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The Capacity of Diatom Species to Survive Ingestion by the Algivorous Minnow, Pimephales Notatus

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THE CAPACITY OF DIATOM SPECIES TO SURVIVE INGESTION BY THE ALGIVOROUS MINNOW, *PIMEPHALES NOTATUS.*

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By

Paul G. Grubach

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The thesis of Paul G. Grubach is hereby accepted:

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I certify that this is the original.

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Author - Paul G. Grubach

IN MEMORIUM

This thesis is dedicated to the memory of the late

Dr. Miles M. Coburn:

outstanding teacher, outstanding scientist, outstanding human being.

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I would like to offer my deepest gratitude to Dr. Jeffrey R. Johansen for his excellent guidance.

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ABSTRACT

The capacity of diatom species to survive gastrointestinal passage through the algivorous minnows *Pimephales notatus* and *Campostoma anomalum* was studied. From a site on the Grand River in northeastern Ohio, 27 minnows and 7 epilithic diatom samples were taken. In order to determine whether diatom taxa varied in digestibility, live/dead ratios of diatom cells taken from the minnows' feces were compared with live/dead ratios of cells taken from immersed rocks. Diatoms that were live/undigested at the time of collection were differentiated from dead/digested cells under light microscopy by noting the presence of chloroplasts and/or lipid droplets. Seventy-seven percent of the diatom taxa observed in the fish feces had at least one frustule in the "living" condition. The results of this study were used to determine the effect of enrichment culture on various diatom taxa, and which taxa have a special resistance to digestion.

Cluster analyses hint that there may be selective dying off as the diatoms pass through the gut. With the use of Student's *t* tests, the percent living of diatoms at the sites was compared to the percent living in the fish, suggesting that *Cymbella affinis*, *Cymbella caespitosa*, *Nitzschia sinuata* var. *tabellaria*, *Cyclotella meneghiniana*, *Navicula capitatoradiata*, *Navicula veneta*, and the *Cymbella*, *Reimeria* and *Gomphonema* genera, survive gut passage more frequently than other species and genera.

Achnanthes linearis and *A. minutissima* appear to be less resistant to digestion than other species, but also less accessible to the piscine grazers than are certain *Cymbella* species. The ratios of living to dead cells at the sites and in the fish indicate that certain *Cymbella* species are more resistant to digestion than are *Achnanthes linearis* and *A. minutissima*.

Diatom species that are readily available to grazers are more resistant to digestion, and vice versa. Diatoms that are less available to grazers are less resistant to digestion.

INTRODUCTION

Grazers and the Structuring of Benthic and Planktonic Algal Communities in Lakes

In open water, lake environments, it is known that microscopic grazers play an important role in the organization of planktonic algal communities (Porter 1977). Zooplankton can control the population of some algae: small naked green algae, nannoflagellates, cryptomonads and certain diatoms are eaten, digested and suppressed by the grazers. Large unicellular desmids, dinoflagellates, filamentous diatoms, and colonial blue-green algae are unaffected by grazing pressure. Rarely found in the digestive tracts of grazers, these are the algae that are not eaten or are actively rejected. Another category of algae, those that survive the digestive process, are of special interest to this study. Certain large green and blue-green algae, particularly those with durable cell walls and gelatinous sheaths, pass unharmed through the guts of zooplankton and even gain some nutrients, carbon and phosphorous compounds from the host during passage. Their numbers are increased by the presence of grazers. The effect of grazing is determined by the proportions of suppressed, unaffected and increased algae, with the end result being that the phytoplankton attains a specific structure (Porter 1973; 1977).

In general then, in the limnetic zone of lakes, grazing pressure plays a role in determining the relative proportions of algal species and influences seasonal succession from a spring association of largely edible flagellates and diatoms to predominantly inedible ones, such as blue-green algae, in summer. Because the major food source for herbivorous zooplankton is the diatom population, grazing pressure most likely changes diatom species composition and numbers (Horne and Goldman 1994). In regard to open water algae, herbivory can select for certain morphological, chemical, physiological, and life-history adaptations that serve as antiherbivore devices, thereby influencing the structure, succession and evolutionary history of these phytoplankton communities (Porter 1977).

The Great Lakes provide a very recent example as to how grazers can contribute to the structuring of algal communities. In Lake Erie and Lake St. Clair, densities of phytoplankton populations are largely affected by the availability of organic and inorganic nutrients and by herbivorous predation and parasitism (Bolsenga and Herdendorf 1993). Although the extent of change that zebra mussels (*Dreissena polymorpha*) can exert on the species composition of the phytoplankton is still unresolved, the increase in grazing pressure by this mollusk has been directly linked to a decrease in phytoplankton over time. Phytoplankton levels in Lake Erie, for example, went down 62-92% (Leach 1993), and planktonic diatoms plummeted 85% despite sufficient nutrients for growth (Holland 1993). By removing large amounts of suspended matter, abundant zebra mussels have the capacity to alter transparency, phytoplankton abundance and the composition of the entire algal community, thereby changing the ecosystem and aquatic food web (Holland 1993). In Lake Huron, for example, zebra mussel feeding has produced a shift from benthic diatoms to a flora dominated by filamentous green algae. As the mollusks infested Saginaw bay, the periphyton switched from a diatom dominant community to a community dominated by mostly *Spirogyra* sp.and *Mougeotia* sp. (Lowe and Pillsbury 1995).

The foregoing scenarios clearly show how herbivores contribute to the structuring of planktonic and benthic algal communities in lake environments. Yet, in the periphyton of stream environments, how herbivores contribute to the structuring of algal communities is less understood.

Do Herbivores Structure Stream Periphyton?

The primary algal components of the periphyton of Ohio streams are diatoms. Are some diatoms being digested and suppressed by grazers in these streams, as they are in the in the limnetic and benthic zone of lakes? Are some stream diatoms passing through the guts of herbivores undigested? Are vertebrate and invertebrate grazers playing an important

role in structuring these lotic diatom communities, just as microscopic and macroinvertebrate grazers play a role in structuring benthic and planktonic algal communities in lakes? In the early 1990s, these questions were being contemplated in the John Carroll University laboratory of Jeffrey Johansen.

It was established that in lake environments, diatoms were being removed by zebra mussels, and large green and blue-green algae could pass through the guts of invertebrate grazers and remain in viable condition: these two factors played a role in determining the organization of the algal community in lakes. It was then asked: is a similar process taking place with the diatom community in the periphyton of Ohio streams? In this context it was also known, for example, that grazing in streams by the stoneroller minnow, *Campostoma anomalum*, can alter the taxonomic composition of algal communities as well as permit the maintenance of low standing crops of algae on rock substrates (Gregory 1983).

This line of thought generated other questions. Are some diatoms being digested and removed by stream grazers, and are some remaining viable after gut passage? Could variation in diatom ability to resist digestion ultimately determine the organization of diatom community structure in streams?

Diatom Resistance to Digestion, Dispersal and Natural Selection

Being primary producers, diatoms have a ubiquitous distribution in lotic systems, and are important both directly and indirectly as food materials for vertebrates and invertebrates. Peterson (1987) hypothesized that diatoms that survive gut passage through grazers should be more prone to downstream displacement than cells attached to the substratum. Organisms that consume diatoms migrate upstream and downstream: they defecate upstream and downstream. Those diatoms that successfully resist digestion will form future diatom communities in different stream areas after they are expelled in herbivore feces. Grazing, therefore, may provide an important vehicle for diatom

dispersal. By extension then, whether nor not diatom species have a special resistance to digestion may be an important determinant of the diatom community structure in streams. This in turn, may influence the entire food chain.

In regard to herbivory, if grazers ingest significant portions of the diatoms in a stream, one could assume that those that remain viable after gut passage would be favored over those that do not remain viable after gut passage. Differences in the efficiency with which diatom taxa are digested could also alter algal dominance patterns in a manner similar to that observed when taxa are selectively ingested (Peterson 1987). For example, if all diatom community members are grazed indiscriminately but some taxa are less digestible than others, resistant taxa should be favored in communities where grazing pressure is high. Of course, it is possible that in some or even a majority of lotic systems herbivory is not a strong enough selective force to have a measurable effect.

Diatom Vulnerability to Ingestion and Digestion: A Hypothesis

It was further suggested that there is another way in which grazers may structure diatom communities in streams. Hill and Knight (1988) found that diatoms in the loose, upper layer of periphyton were generally affected by grazing more than those in the adnate layer. Therefore, small, adnately attached, understory diatoms and large over story species may have evolved two different sets of defense mechanisms against vertebrate and invertebrate grazers.

The small adnately attached taxa may defend against predation by being less accessible to grazing organisms and having a faster rate of division to maintain their populations in the face of grazing pressure from herbivores. This may be their overall strategy against grazing in general. By contrast, large over story species may be unable to divide at the rate they are being ingested and they are accessible to grazing fish *and* invertebrates. Thus, the selection pressure on them to remain viable after gut passage may

be greater than it is on small adnate taxa.

This theoretical perspective was developed even further in the late 1990s and early years of this 21st century. Peterson et al. (1998) hypothesized that there is a trade-off between ingestion and digestion resistance; natural selection should strongly favor digestion resistance in taxa that are highly vulnerable to ingestion by grazers. Diaz Villanueva et al. (2003) observed a similar pattern. Species that were highly susceptible to ingestion were also the most resistant to digestion and vice versa. Species that were less susceptible to ingestion were the least resistant to digestion.

Summarizing the current paradigm, Peterson et al. (1998) pointed out that in stream ecosystems subjected to intense grazing, interspecific variation among diatoms and other algae in vulnerability to ingestion *and* digestion should affect the community structure and functioning of benthic algae. That is to say, both diatom vulnerability to ingestion and digestion are equally important variables.

An Early Viewpoint on Diatom Resistance to Digestion and the Resulting Question

In streams, the herbivore community consists of insects and algivorous fish. By the mid 1990s there were a number of papers that touched upon the ecological significance of the capacity of diatom species to survive ingestion by aquatic insects. Marker et al. (1988), for example, found that more than 50% of the living diatom cells ingested by chironomid larvae were assessed to have been degraded in the gut. Burton et al. (1994) seemed to rule out the possibility that changes in the relative abundances of *A. minutissima* and *C. placentula* could be due to one of the species being able to survive gut passage through the Trichopteran *Glossoma nigrior* better than the other. In a study similar to mine, Peterson (1987) found that between 40% and 52% of the diatoms eliminated in caddisfly feces were characterized as live cells, whereas 73% of the diatoms collected from epilithic habitats were considered living.

Although there was evidence to demonstrate that some diatoms could pass unharmed through the guts of insects, there was scant evidence to show that they could survive passage through the gastrointestinal tracts of fish. For example, Fish (1951) found that for a population of *Tilapia exculenta* in Lake Victoria (Uganda) only the diatom members of the phytoplankton were digested, whereas the cyanobacteria and green algae passed undigested through the gut. Velasquez (1939) reported that diatoms as a group, as compared to other algae, are marginal in their resistance to digestion. Using culturing techniques he found only four diatom groups to be viable after gut passage through the digestive tracts of *Dorosoma cepedianum* specimens (Gizzard Shad)--*Cyclotella meneghiniana*, two unknown *Navicula* species and a *Diatoma* species.

From the foregoing discussion, the reader can see that a theoretical perspective linking diatom resistance to digestion and the structure of diatom communities in streams was built up by the early to mid 1990s, and developed further from the late 1990s onward. It was known that some diatoms could pass through the guts of insects and remain in viable condition, and this in turn could contribute to the structuring of diatom communities in streams. However, it was unclear as to whether or not diatoms could pass through the gastrointestinal tracts of fish and remain in viable condition, and to what extent this would affect the structuring of the diatom community in streams. After all, a diatom has a much longer distance to travel in the gastrointestinal tract of a fish as compared to the gut of an insect. So now, the question was raised: can diatoms pass through the gastrointestinal tracts of fish without suffering mortality?

The Thesis of Thomas Knobloch

Out of all of this work and hypothesizing, there emerged three important questions in the Johansen laboratory. Are some diatoms able to survive gut passage through a fish and then reproduce? Do diatom species differ in their ability to survive gut passage through

fish? And if they do, does it appear that an ability to avoid ingestion is negatively correlated with the ability to resist digestion?

Graduate student Thomas Knobloch devoted his 1991 thesis to this issue: the ability of diatom species to survive gut passage through the bluntnose (*Pimephales notatus*) and stoneroller (*Campostoma anomalum*) minnows. He compared the diatom flora in minnow feces to the flora in enrichment culture in order to determine the species-specific frequency of survival, and found that some diatom species were more resistant to the digestive process than others (Knobloch 1991). To this writer's knowledge, he was the first researcher to do this. Due to the importance of Knobloch's study for my thesis, a more thorough discussion of his findings is warranted.

It was inferred that many species of diatoms can pass through the gut of a minnow and come out viable, because of all of the species removed from the fish a significant number grew in culture; 74 of 203 taxa (36%) were identified in enrichment culture. When the frequency of occurrence in minnow feces was regressed against the frequency of survival in enrichment culture, several diatom species were identified as surviving more frequently than would be expected from their frequency in the feces. Those species that appeared to be the most resistant to digestion were: *Achnanthes linearis, Cocconies placentula* var. *lineate, Cyclotella meneghiniana, Melosira varians, Navicula capitatoradiata, Denticula keutzingiana,* and *Nitzschia palea*.

Knobloch (1991) had more important results that are somewhat surprising. Differences in diatom species composition in the intestine of the bluntnose and stoneroller minnows could not be detected, suggesting that within a particular site they were grazing from the substrates in an unselective fashion. No differences were found in the number of diatom taxa that were able to survive gut passage through the two species of algivorous minnows. Finally, no differences in diatom survival ability could be linked to fish size.

The Concepts Underlying the Present Thesis

Knobloch did indeed demonstrate that diatoms can survive gut passage through fish and remain in viable condition. Any diatom taken from the terminal segment of a fish intestine that then proliferates in culture has certainly survived the digestive process. However, his results were not entirely convincing on another count. He did not really provide compelling evidence of differential ability to resist digestion because of the confounding effect of culturing.

It is possible that the species that were found to have a special resistance to digestion really do not have any special resistance. It may be that the minnows consumed enormous numbers of a particular diatom species and only a very few specimens survived gut passage. These very few specimens then proliferated very rapidly in his cultures and led him to falsely conclude that they had a special resistance to digestion when in fact they do not.

