3, NH_4, Turbidity, Conductivity, Dissolved Oxygen, Temperature

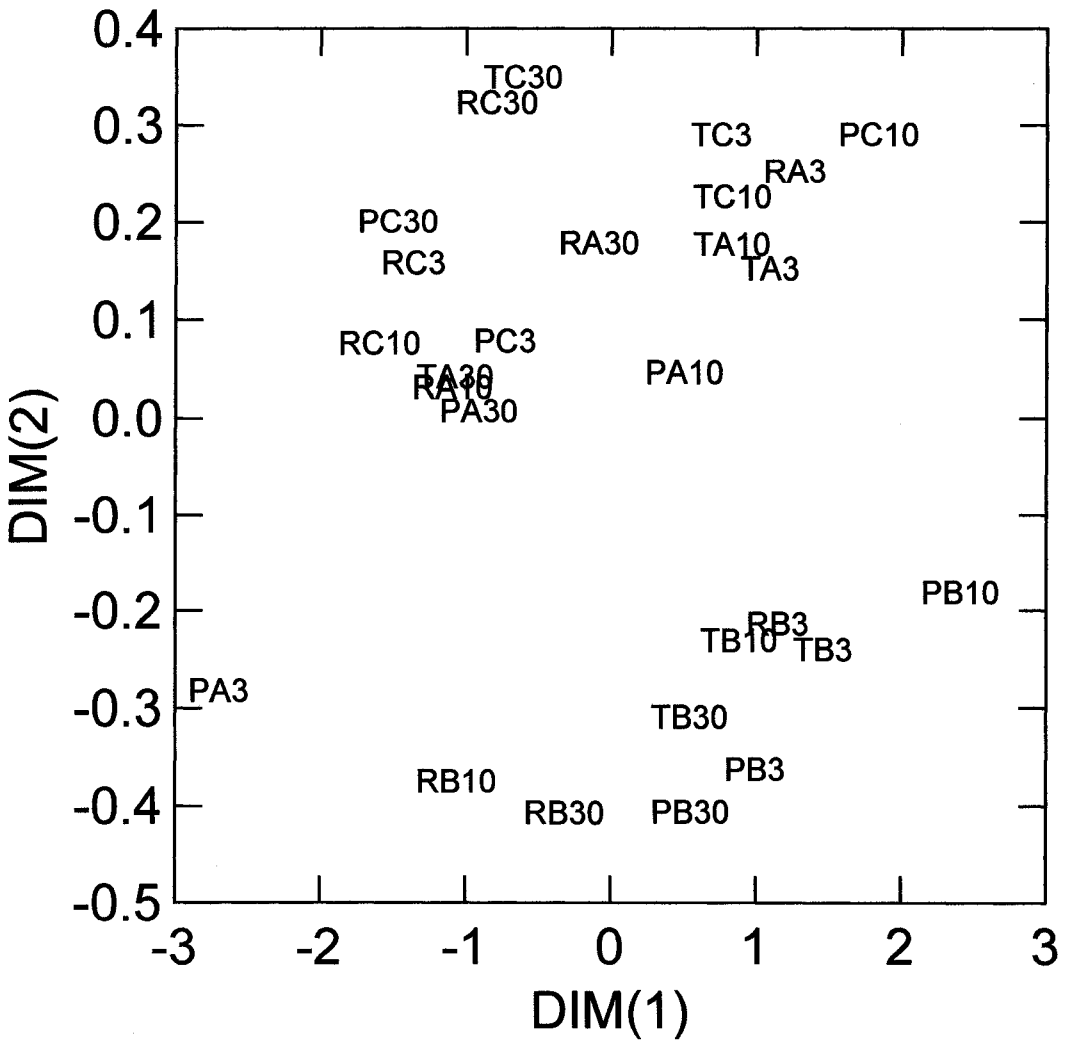

Figure 23.b. Cluster Analysis Grouped by Sample Type (Tile, Rock, Phytoplankton), Site (A, B, C) and Date (8/3, 8/30, 10/10) Using Standardized Species Data
*3 outlier variables (RC3, RA3, PC30) were excluded by decreasing scale of x and y-axis.

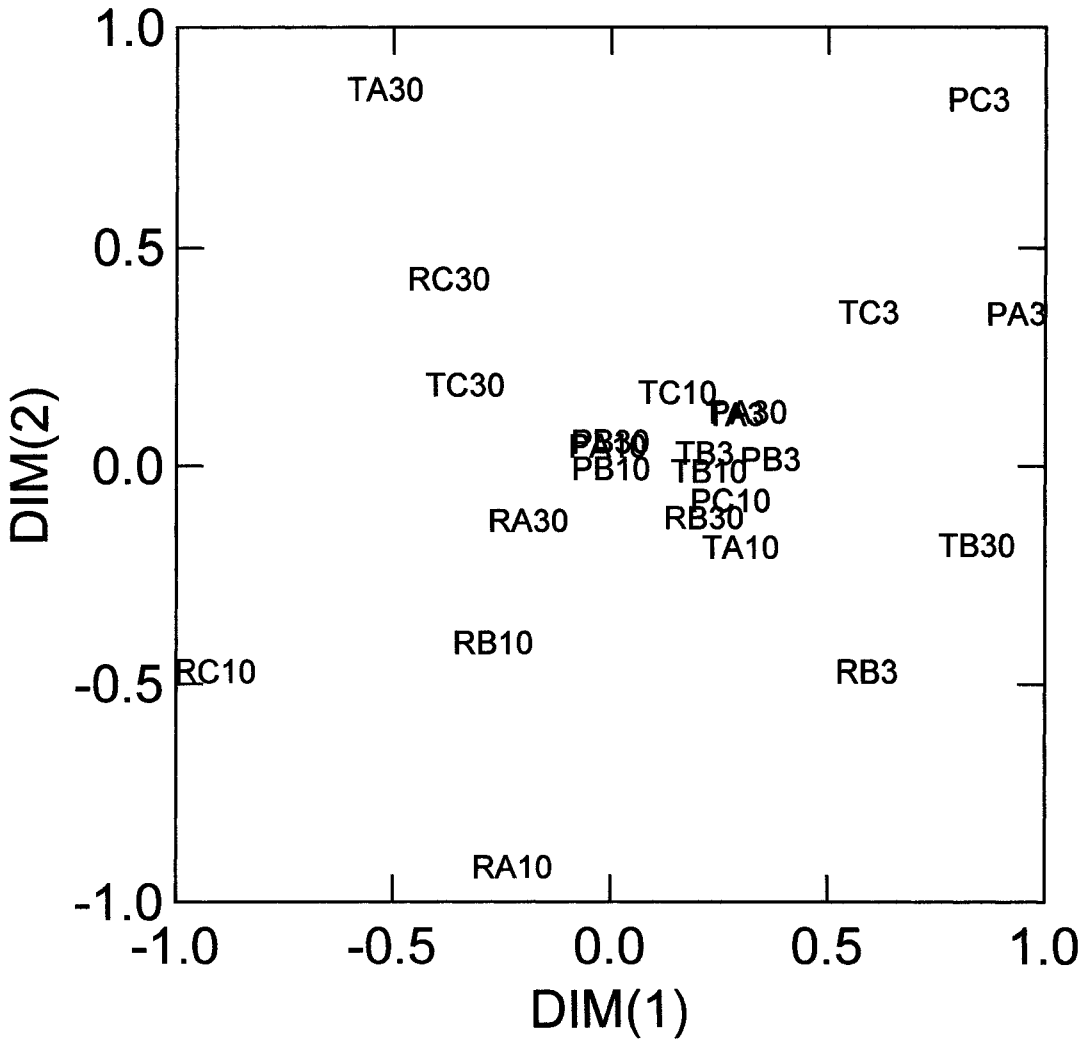

Figure 23.c. Cluster Analysis Grouped by Sample Type (Tile, Rock, Phytoplankton), Site (A, B, C) and Date (8/3, 8/30, 10/10) Using Standardized Species and Water Chemistry Data
*3 outlier variables (RC3, RA3, PC30) were excluded by decreasing scale of x and y-axis.

