Population and Community Changes of Attached-algae to Zinc Stress Alone and in Combination with Selected Environmental Variables

by

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(ABSTRACT)

Four experiments were performed to test the feasibility of using taxonomic composition and abundance of attached algae to identify treatments of zinc (Zn) alone and in combination with treatments of phosphate, snail grazing, and pH.

In the experiment presented in chapter 2, three treatments of zinc (0.05, 0.5, 1.0 mg $Zn \bullet l^{-1}$) and a control could be identified by different algal communities in outdoor, flow-through, stream mesocosms. Established communities were continuously exposed to Zn, and samples were collected on days 0, 2, 5, 10, 20, and 30 after treatment began. Experiments were conducted in spring, summer, and fall 1984. Control stream mesocosms could be identified by diatoms in all seasons. The 0.05 mg $Zn \bullet l^{-1}$ treatment could by identified by certain diatom taxa being more abundant than in the control in all seasons and by a filamentous green-alga in summer and fall. The 0.5 mg $Zn \bullet l^{-1}$ treatment could be identified a filamentous green alga in fall. The 1.0 mg $Zn \bullet l^{-1}$ treatment could be identified by unicellular green-algae in all seasons and by a filamentous blue-green alga in summer. A similarity index (SIMI) indicated that Zn stressed samples generally became less similar to control samples as Zn concentration increased from 0.05 to 1.0 mg $Zn \bullet l^{-1}$. Total biovolume-density of all taxa responded more slowly than did individual taxa in spring and failed to distinguish between Zn treatments in summer and fall. Zn bound to periphyton (microbial community on solid substrates) was more reliable than total Zn in water for identifying Zn treatments. Zn treatments as low as 0.05 mg $Zn \bullet l^{-1}$ changed algal species composition. This conflicts with the criterion (0.047 mg $Zn \bullet l^{-1}$) of the U. S. Environmental Protection Agency for the 24-hour average of total recoverable Zn.

In the experiment presented in chapter 3, individual and combined effects of phosphate (P) and zinc (Zn) on the abundance of dominant algae and protozoa in a community were observed. Nutrient-diffusing artificial substrates were colonized in Douglas Lake, Michigan, and then placed in laboratory microcosms containing one of five Zn treatments (control, 0.1, 1.0, 3.0, and 10.0 mg $Zn \bullet l^{-1}$). After one week of exposure in the laboratory the substrates were scraped and algal and ciliated protozoan abundances determined. Ten of thirteen algae and five of eight ciliated protozoa responded to experimental treatments. Some algae (diatoms and green algae) and ciliated protozoa were stimulated by high P, some stimulated by intermediate P, and some inhibited by high P. One alga and four protozoa responded positively to Zn. Two algae and three protozoa responded to a significant interaction between P and Zn so that abundances were from 3 to 19 times higher than the added effects of individual P and Zn treatments. Total algal abundance was increased by high P and total protozoan abundance was increased by intermediate P but at control levels for high P. The number of protozoan species was increased by P. Total algal abundance was increased by combinations of Zn P and the number of protozoan species was decreased by Zn P. Altered abundance by combinations of Zn and P had not been demonstrated for a community of algae and protozoa previously. Although concentrations of Zn were initially above the level considered safe by the U.S. Environmental Protection Agency, many factors may prevent Zn stress.

In the experiment presented in chapter 4, effects of 0.5 mg $Zn \bullet l^{-1}$ and snail grazing (400 snails m^{-2}) on density of dominant algal taxa were examined using established (12-day colonization) periphyton communities in flow-through stream mesocosms with four treatments (Zn, snails, Zn and snails, control) for 30 days. Grazing and Zn similarly reduced the abundance of 5 of 10 dominant taxa during the first 10 days of treatment. Temperature may play a very important role in determining the effect of snail grazing on attached algal communities. Cold temperatures ($<$ 15 C) may have inhibited snail grazing to the extent that abundance of four taxa increased to levels found in non-snail treatments. However, one diatom was more than twice as abundant in snail treatment over non-snail treatment -- apparently stimulated by the presence of snails during cold conditions; and two diaoms remained at low abundance in snail treatment despite rapid growth in non-snail treatment -- apparently inhibited or selected as a food source by snails during cold con-

ditions. No algal taxa replaced the diatoms inhibited by 0.5 mg $Zn \bullet l^{-1}$ in this October-November, 1984, experiment by day 10. This is in contrast to an experiment performed one month earlier, in September-October, in which a community characteristic of this treatment developed by day 5. Testing individual and combined variables that affect attached algal communities will enhance understanding of population dynamics in algal ecology and pollutant assessment.

In the experiment presented in chapter 5, attached-algal communities were employed to test the US Environmental Protection Agency's (USEPA) guidelines for zinc (Zn) and pH. The experiment was designed to determine whether algal community composition and abundance would be altered by (a) ph 6 or 9, (b) 0.05 mg $Zn \bullet l^{-1}$, or (c) the combination of ph 6 or 9 and 0.05 mg $Zn \bullet l^{-1}$. Stream mesocosms were continuously supplied with natural water from the New River, VA, USA. Established (12-day colonization) communities on artificial substrates were sampled on days 0, 5, 10, 20, and 30 after treatment began on 9 July 1985. Zinc and pH treatments changed algal community composition from diatoms and a filamentous glue-green alga to different diatom taxa, green algae, or a coccoid blue-green alga. Total algal abundance was moderately increased by pH 6 treatment. Treatments of pH 6 and 0.05 mg $Zn \bullet l^{-1}$ significantly altered attached-algal community composition even though these levels are considered "safe" by the USEPA. The pH 9 treatment did not significantly alter community composition, most likely because ambient pH was near this level.

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1.0 Prologue

Attached algae provide a community of interacting organisms of which some are sensitive, some are resistant, and some intermediate in tolerance to stress. These individual tolerances may provide a yardstick with which to identify the intensity and potential damage from anthropogenic and natural disturbances.

Single species tests have been very useful for determining the effects of poisonous compounds within organisms (Subcommittee on Zinc, 1979). However, single species tests have been inadequate for predicting the effect of poisonous compounds on population interactions and community structure (National Research Council, 1981). In addition, these tests are usually run under controlled laboratory conditions so that any change in toxicity (synergism or inhibition) caused by the environment remains undetermined. Despite these facts, data on the response of a community to poisonous compounds are distressingly scarce for the importance of this problem.

Communities of attached algae are ideal for analyzing population and community responses because competition, predation, and environmental variables all affect the abundance of algae (Round, 1981). This attached group of organisms must react to a poison because algae do not have the avoidance capability of fish and other higher organisms. Algal growth rates are rapid so that effects of poisons can be tested over several generations in a short time period (Eppley, 1977). These unicellular organisms are in intimate contact with their environment so that a rapid response

to stress usually occurs (Cairns, 1982). Diatoms, which comprise a major portion of the natural communities in this dissertation, respond to similar concentrations of poisons as fish and invertebrates (Patrick *et al.*, 1968). Attached algae are easily collected, processed, enumerated, and analyzed. Significant effects at this level of the food chain could profoundly affect higher trophic levels (Patrick, 1978).

Zinc (Zn) is an essential nutrient for all forms of life, but excessive amounts of Zn in the aquatic environment are of particular concern (Subcommittee on Zinc, 1979). The response of attached algal communities to Zn will be influenced by biological, chemical, and physical characteristics of the environment. The goal of this research was to determine if the abundance of attached algal taxa would be useful for distinguishing between different treatments of Zn (Chapter 2) and for distinguishing Zn stress from other chemical or biological stress. To do this, natural communities of attached algae on artificial substrates were exposed to Zn stress alone and in combination with nutrient enrichment of phosphate (Chapter 3), grazing by snails (Chapter 4), or extreme pH (Chapter 5).

2.0 Algal-periphyton population and community changes from zinc stress in stream mesocosms

2.1 Introduction

Simulated habitats are reliable systems for testing safe concentrations of hazardous substances so long as these systems are environmentally realistic and include many interacting species (Cairns, 1981; National Research Council, 1981). Odum (1984) suggests that confined experimental systems that are continuous with the natural environment be referred to as mesocosms. Periphyton (microbial community associated with a substrate) form a community of interacting organisms; some are sensitive, some are resistant, and some are intermediate in tolerance to stress. These individual tolerances may provide a yardstick for identifying the intensity and potential damage from anthropogenic wastes.

A general survey of surface waters in the United States showed that the mean concentration of total recoverable zinc (Zn) was approximately 0.064 mg l^{-1} with a maximum level of 1.183 mg $Zn \bullet l^{-1}$ (Subcommittee on Zinc, 1979). This mean level is higher than the criterion of the U. S. Environmental Protection Agency (USEPA) for the 24-h average of total recoverable Zn (0.047

mg $Zn \bullet l^{-1}$). The criterion may be adjusted slightly to account for water hardness (Environmental Criteria and Assessment Office, 1980). The Zn concentrations used in this experiment were set near the USEPA criterion and near the maximum measured in U.S. waterways.