Likewise with those species that were found to be relatively easy to digest. It is possible that a disproportionate number of a particular species survived the digestive process. Yet, the surviving frustules did not propagate well in culture, thus giving one the false impression that this species is not resistant to digestion.

My thesis was originally conceived as a response to these problems associated with Knobloch's study. My study avoided the drawbacks associated with studying diatom clones created in culture. I compared the live/dead ratios of diatoms found on rocks to the live/dead ratios of diatoms found in the posterior portion of minnows' intestines, and then inferred which species were especially resistant to digestion.

Of course, with my study the central questions remain the same. Do diatoms differ in their ability to resist digestion? If so, does it appear that the ability to avoid ingestion is negatively correlated with the ability to resist digestion? Yet, unlike Knobloch's study, I examined what appeared to be "live" and "dead" diatoms in the posterior portion of the

minnows's intestines after the fish were fixed in formalin. If the diatom frustule contained a chloroplast or lipid droplet, it was assumed to be alive. If they lacked them, they were assumed to be dead.

The Four Objectives to the Present Study

The primary intent of this study is multifaceted. First, this thesis will examine the species-specific capacity of diatoms to survive ingestion and passage through the gastrointestinal tracts of the minnows *C. anomalum* and *P. notatus*, an area of stream ecology that is largely unexplored.

Second, my results will be compared with the complementary study of Knobloch (1991). Whereas I compared the diatom taxa in the hindgut of minnows to epilithic diatom samples, Knobloch compared the diatom flora in enrichment culture to the flora in minnow feces to determine the species-specific frequencies of survival. The comparison of both studies will help to discern the effect of enrichment culture on various diatom taxa, and ultimately, which diatom taxa, if any, have a special resistance to digestion. A major drawback of Knobloch's study was the possibility that taxa frequently encountered in enriched media simply proliferate well in culture and have no special resistance to digestion.

Third, my results will be compared to other published studies to see if there are areas of agreement and/or disagreement concerning the species-specific capacity of diatoms to survive ingestion. Finally, this study will empirically test the hypothesis about diatom vulnerability to ingestion and digestion: Species that are highly susceptible to ingestion should be the most resistant to digestion and vice versa. Species that are less susceptible to ingestion should be the least resistant to digestion. Hopefully, this thesis will aid future investigators in determining whether or not a diatom's ability to survive gut passage through grazers is a determinant of diatom and algal community structure in streams.

MATERIALS AND METHODS

Study Area and Collection Techniques

Water samples, diatom scrapings from rocks, and minnows were collected on November 12, 1994, near the confluence of Paine Creek and the Grand River (41 43'N; 081 10'W). This stretch of Paine Creek and the Grand River, approximately 2.4 miles from Paine Falls, runs roughly parallel to Sealy Road and is a part of Lake Metro Parks (Fig. 1). It is identical to Knobloch's (1991) study location.

The study site, approximately 65 meters in length and 8 to 10 meters across, had the size and canopy characteristics of a 3rd order stream (Fig. 2). Ranging in depth from 5 to 80 centimeters, it contained pools, runs and riffles. Riffle substrates were diverse, including boulders, cobble, gravel, and sand. The water was clear and the bottom was composed (for the most part) of sand intermixed with flat stones. Sand predominated the substrata; cobble was only exposed in riffles. Most pools were less than 80 cm deep, separated by shallow riffles 10 to 20 centimeters deep. Rocks and rubble on the streambed were slippery due to a layer of diatom growth at the time of sampling.

Adjacent to the stream was a well-developed riparian zone composed primarily of deciduous hardwoods--Maple, Oak and Wild Grape. The watercourse was wide enough not to be heavily shaded by riparian vegetation and was shallow enough for most of its length to allow light penetration to the substratum. Much of this segment of Paine Creek is exposed to direct sunlight during the dry season (April-November).

Composite periphyton samples were taken from submerged rocks for comparison with diatom genera in the hindgut of bluntnose and stoneroller minnows. Specifically, diatom samples were taken from rocks at seven sites (2 samples per site), ranging from 6 to 11 meters apart. Stones were selected from the substrate that appeared to have been in their

Figure 1. Aerial photo of confluence of Paine Creek with the Grand River.

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present position for some time. Diatom sampling was done by gently scraping algae from submerged rocks into small plastic, opaque collection jars containing a 3% glutaraldehyde solution.

The site descriptions for samples collected from natural substrates are as follows: 1) near the mouth of Paine Creek; 2) upstream of site 1 in the middle of a pool; 3) further upstream from site 2 in a pool below a riffle; 4) a shallow riffle; 5) in a pool with leaf litter; 6) in a riffle; 7) between site 1 and site 2 (Fig. 3). The natural substrates were sampled first from the seven sites (numbers in squares, Fig. 3) to avoid disruption of the substrate. Then the fish were sampled from eight seining locations that were not identical to the substrate sites (numbers in circles, Fig. 3).

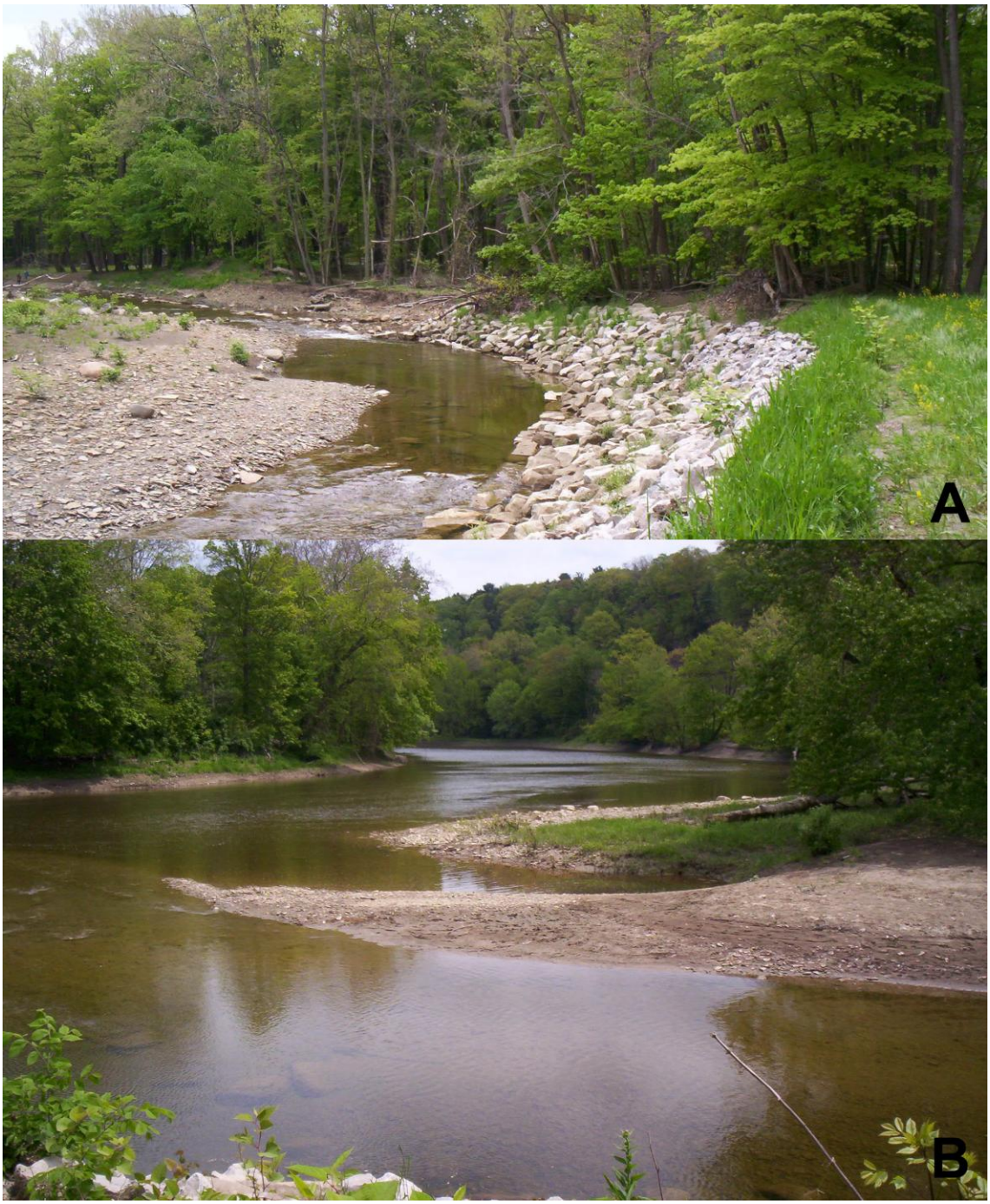

Figure 2. Study site at Paine Creek. A. Upstream portion of Paine Creek showing bend in stream and concrete reinforcement of bank. B. Confluence of Paine Creek (lower portion of photo) with the Grand River.

Two representatives of *C. anomalum* and 32 specimens of *P. notatus* were collected adjacent to these sites using a fine mesh seine. Immediate preservation of fishes in 10% formalin was essential to halt the processes of digestion and preserve the intestinal contents intact. All specimens were properly labeled, kept cool and returned to the laboratory, where they were kept under refrigeration until processing.

Preparation of Permanent Microscope Slides

Concentrated algal samples from the rock scrapings were mounted in Taft's Syrup Mountant (TSM, see Stevenson 1984) for viewing by light microscopy. After each fish was measured to the nearest 1.0 mm standard length, its skin was cut off from the anal pore to the throat. The intestine was pulled out and the most posterior 1 cm portion containing fecal material was excised. Squeezing and maceration removed the contents of this terminal segment. There was no material in the intestinal tract of seven specimens, and these were discarded. Consequently, twenty-seven fish were used in this study, 26 *P. notatus* and 1 *C. anomalum*. Ranging from 31.8 mm to 101.4 mm, their average length was 45.1 mm $\pm$ 14.5 mm (SD).

Like the algal rock scrapings, all gut content samples were mounted in TSM (Stevenson 1984). One drop of 10% TSM was put on a cover slip and the 1 cm portion of diatom containing feces was mixed with the TSM. An additional drop or two of TSM was then applied. Using two tweezers, the material from the intestine was torn out and spread out on the cover slip, after which more 10% TSM was applied. The coverslip was kept in total darkness and at room temperature overnight. The next day more 10% TSM was put on the coverslip.

On the third day the cover slip was mounted on a slide. The slides were pre-heated and then a coverslip with diatoms and 100% TSM was inverted onto it. The inverted cover glass was centered on the microscope slide and tapped with a pencil to disperse trapped air

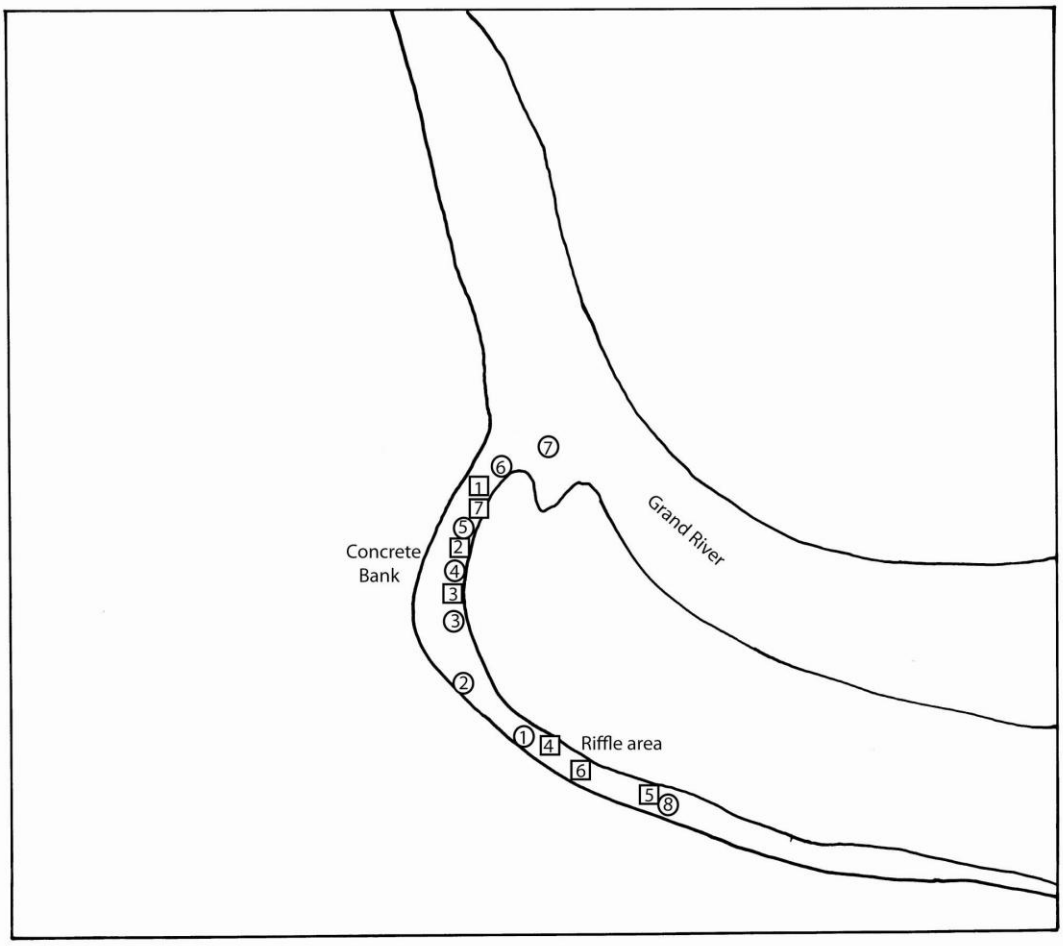

Figure 3. Map of study site. Fish samples are indicated by circles, substrate samples are indicated by squares. Paine Creek flows into the Grand River.

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bubbles, remove excess water and imbed the cover glass into the mounting medium. Finally, two coats of nail polish were applied to the margins of the cover glass to prevent further desiccation. The left margin of each microscope slide was labeled appropriately.