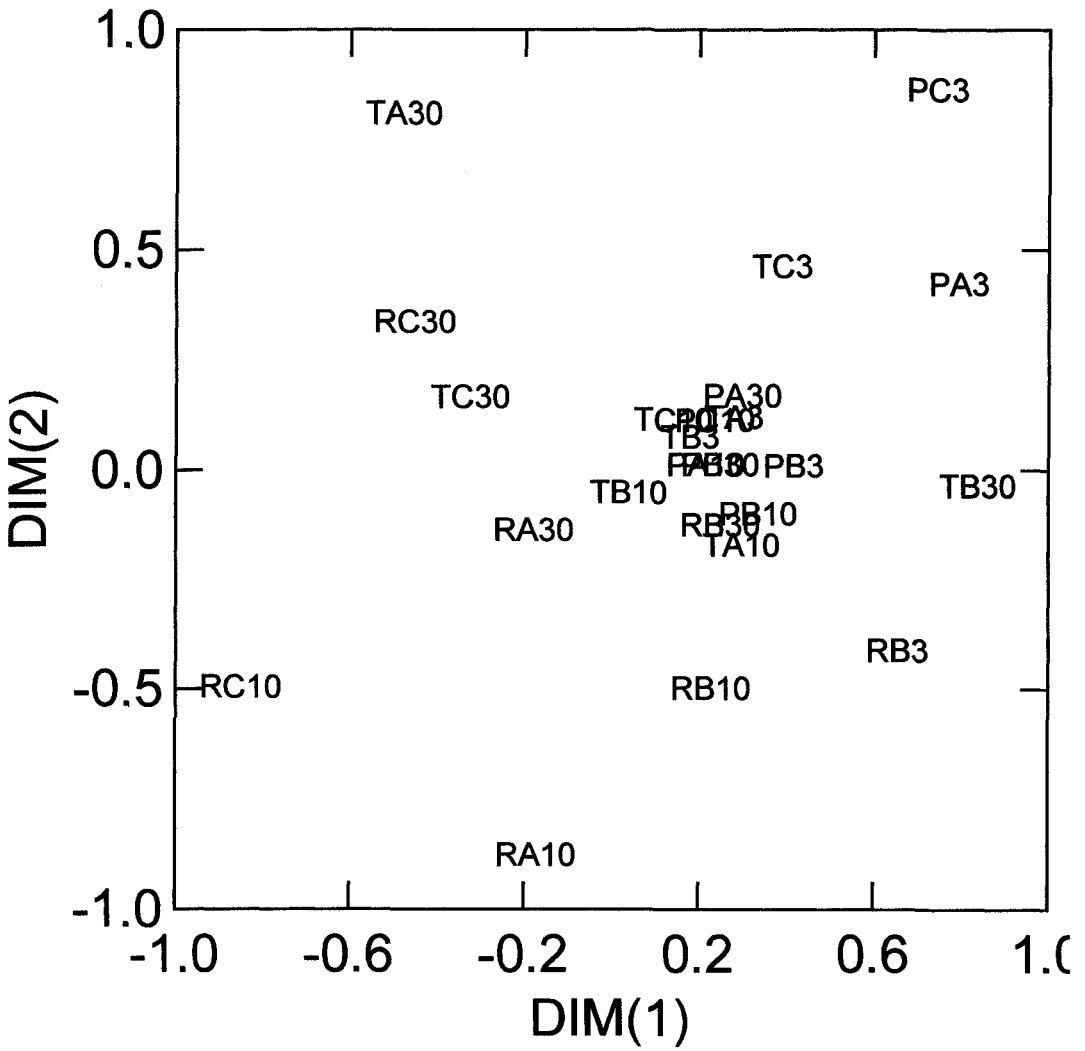

Table 1. Overall number of species and genera present at each site (across all dates and methods) and each method (across all dates and sites)

	All	Site A	Site B	Site C	Tile	Rock	Phyto
# Species	98	58	52	75	55	56	66
# Genera	21	14	13	18	15	16	14

Table 2. Cell density (#/mm^2 or #/mL) for sites (A,B,C) averaged across all dates and cell density (#/mm^2 or #/mL) for dates (8/3,8/30,10/10) averaged across all sites

Site	Date	Tile (#/mm^2)	Rock (#/mm^2)	Phytoplankton (#/mL)
All	All	2455	14482	24
A	All	3911	25399	29
B	All	1782	6808	10
C	All	1673	11240	34
All	8/3/00	303	26082	24
All	8/30/00	2600	5717	30
All	10/10/00	4462	11649	18

Table 3. Genera density (#/mm^2 or #/mL) summed across all sites, all dates, all methods

Genera	Total # Cells/mm^2 or mL
Nitzschia	58341
Amphora	18258
Rhoicosphenia	16687
Cocconeis	16002
Achnanthes	14737
Navicula	13001
Fragilaria	7797
Gomphonema	3568
Cyclotella	1389
Surirella	1158
Cymbella	812
Melosira	391
Gyrosigma	271
Cymatopleura	100
Synedra	60
Aulacoseira	38
Pleurosira	16
Pinnularia	11
Bacillaria	11
Tryblionella	3
Thalassiosira	3

Table 4. Genera density (#/mm^2 or #/mL) by site and method averaged across all dates (8/3, 8/30, 10/10)

	Tile (#/mm^2)			Rock (#/mm^2)			Phytoplankton (#/mL)		
Genus	**Site A**	**Site B**	**Site C**	**Site A**	**Site B**	**Site C**	**Site A**	**Site B**	**Site C**
Achnanthes	252	306	106	2343	2780	4033	3	2	1
Amphora	255	137	98	8508	507	2656	6	1	3
Aulacoseira							1		24
Bacillaria						7			
Cocconeis	1522	2539	2495	727	2865	510	3	5	1
Cyclotella	30	20	12	45	107	700	4	2	6
Cymatopleura						67			
Cymbella	320	29	2	95	9	85	1		
Fragilaria	37	15	4	5036	32	71	3		
Gomphonema	206	34	32	1533	398	172	2	1	1
Gyrosigma			27			152			1
Melosira				100	26	134			
Navicula	999	102	261	4438	537	2302	12	2	14
Nitzschia	3279	315	191	18251	6132	10695	14	3	14
Pinnularia		7							
Pleurosira					11				
Rhoicosphenia	921	37	68	9322	213	554	7	1	1
Surirella			29	399		343			1
Synedra		22	18						
Thalassiosira							2		
Tryblionella			2						
# of Genera	**10**	**12**	**14**	**12**	**12**	**15**	**12**	**8**	**11**

Table 5. Relative abundance (%) of species comprising >4% of the community

		Site A			Site B			Site C		
Genus	**Species**	Tile	Rock	Phyto	Tile	Rock	Phyto	Tile	Rock	Phyto
Achnanthes	helvetica		5			11			16	
Achnanthes	lanceolata				5	6	4			
Achnanthes	minutissima			4						
Amphora	ovalis						4			
Amphora	perpusilla		15	8					10	
Aulacoseira	alpigena									35
Cocconeis	placentula	19			71	21	27	73		
Cyclotella	meneghiniana			5			10			
Cymbella	tumida	4								
Fragilaria	fasciculata		10	5						
Gomphonema	parvulum						6			
Navicula	cryptocephala			4						6
Navicula	tripunctata	11								
Navicula	trivialis							4		5
Navicula	viridula			8					4	4
Nitzschia	amphibia				6	6	6			
Nitzschia	dissipata	24	12							
Nitzschia	inconspicua	16	23	18		38	9		43	
Nitzschia	palea									17
Rhoicosphenia	curvata	12	18	12			5			

Table 6. Mill Creek summary table: cell density, species richness, evenness, dominance by date, site and sampling method

Date (2000)	8/3	8/3	8/3	8/30	8/30	8/30	10/10	10/10	10/10
Site	A	B	C	A	B	C	A	B	C
Tile									
Cell Density (#/mm^2)	149	192	568	1920	2712	3169	9663	2440	1281
Richness (S)	16	14	18	25	20	22	18	18	18
Diversity (H')	0.73	0.60	0.44	0.95	0.44	0.60	0.92	0.67	0.52
Evenness (E)	0.60	0.52	0.35	0.68	0.34	0.45	0.73	0.54	0.42
Dominance (I)	0.35	0.46	0.64	0.19	0.66	0.51	0.16	0.39	0.57
Rock									
Cell Density (#/mm^2)	45664	12199	20382	12023	2986	2141	18508	5240	11198
Richness (S)	15	16	27	19	23	23	25	25	28
Diversity (H')	0.89	0.70	0.91	0.93	0.92	0.86	1.06	1.02	0.92
Evenness (E)	0.76	0.58	0.63	0.72	0.67	0.63	0.76	0.73	0.64
Dominance (I)	0.16	0.32	0.24	0.16	0.18	0.22	0.12	0.15	0.22
Phytoplankton									
Cell Density (#/mL)	38	8	25	36	17	38	12	3	39
Richness (S)	37	17	27	24	20	27	20	9	12
Diversity (H')	1.28	1.09	1.29	1.12	1.05	1.05	1.19	0.88	0.22
Evenness (E)	0.82	0.89	0.90	0.81	0.80	0.73	0.91	0.92	0.21
Dominance (I)	0.08	0.10	0.06	0.13	0.15	0.17	0.07	0.13	0.83