Say *et al.* (1977a) indicated that algae may be much more sensitive in field than in laboratory exposures to Zn. Williams & Mount (1965) found that the number of algal species decreased and the density of fungi increased as Zn increased from 1 to 9 mg l^{-1} in periphyton communities. Comparison of other methods for testing algal communities can be made from Taub (1974), Harding & Whitton (1976), Claesson (1984), Gilfillan *et al.* (1984), Pritchard & Bourquin (1984), and Kimball & Levin (1985).

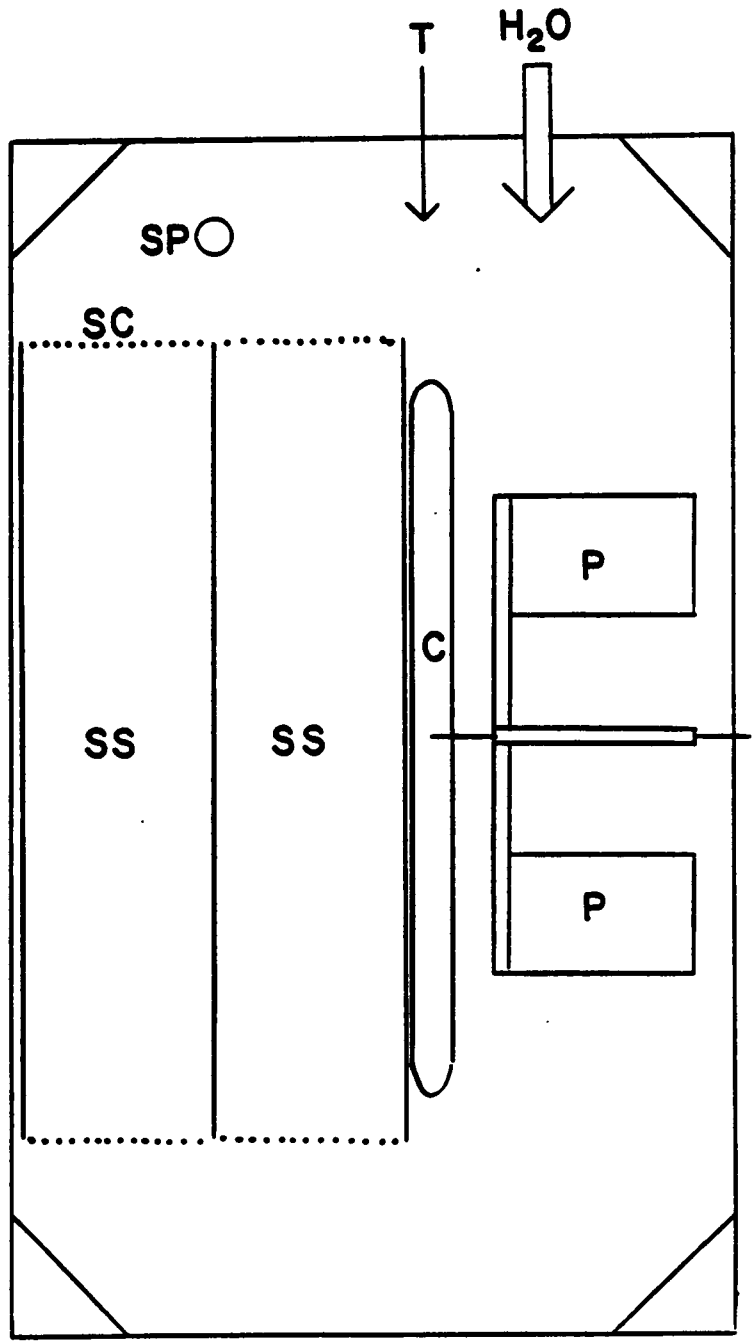
Algal-periphyton were monitored at population and community levels of biological organization to chronic (30 d) Zn exposures in outdoor, flow-through stream mesocosms continuously replenished with natural river water. Population level analysis tested differences in biovolume-density of individual taxa to identify stress. Community level analyses used differences in total biovolume-density and a similarity index that compared overall taxonomic composition to identify stress. These variables were observed during spring, summer, and fall seasons. The spring experiment also tested individual and combined effects of Zn and snail grazing on periphyton.

2.2 Materials and methods

2.2.1 Mesocosms

The three experiments occurred along the New River at the Glen Lyn Plant of the Appalachian Power Company in Giles County, Virginia. Stream mesocosms (Fig. 2-1) were constructed from plywood and painted with a non-toxic chemical resistant paint (Farris, 1986). Plastic paddle wheels maintained a current of approximately 14 cm sec^{-1}. Natural river water was con-

Figure 2-1. Top view of one stream mesocosm with two sub-streams (ss), central longitudinal wall (c) that divides the stream into an oval course, paddle wheel (p) that causes the stream to flow clockwise in the picture, overflow stand pipe (sp), tubing from which Zn drips, and the faucet (H_2O) controls flow rate of natural river water. The sub-streams have a screen (sc) at the entrance and exit to prevent snail passage. The dimensions are: each sub-stream is 11.5 x 49 cm, paddle wheel channel is 18.4 cm wide, and the stream is 76.2 cm long.


T
H_2O
SP
SC
SS
SS
C
P
P

tinuously supplied at 1.2 l min^{-1} for each mesocosm by a pump submersed in the New River. Peristaltic pumps delivered concentrated Zn solutions (as $ZnSO_4$) from separate carboys for each mesocosm. Three replicate mesocosms were used for each Zn treatment. Three treatments (control, 0.05, 1.0 mg $Zn \bullet l^{-1}$) were used in spring (28 April to 28 May) and summer (23 June to 23 July) of 1984 for a total of 9 mesocosms, and a 0.5 mg $Zn \bullet l^{-1}$ treatment was added in fall (7 September to 7 October) of 1984 for a total of 12 mesocosms. Periphyton communities colonized stream mesocosms for 16 d before Zn exposure began in spring and for 10 d in summer and fall. Sampling occurred when Zn exposures began (day 0) and on days 2, 5, 10, 20, and 30.

2.2.2 Artificial substrates

At least three randomly selected artificial substrates were pooled to form one sample per mesocosm. Unglazed hexagonal tiles (5.85 cm^2) covered the floor of each chamber and were used as an artificial substrate for periphyton in spring. The unglazed tiles were replaced with vertical glass rods (5.38 cm^2) during summer and fall experiments because detrital accumulation (approx. 0.25 cm) on the tiles made enumeration difficult.

2.2.3 Algae sampling, processing, & enumeration

Samples were collected from mesocosms on all sampling days. Artificial substrates were scraped with a razor blade and rinsed with distilled water. The resulting sample was homogenized for 10 s in a blender to shorten algal filaments. An 18-ml sample was drawn from this slurry and placed in a vial containing 2 ml of 37% formaldehyde. Algae that were alive (containing chloroplasts) at the time of collection were enumerated using a Palmer-Maloney plankton counting chamber under 400x total magnification. Diatom identifications were confirmed by examining cleaned specimens at 1000x total magnification (Cleve-Euler, 1951-1955; Hustedt, 1930; Patrick &

Reimer, 1966, 1975). Counting proceeded by making one pass through the longest axis of the counting chamber and stopping when the 500th organism was counted. If less than 500 organisms were observed then another sample was prepared and counted in the same manner. Dense samples of algae were diluted (to 1/2, 1/4, or 1/8) by adding distilled water to 5 drops of sample.

2.2.4 Biovolume-density

Biovolume was estimated using mensuration formulas that approximate the geometric shape of each taxon (Beyer, 1981). Direct measurements were made of dominant taxa and median dimensions from the literature were used for less abundant taxa (Prescott, 1962; Patrick & Reimer, 1966, 1975). Algal density per square centimeter was estimated using the standard methods (APHA, 1981). This resulting biovolume-density allows easy comparison of dominance between different taxa. Total biovolume-density was computed by summing over all taxa observed in a sample.

2.2.5 Snail grazing

Periphyton response to Zn stress and snail grazing was examined in spring. A split-split plot design was used with two side-by-side screen chambers in each mesocosm (Fig. 2-1). One chamber contained 10 snails and the other chamber contained none. The chambers were discontinued in summer and fall experiments because screens clogged with periphyton so that current speeds were reduced; however, snails were still placed in the mesocosms. The number of snails per mesocosm was 80 in summer and 120 in fall.

2.2.6 Water chemistry

Alkalinity, hardness, pH, temperature, and total Zn were determined for each mesocosm on all sampling days (APHA, 1981). Samples were collected underwater in plastic containers, refrigerated, and analyzed within 2 d. Total Zn was analyzed using the direct aspiration method for atomic absorption spectrometry (USEPA, 1979).

2.2.7 Zinc concentration of periphyton

Periphyton samples were processed for Zn analysis by obtaining the dry weight from 5.0 ml of slurry filtered on to a 0.45 μm metricel membrane filter (Valdes *et al.* 1982). This sample was placed overnight in a 20 ml vial with 3.0 ml of instra-analyzed nitric acid and then heated for 0.5 h at a temperature just below boiling. After cooling, 200 μl of 30% hydrogen peroxide were added and the sample was heated for 10 min. This solution was cooled and diluted to 20 ml with deionized distilled water. The Zn concentration was measured using the direct aspiration method for atomic absorption spectrometry (USEPA, 1979).