All diatoms were identified using a photomicroscope with Nomarski DIC optics. To determine living and dead population numbers, valves were counted using an oil immersion lens at 1000x magnification. Diatoms were scored as having been dead or

digested (no chloroplasts and/or lipid droplets) at the time of collection or as live or undigested (chloroplasts and/or lipid droplets) at the time of collection.

From each slide at least 400 individual diatoms were counted from randomly selected areas unless there were fewer than 400 frustules on the entire slide, in which case all the diatoms on the slide were counted. Broken diatom valves were enumerated if more than one half of the valve was intact. Most diatoms were identified to species or varietal rank. Those that could not be identified to the species level were put into a genus, or at least listed as centric or pennate.

Several texts were frequently consulted while attempting to establish unknown diatom specimen identity. These references included Collins and Kalinsky (1977), Knobloch (1991), Kramer and Lange-Bertalot (1988a, b), Patrick and Reimer (1966, 1975), and Simonsen (1987a, b, c).

Data Analysis

After the species-specific numbers of living and dead diatoms at each site and for each fish were enumerated, live and dead percent densities were determined by dividing the living and dead counts by the grand total of diatoms observed at the site or in the fish. The number of diatom species and varietal ranks observed at the sites and in the fish were determined. From this data the percentages of species with at least one frustule in the "living" condition (with cytoplasmic inclusions) were calculated for the sites and fish.

Similarity indices (Pielou 1984) and important species values (ISI indices) were generated for the sampling sites and fish specimens utilizing the Ecology Program Library Software (Evensen, unpublished). Sampling sites and fish specimens were clustered using Ruzicka's similarity and an UPGMA clustering algorithm (Pielou 1984).

Cluster analyses were performed on the dead flora for the sites and fish combined, the living flora for the sites and fish combined, the living and dead flora at all sites and in all

fish, the living and dead flora of the fish, and the living and dead flora of the sites. The Important Species Indices (ISI) for the living and dead flora at the sites, in the fish, and for the sites and fish combined were calculated. The computer program did this by finding the taxa-specific average density for each diatom category (the average density), and the percentage of sites and/or fish in which each species was found (the percent presence). These two values were then multiplied together, the product being the ISI index. For the taxon to be entered into Table 1 or 2 it must have scored at least 0.30 in either the "sites," "fish" or "combined" category in either the living or dead flora. Shannon-Wiener and Hill's Diversity Indices (Ludwig and Reynolds 1988) for all 34 specimens were calculated using the Ecology Program Library Software (Table 3).

All taxa categories that had a "combined ISI" of at least 0.30 in either Table 1 or 2 were included in Table 4. This table shows the percent living for these top 20 taxa categories at the 7 sites and in the 27 fish. A percent value was listed only if there were at least 5 frustules of a taxon in a particular fish or at a site. The site and fish means were also determined for all 34 samples. This table enabled me to statistically examine the species-specific live ratios of diatoms both on epilithic substrata and after passage through grazer digestive tracts.

Using a Student's t-test, statistical comparisons between living diatom taxa taken from stones and observed in intestinal material (Table 4) were performed on species-specific (Table 5), genera-specific (Table 6), pennate-specific and centric-specific live diatom cell percentages. The taxon-specific percent die offs were determined by computing the difference between the mean percent living at the sites and in the fish, and then dividing it by the mean percent living at the sites and multiplying by 100. These analyses were used to determine to what extent diatom viability was affected by gut passage and whether these effects differed interspecifically. The replicate size was considered large enough only if

there were at least 5 sites and 10 fish (Table 5), each with at least 5 specimens of the taxon.

The species-specific average percent densities of living and dead diatoms at the sites and in the fish for selected *Achnanthes* and *Cymbella* species were tabulated (Table 7). These values were then used to compute the ratios of species-specific average percent densities of living to dead diatoms for *Achnanthes* and *Cymbella* taxa at the sites and in the fish (Table 8).

Table 1. Important species index for most important species in study area based on counts of diatoms having inclusions (i.e., live diatoms). "Combined" is a value based upon all samples. The samples below the line break had "combined" ISI less than 1.00. An * after a taxon name means that the taxon had a combined ISI above 1.00 in the dead species list (Table 2). Only taxa with an ISI above 0.30 in at least one of the categories are included in the list.

Diatom Species	**Sites**	**Fish**	**Combined**
*Achnanthes linearis**	12.38	2.34	4.29
Pennate spp.*	4.46	3.66	3.86
Cymbella caespitosa	1.36	3.04	2.69
Nitzschia sinuata var. *tabellaria**	2.05	2.58	2.48
*Achnanthes minutissma**	6.62	1.32	2.34
Nitzschia spp.*	4.71	1.37	1.98
Cymbella spp.*	1.14	2.08	1.89
*Cymbella affinis**	1.63	1.87	1.83
*Cyclotella meneghiniana**	0.56	1.73	1.50
*Denticula kuetzingiana**	3.02	1.43	1.74
Melosira varians	0.02	1.70	1.33
Navicula capitatoradiata	1.28	1.09	1.14
Cymbella microcephala	1.16	0.79	0.86
Navicula veneta	1.69	0.63	0.81
Cymbella silesiaca	0.16	0.79	0.65
Nitzschia dissipata	0.77	0.36	0.44
Nitzschia palea	0.51	0.27	0.31
Fragilaria vaucheriae	0.73	0.23	0.31
Navicula spp.	0.25	0.31	0.31
Cymbella delicatula	0.45	0.15	0.20
Synedra cf. *tenera*	0.34	0.01	0.04
Amphipleura pellucida	0.39	0.00	0.03

Table 2. Important species index for most important species in study area based on counts of diatoms lacking inclusions (i.e., dead diatoms). "Combined" is a value based upon all samples.

Diatom Species	Site	Fish	Combined
Achnanthes linearis	13.75	10.44	11.12
Achnanthes minutissima	9.26	8.15	8.38
Nitzschia sinuata var. *tabellaria*	2.68	4.56	4.18
Pennate spp.	2.24	4.67	4.17
Denticula kuetzingiana	2.89	3.39	3.29
Cymbella affinis	2.47	2.97	2.88
Cymbella spp.	0.66	2.14	1.83
Nitzschia spp.	1.60	1.84	1.79
Cymbella microcephala	1.18	1.84	1.72
Cyclotella meneghiniana	0.27	1.74	1.47
Navicula capitatoradiata	0.31	0.69	0.64
Nitzschia dissipata	0.42	0.63	0.59
Navicula veneta	0.39	0.62	0.58
Reimeria sinuata	0.69	0.51	0.55
Cymbella caespitosa	0.54	0.46	0.47
Rhoicosphenia curvata	0.17	0.50	0.45
Melosira varians	0.01	0.55	0.44
Gomphonema spp.	0.25	0.45	0.42
Cymbella delicatula	0.47	0.33	0.36
Fragilaria vaucheriae	0.18	0.35	0.32
Navicula spp.	0.18	0.33	0.31
Cocconeis placentula var. *euglypta*	0.17	0.30	0.28
Nitzschia inconspicua	0.48	0.23	0.27
Nitzschia microcephala	0.42	0.24	0.27
Achnanthes deflexa	0.32	0.22	0.24

Table 3. Diversity Indices for the sites and fish. The calculated mean of Diversity Indices for sites and fish are on the right.

Sample	**Shannon-Wiener H**	**Hill's**	
Site 1	2.7705	0.9821	
Site 2	2.8266	0.9832	
Site 3	2.7370	0.9824	**H**=2.5661
Site 4	2.2479	0.9546	**Hill's**=.9722
Site 5	2.6481	0.9771	
Site 6	2.2191	0.9532	
Site 7	2.5132	0.9731	
Fish 1.1	2.2656	0.9575	
Fish 2.1	2.1445	0.9483	
Fish 3.1	2.5865	0.9811	
Fish 3.3	2.4016	0.9771	**H**=2.4915
Fish 3.4	2.3857	0.9694	**Hill's**=.9731
Fish 4.1	2.5462	0.9794	
Fish 4.2	2.3932	0.9654	
Fish 4.3	2.4249	0.9735	
Fish 5.1	2.3852	0.9723	
Fish 5.3	2.7545	0.9832	
Fish 5.4	2.7319	0.9833	
Fish 5.5	2.5506	0.9793	
Fish 5.6	2.3759	0.9690	
Fish 6.1	2.5018	0.9709	
Fish 6.2	1.1510	0.8889	
Fish 6.3	2.9203	0.9886	
Fish 6.4	2.7382	0.9806	
Fish 6.5	2.6417	0.9826	
Fish 6.7	2.0366	0.9608	
Fish 6.8	2.8368	0.9859	
Fish 6.10	2.8119	0.9866	
Fish 7.1	2.5092	0.9829	
Fish 7.2	2.5966	0.9815	
Fish 7.3	2.7023	0.9834	
Fish 8.1	2.7564	0.9839	
Fish 8.2	2.4032	0.9740	
Fish 8.4	2.7170	0.9833	

Table 4. Percent living in all 34 samples for the top 20 species. A "0/0" means that the species was not seen in either the living or dead condition in that particular fish or site. A dashed line indicates that there were not at least five frustules of that species in that fish or site. All values were rounded off to nearest tenth. Species codes are: **Acli**, *Achnanthes linearis*; **Acmi**, *Achnanthes minutissima*; **Cyme**, *Cyclotella meneghiniana*; **Cy**sp, *Cymbella* spp; **Cyaf**, *Cymbella affinis*; **Cyca**, *Cymbella caespitosa*; **Cyde**, *Cymbella delicatula*; **Cymi**, *Cymbella microcephala*; **Cysi**, *Cymbella silesiaca*; **Deku**, Denticula kuetzingiana.

Sample	**Acli**	**Acmi**	**Cyme**	**Cy**sp	**Cyaf**	**Cyca**	**Cyde**	**Cymi**	**Cysi**	**Deku**
Site 1	33.7	37.4	84.6	61.5	21.3	60.4	00.0	42.9	50.0	50.0
Site 2	28.5	24.9	57.1	51.2	28.3	63.7	23.5	47.9	47.1	45.0
Site 3	30.7	28.7	75.0	70.8	41.4	71.4	----	26.7	----	45.7
Site 4	67.9	61.2	----	55.6	63.6	85.7	77.3	67.7	0/0	82.0
Site 5	16.7	15.8	86.7	66.7	25.6	71.4	42.9	16.7	60.0	32.6
Site 6	63.5	61.2	----	79.0	52.9	----	52.9	75.9	0/0	78.4
Site 7	50.9	40.6	71.4	54.6	20.7	76.5	14.3	57.1	66.7	31.4
Fish 1.1	80.0	----	57.1	94.1	----	95.8	0/0	----	100.0	----
Fish 2.1	16.7	33.3	----	71.4	----	62.5	0/0	----	----	----
Fish 3.1	12.2	6.2	----	10.0	32.1	55.6	----	9.1	33.3	15.4
Fish 3.3	16.0	21.8	75.0	75.0	31.8	70.0	----	20.0	----	56.8
Fish 3.4	20.9	7.1	0/0	41.7	7.7	60.0	----	7.4	----	20.0
Fish 4.1	35.1	37.7	69.7	79.6	41.9	87.3	----	41.7	44.4	51.5
Fish 4.2	7.5	5.1	60.0	28.6	21.4	62.5	80.0	7.7	----	20.7
Fish 4.3	23.0	11.7	----	57.1	37.5	80.0	22.2	33.3	----	53.9
Fish 5.1	13.0	10.0	63.8	50.0	33.3	81.0	----	8.3	----	22.7
Fish 5.3	22.0	25.0	----	66.7	32.1	----	50.0	55.6	----	29.0
Fish 5.4	13.2	4.1	60.0	30.8	27.3	100.0	42.9	43.8	----	25.0
Fish 5.5	32.5	17.9	----	80.0	47.4	----	63.6	60.0	----	20.0
Fish 5.6	21.4	19.4	16.7	40.0	15.8	100.0	----	46.2	56.3	25.0
Fish 6.1	39.4	28.6	59.5	66.7	44.4	88.9	0/0	0/0	100.0	28.6
Fish 6.2	----	----	----	----	0/0	----	0/0	0/0	0/0	----
Fish 6.3	3.3	23.1	15.9	16.7	28.6	25.0	----	----	----	28.6
Fish 6.4	14.0	5.9	18.2	33.3	21.7	----	----	25.0	----	24.0
Fish 6.5	25.8	29.4	22.2	----	----	----	----	----	----	----

Table 4. Continued.

Sample	Acli	Acmi	C.me	Cysp	Cyaf	Cyca	Cyde	Cymi	Cysi	Deku
Fish 6.7	00.0	----	0/0	36.4	69.2	100.0	----	----	----	----
Fish 6.8	22.5	14.3	45.8	66.7	53.3	81.8	----	33.3	----	40.6
Fish 6.10	16.0	13.7	40.7	33.3	44.4	84.6	0/0	16.7	----	44.0
Fish 7.1	33.3	----	----	----	0/0	----	0/0	----	----	40.0
Fish 7.2	10.4	14.3	63.3	70.8	6.3	76.2	----	00.0	58.3	16.7
Fish 7.3	20.6	4.0	53.9	41.2	30.3	66.7	16.7	30.0	60.0	13.8
Fish 8.1	23.1	31.6	88.5	84.6	69.0	95.1	----	37.0	100.0	62.5
Fish 8.2	37.2	17.8	83.3	63.6	45.8	73.2	----	12.5	----	28.6
Fish 8.4	22.4	15.4	83.3	75.0	55.6	82.6	----	53.3	----	30.0
Site mean	41.7	38.6	75.0	62.8	36.3	71.5	35.1	47.8	55.9	52.2
Fish mean	22.4	17.3	54.3	54.7	36.2	77.6	49.9	28.5	69.0	31.7

Table 4. **Continued.** The percent living in all 34 samples for the top 20 species. A "0/0" means that the species was not seen in either the living or dead condition in that particular fish or site. A dashed line indicates that there were not at least five frustules of that species in that fish or site. All values were rounded off to the nearest tenth. Species codes are: **Frva**, *Fragilaria vaucheriae*; **Meva**, *Melosira varians*; **Na**sp, *Navicula* spp.; **Naca**, *Navicula capitatoradiata*; **Nave**, *Navicula veneta*; **Ni**sp, *Nitzschia* spp.; **Nidi**, *Nitzschia dissipata*; **Nipa**, *Nitzschia palea*; **Nisit**, *Nitzschia sinuata* var. *tabellaria*; **Pe**, Pennate spp.