Table 7. Density, richness, diversity, evenness, dominance averaged across sites and dates by method

	Average	8/3/00	8/30/00	10/10/00	Site A	Site B	Site C
Tile							
Cell Density (#/mm^2)	2455.00	303.07	2600.29	4461.65	3910.60	1781.61	1672.80
Richness (S)	18.78	16.00	22.33	18.00	19.67	17.33	19.33
Diversity (H')	0.65	0.59	0.66	0.71	0.86	0.57	0.52
Evenness (E)	0.51	0.49	0.49	0.56	0.67	0.47	0.41
Dominance (I)	0.44	0.48	0.45	0.37	0.23	0.50	0.57
Rock							
Cell Density (#/mm^2)	14482.37	26081.84	5716.74	11648.54	25398.52	6808.23	11240.35
Richness (S)	22.33	19.33	21.67	26.00	19.67	21.33	26.00
Diversity (H')	0.91	0.83	0.90	1.00	0.96	0.88	0.90
Evenness (E)	0.68	0.66	0.68	0.71	0.75	0.66	0.63
Dominance (I)	0.20	0.24	0.19	0.17	0.15	0.22	0.23
Phytoplankton							
Cell Density (#/mL)	24.09	23.72	30.17	18.37	28.73	9.56	33.97
Richness (S)	21.44	27.00	23.67	13.67	27.00	15.33	22.00
Diversity (H')	1.02	1.22	1.07	0.76	1.20	1.01	0.85
Evenness (E)	0.78	0.87	0.78	0.68	0.85	0.87	0.61
Dominance (I)	0.19	0.08	0.15	0.34	0.09	0.12	0.35

Table 8. Percent similarity of diatom assemblage data between methods and sites

	Tile	Rock	Phyto	TileA	TileB	TileC	RockA	RockB	RockC	Phyto A	Phyto B	Phyto C
Tile	100											
Rock	41	100										
Phyto	37	44	100									
Tile A	69	50	35	100								
Tile B	61	17	20	32	100							
Tile C	62	21	27	33	83	100						
Rock A	40	78	35	51	10	13	100					
Rock B	46	59	33	48	45	35	40	100				
Rock C	23	67	39	27	11	17	49	61	100			
Phyto A	37	63	65	47	16	22	58	37	51	100		
Phyto B	55	33	47	44	49	42	25	54	29	43	100	
Phyto C	15	20	65	13	11	20	16	16	26	34	26	100

Table 9. Mill Creek physical parameters by site and date

TURBIDITY (NTU)				
Site	**August 3**	**August 30**	**October 10**	**Average**
A	6.50	7.63	10.07	8.06
B	3.29	9.65	1.78	4.91
C	37.85	68.85	24.95	43.88
CONDUCTIVITY (uS)				
Site	**August 3**	**August 30**	**October 10**	**Average**
A	815	710	880	802
B	1205	1190	2560	1652
C	380	455	658	497
TEMPERATURE (°C)				
Site	**August 3**	**August 30**	**October 10**	**Average**
A	21.80	N/A	N/A	21.80
B	22.70	22.80	18.40	21.30
C	23.20	23.90	10.60	19.23
DISSOLVED OXYGEN (mg/L)				
Site	**August 3**	**August 30**	**October 10**	**Average**
A	5.93	N/A	N/A	5.93
B	5.86	4.50	8.16	6.17
C	5.49	6.65	9.69	7.28

Table 10. Mill Creek nutrient data by site and date

NH_4 ug/L				
Site	**August 3**	**August 30**	**October 10**	**Average**
A	46.43	49.63	51.22	42.91
B	1570.60	2023.43	143.39	1261.01
C	95.44	95.97	93.84	85.36
NO_3 ug/L				
Site	**August 3**	**August 30**	**October 10**	**Average**
A	N/A	182.08	342.90	292.65
B	1549.11	1910.97	2594.49	1862.72
C	342.90	182.08	463.52	383.11
PO_4 ug/L				
Site	**August 3**	**August 30**	**October 10**	**Average**
A	131.80	123.28	92.46	128.79
B	1952.13	1758.69	1473.44	1640.79
C	54.43	60.98	95.74	67.54

Table 11. Pearson correlation matrix (correlation of variables at all sites, all dates for all methods; n=27)

	Dens	Rich	Divers	Even	Dom	NO_3	PO_4	NH_4	Turb	Cond	DO	Temp
Temp	-0.052	0.198	0.348	0.310	-0.293	0.038	0.251	0.371	0.226	-0.117	**-0.883**	1.000
DO	0.039	-0.171	-0.267	-0.210	0.222	-0.099	-0.374	**-0.654**	0.001	0.186	1.000	
Cond	-0.096	-0.396	0.090	0.290	-0.168	**0.933**	**0.679**	0.136	**-0.719**	1.000		
Turb	0.092	**0.487**	0.034	-0.140	0.062	**-0.836**	**-0.816**	**-0.531**	1.000			
NH_4	-0.070	-0.207	0.036	0.081	-0.038	**0.468**	**0.795**	1.000				
PO_4	-0.122	**-0.424**	0.096	0.261	-0.149	**0.868**	1.000					
NO_3	-0.105	-0.397	0.105	0.290	-0.175	1.000						
Dom	0.391	-0.362	**-0.965**	**-0.967**	1.000							
Even	**-0.417**	0.232	**0.935**	1.000								
Divers	-0.235	**0.544**	1.000									
Rich	**0.432**	1.000										
Dens	1.000											

Table 12. One-way ANOVA results (Squared Multiple R, F-ratio, p-value)

(Testing for differences in density, richness, diversity, evenness, dominance between methods at each site)

Factor = Method	**Site A**			**Site B**			**Site C**		
Dependent Variable	**p**	**R**	**F**	**p**	**R**	**F**	**p**	**R**	**F**
L10 Density	**0.002**	0.867	19.543	**0.001**	0.912	31.258	**0.001**	0.915	32.258
Richness	0.346	0.298	1.274	0.337	0.304	1.313	0.375	0.279	1.16
H' Diversity	**0.015**	0.752	9.117	**0.018**	0.74	8.549	0.368	0.283	1.185
Evenness	**0.016**	0.75	8.979	**0.003**	0.849	16.851	0.399	0.264	1.075
L10 Dominance	**0.044**	0.646	5.484	**0.004**	0.843	16.097	0.271	0.353	1.635

Table 13. One-way ANOVA results (Squared Multiple R, F-ratio, p-value)

(Testing for differences in density, richness, diversity, evenness, dominance between sites for each method)

Factor = Site	**Tile Method**			**Rock Method**			**Phyto Method**		
Dependent Variable	**p**	**R**	**F**	**p**	**R**	**F**	**p**	**R**	**F**
L10 Density	0.978	0.007	0.022	0.236	0.382	1.854	**0.056**	0.617	4.826
Richness	0.695	0.114	0.387	0.248	0.372	1.774	0.268	0.355	1.655
H' Diversity	**0.015**	0.753	9.152	0.658	0.13	0.449	0.493	0.21	0.797
Evenness	**0.016**	0.75	8.991	**0.052**	0.627	5.043	0.332	0.307	1.331
L10 Dominance	**0.015**	0.755	9.248	0.144	0.476	2.725	0.504	0.204	0.77

Table 14. Two-way ANOVA results (Squared Multiple R, F-ratio, p-value)

(Testing for any interaction between site and method for density, richness, diversity, evenness, dominance)

Factor = Site & Method	**Site**			**Method**			**Interaction**		
Dependent Variable	**p**	**R**	**F**	**p**	**R**	**F**	**p**	**R**	**F**
L10 Density	0.215	0.898	1.678	**0.000**	0.898	76.198	0.724	0.898	0.517
Richness	0.196	0.380	1.785	0.388	0.380	0.998	0.283	0.380	1.372
H' Diversity	**0.055**	0.562	3.426	**0.005**	0.562	7.125	0.732	0.562	0.507
Evenness	**0.016**	0.639	5.211	**0.002**	0.639	8.820	0.451	0.639	0.964
L10 Dominance	**0.022**	0.644	4.775	**0.001**	0.644	10.717	0.811	0.644	0.393

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Appendix I: Diatom valve density (#valves/mm^2) by sampling method, date, and site