2.2.8 Statistics

Statistical analysis was performed on taxa with average relative abundance greater than 1% over all samples. The spring experiment was set up as a split-split plot design without replicated groups, and summer and fall experiments were set up as split plot designs without replicated groups (Milliken & Johnson, 1984). The spring design provided testing for effects of Zn alone, snails alone, and an interaction between Zn and snails. Duncan's New Multiple Range Test determined if biovolume-density differed among treatments. Zn bound to periphyton was $\log_{10}$ transformed

before statistical analysis because means were as much as two orders of magnitude apart so that variances were correlated with means (Sokal & Rohlf, 1981). The statistical level of significance is 0.05.

A randomization procedure was used to test for differences in SIMI between treatments because dependence between similarity scores makes standard statistical procedures inappropriate. Since there is no standard reference for this application of randomization procedures, this method is included in Appendix A.

2.3 Results

Treatments as low as 0.05 mg $Zn \bullet l^{-1}$ significantly changed taxonomic composition in mesocosms for each season (Table 2-1; Fig. 2-2). Diatoms dominated the control treatment in all three seasons. Filamentous green-algae had increased relative abundance in 0.05 and 0.5 mg $Zn \bullet l^{-1}$ treatments in summer and fall, and diatoms different than those diatoms in the control increased in relative abundance in 0.05 mg $Zn \bullet l^{-1}$ in spring and summer. Unicellular green-algae increased in relative abundance in 1.0 mg $Zn \bullet l^{-1}$ in all seasons, and a filamentous blue-green alga increased in relative abundance in 1.0 mg $Zn \bullet l^{-1}$ in summer. From this point on, taxa will be referred to as "identifying" a treatment if their biovolume-density in that treatment was significantly higher than in other treatments. Water quality analysis indicated that Zn did not significantly alter alkalinity, hardness, or pH (Table 2-2).

2.3.1 Spring

Zn treatments changed community composition from diatoms to green algae and different diatom taxa (Table 2-1, Fig. 2-2). The most substantial differences between treatments occurred

Table 2-1. Results of Duncan's Multiple Range Test between treatments for the dominant taxa during three seasons. Underlined means are not significantly different at the 0.05 level of significance. Treatments with higher means are on the left, and those with lower means are on the right. ACHNANTH = *Achnanthes* spp., COPLAC = *Cocconeis placentula*, CYMINUSI = *Cymbella minuta* var. *silesiaca*, FRAGILAR = *Fragilaria* sp., FRRUMPSC = *Fragilaria rumpens* var. *scotica*, GOMPHONE = *Gomphonema* spp., MEVARI= *Melosira varians*, NACRYPIN = *Navicula cryptocephala* var. *intermedia*, NAVICULA = naviculoid diatoms, NILINE = *Nitzschia linearis*, NISP1 = *Nitzschia* sp. 1, NISP2 = *Nitzschia* sp. 2, NITZSCHIA = *Nitzschia* spp., OOELLI = *Oocystis elliptica*, COCCOID = coccoid green algae, SCENEDES = *Scenedesmus* spp., SURIRELL = *Surirella* spp., PHORMIDI = *Phormidium* sp., ULVARI = *Ulothrix variabilis*.

A) Spring, 1984

TAXON	DAY 0	2	5	10	20	30
ACHNANTH						
FRAGILAR						
NAVICULA			C .05 1.0	C>.05 1.0	C>.05 1.0	C>.05 1.0
NILINE				C>.05>1.0	C>.05 1.0	C>.05 1.0
NISP1				C .05>1.0	C .05 1.0	C .05>1.0
SURIRELL				C .05 1.0		
NISP2				1.0>.05 C		
ULVARI					C>.05 1.0	
MEVARI					C>.05 1.0	C>.05 1.0
CYMINUSI					.05>C 1.0	.05>C 1.0
COCCOID						1.0>.05 C
TOTAL BIOVOLUME					C>.05>1.0	C .05 1.0

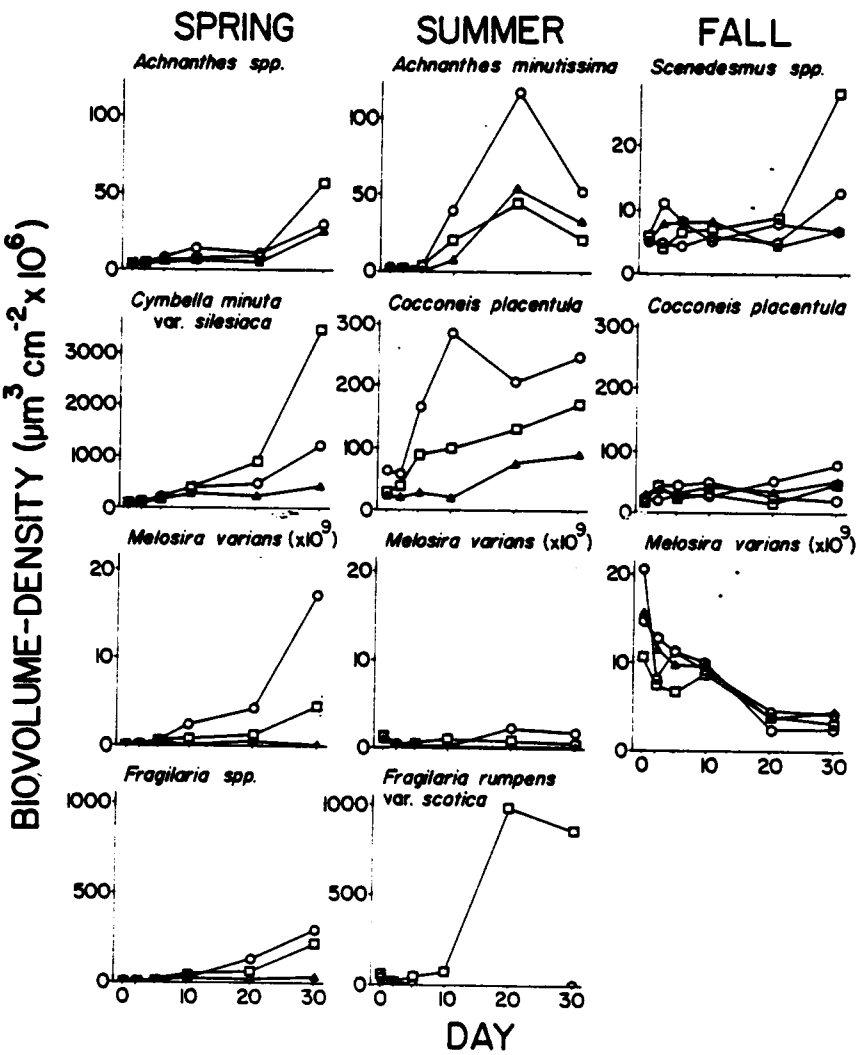
B) Summer, 1984

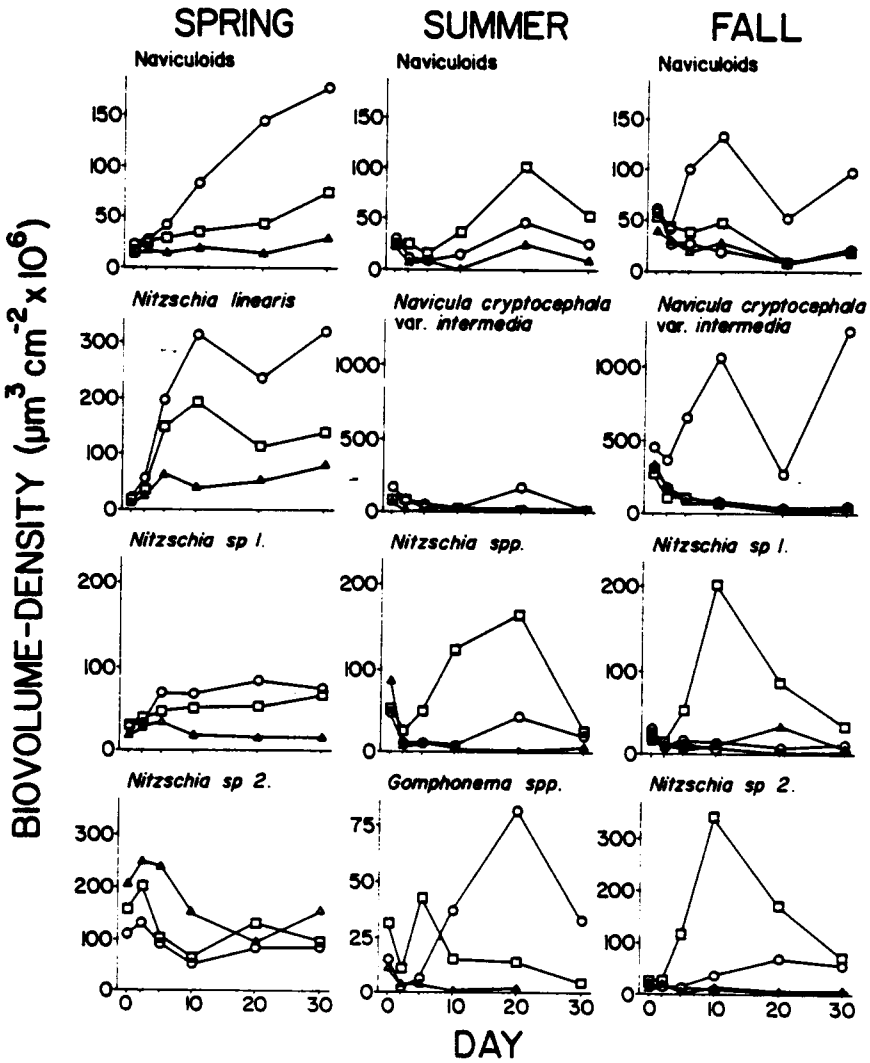
TAXON	DAY 0	2	5	10	20	30
ACHNANTH						
FRRUMPSC						
PHORMIDI		.05 C>1.0				1.0>.05 C
COPLAC	C .05 1.0		C>.05 1.0	C>.05>1.0		
NACRYPIN			C>.05 1.0		C>.05 1.0	
ULVARI			.05 C 1.0		.05>1.0 C	.05>1.0 C
COCCOID			1.0>.05 C	1.0>.05 C	1.0>.05 C	
NAVICULA				.05 C 1.0	.05>C 1.0	.05 C 1.0
GOMPHONE					C>.05 1.0	
NITZSCHI					.05>C 1.0	.05 C 1.0
MEVARI						C .05 1.0
OOELLI						1.0>.05 C
TOTAL BIOVOLUME						