Sample	**Frva**	**Meva**	**Na**sp	**Naca**	**Nave**	**Ni**sp	**Nidi**	**Nipa**	**Nisit**	**Pe**sp
Site 1	76.5	100.	33.3	79.6	79.6	72.0	61.1	67.9	30.4	62.6
Site 2	42.4	72.7	50.0	80.8	63.2	71.6	60.9	77.6	43.5	65.3
Site 3	100.0	0/0	66.7	84.2	72.2	81.4	70.0	55.6	50.0	69.8
Site 4	100.0	0/0	----	87.5	100.	75.8	100.	----	73.7	83.3
Site 5	----	0/0	----	60.0	66.7	73.6	43.8	83.3	29.8	60.7
Site 6	66.7	0/0	----	88.9	95.2	79.2	90.0	0/0	77.3	89.3
Site 7	57.1	----	50.0	85.7	81.0	73.2	42.9	100.	35.3	67.8
Fish 1.1	----	92.7	100.	91.5	92.9	98.7	100.	----	----	92.3
Fish 2.1	57.1	97.2	----	----	----	40.0	----	----	----	52.6
Fish 3.1	0/0	0/0	----	0/0	0/0	23.5	00.0	0/0	33.3	30.0
Fish 3.3	----	60.0	----	----	57.1	25.0	----	----	42.9	23.1
Fish 3.4	00.0	----	----	0/0	----	12.5	----	----	37.3	10.7
Fish 4.1	20.0	0/0	38.5	69.2	45.2	36.8	40.0	0/0	57.7	62.9
Fish 4.2	----	----	----	----	----	9.1	----	0/0	39.5	50.0
Fish 4.3	----	0/0	----	50.0	----	36.8	00.0	0/0	50.0	55.9
Fish 5.1	00.0	0/0	00.0	----	----	----	----	0/0	46.0	18.9
Fish 5.3	0/0	0/0	----	----	----	57.1	----	----	35.9	61.0
Fish 5.4	----	0/0	0/0	----	20.0	60.0	0/0	----	42.6	44.4
Fish 5.5	100.0	0/0	----	----	----	80.0	0/0	----	50.0	57.1
Fish 5.6	28.0	88.5	60.0	60.0	66.7	13.8	----	0/0	54.8	30.6
Fish 6.1	00.0	82.3	66.7	87.5	71.4	56.3	66.7	----	28.6	68.0
Fish 6.2	0/0	----	0/0	0/0	0/0	0/0	0/0	----	----	----
Fish 6.3	----	28.6	71.4	50.0	----	29.6	14.3	20.0	46.2	43.5
Fish 6.4	----	----	----	76.9	54.6	56.3	----	100.	36.8	68.8
Fish 6.5	0/0	----	----	----	42.9	20.0	----	----	50.0	37.0

Table 4. Continued.

Sample	**Frva**	**Meva**	**Na**sp	**Naca**	**Nave**	**Ni**sp	**Nidi**	**Nipa**	**Nisit**	**Pe**sp
Fish 6.7	0/0	0/0	0/0	----	----	----	0/0	0/0	----	31.3
Fish 6.8	79.3	50.0	00.0	31.6	71.4	18.2	16.7	----	62.5	33.3
Fish 6.10	----	75.0	60.0	50.0	37.5	64.3	33.3	----	42.5	36.7
Fish 7.1	0/0	0/0	----	----	0/0	----	----	----	----	40.0
Fish 7.2	----	83.3	33.3	46.2	14.3	14.3	37.5	----	36.4	35.9
Fish 7.3	0/0	50.0	----	72.7	100.	75.0	----	----	31.8	52.8
Fish 8.1	0/0	0/0	----	71.4	53.6	66.7	83.3	----	26.1	87.5
Fish 8.2	----	0/0	71.4	36.4	----	00.0	16.7	----	14.3	34.7
Fish 8.4	0/0	0/0	100.	100.	85.7	77.8	40.0	----	34.7	65.9
Site mean	73.8	86.4	50.0	80.9	79.7	75.3	66.9	76.9	48.6	71.3
Fish mean	35.6	70.8	54.7	63.8	58.1	42.3	37.4	60.0	40.9	47.1

Table 5. Student's t-test done by comparing sites and fish for species from Table 4, the percent living for the top 20 species. "S/F" is the number of sites in which group occurs in sufficient quantities to score over the number of fish in sufficient quantities to score. Replicate size was considered large enough only if there were at least 5 sites and 10 fish with at least 5 specimens of the taxon. Six of the twenty taxa were consequently excluded from analysis.

Taxon	**S/F**	**Mean % Living**		**% Die Off**	**P**
		Site	**Fish**		
Cymbella affinis	7/22	36.3	36.2	.28	.996
Cymbella caespitosa	6/21	71.5	77.6	-8.5	.447
Cymbella spp.	7/24	62.5	54.7	12.9	.379
N. sinuata var. *tab.*	7/22	48.6	40.9	15.8	.202
Cyclotella meneghin.	5/18	75.0	54.3	27.6	.072
Nav. capitatoradiata	7/14	80.9	63.8	21.1	.055
Navicula veneta	7/14	79.7	58.1	27.1	.051
Nitzschia dissipata	7/12	66.9	37.4	44.1	.044*
Cymb. microcephala	7/19	47.8	28.5	40.4	.032*
Achnanthes linearis	7/26	41.7	22.4	46.3	.009**
Dentic. kuetzingiana	7/22	52.2	31.7	39.3	.006**
pennate spp.	7/26	71.3	47.1	33.9	.004**
Nitzschia spp.	7/23	75.3	42.3	43.8	.004**
Achnanthes minutissma	7/23	38.6	17.3	55.2	.000**

Table 6. t-test done by comparing sites and fish for genera. "S/F" is the number of sites in which group occurs in sufficient quantities to score over the number of fish in sufficient quantities to score.

Taxon	S/F	Mean % Living		% Die Off	P
		Site	Fish		
Cocconeis	3/11	14.4	14.2	1.4	.988
Cymbella and Reimeria	7/26	53.4	49.7	6.9	.627
Gomphonema	6/20	39.3	30.2	23.2	.400
Centrics (All centrics)	5/19	78.2	62.4	20.2	.148
Surirella	7/27	21.8	5.7	73.9	.129
Fragilaria	6/12	66.9	43.8	34.5	.128
Synedra	7/9	61.1	42.0	31.3	.091
Cyclotella and Cyclostephanos	5/18	74.8	53.7	28.2	.068
Pennate (All pennates)	7/27	52.0	38.4	26.2	.035*
Navicula	7/24	70.1	48.7	30.5	.008**
Achnanthes	7/26	40.3	21.0	47.9	.006**
Denticula	7/23	52.2	31.8	39.1	.005**
Nitzschia	7/25	63.2	42.1	33.4	.002**

Table 7. The species-specific average percent densities of living (having inclusions) and dead (lacking inclusions) diatoms for some important taxa.

Diatom Species	Living sites	Living fish	Dead sites	Dead fish
Achnanthes linearis	12.38	2.53	13.75	10.44
Achnanthes minutissima	6.62	1.42	9.26	8.15
Cymbella spp.	1.14	2.16	0.66	2.14
Cymbella affinis	1.63	2.11	2.47	3.34
Cymbella delicatula	0.53	0.31	0.47	0.46
Cymbella silesiaca	0.22	1.01	0.14	0.42
Cymbella caespitosa	1.36	3.04	0.62	0.65

Table 8. The ratio of species-specific average percent densities of living (having inclusions) to dead (lacking inclusions) diatoms for some important taxa.

Diatom Species	Ratio living/dead sites	fish
Achnanthes linearis	0.90	0.24
Achnanthes minutissima	0.71	0.17
Cymbella spp.	1.73	1.01
Cymbella affinis	0.66	0.63
Cymbella delicatula	1.13	0.67
Cymbella silesiaca	1.57	2.40
Cymbella caespitosa	2.19	4.68

RESULTS

A total of 142 and 156 different taxa were observed at the sites and in the fish, respectively. (See Appendix A for a full list of species.) For the sites, 79.6% of those taxa had at least one frustule with cytoplasmic inclusions, while in the fish 77.6% of the taxa observed had at least one frustule in the "living" condition.

Important Species Indices (ISI) were calculated for both living (Table 1) and dead (Table 2) diatoms. Both living and dead diatom floras show some similarity in important species present, *Achnanthes linearis* being the most important species for both the living and dead. Furthermore, 18 of 22 of the most important species for the living flora were also part of the most important species for the dead flora.

The comparison of diatom community diversity between the sites and fish was determined using Shannon-Weiner and Hill's Diversity Indices (Table 3). The diversity indices for the sites and fish look very similar, indicating that the species richness and species evenness found at the sites reflects that observed in the fish.

A cluster analysis based on all the living diatom taxa from the sites and fish showed the sites clustered together more tightly than the living floras from the fish. With UPGMA clustering algorithm, riffle sites 4 and 6 (Fig. 3) were the most similar of any pair of comparisons (Fig. 4). The remaining sites (2, 3, 1, 5, 7), all in shallow pools where leaf litter accumulation was observed (Fig. 3), formed a separate but also very tight cluster (Fig. 4). Fish 6.5 (captured near pool site 1) was the only fish that fell in with the cluster containing the five pool sites, and it was less than 50% similar to the site cluster. The sites are more similar to each other than they are to the fish, and more similar in general than the fish are to each other. There is more variability in the living flora obtained from the fish than can be found on the substrates.

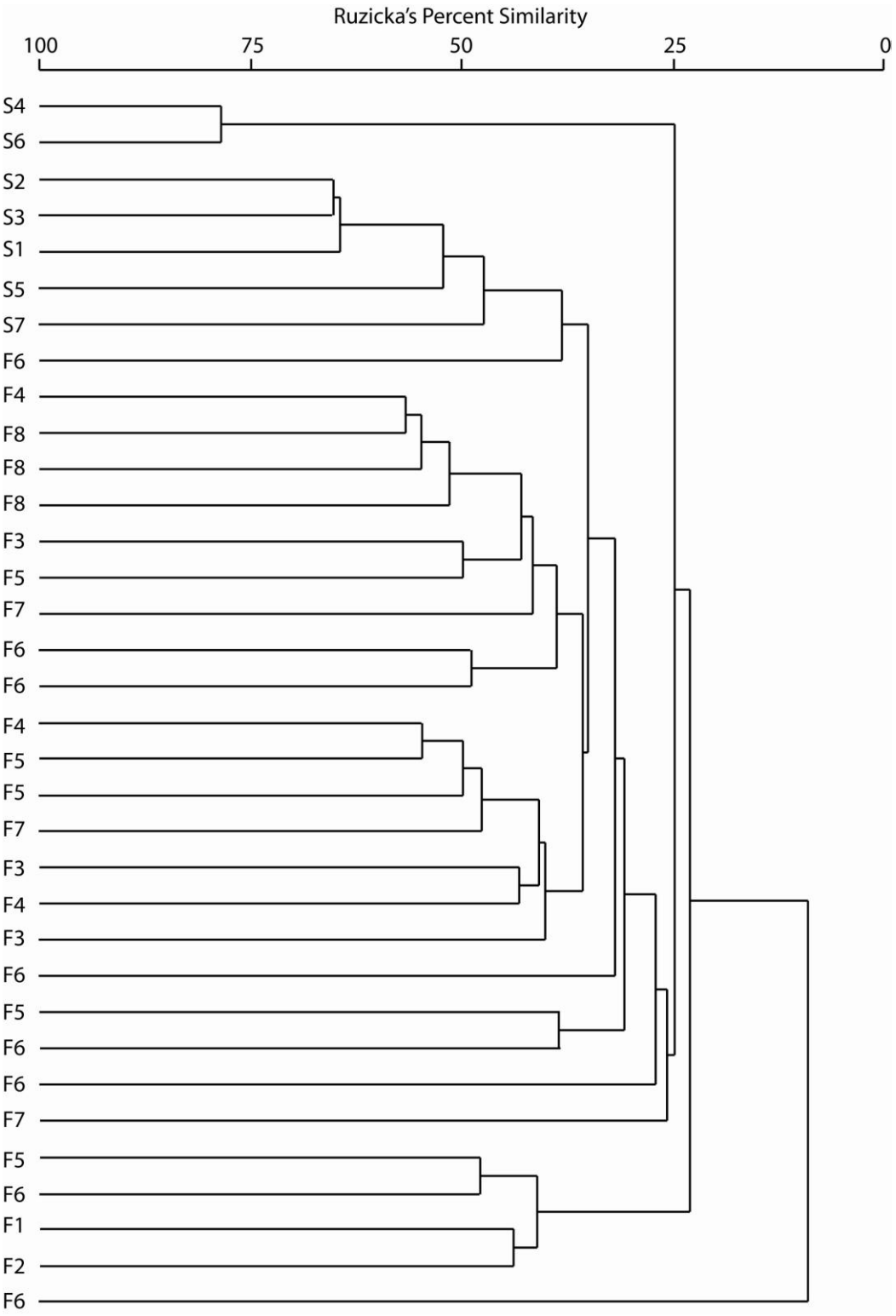

Figure 4. Cluster of living floras from fish (F) and natural substrates (S).

One concern when collecting samples was that the flora in the fish from the various sites would, perhaps, be similar to the sites they were taken from. If so, this would require blocking by site in any statistical analysis. However, cluster analysis of the living diatom floras (Fig. 4) when compared to where the fish were sampled (Fig. 3) demonstrates that there is not a close relationship between the minnows and the sites they were taken from. Furthermore, the fish from the same sites did not typically cluster together, further supporting the idea that the fish were feeding at a variety of sites over the course of a day.

Given this finding, we consider the fish to be independent samples from Paine Creek and they were treated as such in all statistical analyses. The sites were also considered as independent samples.

Although I only had 34 samples (7 natural substrates, 27 fish), the living and dead diatom floras could be tabulated separately to give 68 "floras." A cluster of the dead diatom floras from the sites and fish was run, but is not shown since it was very similar to that for the living floras (Fig. 4).