Sample Type:		Tile	Tile	Tile	Tile	Tile	Tile	Tile
Sample Date:		8/3/00	8/3/00	8/3/00	8/30/00	8/30/00	8/30/00	10/10/00
Sample Site:		A	B	C	A	B	C	A
Genus	Species							
Achnanthes	conspicua	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Achnanthes	delicatula	0.00	7.93	11.90	50.30	0.00	0.00	0.00
Achnanthes	helvetica	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Achnanthes	hungarica	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Achnanthes	hustedtii	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Achnanthes	lanceolata	2.97	22.80	29.74	33.53	162.72	68.30	147.53
Achnanthes	minutissima	2.97	7.93	0.00	243.10	75.94	81.96	258.18
Achnanthes	oblongella	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Achnanthes	pinnata	0.00	0.00	0.00	16.77	0.00	0.00	0.00
Amphora	ovalis	0.00	0.00	5.95	0.00	119.33	0.00	110.65
Amphora	ovalis pediculus	4.46	1.98	44.62	117.36	21.70	81.96	110.65
Amphora	perpusilla	29.00	0.00	5.95	25.15	21.70	109.28	368.83
Aulacoseira	alpigena	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Aulacoseira	italica	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacillaria	paradoxa	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cocconeis	pediculus	0.00	0.00	11.90	0.00	0.00	122.94	0.00
Cocconeis	placentula	171.03	257.79	907.21	964.00	4382.62	4494.31	3430.16
Cocconeis	klamathensis	0.00	0.00	5.95	0.00	0.00	0.00	0.00
Cocconeis	rugosa	0.00	0.00	20.82	0.00	0.00	0.00	0.00
Cyclotella	atomus	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cyclotella	meneghiniana	0.00	0.00	8.92	16.77	0.00	27.32	73.77
Cyclotella	pseudostelligera	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cyclotella	stelligera	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cymatopleura	solea	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cymbella	norvegica	0.00	0.00	0.00	0.00	86.78	0.00	0.00
Cymbella	prostrata	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cymbella	silesiaca	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cymbella	sinuata	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cymbella	tumida	0.00	0.00	5.95	0.00	0.00	0.00	958.97
Cymbella	turgidula	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Fragilaria	fasciculata	0.00	0.00	0.00	0.00	0.00	0.00	110.65
Fragilaria	vaucherae	0.00	0.00	0.00	0.00	21.70	0.00	0.00
Gomphonema	augur	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Gomphonema	angustum	0.00	3.97	0.00	16.77	0.00	0.00	0.00
Gomphonema	gracile	0.00	0.00	0.00	16.77	0.00	0.00	0.00
Gomphonema	minutum	0.00	0.00	0.00	50.30	0.00	0.00	0.00
Gomphonema	olivaceum	0.00	0.00	0.00	0.00	0.00	0.00	258.18
Gomphonema	parvulum	2.97	33.71	0.00	33.53	65.09	81.96	221.30
Gomphonema	subclavatum	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Gomphonema	truncatum	1.49	0.00	0.00	16.77	0.00	0.00	0.00
Gyrosigma	acuminatum	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Gyrosigma	scalproides	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Gyrosigma	spencerii	0.00	0.00	0.00	0.00	0.00	81.96	0.00

Sample Type:		Tile	Tile	Tile	Tile	Tile	Tile	Tile
Sample Date:		8/3/00	8/3/00	8/3/00	8/30/00	8/30/00	8/30/00	10/10/00
Sample Site:		A	B	C	A	B	C	A
Genus	Species							
Hantzschia	amphioxys	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Melosira	varians	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Navicula	angusta	0.00	0.00	0.00	33.53	0.00	0.00	0.00
Navicula	capitoradiata	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Navicula	cincta	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Navicula	cryptocephala	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Navicula	cryptotenella	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Navicula	cuspidata	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Navicula	decussis	0.00	0.00	0.00	0.00	0.00	27.32	0.00
Navicula	goeppertiana	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Navicula	lanceolata	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Navicula	menisculus	4.46	6.94	14.87	33.53	43.39	0.00	73.77
Navicula	minima	10.41	12.89	0.00	0.00	0.00	0.00	0.00
Navicula	pseudoreinhardtii	0.00	0.00	0.00	50.30	0.00	0.00	0.00
Navicula	pupula	2.97	0.00	5.95	0.00	0.00	0.00	0.00
Navicula	radiosa	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Navicula	reichardtiana	0.00	0.00	0.00	0.00	21.70	0.00	0.00
Navicula	salinarum	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Navicula	sp1	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Navicula	symmetrica	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Navicula	tripunctata	0.00	0.00	0.00	0.00	0.00	27.32	2618.72
Navicula	trivialis	0.00	0.00	0.00	0.00	0.00	382.49	0.00
Navicula	veneta	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Navicula	viridula	5.95	2.97	32.72	16.77	21.70	54.64	147.53
Nitzschia	amphibia	0.00	5.95	5.95	33.53	97.63	54.64	331.95
Nitzschia	angustatula	0.00	0.00	0.00	16.77	0.00	0.00	0.00
Nitzschia	angustiforaminata	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Nitzschia	calida	0.00	0.00	0.00	0.00	21.70	0.00	0.00
Nitzschia	closterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Nitzschia	constricta	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Nitzschia	dissipata	5.95	0.00	5.95	310.16	21.70	13.66	5200.56
Nitzschia	fonticola	0.00	0.00	0.00	33.53	21.70	27.32	0.00
Nitzschia	frustulum	11.90	5.95	0.00	167.65	0.00	27.32	0.00
Nitzschia	gracilis	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Nitzschia	inconspicua	18.59	7.93	0.00	1257.39	43.39	150.27	2397.42
Nitzschia	levidensis	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Nitzschia	macilenta	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Nitzschia	microcephala	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Nitzschia	palea	0.00	5.95	5.95	50.30	0.00	136.61	0.00
Nitzschia	reversa	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Nitzschia	umbonata	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Nitzschia	viridula	1.49	0.00	0.00	0.00	0.00	0.00	0.00
Nitzschia	wuellerstorffii	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pinnularia	gibba	0.00	0.00	0.00	0.00	21.70	0.00	0.00
Pleurosira	laevis	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Sample Type:		Tile	Tile	Tile	Tile	Tile	Tile	Tile
Sample Date:		8/3/00	8/3/00	8/3/00	8/30/00	8/30/00	8/30/00	10/10/00
Sample Site:		A	B	C	A	B	C	A
Genus	Species							
Rhoicosphenia	curvata	20.82	0.00	0.00	234.71	86.78	204.91	2508.07
Surirella	angusta	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Surirella	minuta/pinnata	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Surirella	ovalis	0.00	0.00	0.00	0.00	0.00	27.32	0.00
Surirella	ovata	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Surirella	suecica	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Synedra	demerarae	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Synedra	ulna	0.00	0.00	0.00	0.00	65.09	54.64	0.00
Thalassiosira	weisflogii	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Tryblionella	calida	0.00	0.00	5.95	0.00	0.00	0.00	0.00

Sample Type:		Tile	Tile
Sample Date:		10/10/00	10/10/00
Sample Site:		B	C
Genus	Species		
Achnanthes	conspicua	0.00	0.00
Achnanthes	delicatula	0.00	0.00
Achnanthes	helvetica	0.00	0.00
Achnanthes	hungarica	0.00	0.00
Achnanthes	hustedtii	196.71	0.00
Achnanthes	lanceolata	344.25	125.14
Achnanthes	minutissima	98.36	0.00
Achnanthes	oblongella	0.00	0.00
Achnanthes	pinnata	0.00	0.00
Amphora	ovalis	24.59	0.00
Amphora	ovalis pediculus	36.88	26.35
Amphora	perpusilla	184.42	19.76
Aulacoseira	alpigena	0.00	0.00
Aulacoseira	italica	0.00	0.00
Bacillaria	paradoxa	0.00	0.00
Cocconeis	pediculus	0.00	0.00
Cocconeis	placentula	2975.26	1923.21
Cocconeis	klamathensis	0.00	0.00
Cocconeis	rugosa	0.00	0.00
Cyclotella	atomus	0.00	0.00
Cyclotella	meneghiniana	61.47	0.00
Cyclotella	pseudostelligera	0.00	0.00
Cyclotella	stelligera	0.00	0.00
Cymatopleura	solea	0.00	0.00
Cymbella	norvegica	0.00	0.00
Cymbella	prostrata	0.00	0.00
Cymbella	silesiaca	0.00	0.00
Cymbella	sinuata	0.00	0.00
Cymbella	tumida	0.00	0.00
Cymbella	turgidula	0.00	0.00
Fragilaria	fasciculata	24.59	13.17
Fragilaria	vaucherae	0.00	0.00
Gomphonema	augur	0.00	0.00
Gomphonema	angustum	0.00	0.00
Gomphonema	gracile	0.00	13.17
Gomphonema	minutum	0.00	0.00
Gomphonema	olivaceum	0.00	0.00
Gomphonema	parvulum	0.00	0.00
Gomphonema	subclavatum	0.00	0.00
Gomphonema	truncatum	0.00	0.00
Gyrosigma	acuminatum	0.00	0.00
Gyrosigma	scalproides	0.00	0.00
Gyrosigma	spencerii	0.00	0.00
Hantzschia	amphioxys	0.00	0.00
Melosira	varians	0.00	0.00