C) Fall, 1984

TAXON	DAY 0	2	5	10	20	30
COPLAC					C 1.0 .5 .05	C 1.0 .05 .5
MEVARI	C 1.0 .5 .05					
NACRYPIN		C .5 1.0 .05	C >.5 .05 1.0	C >1.0 .05 .5	C 1.0 .5 .05	C >1.0 .5 .05
NAVICULA			C >.05 .5 1.0	C >.05 1.0 .5	C >.5 .05 1.0	C >1.0 .05 .5
NISP1	C .05 .5 1.0		.05 C .5 1.0	.05> C 1.0 .5	.05> C 1.0 .5	.05> C 1.0 .5
NISP2			.05 >C .5 1.0	.05> C 1.0 .5	.05> C >1.0 .5	.05 C >1.0 .5
OOELLI		1.0>.05 .5 C			1.0>.5 C .05	1.0>.5 .05 C
COCCOID	.05 C .5 1.0		.5 .05 1.0 C	.5 1.0 .05 C	1.0>.5 .05 C	1.0>.5 .05 C
SCENEDES		.5 1.0 C .05				
PHORMIDI	.05 C 1.0 .5	.05 C .5 1.0	C> .05 .5 1.0	.05 C .5 1.0	.5 >1.0 C .05	
ULVARI			.5 .05 1.0 C	.5 .05>1.0 C	.5 >.05 1.0 C	.5 .05>1.0 C
TOTAL BIOVOLUME	C 1.0 .5 .05					

Figure 2-2. Mean biovolume-density (n = 3) of dominant algae on sampling days in three seasons. Spring data are the mean of snail and nonsnail treatments. All taxa have biovolume-density ($\mu m^3\ cm^{-2}$) x 10^6 except for *Melosira varians* that is x 10^9. Zinc treatments are Control ○, 0.05 mg $Zn \bullet l^{-1}$ □, 0.5 mg $Zn \bullet l^{-1}$ ⬡, and 1.0 mg $Zn \bullet l^{-1}$ △.


SPRING
SUMMER
FALL
Achnanthes spp.
Achnanthes minutissima
Scenedesmus spp.
Cymbella minuta var. silesiaca
Cocconeis placentula
Cocconeis placentula
Melosira varians (x10^9)
Melosira varians (x10^9)
Melosira varians (x10^9)
Fragilaria spp.
Fragilaria rumpens var. scotica
BIOVOLUME-DENSITY (μm^3 cm^-2 x 10^6)
DAY


SPRING
SUMMER
FALL
Naviculoids
Naviculoids
Naviculoids
Nitzschia linearis
Navicula cryptocephala var. intermedia
Navicula cryptocephala var. intermedia
Nitzschia sp I.
Nitzschia spp.
Nitzschia sp I.
Nitzschia sp 2.
Gomphonema spp.
Nitzschia sp 2.
BIOVOLUME-DENSITY (µm³ cm⁻² x 10⁶)
DAY

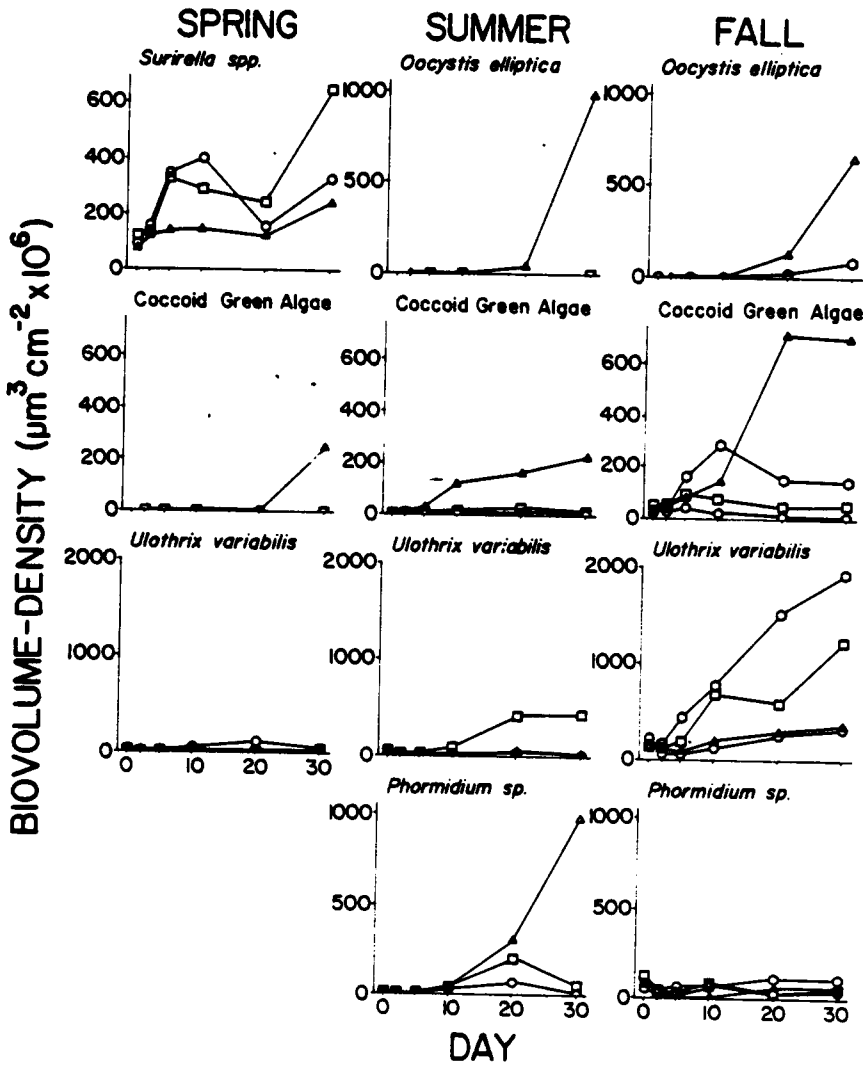

SPRING
SUMMER
FALL
Surirella spp.
Oocystis elliptica
Oocystis elliptica
Coccoid Green Algae
Coccoid Green Algae
Coccoid Green Algae
Ulothrix variabilis
Ulothrix variabilis
Ulothrix variabilis
Phormidium sp.
Phormidium sp.
BIOVOLUME-DENSITY (µm³cm⁻²x10⁶)
DAY

Table 2-2. Means (± 1 SE) for selected water chemistry variables (n = 15) in the New River, Virginia, during three seasons in 1984.