For the sake of illustration, assume that the living diatom species at the sites were consumed by the fish in direct proportion to their frequencies at the sites and there was an equal die-off applied to all species during digestion. If these two conditions were met, there would be 100% similarity between the living floras of the sites and living floras of the fish. Even if we relax these stringent conditions and assume a nearly equal die-off applied to all species during digestion, then the site and fish floras would still be very similar. However, the results (Fig. 4: Fig. 5) seem to suggest that there was preferential die-off. The living floras from the sites and fish separate from each other.

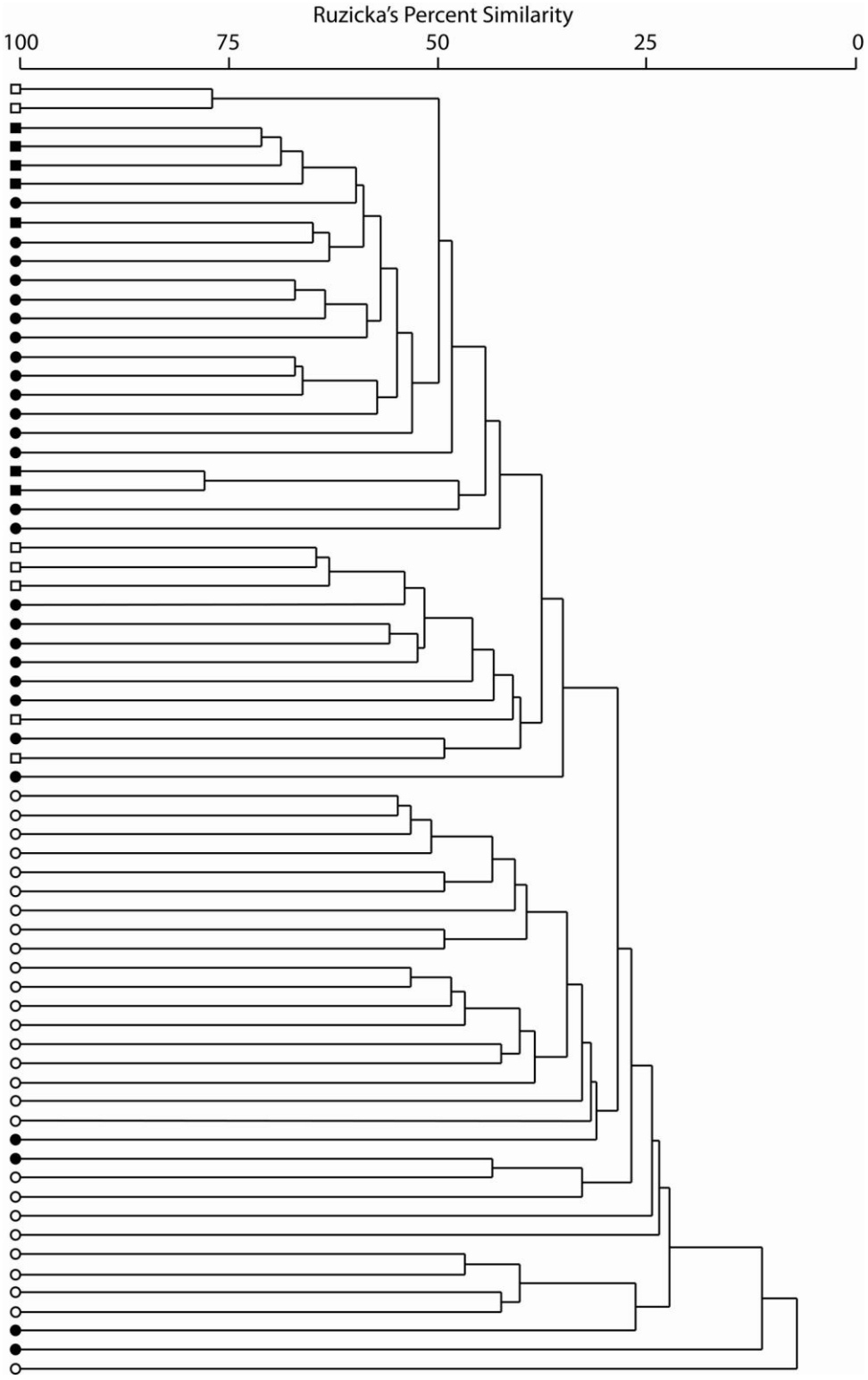

Figure 5. Cluster of living (clear) and dead (black) floras of fish (○) and sites (□).

The “dead” diatom floras of the sites showed the same two clusters as seen in the cluster of only living diatoms (Fig. 4). Interestingly, the living diatom floras of the sites were more dispersed than the “dead” diatom flora (Fig. 5). The dead floras of the sites tend to have more internal similarity than the living floras of the sites, as indicated by the shorter length of the branches. Exceptional outliers (all fish) are at the bottom of the cluster (Fig. 5). Note also the very interesting observation that the living floras of five sites are similar to the dead floras of several fish.

There were similar results for two other clusters not shown. For the total flora in the fish, there is good separation between the dead and living diatom floras. For the living and dead flora at the sites, there is also good separation between the living and dead.

Table 4 is the percent living diatom taxa in all 34 samples for the taxa with an ISI above 0.30 for at least one community (Table 1 and Table 2). Most taxa had a higher percentage of “living” at the sites than in the fish, which is what one would expect if the taxa died off as a result of ingestion. Four taxa were an exception to this finding: *Cymbella caespitosa*, *C. delicatula*, *C. silesiaca*, and *Navicula* species.

Student's t-tests were done based upon data in Table 4, comparing the percent living at the sites to the percent living in the fish for individual species, genera and pennate and centric categories. They display an important point. All the listed taxa had at least some frustules that survived gut passage. Hence, saying that "a species does or does not survive gut passage well" is a relative judgment based upon statistics, meaning that it has a greater or lesser probability of survival as compared to other species.

If there is no significant difference between the mean percent living in the sites and fish ($p>.05$), it could mean one of two things. Either the replicate size was not large enough or there was in fact no significant difference between the two variables. The latter case

suggests that the taxon survives gut passage well. If, in addition, there is a small percent die off, then one may be even more confident that the taxon is especially resistant to digestion. If however there is a significant difference between said variables ($p<.05$) and a large percent die off, then the taxon probably lacks the capacity to survive gut passage well.

In Table 5, 14 taxa meeting the criterion of large enough replicate size, at least 5 sites and 10 fish, are shown. The top two species, *C. affinis* and *C. caespitosa*, show no significant difference between the sites and fish, and the percent die off is minuscule, being negative in the case of *C. caespitosa*. This suggests that these two taxa have a special resistance to digestion. *Cymbella* spp. and *Nitzschia sinuata* var. *tabellaria* also show no significant difference between sites and fish and the percent die off is relatively low, indicating that they survive gut passage better than other taxa further down the list.

The next three taxa, *C. meneghiniana*, *N. capitatoradiata* and *N. veneta*, have a significance level slightly greater than .05 and a percent die off of approximately 25%, enabling one to conclude that they probably have an intermediate capacity to survive gut passage.

Finally, in the last seven taxa there is a significant difference ($P<.05$) between the mean percent living in the sites and fish, and a relatively large percent die off. This suggests that these taxa are being seriously affected by gut passage, as they probably are more easily digested than taxa further up the list.

Table 6 implies that some genera survive gut passage much better than others. The difference between the mean percent living in the sites and fish are not significant ($P>.05$) for *Cocconeis*, *Cymbella, Reimeria, Gomphonema*, "all centrics," *Surirella, Fragilaria, Synedra*, *Cyclotella* and *Cyclostephanos*. The difference is significant for "all pennates," *Navicula, Achnanthes, Denticula*, and *Nitzschia*.

Still, some of these results must be viewed with caution. In the case of *Cocconeis*

and *Synedra*, the replicate size did not meet the criteria of at least 5 sites and 10 fish. Replicate size and the standard deviation of abundances could be the reason some analyses were not significant.

The average percent density of living specimens of *Achnanthes linearis*, *A. minutissima* and *Cymbella delicatula* at the sites is greater than in the fish (Table 7). By contrast, for *Cymbella* spp., *C. affinis*, *C. silesiaca* and *C. caespitosa*, the average percent densities of living specimens is somewhat greater in the fish than at the sites.

Furthermore, the average percent densities of dead *Achnanthes linearis* and *A. minutissima* diatoms are somewhat greater at the sites than in the fish. Just the opposite is the case for *Cymbella* spp., *C. affinis*, *C. silesiaca* and *C. caespitosa*. The average percent densities of dead diatoms are somewhat greater in the fish than at the sites. For *C. delicatula*, the two variables are almost equal.

If there is a significant die-off for *Achnanthes linearis* and *Achnanthes minutissima*, we should expect the ratio of species-specific average percent densities of living to dead would be greater at the sites than in the fish—and this is what we observe (Table 8). For *Cymbella affinis*, *Cymbella silesiaca*, and *Cymbella caespitosa*, the ratio of living to dead in the fish is almost equal to or greater than the ratio of living to dead at the sites. This is consistent with the hypothesis that these taxa are resistant to digestion. However, for *Cymbella* spp and *Cymbella delicatula*, the ratio of living to dead at the sites is somewhat greater than the ratio of living to dead in the fish (Table 8).

DISCUSSION

The evidence from this study indicates that some diatom taxa resisted digestion in the gut of the algivorous minnows better than others. Both cluster analyses are consistent with the hypothesis of an unequal die-off during gut passage (Figures 4 and 5). The Student's t-tests demonstrated that some species and genera are more resistant to digestion

than others (Tables 5 and 6). Tables 7 and 8 both support the idea that certain species are more resistant to digestion than others. Before we discuss these results in greater detail, something in defense of the methodology of this thesis should be said.

Possible Problems With This Thesis and a Response

It may be argued that the length of the fish may influence the results of a study of this nature. The probability of a diatom dying is directly related to the length of the fish: larger fish have longer intestines than smaller fish, and thus, the probability of a diatom being digested is greater in larger fish.

Knobloch (1991) addressed this issue. When *P. notatus* and *C. anomalum* specimens were ordered by standard length, no clear relationship between size and number of living diatom species was detected. Fish size did not correlate with total number of species in the feces, or the percentages of those species identified in living preparations. For this reason, the length of the minnow specimens and the probability of a diatom being digested were not dealt with in this study.

It may further be argued that *P. notatus* and *C. anomalum* utilize diatoms differently, and thus, the one *C. anomalum* specimen should not have been included in our analysis. Once again, Knobloch (1991) put forth evidence that undermines this viewpoint. A cluster analysis did not separate these fishes by species at a particular site. This suggests that these two minnow species were ingesting the same diatoms in the same relative abundances at each of the sites they were taken from. He also could not find any clearly discernable relationship between fish species and the frequency of living diatoms. His findings support the view that *P. notatus* and *C. anomalum* specimens may be used interchangeably in my study of diatom ability to resist digestion as they pass through the gastrointestinal tracts of these fishes.

Sellman et al. (2001) found in a cluster analysis that diatom species composition clustered by fish species, with common shiners (*Luxilus cornutus*) being a totally exclusive cluster, while natural substrates were clustered within the stoneroller and bluntnose clusters. In addition, samples from natural substrates had an internal similarity not significantly different from their similarity to samples in stoneroller and bluntnose guts. This suggests that *P. notatus* and *C. anomalum* utilize diatom species from the substrata in a similar fashion, as they were efficient collectors of diverse representative diatom samples.

Although Rosati et al. (2003) found that *C. anomalum*, *P. notatus*, and *Semotilus atromaculatus* did not collect equally representative samples of diatoms, they concluded that all three species are representative samplers of diatoms in mid-order streams, and these fish samplers seem to be interchangeable. This finding would have direct bearing on my study: the one *C. anomalum* specimen can be viewed as interchangeable with a *P. notatus* specimen.

The Cluster Analyses

As the first cluster analysis shows (Fig. 4), the living flora of the sites form a tighter cluster that is separate from the cluster of the living flora of the fish. This finding is consistent with the hypothesis that there is an unequal die-off operating on the diatom flora as they pass through the gut. In order to see that this is so, assume that the fish consumed the living diatom flora in direct proportion to their frequency on the rocks, and there was an equal die-off applied to all species during digestion. If these stringent conditions were met, the living flora of the sites would form a tight cluster with the living flora of the fish.

In the second cluster analysis (Fig. 5), the living flora from the sites, the dead flora from the sites, and the dead flora from the fish form a cluster that is separate from the cluster of living flora of the fish. Once again, this is consistent with the hypothesis of an

unequal die-off. In order to understand why this is so, it must be realized that the living flora of the sites form a somewhat tight cluster with the dead flora of the sites: this suggests that there is an even distribution of the living and dead floras for the sites. However, note that the dead flora from the fish are separated from the living flora of the fish: this suggests an uneven distribution of the living and dead, which is consistent with an uneven die-off.

Tables 7 and 8

In Table 7, we see that the average percent densities of living valves are higher at the sites than in the fish for *Achnanthes linearis* and *Achnanthes minutissima.* Perhaps this means that the fish have a hard time scraping the living frustules off of the rocks. Alternatively, it could mean that there is a significant die-off of these species during digestion. Both interpretations are consistent with the hypothesis that if a species is difficult to ingest it should be less resistant to digestion.

Just the opposite is the case for *Cymbella* spp., *Cymbella affinis, Cymbella silesiaca,* and *Cymbella caespitosa*. The average percent densities are higher in the fish than at the sites (Table 7). This could mean that these species are easy for the fish to ingest, or alternatively, these species are relatively more resistant to digestion than other species, such as *Achnanthes linearis* and *A. minutissima*. Both interpretations are consistent with the hypothesis that if a species is relatively easy to ingest, it should be more resistant to digestion.

If *Achnanthes linearis* and *Achnanthes minutissima* are relatively less resistant to digestion than other species, we should expect the ratio of species-specific average percent densities of living to dead would be greater at the sites than in the fish—and this is what we observe (Table 8). However, for *Cymbella silesiaca* and *Cymbella caespitosa*, the ratio of the species-specific percent densities of living to dead in the fish is greater than that of the sites. For *Cymbella affinis*, the two ratios are almost the same. These findings are

consistent with the view that these species are relatively more resistant to digestion than others.