Sample Type:		Tile	Tile
Sample Date:		10/10/00	10/10/00
Sample Site:		B	C
Genus	Species		
Navicula	angusta	0.00	0.00
Navicula	capitoradiata	0.00	0.00
Navicula	cincta	0.00	0.00
Navicula	cryptocephala	0.00	72.45
Navicula	cryptotenella	0.00	0.00
Navicula	cuspidata	0.00	0.00
Navicula	decussis	0.00	0.00
Navicula	goeppertiana	0.00	0.00
Navicula	lanceolata	0.00	0.00
Navicula	menisculus	122.94	52.69
Navicula	minima	0.00	0.00
Navicula	pseudoreinhardtii	24.59	0.00
Navicula	pupula	0.00	0.00
Navicula	radiosa	0.00	0.00
Navicula	reichardtiana	0.00	0.00
Navicula	salinarum	0.00	72.45
Navicula	sp1	0.00	0.00
Navicula	symmetrica	49.18	0.00
Navicula	tripunctata	0.00	13.17
Navicula	trivialis	0.00	0.00
Navicula	veneta	0.00	0.00
Navicula	viridula	0.00	26.35
Nitzschia	amphibia	565.55	26.35
Nitzschia	angustatula	0.00	0.00
Nitzschia	angustiforaminata	0.00	0.00
Nitzschia	calida	0.00	0.00
Nitzschia	closterium	0.00	0.00
Nitzschia	constricta	0.00	39.52
Nitzschia	dissipata	49.18	32.93
Nitzschia	fonticola	12.29	0.00
Nitzschia	frustulum	12.29	0.00
Nitzschia	gracilis	0.00	0.00
Nitzschia	inconspicua	73.77	46.10
Nitzschia	levidensis	0.00	0.00
Nitzschia	macilenta	0.00	0.00
Nitzschia	microcephala	0.00	0.00
Nitzschia	palea	0.00	0.00
Nitzschia	reversa	0.00	0.00
Nitzschia	umbonata	0.00	0.00
Nitzschia	viridula	0.00	0.00
Nitzschia	wuellerstorffii	0.00	0.00
Pinnularia	gibba	0.00	0.00
Pleurosira	laevis	0.00	0.00
Rhoicosphenia	curvata	24.59	0.00
Surirella	angusta	0.00	13.17

Sample Type:		Tile	Tile
Sample Date:		10/10/00	10/10/00
Sample Site:		B	C
Genus	Species		
Surirella	minuta/pinnata	0.00	26.35
Surirella	ovalis	0.00	0.00
Surirella	ovata	0.00	19.76
Surirella	suecica	0.00	0.00
Synedra	demerarae	0.00	0.00
Synedra	ulna	0.00	0.00
Thalassiosira	weisflogii	0.00	0.00
Tryblionella	calida	0.00	0.00

Appendix II: Valve density (#valves/mm^2) of diatom species on rocks by date and site

Sample Type:		Rock	Rock	Rock	Rock	Rock	Rock
Sample Date:		8/3/00	8/3/00	8/3/00	8/30/00	8/30/00	8/30/00
Sample Site:		A	B	C	A	B	C
Genus	Species						
Achnanthes	conspicua	0.00	0.00	401.62	0.00	0.00	0.00
Achnanthes	delicatula	0.00	0.00	0.00	0.00	0.00	0.00
Achnanthes	helvetica	0.00	0.00	7329.55	3815.38	1414.40	710.01
Achnanthes	hungarica	0.00	0.00	401.62	0.00	0.00	0.00
Achnanthes	hustedtii	0.00	100.40	0.00	0.00	104.77	0.00
Achnanthes	lanceolata	0.00	2008.10	200.81	0.00	340.50	43.03
Achnanthes	minutissima	0.00	853.44	0.00	0.00	0.00	0.00
Achnanthes	oblongella	0.00	0.00	0.00	0.00	0.00	0.00
Achnanthes	pinnata	0.00	0.00	0.00	0.00	0.00	0.00
Amphora	ovalis	481.94	0.00	200.81	200.81	104.77	0.00
Amphora	ovalis pediculus	963.89	100.40	0.00	401.62	26.19	53.79
Amphora	perpusilla	13735.38	200.81	3012.15	2911.74	248.83	376.52
Aulacoseira	alpigena	0.00	0.00	0.00	0.00	0.00	0.00
Aulacoseira	italica	0.00	0.00	0.00	0.00	0.00	0.00
Bacillaria	paradoxa	0.00	0.00	0.00	0.00	0.00	21.52
Cocconeis	pediculus	481.94	0.00	200.81	0.00	26.19	0.00
Cocconeis	placentula	963.89	4719.03	1004.05	0.00	1741.81	43.03
Cocconeis	klamathensis	0.00	0.00	0.00	0.00	0.00	0.00
Cocconeis	rugosa	0.00	0.00	0.00	0.00	0.00	0.00
Cyclotella	atomus	0.00	0.00	0.00	0.00	0.00	0.00
Cyclotella	meneghiniana	0.00	200.81	2008.10	0.00	26.19	21.52
Cyclotella	pseudostelligera	0.00	0.00	0.00	0.00	0.00	0.00
Cyclotella	stelligera	0.00	0.00	0.00	0.00	0.00	0.00
Cymatopleura	solea	0.00	0.00	200.81	0.00	0.00	0.00
Cymbella	norvegica	0.00	0.00	0.00	0.00	0.00	0.00
Cymbella	prostrata	0.00	0.00	0.00	0.00	0.00	0.00
Cymbella	silesiaca	0.00	0.00	0.00	0.00	0.00	0.00
Cymbella	sinuata	0.00	0.00	0.00	0.00	0.00	21.52
Cymbella	tumida	0.00	0.00	0.00	150.61	26.19	0.00
Cymbella	turgidula	0.00	0.00	0.00	0.00	0.00	21.52
Fragilaria	fasciculata	10120.81	0.00	0.00	100.40	0.00	0.00
Fragilaria	vaucherae	0.00	0.00	0.00	0.00	0.00	0.00
Gomphonema	augur	0.00	0.00	0.00	0.00	0.00	0.00
Gomphonema	angustum	0.00	301.21	0.00	0.00	0.00	0.00
Gomphonema	gracile	0.00	0.00	0.00	0.00	0.00	0.00
Gomphonema	minutum	0.00	0.00	0.00	0.00	0.00	21.52
Gomphonema	olivaceum	0.00	0.00	0.00	0.00	0.00	0.00
Gomphonema	parvulum	2409.72	803.24	200.81	100.40	26.19	21.52
Gomphonema	subclavatum	0.00	0.00	200.81	0.00	0.00	0.00
Gomphonema	truncatum	481.94	0.00	0.00	0.00	0.00	0.00
Gyrosigma	acuminatum	0.00	0.00	0.00	0.00	0.00	21.52
Gyrosigma	scalproides	0.00	0.00	200.81	0.00	0.00	21.52
Gyrosigma	spencerii	0.00	0.00	0.00	0.00	0.00	0.00