Season	Nominal zinc concentration (mg l^{-1})	Actual total zinc concentration (mg l^{-1})	Temp (C)	pH	Hardness (mg l^{-1} as $CaCO_3$)	Alkalinity (mg l^{-1} as $CaCO_3$)
Spring	Control	–*	25.1 (± 1.8)	8.39 (± 0.30)	71.1 (± 13.5)	49.8 (± 7.1)
	0.05	0.043 (± 0.060)	–	8.31 (± 0.38)	70.7 (± 14.6)	49.5 (± 5.9)
	1.0	0.819 (± 1.014)	–	8.06 (± 0.26)	72.3 (± 10.6)	49.8 (± 6.1)
Summer	Control	0.028 (± 0.016)	25.5 (± 0.8)	8.44 (± 0.10)	70.2 (± 3.65)	47.6 (± 1.5)
	0.05	0.035 (± 0.012)	–	8.42 (± 0.09)	70.5 (± 4.2)	48.5 (± 1.3)
	1.0	1.101 (± 0.955)	–	8.08 (± 0.08)	70.8 (± 2.7)	48.4 (± 1.4)
Fall	Control	0.094 (± 0.228)	20.6 (± 1.9)	8.31 (± 0.12)	88.8 (± 2.3)	56.2 (± 0.7)
	0.05	0.087 (± 0.109)	–	8.27 (± 0.09)	88.3 (± 2.6)	55.5 (± 0.8)
	0.50	0.504 (± 0.286)	–	8.20 (± 0.05)	87.1 (± 2.6)	59.7 (± 1.1)
	1.0	0.975 (± 0.299)	–	8.14 (± 0.02)	88.3 (± 2.3)	56.1 (± 0.7)

* Below detecton limits of 0.02 mg Zn l^{-1}.

on day 30, although biovolume-density was reduced on day 10 for two diatoms by 0.05 mg $Zn \bullet l^{-1}$ and for two other diatoms by 1.0 mg $Zn \bullet l^{-1}$. The control could be identified by the diatoms *Melosira varians* C.A. Ag. ($P = 0.004$), naviculoids ($P = 0.004$), *Nitzschia linearis* W. Smith ($P = 0.012$). These taxa had reduced biovolume-density from 0.05 mg $Zn \bullet l^{-1}$. The 0.05 mg $Zn \bullet l^{-1}$ treatment could be identified by the diatom *Cymbella minuta* var. *silesiaca* (Bleisch *ex* Rabh.) Reim., which had significantly higher biovolume-density in 0.05 mg $Zn \bullet l^{-1}$ over control and 1.0 mg $Zn \bullet l^{-1}$ treatments ($P = 0.018$). The diatoms *Nitzschia* sp. 1 (approximately 30% *N. palea* [Kuetz.] W. Smith and 70% *N. dissipata* [Kuetz.] Grun.) ($P = 0.046$) and *Surirella* spp. ($P = 0.037$) were equally abundant in control and 0.05 mg $Zn \bullet l^{-1}$ but were reduced in abundance by 1.0 mg $Zn \bullet l^{-1}$. The 1.0 mg $Zn \bullet l^{-1}$ treatment could be identified by the presence of a coccoid green-alga, similar in morphology to *Chlorella*, that had significantly higher biovolume-density in 1.0 mg $Zn \bullet l^{-1}$ ($P = 0.072$). The diatoms *Achnanthes minutissima* Kuetz., *Fragilaria* spp., and *Nitzschia* sp. 2 (approximately 90% small forms of *N. palea*), and the filamentous green-alga *Ulothrix variabilis* Kuetz. did not respond significantly to any Zn treatment.

Total biovolume-density and similarity (SIMI) in overall taxonomic composition were examined as community variables in spring. Total biovolume-density was reduced ($P < 0.0001$) in Zn-treated mesocosms on days 20 and 30 (Table 2-1). Differences in community similarity between treatments occurred from day 10 to 30 ($P < 0.05$). Figure 2-3 shows that Zn-treated communities became less similar to the control as Zn concentration increased.

Snail grazing or an interaction between Zn and snail grazing did not significantly affect biovolume-density of any taxon on tile substrates. The maximum Zn concentration in periphyton (μg Zn g^{-1} dry wt periphyton) differed among all treatments ($P < 0.0001$) and was approximately 270 in the control, 1200 in 0.05 mg $Zn \bullet l^{-1}$, and 6200 in 1.0 mg $Zn \bullet l^{-1}$ (Fig. 2-4).

Figure 2-3. Similarity (SIMI) scores for algal periphyton communities in three seasons. ○ = mean SIMI among control samples (n = 3); □ = mean SIMI between control and 0.05 mg $Zn \bullet l^{-1}$ samples (n = 9); ⬡ = mean SIMI between control and 0.5 mg $Zn \bullet l^{-1}$ samples (n = 9); and △ = mean SIMI between control and 1.0 mg $Zn \bullet l^{-1}$ samples (n = 9).

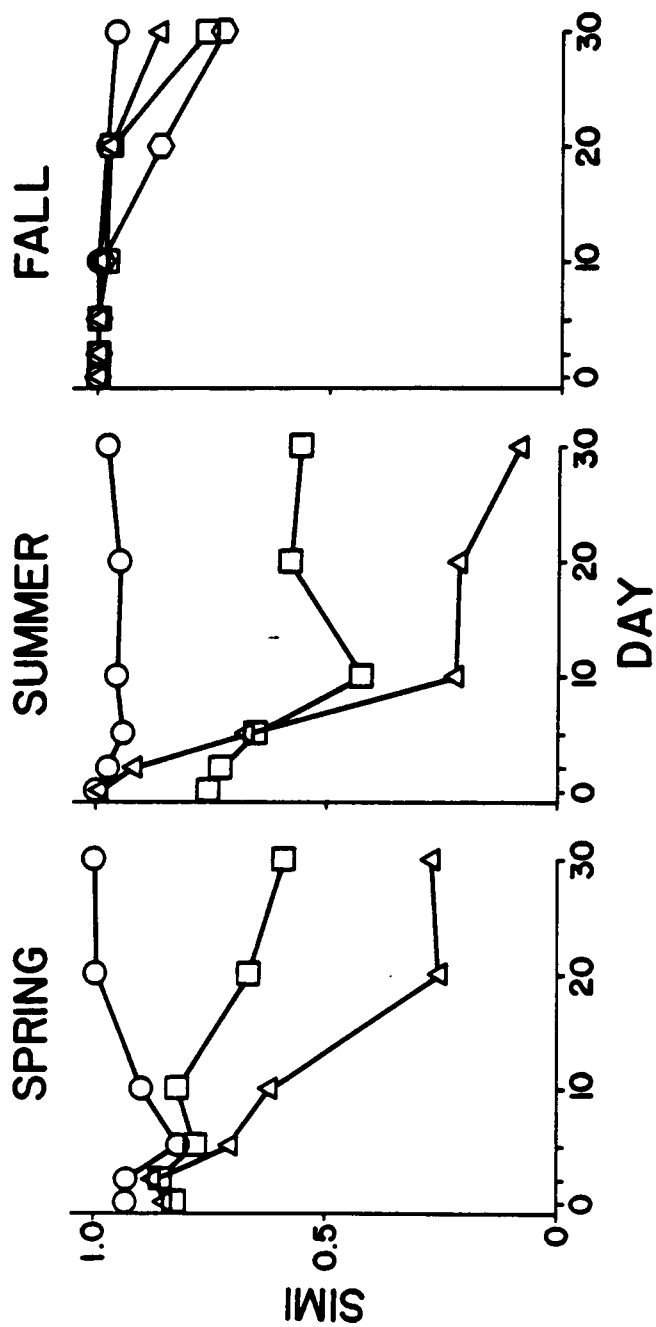
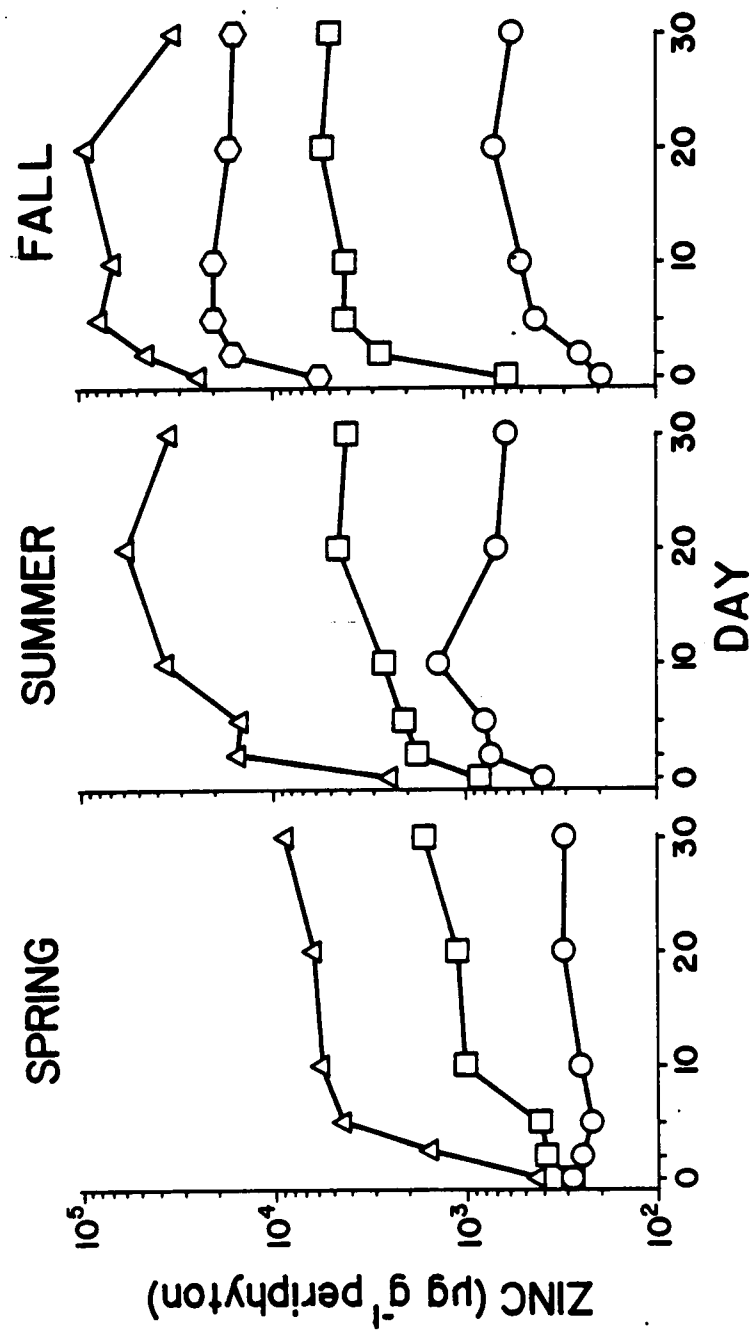