Comparison with Knobloch

Knobloch (1991) found that 74 of his 203 taxa (36%) were able to survive passage through the gastrointestinal tract at least some of the time (pp.vii, 24) while my percentages are about twice as high. 77.6% of the 156 taxa in my study had cytoplasmic inclusions following gut passage. Perhaps my percentages are higher because some frustules were classified as "living" (i.e., they had cytoplasmic inclusions) even though they were dead or were in the process of dying. Furthermore, some taxa may be highly, moderately or marginally resistant to digestion but do not proliferate well in culture, and thus, would have a low or zero survival frequency in Knobloch's study. After all, Estes and Dute (1994) pointed out that investigators who rely upon diatom clones created in culture must exercise caution. Pooling the results of both studies would suggest somewhere between 36% and 78% of the diatom taxa are actually able to survive gut passage at least some of the time.

Knobloch's (1991) most important species as determined by ISI values were *Achnanthes linearis*, *Nitzschia sinuata* var. *tabellaria*, *Navicula capitatoradiata* and *Denticula kuetzingiana*, all having important species values greater than 4.0 (p.23). His most important species, *A. linearis*, had an ISI of 25.07.

My most important species showed a more even spread. For the living diatoms, I had 12 taxa with Important Species values greater than 1.00 but less than 5.00 in the "combined" category (Table 1). For the dead diatoms, I had 10 taxa with Important Species values greater than 1.00 but less than 12.00 in the "combined" category (Table 2).

Like Knobloch, *Achnanthes linearis* was my most important species. Twelve of 17 species listed on his "Important Diatom Species" table (Knobloch 1991, p.23) were also among my most important living or dead diatom species. A comparison of all of his diatom

taxa (Ibid, pp.13-22) with mine shows a distinct overlap. Ergo, the diatom flora and dominance patterns of my study are similar to that of Knobloch (1991), suggesting that our fish specimens were eating similar things in the different years.

According to Knobloch (p.34), when diatom floras in the minnow intestines were regressed against diatom floras present in enrichment culture, *Achnanthes linearis*, *Cyclotella meneghiniana*, *Denticula kuetzingiana*, *Melosira varians*, and *Navicula capitatoradiata* were among the outliers, suggesting that these survived gut passage better than other taxa. Although I did not do a t-test on *M. varians*, Table 4 hints that it has a high survival rate. Similarly, it was a minor component of Knobloch's flora but it had a fairly good survival rate of 55% (p.28). Interestingly enough, Nicotri (1977) also found *Melosira* spp. hard to digest. The other species will be discussed below.

To be sure, there are more similarities between the studies of Knobloch and myself. Both of us found that *Cyclotella meneghiniana* and *Navicula captitatoradiata* had relatively high survival rates, *Navicula veneta* had an intermediate capacity to survive ingestion, and *Cymbella microcephala* had a lower rate of survival.

I found *Denticula kuetzingiana* and *Nitzschia dissipata* to have die-off rates of 39.3% and 44.1%, respectively, whereas Knobloch found them to have commensurate average survival rates of 70% and 55% (pp. 27, 28). Yet, my study found the difference between the mean percent living at the sites and in the fish to be significant for both species (Table 5), which suggests that they do not survive gut passage well. Knobloch's regression analysis (p.34) found *Denticula kuetzingiana* to be an outlier, suggesting that it survives gut passage better than other taxa.

Knobloch's regression analysis also found *Fragilaria vaucheriae* to be seriously affected by gut passage, as it had it a very low rate of survival. Although I did not do a t-test on this species, Table 4 also suggests that it experienced a relatively large die-off.

There is an important difference between our studies. Knobloch found that *A. linearis* survived gut passage far better than other taxa--exhibiting an average survival frequency of 95% (p.27)--whereas I found that it had a relatively high percent die off (46.3%). It could be that Knobloch's finding is an artifact of culturing. *A. linearis* may have been so abundant in the stream that at least one or a few survived ingestion, and then proliferated extremely well in his cultures.

In contrast to my findings, Knobloch found no genera being over or under represented among the living taxa (p.35). Table 6 suggests that *Cocconeis, Cymbella, Reimeria* and *Gomphonema* survived ingestion better than other genera, whereas *Achnanthes, Denticula* and *Nitzschia* displayed a high percent die-off, and the difference between the percent living at the sites and in the fish was significant at the one percent level. Other genera seem to be in between these two extremes. (Note: In the case of *Cocconeis*, the replicate size did not meet the criterion of at least 5 sites and 10 fish.)

According to my results, *Cymbella affinis* and *Cymbella caespitosa* survived ingestion better than all other species (P=.996 and P=.447, respectively). However, Knobloch (p.28) found them to have low average survival frequencies, 30% and 25%, respectively. Because both species were relatively significant components of his diatom flora in the feces (as evidenced by ISI values > 1.00), one may rule out the possibility that there simply were too few frustules available to proliferate well in culture. A much better explanation for the difference may be that these Cymbelloid species respond poorly to culturing.

I found *Nitzschia sinuata* var. *tabellaria* to pass through the gut somewhat well, having only a 15.8% die off rate (P=.202). However, Knobloch found that it was easier to digest, as it had an average survival rate of only 45% (p.27). One explanation for this difference may be that it has only a marginal ability to proliferate in culture.

Finally, I found that centric diatoms as a group are more resistant to digestion than are pennates (Table 6). Knobloch's study says nothing about this.

Comparison to Published Studies

Summarizing the current paradigm, Peterson et al. (2003) pointed out that diatom species differ in their ability to grow in culture from grazer feces, and in the degree to which chloroplast condition within frustules is degraded by passage through grazer guts; and finally, there is interspecific variation in diatom digestibility.

Devercelli and Williner (2006) provided evidence that certain diatom taxa (such as a *Denticula* sp. and *Navicula* spp.) from an Argentina stream exhibited digestion resistance and reproductive stimulation following gut passage through crabs, *Aegla uruguayana*. I found the *Denticula* and *Navicula* genera to be among the taxa that are the least resistant to digestion (Table 6).

Nocotri (1977) noted that *Achnanthes* spp. adhere to rock surfaces and therefore may be less affected by grazing than are other diatoms. This is supported by Power (1990) who found that grazing armored catfish scoured bedrock substrata and depleted algae, leaving sparse standing crops of adnate diatoms, primarily *Achnanthes* spp. Hill and Knight (1988) also found *A. minutissima* to be relatively less accessible to insect grazers than other taxa. Finally, Kawamura et al. (1992) found that species with a filamentous form or low adhesive strength are more prone to being grazed than are diatoms that are tightly attached to the substrata.

Apparently *C. anomalum* and *P. notatus* also have some difficulty scraping living *A. linearis* and *A. minutissima* frustules off rocks. It is seen in Table 7 that the average percent densities of living valves for both species were considerably higher at the sites (12.38%, 6.62%) than in the fish (2.53%, 1.42%). In Table 8, the ratio of average percent densities of living to dead diatoms is greater at the sites than in the fish.

There are other points of agreement between Nicotri's (1977) study of marine taxa and my freshwater lotic study. First, he found significant differences in the susceptibility to digestion of various diatoms. Secondly, *Achnanthes* spp. did not survive gut passage well. Finally, diatom digestion was accomplished by chemical degradation rather than mechanical grinding, for the siliceous tests of diatoms in the feces were mostly whole and unharmed. This agrees with my observations, as I found the majority of diatoms under light microscopy to have their silica tests intact, despite the fact that they lacked living material like chloroplasts and lipid droplets.

Indeed, Hamm et al. (2003) noted that diatoms can survive gut passage if they escape being crushed, and they hypothesized that frustules have evolved as effective armor against predators because extraordinary force is required to crack them.

Velasquez (1939) reported that diatoms as a group, as compared to other algae, are marginal in their resistance to digestion. Using culturing techniques he found only four diatom groups to be viable after gut passage through the digestive tracts of *Dorosoma cepedianum* specimens (Gizzard Shad)--*Cyclotella meneghiniana*, two unknown *Navicula* species and a *Diatoma* species. Interestingly, I found that *Cyclotella meneghiniana* had a somewhat better than average capacity to survive gut passage.

Although Peterson (1987) concluded for statistical reasons that his diatom taxa did not differ in digestibility, he did report that percentages of dead frustules were significantly higher in fecal material than in pre-grazed communities for *A. minutissima*. This suggests that it experienced considerable die off during gut passage through larval caddisflys, a finding that is congruent with my overall results. However, my particular data on this matter was just the opposite of his. Percentages of dead frustules were higher at the pre-grazed sites than in the fecal material (Table 7).

He also reported that small, adnate diatom species like *A. minutissima* are less

accessible to insect grazers. Once again, my study also suggests (Tables 7 and 8) that living *A. minutissima* specimens are not readily accessible to algivorous minnow grazers, for the average percent density of living diatoms was considerably greater at the sites (6.62%) than in the fish (1.42%), and the ratio of the average percent density of living to dead valves was greater in the natural substrates from sites than in the fish.

In a later study Peterson et al. (1998) found that *A. minutissimum* cells in periphyton exposed to heavy grazing pressure by caddis larvae and mayfly nymphs in a small montane stream were efficiently converted to "dead cells," suggesting low resistance to digestion. Once again, this finding parallels mine somewhat, as this taxon appears to be very digestible (Table 5).

In their 2003 gut passage study, Peterson et al. concluded that *Achnanthes lanceolata* and *Synedra ulna* represent the "lower end" of a digestion-resistance spectrum, with *S. ulna* at the non-resistant extreme. Interesting enough, I found that *Achnanthes* taxa in general had a low resistance to digestion (Table 6).

In laboratory streams, Colletti et al. (1987) found that small, adnately attached species like *A. minutissima* and *Cocconeis placentula* var. *euglypta* were less accessible to mayfly grazers than large over story species like *Nitzschia dissipata*, *Cymbella affinis* and *Synedra ulna*. This finding may also apply to algivorous minnows. For previously stated reasons, living *A. minutissima* frustules are not readily accessible to these piscene grazers. By contrast, Table 7 shows that *Cymbella* spp., *C. affinis*, *C. silesiaca*, and *C. caespitosa* have a higher average density of living frustules in the fish than at the sites. This could mean that algivorous fish grazers are able to take overstory species with greater ease than small, adnately attached taxa.

In a study of *Asellus aquaticus* and *Gammarus pulex* species (Crustacea, Isopoda) in three rivers in southwestern England, Moore (1975) had some findings that are in

agreement with mine, and one that differed. Compared to other taxa, *A. minutissima* had the lowest survival rate (1.5-1.4%) in the gut while *Cymbella* spp. had the highest (18-62%). During one month of his study 44% to 66% of the specimens of *Cymbella affinis* ingested by the isopods possessed intact chloroplasts after gut passage. He concluded however that *A. minutissima* was highly accessible to the isopod grazers. My findings suggest this taxon was less accessible to the fish as compared to other taxa (Table 7 and 8).

In laboratory streams at high velocity, *A. minutissima* and *Nitzschia* spp. were found to be easily degraded in the gut of Chironomid larvae, having 85%-90% digestion per passage and 65%-85% digestion per passage, respectively. At low velocity the digestion rates of *Nitzschia* spp. were less, 40%-80% (Marker et al. 1988). Once again, these results parallel mine (Tables 5 and 6), as both taxa were relatively easy to digest.

Diaz Villanueva et al. (2003) observed a pattern that is congruent with my results. Species that were highly susceptible to ingestion, like *Cymbella silesiaca*, were also the most resistant to digestion and vice versa. Species that were less susceptible to ingestion were the least resistant to digestion.

The Hypothesis Concerning Diatom Vulnerability to Ingestion and Digestion

When the results of my study are pooled with all the other aforementioned studies, it can be stated with a fair degree of confidence that diatom taxa differ in their capacity to remain viable after gastrointestinal passage through vertebrate and invertebrate grazers. Three ecological questions remain. In at least some lotic systems, does natural selection favor those diatoms that have a high resistance to digestion and penalize those that have low resistance? Does differential survivability following ingestion play a role in determining diatom community structure in streams? Are some diatoms favored by their stronger adhesion to the substrate?

Diatoms form the major dietary component of a large number of aquatic animals

such as herbivorous cyclopoid copepods, various insect larvae, planktonic crustacea and microherbivorous fish (Calow 1973). From this one could assume, prima facie, that the capacity to remain viable after gut passage would be one of an array of defense mechanisms diatom prey species have evolved to compensate for heavy predation pressure.

However, the Devil's Advocate may counter: A superficial examination of all the data from my study, Knobloch's (1991) and others concerning *A. linearis* and *A. minutissima* may suggest that natural selection does not overall penalize taxa which are highly digestible, and the capacity to survive ingestion does not play a role in determining diatom community structure. For, if in the past, natural selection penalized taxa that have a low resistance to digestion, why were *A. linearis* and *A. minutissima* the most prevalent taxa in Knobloch's, mine and other studies? Apparently, selective forces from lower survivability did not condemn them to extinction.

We rebut the Devil's Advocate. Hill and Knight (1988) found that diatoms in the loose, upper layer of periphyton were generally affected by grazing more than those in the adnate layer. Therefore, small, adnately attached diatoms like *Achnanthes* spp. and large over story species like *Cymbella affinis* may have evolved two different sets of defense mechanisms against vertebrate and invertebrate grazers.

The small adnately attached taxa may defend against predation by being highly inaccessible to grazers and having a faster rate of division to maintain their populations in the face of grazing pressure from insects and algivorous fish. This may be their overall strategy against grazing in general.

By contrast, large over story species may be unable to divide at the rate they are being ingested and they are accessible to grazing fish *and* invertebrates. Thus, the selection pressure on them to remain viable after gut passage may be greater than it is on small adnate taxa like *Achnanthes* spp. Perhaps this explains why overstory genera such as *Cymbella*

spp. seem to have a special resistance to digestion. They do have a higher degree of silicification, and certain *Cymbella* spp. (e.g. the subgenus *Encyonema*) are additionally encased in mucilage tubes.

My theoretical perspective concurs somewhat with that of Peterson et al. (1998), as he hypothesized that there is an evolutionary trade-off between ingestion and digestion resistance; natural selection should strongly favor digestion resistance in taxa that are highly vulnerable to ingestion by grazers. He found that the diatoms most susceptible to ingestion (small chain-forming *Fragilaria*) were the most resistant to digestion. In contrast, *Achnanthidium* (=*Achnanthes*) *minutissimum*, *Planothidium* (=*Achnanthes*) *lanceolatum* and *Cocconeis placentula* var. *euglypta* were relatively more resistant to ingestion, a finding that parallels mine. Tables 7 and 8 hint that *A. minutissima* was less susceptible to ingestion by grazing fish than other taxa. Selection pressure on these species to develop a resistance to digestion should be much less.