Sample Type:		Rock	Rock	Rock	Rock	Rock	Rock
Sample Date:		8/3/00	8/3/00	8/3/00	8/30/00	8/30/00	8/30/00
Sample Site:		A	B	C	A	B	C
Genus	Species						
Hantzschia	amphioxys	0.00	0.00	0.00	0.00	0.00	0.00
Melosira	varians	0.00	0.00	401.62	301.21	78.58	0.00
Navicula	angusta	0.00	0.00	200.81	0.00	0.00	0.00
Navicula	capitoradiata	0.00	0.00	0.00	0.00	0.00	0.00
Navicula	cincta	0.00	0.00	0.00	0.00	0.00	0.00
Navicula	cryptocephala	0.00	0.00	0.00	200.81	78.58	0.00
Navicula	cryptotenella	0.00	0.00	0.00	1305.26	0.00	21.52
Navicula	cuspidata	0.00	0.00	0.00	0.00	0.00	0.00
Navicula	decussis	0.00	0.00	0.00	0.00	0.00	0.00
Navicula	goeppertiana	0.00	0.00	0.00	0.00	0.00	0.00
Navicula	lanceolata	0.00	0.00	0.00	0.00	0.00	0.00
Navicula	menisculus	1445.83	100.40	401.62	100.40	26.19	32.27
Navicula	minima	0.00	0.00	0.00	0.00	0.00	0.00
Navicula	pseudoreinhardtii	0.00	0.00	0.00	0.00	0.00	0.00
Navicula	pupula	0.00	0.00	0.00	0.00	0.00	0.00
Navicula	radiosa	0.00	0.00	0.00	0.00	26.19	0.00
Navicula	reichardtiana	963.89	0.00	0.00	0.00	78.58	0.00
Navicula	salinarum	0.00	0.00	1004.05	0.00	0.00	0.00
Navicula	sp1	0.00	0.00	0.00	0.00	0.00	0.00
Navicula	symmetrica	0.00	0.00	0.00	0.00	26.19	21.52
Navicula	tripunctata	3373.60	0.00	0.00	903.64	0.00	0.00
Navicula	trivialis	0.00	0.00	200.81	0.00	0.00	387.28
Navicula	veneta	0.00	0.00	0.00	0.00	0.00	0.00
Navicula	viridula	0.00	100.40	1807.29	200.81	0.00	64.55
Nitzschia	amphibia	0.00	1606.48	0.00	100.40	183.35	21.52
Nitzschia	angustatula	0.00	0.00	0.00	0.00	0.00	0.00
Nitzschia	angustiforaminata	0.00	0.00	0.00	0.00	0.00	0.00
Nitzschia	calida	0.00	0.00	0.00	0.00	0.00	0.00
Nitzschia	closterium	0.00	0.00	200.81	0.00	0.00	0.00
Nitzschia	constricta	0.00	0.00	200.81	0.00	26.19	64.55
Nitzschia	dissipata	16627.04	200.81	0.00	1606.48	26.19	0.00
Nitzschia	fonticola	0.00	0.00	0.00	0.00	0.00	0.00
Nitzschia	frustulum	0.00	0.00	401.62	0.00	0.00	0.00
Nitzschia	gracilis	0.00	0.00	0.00	0.00	0.00	0.00
Nitzschia	inconspicua	19518.70	12701.21	17972.47	7229.15	1100.09	1753.50
Nitzschia	levidensis	0.00	0.00	0.00	0.00	0.00	0.00
Nitzschia	macilenta	0.00	0.00	0.00	0.00	0.00	0.00
Nitzschia	microcephala	0.00	0.00	0.00	0.00	26.19	0.00
Nitzschia	palea	0.00	100.40	803.24	502.02	0.00	494.85
Nitzschia	reversa	0.00	0.00	0.00	0.00	0.00	0.00
Nitzschia	umbonata	0.00	0.00	0.00	0.00	0.00	0.00
Nitzschia	viridula	0.00	0.00	0.00	0.00	0.00	0.00
Nitzschia	wuellerstorffii	0.00	0.00	0.00	0.00	0.00	0.00
Pinnularia	gibba	0.00	0.00	0.00	0.00	0.00	0.00
Pleurosira	laevis	0.00	0.00	0.00	0.00	0.00	0.00

Sample Type:		Rock	Rock	Rock	Rock	Rock	Rock
Sample Date:		8/3/00	8/3/00	8/3/00	8/30/00	8/30/00	8/30/00
Sample Site:		A	B	C	A	B	C
Genus	Species						
Rhoicosphenia	curvata	18795.79	301.21	1004.05	3815.38	209.54	21.52
Surirella	angusta	0.00	0.00	0.00	0.00	0.00	0.00
Surirella	minuta/pinnata	0.00	0.00	0.00	0.00	0.00	0.00
Surirella	ovalis	0.00	0.00	200.81	0.00	0.00	0.00
Surirella	ovata	963.89	0.00	0.00	100.40	0.00	0.00
Surirella	suecica	0.00	0.00	401.62	0.00	0.00	0.00
Synedra	demerarae	0.00	0.00	0.00	0.00	0.00	0.00
Synedra	ulna	0.00	0.00	0.00	0.00	0.00	0.00
Thalassiosira	weisflogii	0.00	0.00	0.00	0.00	0.00	0.00
Tryblionella	calida	0.00	0.00	0.00	0.00	0.00	0.00

Sample Type:		Rock	Rock	Rock
Sample Date:		10/10/00	10/10/00	10/10/00
Sample Site:		A	B	C
Genus	Species			
Achnanthes	conspicua	0.00	0.00	0.00
Achnanthes	delicatula	0.00	0.00	0.00
Achnanthes	helvetica	3212.96	2964.59	3012.15
Achnanthes	hungarica	0.00	0.00	0.00
Achnanthes	hustedtii	0.00	459.75	0.00
Achnanthes	lanceolata	0.00	95.12	0.00
Achnanthes	minutissima	0.00	0.00	0.00
Achnanthes	oblongella	0.00	0.00	0.00
Achnanthes	pinnata	0.00	0.00	0.00
Amphora	ovalis	0.00	269.51	70.87
Amphora	ovalis pediculus	1004.05	79.27	779.61
Amphora	perpusilla	5823.48	491.46	3472.83
Aulacoseira	alpigena	0.00	0.00	0.00
Aulacoseira	italica	0.00	0.00	0.00
Bacillaria	paradoxa	0.00	0.00	0.00
Cocconeis	pediculus	468.56	0.00	0.00
Cocconeis	placentula	267.75	2108.50	283.50
Cocconeis	klamathensis	0.00	0.00	0.00
Cocconeis	rugosa	0.00	0.00	0.00
Cyclotella	atomus	0.00	0.00	0.00
Cyclotella	meneghiniana	133.87	95.12	70.87
Cyclotella	pseudostelligera	0.00	0.00	0.00
Cyclotella	stelligera	0.00	0.00	0.00
Cymatopleura	solea	0.00	0.00	0.00
Cymbella	norvegica	0.00	0.00	0.00
Cymbella	prostrata	0.00	0.00	70.87
Cymbella	silesiaca	0.00	0.00	70.87
Cymbella	sinuata	133.87	0.00	70.87
Cymbella	tumida	0.00	0.00	0.00
Cymbella	turgidula	0.00	0.00	0.00
Fragilaria	fasciculata	4886.37	95.12	212.62
Fragilaria	vaucherae	0.00	0.00	0.00
Gomphonema	augur	267.75	0.00	0.00
Gomphonema	angustum	0.00	0.00	0.00
Gomphonema	gracile	0.00	0.00	0.00
Gomphonema	minutum	0.00	0.00	0.00
Gomphonema	olivaceum	401.62	0.00	0.00
Gomphonema	parvulum	669.37	63.41	70.87
Gomphonema	subclavatum	0.00	0.00	0.00
Gomphonema	truncatum	267.75	0.00	0.00
Gyrosigma	acuminatum	0.00	0.00	0.00
Gyrosigma	scalproides	0.00	0.00	212.62
Gyrosigma	spencerii	0.00	0.00	0.00
Hantzschia	amphioxys	0.00	0.00	0.00

Sample Type:		Rock	Rock	Rock
Sample Date:		10/10/00	10/10/00	10/10/00
Sample Site:		A	B	C
Genus	Species			
Melosira	varians	0.00	0.00	0.00
Navicula	angusta	0.00	0.00	70.87
Navicula	capitoradiata	0.00	0.00	0.00
Navicula	cincta	0.00	0.00	0.00
Navicula	cryptocephala	267.75	126.83	566.99
Navicula	cryptotenella	1204.86	221.95	70.87
Navicula	cuspidata	0.00	0.00	0.00
Navicula	decussis	0.00	0.00	0.00
Navicula	goeppertiana	0.00	31.71	0.00
Navicula	lanceolata	0.00	0.00	0.00
Navicula	menisculus	669.37	63.41	354.37
Navicula	minima	0.00	0.00	0.00
Navicula	pseudoreinhardtii	0.00	0.00	0.00
Navicula	pupula	0.00	0.00	0.00
Navicula	radiosa	0.00	0.00	0.00
Navicula	reichardtiana	803.24	158.53	921.36
Navicula	salinarum	0.00	126.83	141.75
Navicula	sp1	0.00	0.00	0.00
Navicula	symmetrica	133.87	221.95	0.00
Navicula	tripunctata	803.24	63.41	70.87
Navicula	trivialis	0.00	0.00	0.00
Navicula	veneta	0.00	0.00	0.00
Navicula	viridula	937.11	158.53	566.99
Nitzschia	amphibia	267.75	634.14	0.00
Nitzschia	angustatula	0.00	0.00	0.00
Nitzschia	angustiforaminata	0.00	0.00	0.00
Nitzschia	calida	0.00	0.00	0.00
Nitzschia	closterium	0.00	0.00	0.00
Nitzschia	constricta	0.00	0.00	354.37
Nitzschia	dissipata	267.75	63.41	141.75
Nitzschia	fonticola	0.00	0.00	70.87
Nitzschia	frustulum	0.00	0.00	0.00
Nitzschia	gracilis	0.00	0.00	0.00
Nitzschia	inconspicua	8367.07	1601.19	9319.93
Nitzschia	levidensis	0.00	0.00	0.00
Nitzschia	macilenta	0.00	0.00	0.00
Nitzschia	microcephala	0.00	0.00	0.00
Nitzschia	palea	267.75	126.83	283.50
Nitzschia	reversa	0.00	0.00	0.00
Nitzschia	umbonata	0.00	0.00	0.00
Nitzschia	viridula	0.00	0.00	0.00
Nitzschia	wuellerstorffii	0.00	0.00	0.00
Pinnularia	gibba	0.00	0.00	0.00
Pleurosira	laevis	0.00	31.71	0.00
Rhoicosphenia	curvata	5354.93	126.83	637.87