SPRING
SUMMER
FALL
SIMI
1.0
0.5
0
0
10
20
30
DAY

Figure 2-4. Mean (n = 3) Zn concentration (μg $Zn \bullet g^{-1}$ periphyton dry weight) on artificial substrates for three seasons. ○ = control, □ = 0.05 mg $Zn \bullet l^{-1}$, ⬡ = 0.5 mg $Zn \bullet l^{-1}$, △ = 1.0 mg $Zn \bullet l^{-1}$.


SPRING
SUMMER
FALL
ZINC (µg g⁻¹ periphyton)
DAY
10⁵
10⁴
10³
10²
0
10
20
30

2.3.2 Summer

Zn treatments changed community composition from diatoms to green algae (Table 2-1; Fig. 2-2). The most substantial differences between treatments occurred on day 30, although two diatoms had reduced biovolume-density from 0.05 mg $Zn \bullet l^{-1}$ on day 5. The control could be identified by the diatoms *Cocconeis placentula* Ehr. ($P = 0.011$), *Gomphonema* spp. ($P < 0.0001$), and *M. varians* ($P = 0.022$) which were reduced in biovolume-density by 0.05 mg $Zn \bullet l^{-1}$. The 0.05 mg $Zn \bullet l^{-1}$ treatment could be identified by the filamentous green-alga *U. variabilis* ($P = 0.045$) and the diatoms *Nitzschia* spp. ($P = 0.014$) and naviculoids ($P = 0.0004$), which had lower density in control and 1.0 mg $Zn \bullet l^{-1}$. The 1.0 mg $Zn \bullet l^{-1}$ treatment could be identified by the unicellular green-alga *Oocystis elliptica* W. West ($P = 0.017$) and a coccoid green-alga similar in morphology to *Chlorella* ($P = 0.024$). *Phormidium* sp., a filamentous blue-green alga, identified 1.0 mg $Zn \bullet l^{-1}$ by day 30 ($P = 0.026$). The diatoms *Achnanthes minutissima*, *Fragilaria rumpens* var. *scotica* (Grun.) A. Cl., and *Navicula cryptocephala* var. *intermedia* Grun. ($P = 0.139$), did not respond significantly to any Zn treatment. Absence of *F. rumpens* var. *scotica* in treatments other than 0.05 mg $Zn \bullet l^{-1}$ suggests that further counting would have revealed a Zn effect by reducing variability caused by its filamentous form.

At the community level, total biovolume-density did not differ among treatments. Differences in community similarity between treatments occurred from day 5 to day 30 ($P < 0.05$). Figure 2-3 shows that Zn-treated communities became less similar to the control as Zn concentration increased from 0.05 to 1.0 mg $Zn \bullet l^{-1}$. The maximum Zn concentration in periphyton (μg Zn g^{-1} dry wt periphyton) differed among all treatments ($P < 0.0001$) and was approximately 830 in the control, 3600 in 0.05 mg $Zn \bullet l^{-1}$, and 42000 in 1.0 mg $Zn \bullet l^{-1}$ (Fig. 2-4).

2.3.3 Fall

Zn treatments changed community composition from diatoms to green or blue-green algae (Table 2-1, Fig. 2-2). The most substantial difference between treatments occurred on day 20, although the biovolume-density of two diatoms was reduced by 0.05 mg $Zn \bullet l^{-1}$ on day 5. Control treatments could be identified by the diatoms *N. cryptocephala* var. *intermedia* ($P < 0.0001$) and naviculoids ($P < 0.0001$) that were reduced in biovolume-density by 0.05 mg $Zn \bullet l^{-1}$. The 0.05 mg $Zn \bullet l^{-1}$ treatment could be identified by *Nitzschia* sp. 1 (primarily large forms of *N. palea*) ($P = 0.003$) and *Nitzschia* sp. 2 (primarily small forms of *N. palea*) ($P < 0.0001$) which were reduced in biovolume-density by 0.5 and 1.0 mg $Zn \bullet l^{-1}$. *Ulothrix variabilis* identified both 0.05 and 0.5 mg $Zn \bullet l^{-1}$ and was reduced in biovolume-density by 1.0 mg $Zn \bullet l^{-1}$ ($P < 0.0001$). The filamentous blue-green alga *Phormidium* sp. did not have consistently higher abundance in one treatment over other treatments during the fall experiment ($P = 0.001$). *Scenedesmus* spp., a green alga, was most abundant in 0.5 mg $Zn \bullet l^{-1}$ on day 2 ($P = 0.083$). The 1.0 mg $Zn \bullet l^{-1}$ treatment had increased relative abundance by *Oocystis elliptica* ($P = 0.001$) and a coccoid green-alga similar in morphology to *Chlorella* ($P = 0.0004$). *Melosira varians* did not respond significantly to any Zn treatment. *Cocconeis placentula* did not differ in biovolume-density between control and 1.0 mg $Zn \bullet l^{-1}$ but did have reduced abundance on different days for 0.05 and 0.5 mg $Zn \bullet l^{-1}$ ($P = 0.014$). Four taxa had significantly different biovolume-density between treatments on day 0.

Total biovolume-density did not differ among treatments. Differences in community similarity between treatments occurred from day 2 to day 30 ($P < 0.05$). Figure 2-3 shows that Zn treated communities had reduced similarity to the control by day 30. The maximum Zn concentration in periphyton (μg Zn g^{-1} dry wt periphyton) differed among all treatments ($P < 0.0001$) and was approximately 530 in the control, 4600 in 0.05 mg $Zn \bullet l^{-1}$, 17000 in 0.5 mg $Zn \bullet l^{-1}$, and 61000 in 1.0 mg $Zn \bullet l^{-1}$ (Fig. 2-4).

2.4 *Discussion*

2.4.1 Population response to zinc stress and snail grazing

Zn treatments as low as 0.05 mg $Zn \bullet l^{-1}$ had communities with increased relative abundance of green and blue-green algae in all three seasons despite this being the "safe" concentration set by USEPA. No claim is made that these algal taxa are "indicator organisms" for Zn only. The same taxa may respond in a similar fashion to other chemicals. Ambient conditions in control treatments could be identified by the diatoms *Cocconeis placentula*, *Gomphonema*, *Melosira varians*, naviculoids (spring and fall), *Navicula cryptocephala* var. *intermedia*, and *Nitzschia linearis*. These taxa are recommended for laboratory and field tests that determine safe concentrations of Zn and poisons for aquatic habitats. The distribution of these taxa throughout the U. S. and Europe (Hustedt, 1930, 1933; Prescott, 1962; Patrick & Reimer, 1966, 1975) would allow levels of stress to be compared among different ecosystems. The 0.05 mg $Zn \bullet l^{-1}$ treatment could be identified by *Cymbella minuta* var. *silesiaca*, naviculoids (summer), *Nitzschia*, and *Ulothrix variabilis*. Rushforth *et al.*, (1981) found *Nitzschia palea* to have relative abundance positively correlated with Zn in natural streams. The 0.5 mg $Zn \bullet l^{-1}$ treatment could be identified by *U. variabilis* with a low abundance of diatoms. The persistence of *U. variabilis* at this concentration suggests that it was more resistant than diatoms to Zn stress. The 1.0 mg $Zn \bullet l^{-1}$ treatment could be identified by coccoid green-algae, *Oocystis elliptica*, and *Phormidium*. Coccoid green-algae and blue-green algae have been associated with habitats of high Zn concentration (Shehata & Whitton, 1981). Extrapolating these results to field situations will be complicated by interactions between Zn and other chemicals (natural or anthropogenic) in water (Cairns, 1977a; Hart & Cairns, 1984) or the potential ability of algae to adapt to changes in stress (Say *et al.*, 1977a).

Diatoms were more sensitive than a filamentous green alga which was more sensitive than unicellular green-algae and a filamentous blue-green alga (Table 2-2). Patrick (1978) suggested this

as a hypothesis for periphyton communities exposed to vanadium or hexavalent chromium in stream mesocosms. Diatoms are more sensitive than coccoid green-algae to Zn stress if values in the literature for individual taxa are compared (Coleman *et al.* 1971; Rachlin & Farran, 1974; Rosko & Rachlin, 1977; Cairns *et al.*, 1978; Rizet *et al.*, 1978; Rachlin *et al.* 1983).