Testing the Hypothesis

One possible method of testing our hypothesis is as follows. We can search the literature and see if various species fit or undermine the theory. More specifically, if a species is observed to be an under story species (or relatively hard to ingest), we should find in our results that it is relatively easy to digest, and vice versa. If a species is observed as an over story species (or relatively easy to ingest), our findings should show that it is relatively hard to digest.

In three streams in southwest Missouri, Fowler and Tabler (1985) found evidence that *C. oligolopis* and *C. anomalum pullum* selectively graze attached stalked genera such as *Gomphonema* and *Cymbella* and are less inclined to ingest more tightly adherent forms such as *Navicula*. Thus, our theory predicts that the *Gomphonema* and *Cymbella* genera should be resistant to digestion, and the *Navicula* genera should be less resistant to digestion. The

results in Table 6 are consistent with this prediction. The mean percent difference between the living at the sites and in the fish for *Gomphonema* and *Cymbella* is not significant, but for *Navicula* it is significant. For *Gomphonema* and *Cymbella* the percent die-off is relatively small, but it is larger for *Navicula*.

Power (1990) found that grazing armored catfish scoured bedrock substrata and depleted algae, leaving sparse standing crops of adnate diatoms, primarily *Achnanthes* spp. Since this genera is somewhat difficult for the predator to ingest, it should relatively easy to digest. The results in Table 6 are consistent with this prediction.

Hoagland et al. (1982) found *Melosira varians* in the over story. Since it should be easy for predators to ingest, our theory correctly predicted it would be relatively hard to digest. They also observed *Nitzschia dissipata* and *N. palea* to be a part of the dense under story. Therefore, since they are relatively hard for algivores to get at, they should be easy to digest. Table 5 shows that *N. dissipata* is relatively easy to digest, as the difference between the mean percent living at the sites and fish is significant, and there is a large percent die off.

Hoagland et al. (1982) also observed unspecified *Nitzschia* species as part of the low profile, dense under story. Once again, according to our theory *Nitzschia* genera should be hard to ingest but easy to digest because it is a part of the under story. Examine Tables 5 and 6. It is seriously affected by gut passage and easy to digest.
The same seems to be true for *Fragilaria vaucheriae*, as it appeared to be an under story species in "a large rosette, and attached by a mucilage pad." Since the periphyton community had a vertical structure, it looked as though to be somewhat protected from grazing by the over story. According to our theory then, it should be easy to digest. And this is what our data suggests. See page 40.

Another researcher observed *Fragilaria vaucheriae* to have a different form of

attachment. Using scanning electron microscope micrographs, Lamb et al. (1987) found *Fragilaria vaucheriae* specimens to be primarily prostrate on rocks, prostrate on other diatoms, and adnate on rocks. According to the theory presented here, this should make them less accessible to grazers, and in turn, less resistant to digestion. Knobloch's (1991) regression analysis also found *Fragilaria vaucheriae* to be seriously affected by gut passage, as it had it a very low rate of survival. Although I did not do a t-test on this species, Table 4 also suggests that it experienced a relatively large die-off.
Hoagland et al. (1982) also make an interesting observation worth mentioning here. They say certain prostrately attached species produce slime "halos." These slime "halos" could protect the prostrately attached diatom from ingestion by insects.

Patrick (1976) noted that populations of *Gomphonema* and *Cymbella* develop upright dendritic colonies in the over story. Since these two genera are considered part of the over story (and thus easy to ingest by algivores), they should be hard to digest. And this is what we found. See Table 6. The difference between the diatom density at the site and in the fish was not significant, and there was a relatively small die-off.
She also found that *Melosira* species drape themselves over the top of the diatom community. This implies they are easy to ingest, as they are a part of the top of the over story, so we should find that they are hard to digest. Table 4 suggests that *M. varians* is relatively hard to digest, as there is not a big difference between the densities of this species at the sites as compared to the fish.

Patrick (1976) also observed *Achnanthes minutissima* forms a pavement growth on the substrate. It is an adnate diatom in the under story, and thus, is hard to ingest, so it should be easy to digest. And this is what we found. Interestingly, she says this diatom has a rapid reproduction rate, and we proposed that being highly inaccessible to insect grazers and having a faster rate of division to maintain their populations in the face of

grazing pressure was their overall defense against grazing in general (see p. 46).

To date, relatively little work has been conducted to evaluate the ecological implications of differential digestibility of diatom taxa and other forms of algae in benthic systems (Peterson et al. 2003). Does interspecific variation in diatoms to remain viable after gut passage through grazers play a role in determining benthic-algal community structure? This is certainly a topic worthy of further study.

LITERATURE CITED

Bolsenga, S.J. and C.E. Herdendorf. **Lake Erie and Lake St. Clair Handbook**. 1993. Wayne State University Press, Detroit.

Burton, M.F., M.P.Oemke, J.M. Molloy. 1994. Effects of grazing by the Trichopteran, **Glossoma nigrior**, on diatom community composition in the Ford River, Michigan, pp.599-608. **In**: J.P. Kociolek (ed.). **Proceedings of the 11th International Diatom Symposium: San Francisco, California, 12-17 August 1990.** California Academy of Sciences, San Francisco.

Calow, P. 1973. The food of **Ancylus fluviatilis** (Mull.), a littoral stone-dwelling herbivore. **Oecologia 13**:113-133.

Collins, G.B.and R.C. Kalinsky. 1977. Studies on Ohio Diatoms. **Bulletin of the Ohio Biological Survey 5**(3): 1-76.

Colletti, P.J., D.W. Blinn, A. Pickart, and V.T. Wagner. 1987. Influence of different densities of the mayfly grazer **Heptagenia criddlei** on lotic diatom communities. **J**. **N**. **Am**. **Benthol**. **Soc**. **6**(4): 270-280.

Devercelli, M. and V. Williner. 2006. Diatom grazing by *Aegla uruguayana* (Decapoda: Anomura: Aeglidae): digestibility and cell viability after gut passage. **Ann. Limnol.-Int. J. Lim. 42** (2): 73-77.

Diaz Villanueva, V. and R. Albarino. 2003. Algal ingestion and digestion by two ephemeropteran larvae from a Patagonian Andean stream, pp. 468-475. **In:** Elda Gaino (Ed) **Research Update on Ephemeroptera & Plecoptera**. Universita di Perugia, Italia.

Estes, A. and R. R. Dute. 1994. Valve abnormalities in diatom clones maintained in long-term culture. **Diatom Reasearch (9)** (2): 249-258.

Fish, G.R. 1951. Digestion in **Tilapia exculenta**. **Nature 167**: 900-901.

Fowler, J.F. and C.A. Tabler. 1985. Food habits and feeding periodicity in two sympatric stonerollers (Cyprinidae). **American Midland Naturalist 113** (2): 217-224.

Gregory, S.V. 1983. Plant-herbivore interactions in stream systems, pp. 157-89. **In**: J.R. Barnes and G.W. Minshall (eds.). **Stream Ecology--Application and Testing of General Ecological Theory**. Plenum, New York.

Hamm, Christian E., Rudolf Merkel, Olaf Springer, Piotr Jurkojc, Christian Maier, Kathrin Prechtel, and Victor Smetacek. 2003. Architecture and material properties of diatom shells provide effective mechanical protection. **Nature 421** : 841-843.

Hill, W.R. and A.W. Knight. 1988. Concurrent grazing effects of two stream insects on periphyton. **Limnol**. **Oceanogra**. **33** (1): 15-26.

Hoagland, K.D., S. C. Roemer, and J. R. Rosowski. 1982. Colonization and Community Structure of Two Periphyton Assemblages, with Emphasis on the Diatoms (Bacillariophyceae). **American Journal of Botany**. **69** (2): 188-213.

Holland, R. E. 1993. Changes in planktonic diatoms and water transparency in Hatchery Bay, Bass Island area, western Lake Erie, since the establishment of the zebra mussel. **Journal of Great Lakes Research** 19(3): 617-624.

Horne, A.J. and C.R. Goldman. 1994. **Limnology: 2nd Edition**. McGraw-Hill. New York.

Kawamura, T. and R. Hirano. 1992. Seasonal Changes in Benthic Diatom Communities Colonizing Glass Slides in Aburatsubo Bay, Japan. **Diatom Research**. **7** (2): 227-239.

Knobloch, T.J. 1991. Viability of diatom species following ingestion by the algivorous minnows, **Campostoma anomalum** and **Pimephales notatus**. Unpublished M.S. Thesis. John Carroll University, University Hgts., Ohio. 118 pp.

Krammer, K. and H. Lange-Bertalot. 1988a. **Bacillariophyceae**. 1. Teil: **Naviculaceae**. In: H. Ettle, J. Gerloff, and H. Heynig (eds.), **Suesswasser- Flora von Mitteleuropa** Vol. 2/1, 876 pp.

Krammer, K. and H. Lange-Bertalot. 1988b. **Bacillariphyceae**. 2. Teil: **Bacillariaceae**, **Epithemiaceae**, **Surirellaceae**. In: H. Ettle, J.Gerloff, and H. Heynig (eds.), **Suesswasser-Flora von Mitteleuropa** Vol. 2/2, 569 pp.

Lamb, M. A. and R. L. Lowe. 1987. Effects of current velocity on the physical structuring of Diatom (Bacillariophyceae) communities. **The Ohio Journal of Science 87** : 72-77.

Leach, J.H. 1993. Impacts of the zebra mussel (*Dreissana polymorpha*) on water quality and fish spawning reefs in western Lake Erie. In: **Zebra mussels: Biology, Impacts, and Control**, T. F. Nalepa and D. W. Schloesser, eds., pp. 381-397. Lewis Publishers, Boca Raton, Florida.

Lowe, R. L., and R. W. Pillsbury. 1995. Shifts in the benthic algal community structure and function following the appearance of zebra mussels (Dreissna polymorpha) in Saginaw Bay, Lake Huron**. Journal of Great Lakes Research** 21(4): 558-566.

Ludwig, J.A. and J.F. Reynolds. 1988. **Statistical Ecology**: **A Primer on Methods and Computing**. John Wiley, New York, 337 pp.

Marker, A.F.H., R.T. Clarke, J.A. Rother. 1988. Changes in an epilithic population of

diatoms, grazed by chironomid larvae, in an artificial recirculating stream, pp.143-149. **In**: F.E. Round (ed.). **Proceedings of the Ninth International Diatom Symposium**: **Bristol, August 24-30, 1986**. Biopress Ltd., Bristol, England.

Moore, J. W., 1975. The role of algae in the diet of *Asellus Aquaticus* L. and *Gammarus Pulex* L. **Journal of Animal Ecology 4**: 719-730.

Nicotri, M.E., 1977. Grazing effects of four marine intertidal herbivores on the microflora. **Ecology 58**: 1020-1032.

Patrick, R. 1976. The Formation and Maintenance of Benthic Diatom Communities. **Proceedings of the American Philosophical Society 120** (6): 475-484.

Patrick, R. and C.W. Reimer. 1966. **The Diatoms of the United States** (Exclusive of Alaska and Hawaii) Volume **1**: **Fragilariaceae**, **Eunotiaceae**, **Achnantheceae**, **Naviculaceae**, Philadelphia: Academy of Natural Sciences of Philadelphia, 618 pp.

Patrick, R. and C.W. Reimer. 1975. **The Diatoms of the United States** (Exclusive of Alaska and Hawaii) Volume 2 Part 1: **Entomonidaceae**, **Cymbellaceae**, **Gomphonemaceae**, **Epithemiaceae**, Philadelphia: Academy of Natural Sciences of Philadelphia, 213 pp.

Peterson, C.G. 1987. Gut passage and insect grazer selectivity of lotic diatoms. **Freshwater Biology 18**: 455-460.

Peterson, C.G., K.A. Vormittag, and H.M. Valett. 1998. Ingestion and digestion of epilithic algae by larval insects in a heavily grazed montane stream. **Freshwater Biology** 40: 607-623.

Peterson, G.C. and T. L. Jones. 2003. Diatom viability in insect fecal material: comparison between two species, *Achnanthidium lanceolatum* and *Synedra ulna.*

Hydrobiologia 501: 93-99.

Pielou, E.C. 1984. **The Interpretation of Ecological Data**: **A Primer on Classification and ordination**. John Wiley, New York, 263 pp.

Porter, K.G. 1973. Selective grazing and differential digestion of algae by zooplankton. **Nature 244**: 179-180.

Porter, K.G. 1977. The plant-animal interface in freshwater ecosystems. **American Scientist 65**: 159-170.

Power, M.E. 1990. Resource enhancement by indirect effects of grazers: armored catfish, algae, and sediment. **Ecology 71**(13): 897-904.

Power, M.E., W.J. Matthews, A.J. Stewart. 1985. Grazing minnows, piscivorous bass, and stream algae: dynamics of a strong interaction. **Ecology 66**(5):1448-1456.

Rosati, T. C., J. R. Johansen, M. M. Coburn. 2003. Cyprinid fishes as samplers of benthic diatom communities in freshwater streams of varying water quality. **Can. J. Fish. Aquat. Sci. 60** (2): 117-125.

Sellman, S.M., Johansen, J.R., and Coburn, M.M. 2001. Using fish to sample diatom composition in streams: are gut contents representative of natural substrates? **In: 16th International Diatom Symposium, 25 August-1 September 2000. Athens and the Aegean Islands**. Edited by A. Economou-Amilli. University of Athens, Athens Greece. pp. 529-536.

Simonsen, R. 1987a. **Atlas and Catalogue of the Diatom Types of Friedrich Hustedt** Volume 1, Catalogue, 525 pp.

Simonsen, R. 1987b. **Atlas and Catalogue Types of the Diatom Types of Friedrich Hustedt** Volume 2, Atlas, Plates 1-395.

Simonsen, R. 1987c. **Atlas and Catalogue of the Diatom Types of Friedrich Hustedt** Volume 3, Atlas, Plates 396-772.

Stevenson R.J. 1984. Procedures for mounting algae in syrup medium. **Transactions of the American Microscopical Society 103**: 320-321.