Sample Type:		Rock	Rock	Rock
Sample Date:		10/10/00	10/10/00	10/10/00
Sample Site:		A	B	C
Genus	Species			
Surirella	angusta	0.00	0.00	0.00
Surirella	minuta/pinnata	0.00	0.00	0.00
Surirella	ovalis	0.00	0.00	0.00
Surirella	ovata	133.87	0.00	283.50
Surirella	suecica	0.00	0.00	141.75
Synedra	demerarae	0.00	0.00	0.00
Synedra	ulna	0.00	0.00	0.00
Thalassiosira	weisflogii	0.00	0.00	0.00
Tryblionella	calida	0.00	0.00	0.00

Appendix III. Valve density (# valves/mL) of diatom species in phytoplankton samples by date and site

Sample Type:		Phyto	Phyto	Phyto	Phyto	Phyto	Phyto
Sample Date:		8/3/00	8/3/00	8/3/00	8/30/00	8/30/00	8/30/00
Sample Site:		A	B	C	A	B	C
Genus	Species						
Achnanthes	conspicua	0.00	0.00	0.00	0.00	0.00	0.00
Achnanthes	delicatula	0.00	0.00	0.00	0.00	0.00	0.00
Achnanthes	helvetica	0.46	0.00	0.00	0.91	0.00	0.00
Achnanthes	hungarica	0.00	0.00	0.00	0.00	0.00	0.00
Achnanthes	hustedtii	0.00	0.46	0.00	0.00	1.14	0.00
Achnanthes	lanceolata	0.00	0.00	0.00	0.00	2.05	0.46
Achnanthes	minutissima	0.46	0.46	0.46	5.69	1.37	0.00
Achnanthes	oblongella	0.00	0.00	0.91	0.00	0.46	0.00
Achnanthes	pinnata	0.00	0.00	0.00	0.00	0.00	0.00
Amphora	ovalis	0.91	0.46	1.82	1.14	1.14	0.46
Amphora	ovalis pediculus	0.00	0.00	0.00	1.14	0.46	1.59
Amphora	perpusilla	3.64	0.00	3.87	7.74	1.82	2.73
Aulacoseira	alpigena	0.00	0.00	0.00	3.19	0.00	0.00
Aulacoseira	italica	0.00	0.00	0.00	0.00	0.00	0.46
Bacillaria	paradoxa	0.00	0.00	0.00	0.00	0.00	0.00
Cocconeis	pediculus	1.37	0.46	0.00	0.00	0.00	0.91
Cocconeis	placentula	2.73	3.19	2.05	1.82	12.07	0.91
Cocconeis	klamathensis	0.00	0.00	0.00	0.00	0.00	0.00
Cocconeis	rugosa	0.00	0.00	0.00	0.00	0.00	0.00
Cyclotella	atomus	0.00	0.00	2.73	1.82	0.00	4.10
Cyclotella	meneghiniana	8.65	3.19	4.55	0.00	1.82	1.82
Cyclotella	pseudostelligera	0.00	0.00	0.46	0.00	0.00	0.00
Cyclotella	stelligera	0.00	0.00	0.91	0.00	0.00	2.05
Cymatopleura	solea	0.00	0.00	0.00	0.00	0.00	0.00
Cymbella	norvegica	0.00	0.00	0.00	0.00	0.00	0.00
Cymbella	prostrata	0.00	0.00	0.00	0.46	0.00	0.00
Cymbella	silesiaca	0.00	0.00	0.00	0.00	0.00	0.00
Cymbella	sinuata	0.00	0.00	0.00	0.00	0.00	0.00
Cymbella	tumida	2.28	0.00	0.91	0.91	0.46	0.00
Cymbella	turgidula	0.00	0.00	0.00	0.00	0.00	0.00
Fragilaria	fasciculata	5.24	0.00	0.00	1.82	0.00	0.00
Fragilaria	vaucherae	0.00	0.00	0.00	0.00	0.00	0.00
Gomphonema	augur	0.00	0.00	0.00	0.00	0.00	0.00
Gomphonema	angustum	0.00	0.00	0.00	0.00	0.00	0.00
Gomphonema	gracile	0.00	0.00	0.00	0.00	0.00	0.00
Gomphonema	minutum	0.91	0.00	0.00	0.00	0.00	0.00
Gomphonema	olivaceum	0.46	0.00	0.00	0.00	0.00	0.00
Gomphonema	parvulum	1.59	0.46	0.91	0.91	2.73	0.91
Gomphonema	subclavatum	0.00	0.00	0.00	0.00	0.00	0.00
Gomphonema	truncatum	0.91	0.00	0.00	0.00	0.00	0.00
Gyrosigma	acuminatum	0.00	0.00	0.00	0.00	0.00	0.00
Gyrosigma	scalproides	0.91	0.00	0.00	0.00	0.00	0.00

Sample Type:		Phyto	Phyto	Phyto	Phyto	Phyto	Phyto
Sample Date:		8/3/00	8/3/00	8/3/00	8/30/00	8/30/00	8/30/00
Sample Site:		A	B	C	A	B	C
Genus	Species						
Gyrosigma	spencerii	0.00	0.00	0.91	0.00	0.00	0.91
Hantzschia	amphioxys	0.00	0.00	0.46	0.00	0.00	0.00
Melosira	varians	0.00	0.00	0.00	0.00	0.46	0.00
Navicula	angusta	0.46	0.00	0.00	0.91	0.46	0.00
Navicula	capitoradiata	0.00	0.46	0.00	0.00	0.00	0.00
Navicula	cincta	0.46	0.00	0.46	0.00	0.00	0.46
Navicula	cryptocephala	0.46	0.00	4.10	3.87	0.46	7.29
Navicula	cryptotenella	0.46	0.00	0.91	0.00	0.00	0.00
Navicula	cuspidata	0.46	0.00	0.00	0.00	0.00	0.00
Navicula	decussis	0.00	0.46	1.14	0.00	0.00	0.00
Navicula	goeppertiana	0.00	0.46	0.00	0.00	0.00	0.00
Navicula	lanceolata	0.46	0.00	0.00	0.00	0.00	0.00
Navicula	menisculus	0.91	0.00	0.91	0.91	0.00	0.00
Navicula	minima	0.00	0.00	0.00	0.00	0.00	0.00
Navicula	pseudoreinhardtii	0.00	0.00	0.00	0.00	0.00	0.00
Navicula	pupula	0.00	0.46	0.00	0.00	0.00	0.00
Navicula	radiosa	0.00	0.00	0.00	0.00	0.00	0.00
Navicula	reichardtiana	0.00	1.37	1.14	1.37	0.00	2.28
Navicula	salinarum	0.91	0.00	0.00	0.00	0.00	2.73
Navicula	sp1	0.00	0.00	0.00	0.00	0.00	0.46
Navicula	symmetrica	0.46	0.46	0.00	0.00	0.00	0.00
Navicula	tripunctata	1.82	0.00	0.00	0.46	0.00	0.00
Navicula	trivialis	1.82	0.00	0.00	0.00	0.00	10.48
Navicula	veneta	0.91	0.00	0.00	0.00	0.00	0.00
Navicula	viridula	10.02	1.37	5.92	2.28	0.00	2.05
Nitzschia	amphibia	0.46	1.82	0.91	0.00	1.82	0.00
Nitzschia	angustatula	0.00	0.00	0.00	0.00	0.00	0.46
Nitzschia	angustiforaminata	0.00	0.00	0.46	0.00	0.00	0.00
Nitzschia	calida	0.00	0.00	0.00	0.00	0.00	0.00
Nitzschia	closterium	0.00	0.00	0.00	0.00	0.00	0.00
Nitzschia	constricta	0.91	0.00	0.00	0.00	0.00	0.00
Nitzschia	dissipata	1.37	0.00	0.00	1.59	0.00	0.46
Nitzschia	fonticola	0.46	0.00	0.00	0.00	0.00	0.00
Nitzschia	frustulum	0.00	0.00	0.00	0.00	0.00	0.00
Nitzschia	gracilis	0.00	0.00	0.00	0.00	0.00	0.91
Nitzschia	inconspicua	5.47	0.91	1.82	22.77	2.28	1.37
Nitzschia	levidensis	0.00	0.00	0.00	0.91	0.00	0.46
Nitzschia	macilenta	0.00	0.00	0.00	0.00	0.00	0.00
Nitzschia	microcephala	0.00	0.00	0.00	0.00	0.00	0.00
Nitzschia	palea	0.46	0.00	5.24	1.82	0.46	27.56
Nitzschia	reversa	0.00	0.00	0.00	0.00	0.00	0.46
Nitzschia	umbonata	0.46	0.00	0.00	0.00	0.00	0.00
Nitzschia	viridula	0.00	0.00	0.00	0.00	0.00	0.00
Nitzschia	wuellerstorffii	0.00	0.00	0.46	0.00	0.00	0.00
Pinnularia	gibba	0.00	0.00	0.00	0.00	0.00	0.00