Snails did not significantly change periphyton abundance in spring. Other experiments have found snails to reduce algal abundance except for the most tightly attached diatoms (Lamberti & Moore, 1984). The non-significant results reported may be due to low snail density or because snails preferred mesocosm walls to detrital accumulation on tile substrates. Patrick (1978) suggests that snail growth rates will be lower when heavy metals cause diatoms to be replaced by less nutritious green algae.

2.4.2 Community response to zinc stress

Zn clearly determined what taxa would increase in abundance, but this does not imply that other variables were not important. Ecosystems are complex so that simple cause-and-effect relationships between a chemical treatment and a species response are often difficult to detect (National Research Council, 1981). The different seasonal responses of algae to treatment as low as 0.05 mg $Zn \bullet l^{-1}$ could depend on changes in the chemical and physical environment (e.g., hardness, pH, temperature) and whether the microhabitat of the organism provided a refuge from stress. Diatoms that are closely attached to substrates may be protected by overlying taxa. Zn stress on individual organisms will affect biological interactions like competition and predation so that reduced biovolume-density of one taxon may be compensated by increased abundance of another taxon.

Although total biovolume-density was poor for identifying Zn stress, the SIMI index performed well. Total biovolume-density did not identify Zn treatments in summer and fall when a decline in one taxon was compensated by an increase in another. The lack of differences in total biovolume-density may be important because diatom taxa that were inhibited by Zn were replaced

by green and blue-green algae. Although total biovolume-density was unaffected in summer and fall, diatoms may be a preferred food source for higher level consumers (Lamberti & Moore, 1984), when green algae are more abundant than diatoms, consumers might be stressed by lower food quality at higher Zn concentrations. Taxonomic changes could be identified with SIMI because it is derived from this data, but changes in total biovolume-density did not correspond to taxonomic changes.

There was a discrepancy between SIMI scores and population densities on day 0 in fall. Although biovolume-density of some taxa differed between treatments, relative abundances between taxa were consistent so that communities did not differ in overall similarity. These differences disappeared by day 2, and effects due to Zn were apparent by consistent differences between treatments from day 10 to day 20. Differences on day 0 are attributed to high variability in the early stages of algal colonization rather than Zn treatment. Although 0.05 mg $Zn \bullet l^{-1}$ appears to have reduced SIMI on day 0 in summer (Fig. 2-3), there were no taxa with significantly different biovolume-density on this day.

2.4.3 Zinc in stream mesocosms

Zn bound to periphyton (bound Zn; Fig. 2-4) was more reliable than total Zn in water (water Zn; Table 2-2) for identifying Zn stress. Bound Zn is a better measure than water Zn for many reasons. Water Zn was often below the detectable limit of flame atomic absorption spectrometry in control and 0.05 mg $Zn \bullet l^{-1}$ treatments, but bound Zn never was. The variability of water Zn often made it impossible to distinguish between control and 0.05 mg $Zn \bullet l^{-1}$ treatments (Table 2-2), yet bound Zn differed significantly between all treatments by day 2. Bound Zn is intimately associated with periphyton and may better indicate concentrations near the cells, whereas water Zn is far removed. Water Zn may be highly variable in space and time from natural and anthropogenic sources so that bound Zn may better represent long-term exposures. Bound Zn was proportional to the nominal Zn concentration, and data from Groth (1971) shows a bound Zn concentration

of 350 x 10^3 μg Zn g^{-1} lake sediment which is comparable to that found in the stream mesocosm periphyton of the control treatment. Algal taxon abundance was consistent with the bound Zn concentration among replicates despite high variability in water Zn.

2.4.4 Conclusions

Treatments as low as 0.05 mg Zn•l^{-1} significantly changed algal community composition from diatoms to green or blue-green algae. Water concentrations fo this treatment ranged above and below the criterion level of 0.047 mg Zn•l^{-1} set by USEPA. This suggests that further testing at the criterion is necessary to set a safe level.

Safe concentrations of Zn can be obtained by using periphyton in stream mesocosms because individual tolerances of diatoms are comparable to fish and other higher organisms (Patrick *et al.*, 1968). Advantages include environmental realism; biological interactions between different taxa; most, if not all, life stages; Zn in natural river water; and chronic exposures.

3.0 Algal and protozoan community response to individual and combined treatments of zinc and phosphate

3.1 Introduction

Macronutrients and essential metallic micronutrients interact to affect algae and protozoa in nature and in the laboratory. Kuwabara (1985) demonstrated zinc (Zn) stress to directly increase phosphorus (P) limitation in a green alga. Stoermer *et al.* (1980) report that heavy metal stress results in increased polyphosphate-body formation in eukaryotic algae. Hart & Cairns (1984) found communities of protozoa from eutrophic lakes to be more resistant than protozoa from oligotrophic lakes to anthropogenic stress. Phosphate and Zn are important variables controlling productivity in aquatic ecosystems (Wetzel, 1983). Phosphate often limits productivity, and Zn, although necessary at low concentrations, may be harmful at high concentrations.

The importance of testing communities of organisms rather than isolated species is gaining increased attention (*eg*, National Research Council, 1981). Periphyton provide an interacting

community composed of algae, protozoa, and other organisms. A new artificial substrate has made it possible to create natural periphytic communities of differing nutrient enrichment which may then be manipulated in the laboratory (Fairchild & Lowe, 1984). These artificial substrates provide replicate communities from controlled nutrient concentrations.

In this experiment natural periphyton communities were developed on substrates of different P enrichment. These communities were exposed to different levels of Zn in the laboratory. The experiment was undertaken to determine whether the density of individual taxa within a community would be affected by (a) P alone, (b) Zn alone, or (c) an interaction between P and Zn so that the end result is different from individual added effects of P and Zn.

3.2 Materials and methods

3.2.1 Artificial substrate

Nutrient-diffusing substrates (clay flower pots) were constructed following the method of Fairchild & Lowe (1984). Pots were 8.8 cm in diameter and 245 ml in volume. The base was sealed with a plastic petri dish, and the small hole at the top was sealed with a #000 stopper. A #10 rubber stopper with a 20 cm wooden dowl was attached to the base of the petri dish to anchor each substrate in the lake bottom. Pots were filled with a 2% agar solution of 0.1 M KH_2PO_4, 0.01 M KH_2PO_4, or a distilled water control. The 45 substrates were distributed randomly on a grid in approximately 1 m of water and placed 50 cm apart. One third of the substrates in each treatment were placed in Douglas Lake, Cheboygan County, Michigan on each day from June 8-10, 1984. Douglas Lake is a well-buffered, hardwater, mesotrophic lake. The substrates were transferred to the laboratory after 21 days of colonization on 29 June-1 July, 1984. A 400 ml plastic beaker was inverted over each substrate under water before transfer so as to not disturb periphyton.

3.2.2 Microcosms

On transfer days, one substrate from each P treatment was placed in a 3 l plastic container having 1.75 l of one of five Zn (as $ZnSO_4$) solutions (control, 0.1, 1.0, 3.0, 10.0 mg $Zn \bullet l^{-1}$). This resulted in three replicate microcosms for each of the 15 treatments. Microcosms were continuously aerated, and a 16:8 light-dark schedule was used. Containers were illuminated at ca. 5,000 lux with daylight equivalent fluorescent lights (Vita-lights, Duro-Test Corporation, North Bergen, N.J.). Substrates were removed after one week and thoroughly scraped with a razor blade and toothbrush and rinsed with distilled water to remove periphyton.

3.2.3 Algae

An 18 ml subsample was collected and 2 ml of 37% formaldehyde were added as a preservative. Each sample was thoroughly mixed and 0.1 ml aliquots examined in a Palmer-Maloney nanoplankton cell. Identification and enumeration of algal taxa proceeded by scanning back-and-forth and proceeding from bottom to top of the counting chamber area until 500 individuals were enumerated. The transect on which the 500th individual occurred was completed so that counts were usually greater than 500. Only individuals alive (containing chloroplasts) during sampling were counted.

3.2.4 Protozoa

A small aliquot (ca. 20 ml) was removed and two-drop subsamples were examined at 200X-450X for living protozoan species. All species identifications were made from living material,

and subsampling continued until an asymptotic number of species had been identified from each sample.

Density of ciliate protozoans was determined from a second subsample. An 85 ml subsample was removed from each collection and centrifuged at 1000 rpm for 10 minutes. The supernatant was decanted and the pellet resuspended in 2% glutaraldehyde in 0.05 M cacodylate buffer to a final volume of 3 ml and stored in small vials. Preserved ciliates were counted by the same technique used for enumerating algae. Ciliates encountered were identified (using species lists from living material) to genus, and the number of individuals in each genus was enumerated. A minimum of 200 individuals were counted from each sample. If one full counting chamber did not produce a count of at least 200, a second complete counting chamber was examined. Two counting chambers were always sufficient to obtain enough individuals for statistical analysis.