Velasquez, G.F. 1939. On the viability of Algae obtained from the digestive tract of the gizzard shad **Dorosoma Cepedianum** (le Sueur). **American Midland Naturalist 22**: 376-412.

APPENDIX A

The Percent Relative Density of Living and Dead Specimens for Each Diatom Species Found at the Sites and in the Fish

Table A. The percent relative density of living and dead specimens for each diatom species found at the sites and in the fish.

DIATOM SPECIES	SITES		FISH	
	LIVE	DEAD	LIVE	DEAD
Achnanthes	0.00	0.05	0.02	0.15
Achnanthes clevei	0.04	0.01	0.01	0.01
Achnanthes deflexa	0.28	0.32	0.04	0.35
Achnanthes laevissima	0.00	0.00	0.00	0.00
Achnanthes lanceolata	0.04	0.10	0.02	0.13
Achnanthes lanceolata var. dubia	0.00	0.03	0.00	0.00
Achnanthes linearis	12.38	13.75	2.53	10.44
Achnanthes linearis f. curta	0.00	0.03	0.00	0.03
Achnanthes microcephala	0.07	0.03	0.01	0.01
Achnanthes minutissima	6.62	9.26	1.42	8.15
Achnanthes orientalis	0.00	0.03	0.00	0.01
Achnanthes pinnata var. japonica	0.00	0.02	0.01	0.00
Amphipleura pellucida	0.39	0.23	0.03	0.13
Amphora	0.01	0.07	0.01	0.17
Amphora bullatoides	0.00	0.02	0.00	0.08
Amphora libyca	0.00	0.00	0.03	0.01
Amphora montana	0.00	0.00	0.00	0.00
Amphora pediculus	0.01	0.02	0.03	0.09
Amphora veneta	0.00	0.00	0.02	0.01
Anomoeneis	0.00	0.00	0.00	0.00
Anomoeneis vitrea	0.06	0.06	0.07	0.20
Aulacosiera ambigua	0.00	0.00	0.02	0.12
Aulacosiera italica	0.00	0.00	0.00	0.00
Bacillaria paradoxa	0.01	0.00	0.00	0.04
Caloneis bacillum	0.04	0.01	0.03	0.00
Centric	0.06	0.01	0.03	0.05
Cocconeis	0.00	0.04	0.05	0.25
Cocconeis pediculus	0.02	0.04	0.03	0.02
Cocconeis placentula	0.00	0.00	0.00	0.07
Cocconeis placentula var. euglypta	0.04	0.24	0.03	0.51
Cocconeis placentula var. lineata	0.01	0.08	0.02	0.06
Cocconeis placentula var. placentula	0.02	0.05	0.03	0.20
Cocconeis placentula raphe valve	0.00	0.00	0.00	0.01
Cocconeis scutellum	0.00	0.05	0.00	0.36
Cyclostephanos dubius	0.00	0.00	0.00	0.04
Cyclostephanos invisitatus	0.00	0.00	0.01	0.06

Table A. Continued.

DIATOM SPECIES	SITES LIVE	SITES DEAD	FISH LIVE	FISH DEAD
Cyclotella	0.00	0.00	0.00	0.00
Cyclotella cryptica	0.00	0.00	0.01	0.01
Cyclotella kuetzingiana	0.02	0.00	0.04	0.13
Cyclotella meneghiniana	2.17	0.27	2.12	1.96
Cymbella	1.14	0.66	2.16	2.14
Cymbella affinis	1.63	2.47	2.11	3.34
Cymbella aspera	0.02	0.00	0.00	0.00
Cymbella brehmii	0.00	0.00	0.04	0.03
Cymbella caespitosa	1.36	0.62	3.04	0.65
Cymbella delicatula	0.53	0.47	0.31	0.46
Cymbella elginensis	0.02	0.00	0.06	0.02
Cymbella hustedtii	0.02	0.00	0.00	0.00
Cymbella microcephala	1.16	1.18	0.97	2.07
Cymbella minuta	0.00	0.00	0.01	0.00
Cymbella naviculaformis	0.00	0.02	0.02	0.00
Cymbella prostrata	0.02	0.01	0.02	0.04
Cymbella silesiaca	0.22	0.14	1.01	0.42
Cymbella triangulum	0.04	0.02	0.07	0.04
Cymbella tumida	0.06	0.10	0.20	0.14
Cymbella turgidula	0.01	0.04	0.04	0.10
Cymatopleura solea	0.00	0.00	0.00	0.00
Denticula kuetzingiana	3.02	2.89	1.54	3.39
Diatom tenue var. elongatum	0.00	0.00	0.00	0.01
Diatom tenue var. elongata	0.00	0.00	0.00	0.01
Diatom vulgare	0.60	0.01	0.09	0.16
Diploneis	0.00	0.01	0.01	0.02
Diploneis elliptica	0.00	0.00	0.00	0.00
Diploneis oblongella	0.00	0.07	0.01	0.01
Diploneis parma	0.01	0.00	0.01	0.00
Epithemia adnata	0.00	0.00	0.00	0.00
Epithemia turgida	0.00	0.00	0.00	0.01
Eunotia	0.00	0.00	0.01	0.00
Eunotia denticulata	0.00	0.00	0.01	0.01
Eunotia exigua	0.00	0.02	0.01	0.03
Eunotia major	0.00	0.01	0.00	0.01
Eunotia musicola	0.00	0.00	0.00	0.00
Eunotia sudetica	0.00	0.01	0.00	0.00
Fragilaria	0.00	0.00	0.01	0.00

Table A. Continued.

DIATOM SPECIES	SITES LIVE	SITES DEAD	FISH LIVE	FISH DEAD
Fragilaria capucina	0.09	0.16	0.09	0.14
Fragilaria capucina var. lanceolata	0.00	0.00	0.02	0.03
Fragilaria capucina var. vaucheriae	0.00	0.00	0.05	0.04
Fragilaria exigua	0.00	0.00	0.00	0.01
Fragilaria vaucheriae	0.85	0.25	0.52	0.62
Frustulia rhomboides	0.00	0.00	0.00	0.00
Frustulia rhomboides var. crassinervia	0.00	0.00	0.02	0.00
Frustulia vulgaris	0.00	0.00	0.41	0.22
Gomphonema	0.24	0.29	0.19	0.64
Gomphonema acimatum	0.00	0.00	0.01	0.00
Gomphonema augur	0.01	0.00	0.00	0.00
Gomphonema brasiliense	0.00	0.00	0.00	0.00
Gomphonema dichotomum	0.00	0.02	0.01	0.08
Gomphonema elavatum	0.00	0.00	0.00	0.00
Gomphonema intracatum	0.07	0.19	0.11	0.24
Gomphonema olivaceum	0.00	0.05	0.00	0.02
Gomphonema olivaceum var. minutissma Hustedt	0.00	0.00	0.00	0.01
Gomphonema parvulum	0.08	0.05	0.09	0.08
Gomphonema simus	0.02	0.03	0.00	0.00
Gomphonema spaeorophorum	0.04	0.12	0.19	0.24
Gomphonema truncatum	0.01	0.04	0.07	0.10
Gomphonema truncatum var. capitata	0.00	0.00	0.00	0.01
Gyrosigma acuminatum	0.00	0.00	0.01	0.00
Gyrosigma attenuatum	0.00	0.00	0.01	0.00
Gyrosigma scalproides	0.02	0.00	0.04	0.08
Gyrosigma spencerii	0.00	0.01	0.09	0.01
Melosira granulata f. alpha	0.00	0.00	0.04	0.04
Melosira granulata f. angustissima	0.00	0.00	0.03	0.04
Melosira varians	0.05	0.03	3.54	1.14
Meridian circulare	0.00	0.01	0.01	0.01
Navicula	0.25	0.21	0.47	0.49
Navicula agrestis	0.00	0.00	0.01	0.00
Navicula accomoda	0.00	0.00	0.02	0.00
Navicula capitata	0.02	0.02	0.03	0.03
Navicula capitatoradiata	1.28	0.31	1.55	0.89
Navicula cryptocephala	0.25	0.07	0.22	0.16
Navicula cryptotenella	0.10	0.08	0.09	0.13
Navicula elginensis	0.00	0.00	0.01	0.02

Table A. Continued.

DIATOM SPECIES	SITES LIVE	SITES DEAD	FISH LIVE	FISH DEAD
Navicula exilis	0.00	0.00	0.02	0.01
Navicula gregaria	0.31	0.20	0.25	0.34
Navicula heimansii	0.00	0.00	0.00	0.01
Navicula insociabilis	0.00	0.00	0.00	0.00
Navicula lanceolata	0.08	0.23	0.02	0.15
Navicula menisculus	0.04	0.04	0.06	0.06
Navicula menisculus var. upsaliensis	0.32	0.21	0.26	0.27
Navicula minima	0.00	0.00	0.00	0.00
Navicula mutica	0.00	0.02	0.00	0.00
Navicula notha	0.02	0.04	0.00	0.01
Navicula phyletta	0.00	0.00	0.05	0.03
Navicula pupula	0.00	0.00	0.07	0.03
Navicula pupula var. pupula	0.00	0.00	0.02	0.00
Navicula radiosa	0.00	0.00	0.00	0.01
Navicula schroeteri	0.06	0.06	0.01	0.06
Navicula seminulum	0.00	0.00	0.00	0.00
Navicula subminiscula	0.00	0.02	0.01	0.02
Navicula tenelloides	0.00	0.00	0.02	0.00
Navicula tenera	0.01	0.00	0.00	0.01
Navicula tripuncta	0.00	0.00	0.00	0.01
Navicula trivialis	0.02	0.01	0.01	0.01
Navicula veneta	1.69	0.46	0.85	0.80
Navicula viridula var. linearis	0.02	0.04	0.00	0.02
Nitzschia	4.71	1.60	1.61	1.91
Nitzschia acicularis	0.06	0.00	0.00	0.00
Nitzschia agnita	0.00	0.00	0.02	0.00
Nitzschia amphibia	0.00	0.03	0.01	0.00
Nitzschia angustatula	0.00	0.01	0.00	0.02
Nitzschia angustaforaminata	0.00	0.00	0.01	0.01
Nitzschia brevissima	0.00	0.00	0.01	0.00
Nitzschia capitellata	0.11	0.02	0.03	0.04
Nitzschia clausii	0.06	0.05	0.05	0.18
Nitzschia constricta	0.04	0.03	0.00	0.04
Nitzschia dibilis	0.00	0.00	0.01	0.00
Nitzschia dissipata	0.77	0.49	0.65	0.85
Nitzschia filiformis	0.00	0.01	0.00	0.00
Nitzschia fonticola	0.05	0.08	0.05	0.03
Nitzschia hantzschiana	0.00	0.03	0.01	0.01

Table A. Continued.

DIATOM SPECIES	SITES LIVE	SITES DEAD	FISH LIVE	FISH DEAD
Nitzschia hungarica	0.02	0.00	0.01	0.00
Nitzschia inconspicua	0.24	0.48	0.08	0.34
Nitzschia intermedia	0.06	0.00	0.00	0.00
Nitzschia levidensis var. victoriae	0.00	0.00	0.01	0.00
Nitzschia linearis	0.01	0.00	0.00	0.01
Nitzschia linearis var. tenuis	0.02	0.00	0.00	0.02
Nitzschia microcephala	0.23	0.49	0.26	0.37
Nitzschia minuta	0.37	0.10	0.05	0.04
Nitzschia nana	0.11	0.12	0.04	0.06
Nitzschia nereidis	0.04	0.02	0.11	0.06
Nitzschia palea	0.59	0.21	0.49	0.20
Nitzschia palea var. capitellata	0.00	0.00	0.00	0.01
Nitzschia palea var. tenuirostris	0.23	0.08	0.01	0.00
Nitzschia paleacea	0.04	0.03	0.02	0.01
Nitzshia pusilla	0.00	0.00	0.00	0.01
Nitzschia rautenbachiae	0.00	0.00	0.00	0.04
Nitzschia recta	0.01	0.01	0.00	0.07
Nitzschia sinuata	0.01	0.00	0.04	0.15
Nitzschia sinuata var. tabellaria	2.05	2.68	2.90	4.73
Nitzschia sociabilus	0.00	0.00	0.00	0.01
Nitzschia subacicularis	0.00	0.00	0.02	0.00
Nitzschia supralitorea	0.07	0.14	0.14	0.11
Nitzschia cf. umbonata	0.00	0.00	0.00	0.01
Pennate	4.66	2.24	3.80	4.67
Pinularia	0.00	0.00	0.03	0.03
Pinnularia acoricola	0.02	0.17	0.06	0.00
Pinnularia ignobilis	0.00	0.00	0.01	0.00
Pinnularia obscura	0.00	0.00	0.01	0.01
Pinnularia viridis	0.02	0.00	0.01	0.00
Reimeria sinuata	0.08	0.69	0.04	0.63
Rhoicosphenia curvata	0.07	0.20	0.14	0.72
Rhopalodia gibba	0.02	0.01	0.00	0.00
Rhopalodia operculata	0.00	0.00	0.00	0.00
Stauroneis agrestis	0.00	0.00	0.00	0.02
Surirella	0.00	0.00	0.00	0.02
Surirella amphioxys	0.02	0.00	0.01	0.02
Surirella angusta	0.02	0.01	0.03	0.03
Surirella brebessonii	0.00	0.00	0.00	0.00

Table A. Continued.

DIATOM SPECIES	SITES LIVE	SITES DEAD	FISH LIVE	FISH DEAD
Surirella brebesonnii var. kuetzingii	0.02	0.02	0.00	0.01
Surilla minuta	0.01	0.00	0.01	0.02
Surirella tenera	0.00	0.00	0.00	0.02
Synedra	0.23	0.08	0.17	0.16
Synedra acus	0.02	0.00	0.00	0.00
Synedra acrus	0.00	0.00	0.00	0.00
Synedra faciculata	0.00	0.00	0.01	0.00
Synedra miniscula	0.16	0.28	0.12	0.23
Synedra pulchella	0.01	0.03	0.07	0.07
Synedra tenera	0.04	0.05	0.07	0.01
Synedra cf. tenera	0.34	0.09	0.04	0.06
Synedra ulna	0.04	0.02	0.04	0.04
Synedra ulna var. contracta	0.05	0.07	0.01	0.10