Sample Type:		Phyto	Phyto	Phyto	Phyto	Phyto	Phyto
Sample Date:		8/3/00	8/3/00	8/3/00	8/30/00	8/30/00	8/30/00
Sample Site:		A	B	C	A	B	C
Genus	Species						
Pleurosira	laevis	0.00	0.00	0.00	0.00	0.00	0.00
Rhoicosphenia	curvata	14.12	0.00	2.73	3.87	1.37	1.14
Surirella	angusta	0.00	0.00	0.00	0.00	0.00	0.00
Surirella	minuta/pinnata	0.00	0.00	0.00	0.00	0.00	0.00
Surirella	ovalis	0.46	0.00	0.00	0.00	0.46	0.00
Surirella	ovata	0.00	0.00	2.28	0.00	0.00	0.00
Surirella	suecica	0.00	0.00	0.00	0.00	0.00	0.00
Synedra	demerarae	0.00	0.46	0.00	0.00	0.00	0.00
Synedra	ulna	0.00	0.00	0.00	0.00	0.00	0.00
Thalassiosira	weisflogii	1.82	0.00	0.00	3.19	0.46	0.00
Tryblionella	calida	0.00	0.00	0.00	0.00	0.00	0.00

Sample Type:		Phyto	Phyto	Phyto
Sample Date:		10/10/00	10/10/00	10/10/00
Sample Site:		A	B	C
Genus	Species			
Achnanthes	conspicua	0.00	0.00	0.00
Achnanthes	delicatula	0.00	0.00	0.00
Achnanthes	helvetica	0.46	0.00	0.00
Achnanthes	hungarica	0.00	0.00	0.00
Achnanthes	hustedtii	0.00	0.00	0.00
Achnanthes	lanceolata	0.46	0.00	0.00
Achnanthes	minutissima	0.00	0.00	0.00
Achnanthes	oblongella	0.00	0.00	0.00
Achnanthes	pinnata	0.00	0.00	0.00
Amphora	ovalis	0.46	0.46	0.00
Amphora	ovalis pediculus	2.28	0.00	0.00
Amphora	perpusilla	1.82	0.00	0.00
Aulacoseira	alpigena	0.00	0.00	71.51
Aulacoseira	italica	0.00	0.00	0.00
Bacillaria	paradoxa	0.00	0.00	0.00
Cocconeis	pediculus	1.37	0.00	0.46
Cocconeis	placentula	0.68	0.46	0.00
Cocconeis	klamathensis	0.00	0.00	0.00
Cocconeis	rugosa	0.00	0.00	0.00
Cyclotella	atomus	0.00	0.00	0.00
Cyclotella	meneghiniana	0.46	0.91	0.68
Cyclotella	pseudostelligera	0.00	0.00	0.00
Cyclotella	stelligera	0.00	0.00	0.00
Cymatopleura	solea	0.00	0.00	0.00
Cymbella	norvegica	0.00	0.00	0.00
Cymbella	prostrata	0.00	0.00	0.00
Cymbella	silesiaca	0.00	0.00	0.00
Cymbella	sinuata	0.00	0.00	0.00
Cymbella	tumida	0.00	0.00	0.00
Cymbella	turgidula	0.00	0.00	0.00
Fragilaria	fasciculata	1.59	0.00	0.00
Fragilaria	vaucherae	0.00	0.00	0.00
Gomphonema	augur	0.00	0.00	0.00
Gomphonema	angustum	0.00	0.00	0.00
Gomphonema	gracile	0.00	0.00	0.00
Gomphonema	minutum	0.00	0.00	0.00
Gomphonema	olivaceum	0.91	0.00	0.00
Gomphonema	parvulum	0.00	0.00	0.46
Gomphonema	subclavatum	0.00	0.00	0.00
Gomphonema	truncatum	0.00	0.00	0.00
Gyrosigma	acuminatum	0.00	0.00	0.00
Gyrosigma	scalproides	0.00	0.00	0.00
Gyrosigma	spencerii	0.00	0.00	0.00
Hantzschia	amphioxys	0.00	0.00	0.00

Sample Type:		Phyto	Phyto	Phyto
Sample Date:		10/10/00	10/10/00	10/10/00
Sample Site:		A	B	C
Genus	Species			
Melosira	varians	0.00	0.00	0.46
Navicula	angusta	0.00	0.46	0.00
Navicula	capitoradiata	0.00	0.00	0.00
Navicula	cincta	0.00	0.00	0.00
Navicula	cryptocephala	2.28	0.46	0.00
Navicula	cryptotenella	0.00	0.00	0.00
Navicula	cuspidata	0.00	0.00	0.00
Navicula	decussis	0.00	0.00	0.00
Navicula	goeppertiana	0.00	0.00	0.00
Navicula	lanceolata	0.00	0.00	0.00
Navicula	menisculus	0.46	0.00	0.00
Navicula	minima	0.00	0.00	0.00
Navicula	pseudoreinhardtii	0.00	0.00	0.00
Navicula	pupula	0.00	0.00	0.00
Navicula	radiosa	0.00	0.00	0.00
Navicula	reichardtiana	1.37	0.46	0.91
Navicula	salinarum	0.46	0.00	0.00
Navicula	sp1	0.00	0.00	0.00
Navicula	symmetrica	0.00	0.00	0.91
Navicula	tripunctata	0.46	0.00	0.00
Navicula	trivialis	0.00	0.00	0.46
Navicula	veneta	0.00	0.00	0.00
Navicula	viridula	1.82	0.00	0.91
Nitzschia	amphibia	0.00	0.00	0.00
Nitzschia	angustatula	0.00	0.00	0.00
Nitzschia	angustiforaminata	0.00	0.00	0.00
Nitzschia	calida	0.00	0.00	0.00
Nitzschia	closterium	0.00	0.00	0.00
Nitzschia	constricta	0.46	0.00	0.00
Nitzschia	dissipata	0.00	0.00	0.00
Nitzschia	fonticola	0.00	0.00	0.00
Nitzschia	frustulum	0.46	0.00	0.00
Nitzschia	gracilis	0.00	0.00	0.00
Nitzschia	inconspicua	3.42	1.82	0.46
Nitzschia	levidensis	0.00	0.00	0.00
Nitzschia	macilenta	0.00	0.46	0.00
Nitzschia	microcephala	0.00	0.00	0.00
Nitzschia	palea	0.00	0.00	0.91
Nitzschia	reversa	0.00	0.00	0.00
Nitzschia	umbonata	0.00	0.00	0.00
Nitzschia	viridula	0.00	0.00	0.00
Nitzschia	wuellerstorffii	0.00	0.00	0.00
Pinnularia	gibba	0.00	0.00	0.00
Pleurosira	laevis	0.00	0.00	0.00
Rhoicosphenia	curvata	3.19	1.37	0.00

Sample Type:		Phyto	Phyto	Phyto
Sample Date:		10/10/00	10/10/00	10/10/00
Sample Site:		A	B	C
Genus	Species			
Surirella	angusta	0.00	0.00	0.00
Surirella	minuta/pinnata	0.00	0.00	0.00
Surirella	ovalis	0.00	0.00	0.00
Surirella	ovata	0.00	0.00	0.00
Surirella	suecica	0.00	0.00	0.00
Synedra	demerarae	0.00	0.00	0.00
Synedra	ulna	0.00	0.00	0.00
Thalassiosira	weisflogii	0.00	0.00	0.46
Tryblionella	calida	0.00	0.00	0.00