3.2.5 Biovolume-density

Numbers of individuals per taxon were converted to biovolume-density ($\mu m^3\ cm^{-2}$) by estimating the size of the mean individual of each taxon and converting abundances to densities on the substrate (APHA, 1981). Biovolumes of dominant algae were estimated by measuring 10 randomly encountered individuals, and median measurements for rare taxa were taken from reported values in the literature (Prescott, 1962; Patrick & Reimer, 1966, 1975). Median protozoan biovolume for each taxon was estimated by averaging biovolumes of all congeneric protozoan species examined in this experiment from reported values in the literature (Kahl, 1930-1935; Gates *et al.*, 1982). These dimensions were used in mensuration formulas to determine volumes of uniform geometric shapes (cylinders, spheres, prolate spheroids, rectangular prisms, etc.) that approximated respective taxon (Beyer, 1981).

3.2.6 Statistics

The statistical model was a two factor factorial in a randomized complete block design (Lentner & Bishop, 1986). Specifically, analysis of variance (ANOVA) was used to test whether periphyton biovolume-density was affected by P, Zn, or an interaction between P and Zn. If $0.05 < p \leq 0.1$ then the difference between group means was considered moderately significant, and if $p \leq 0.05$ the difference between group means was considered highly significant. Fisher's LSD and Duncan's New Multiple Range Test (SAS, 1982) indicated which treatments differed in biovolume-density. Taxa were considered dominant and used for statistical analysis if the mean cell count for that taxon was greater than 1% of the total cell counts.

3.3 Results

3.3.1 Population response to phosphate

Of ten algal taxa that responded to P (Fig. 3-1) six are diatoms and four are green algae. *Cyclotella* spp., a planktonic centric diatom, was less abundant in 0.1 M P ($P < 0.001$). This taxon was primarily composed of two species, *C. comensis* Grun. (80%) and *C. michiganiana* Skv. (20%), which could not be distinguished in the Palmer-Maloney cell.

The filamentous green-alga *Stigeoclonium nanum* Kuetz. is composed of an extensive prostrate portion of subglobose cells giving rise to short-tufted filaments, and morphology of this alga suggests that it is a juvenile or growth form of another species (Prescott, 1962). Since the complete life-cycle of this alga was not observed, it may be a form of *S. tenue* observed by Fairchild *et al.* (1985). The prostrate cells of *S. nanum* were more abundant in 0.01 M P than in the other two

Figure 3-1. Biovolume-density (μm^3 cm^{-2}) for the 13 dominant algal taxa. Shown are means (n = 3) for each Zn treatment (○ = control, □ = 0.01 M KH_2PO_4, △ = 0.1 M KH_2PO_4).

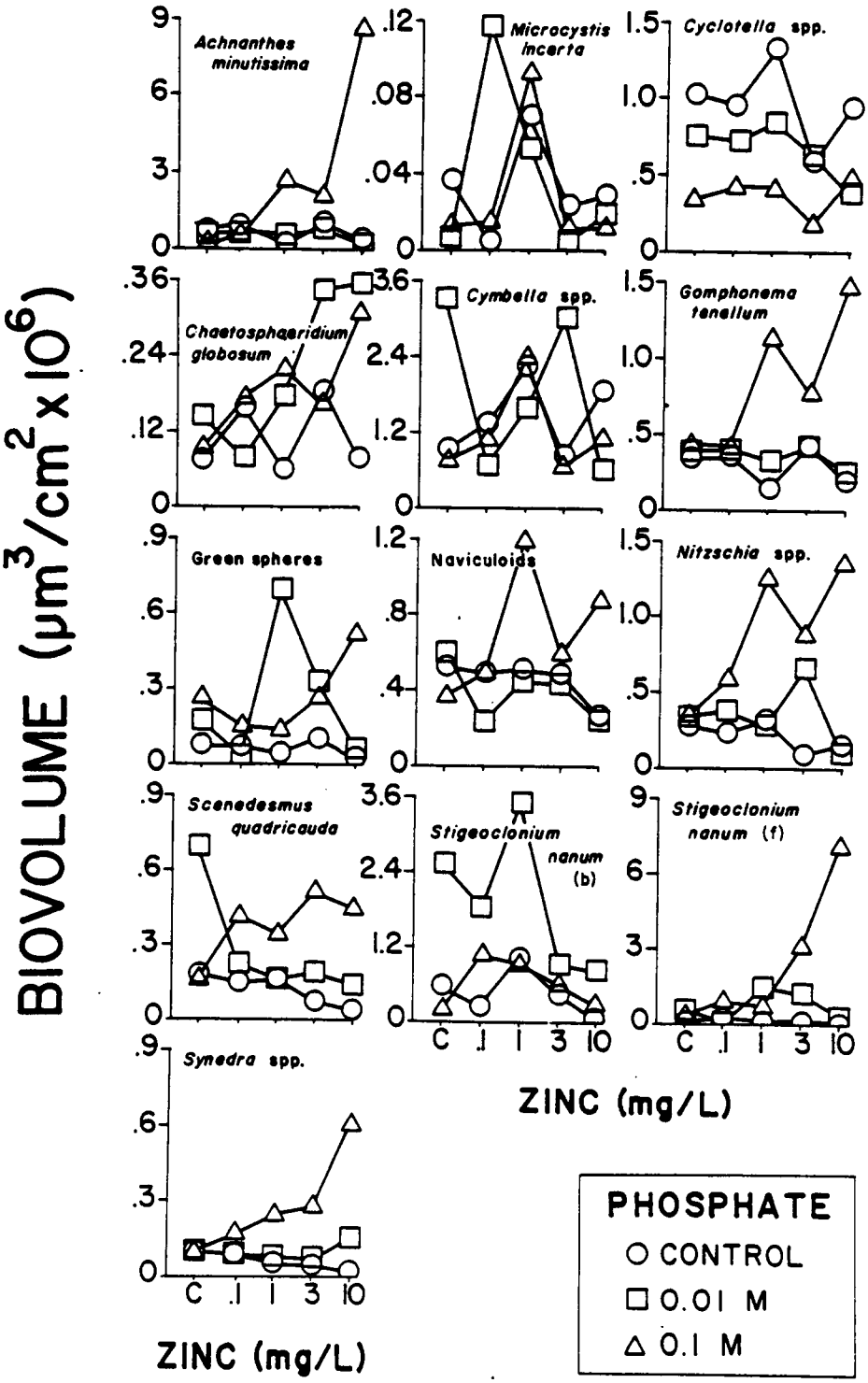

BIOVOLUME (µm³/cm² x 10⁶)
Achnanthes minutissima
Microcystis incerta
Cyclotella spp.
Chaetosphaeridium globosum
Cymbella spp.
Gomphonema tenellum
Green spheres
Naviculoids
Nitzschia spp.
Scenedesmus quadricauda
Stigeoclonium nanum (b)
Stigeoclonium nanum (f)
Synedra spp.
C .1 1 3 10
ZINC (mg/L)
PHOSPHATE
CONTROL
0.01 M
0.1 M

Table 3-1. Duncan's New Multiple Range Test indicating treatments where dominant taxa differed significantly in biovolume-density. Asterisks indicate results of Fisher's LSD test. *Stigeoclonium nanum* reported as prostrate (b) and filamentous (f).

Taxon	Phosphate (M)			Zinc (mg l^{-1})				
Algae								
Cyclotella spp.	C	.01	0.1					
Coccoid green algae	0.1	.01	C					
Gomphonema tenellum	0.1	.01	C					
Naviculoid diatoms	0.1	C	.01	1.0	10	C	3.0	0.1*
Nitzschia spp.	0.1	.01	C					
Scenedesmus spp.	0.1	.01	C*					
Stigeoclonium nanum (b)	.01	0.1	C					
Stigeoclonium nanum (f)	0.1	.01	C	10	3.0	1.0	0.1	C
Synedra spp.	0.1	.01	C					
Protozoa								
Aspidisca spp.	0.1	.01	C	3.0	0.1	1.0	C	10
Colpidium spp.	.01	C	0.1	10	C	1.0	3.0	0.1
Cyclidium spp.	0.1	.01	C	0.1	3.0	10	1.0	C
Leptopharynx spp.	.01	C	0.1					
Vorticella spp.	.01	0.1	C	3.0	1.0	C	10	0.1

treatments ($P = 0.028$, Table 3-1). Also more abundant in 0.1 M P are the filamentous portion of *S. nanum* ($P < 0.001$) and the diatoms *Gomphonema tenellum* Kuetz. ($P = 0.002$), naviculoids ($P = 0.032$), *Nitzschia* spp. ($P < 0.001$), and *Synedra* spp. ($P = 0.021$, Table 3-1). Examination of acid-cleaned material indicated that *Synedra* was composed of approximately 85% of the single cell form of *Fragilaria vaucheriae* (Kuetz.) Peters (which often occurs singly and is considered to be a transitional species between *Synedra* and *Fragilaria* (Patrick & Reimer, 1966)) and of approximately 15% other *Synedra* species. Displaying moderately significant increased biovolume-density in 0.1 M P were *Achnanthes minutissima* Kuetz. ($P = 0.102$), green coccoid algae ($p = 0.082$), and *Scenedesmus* spp. ($P = 0.093$).

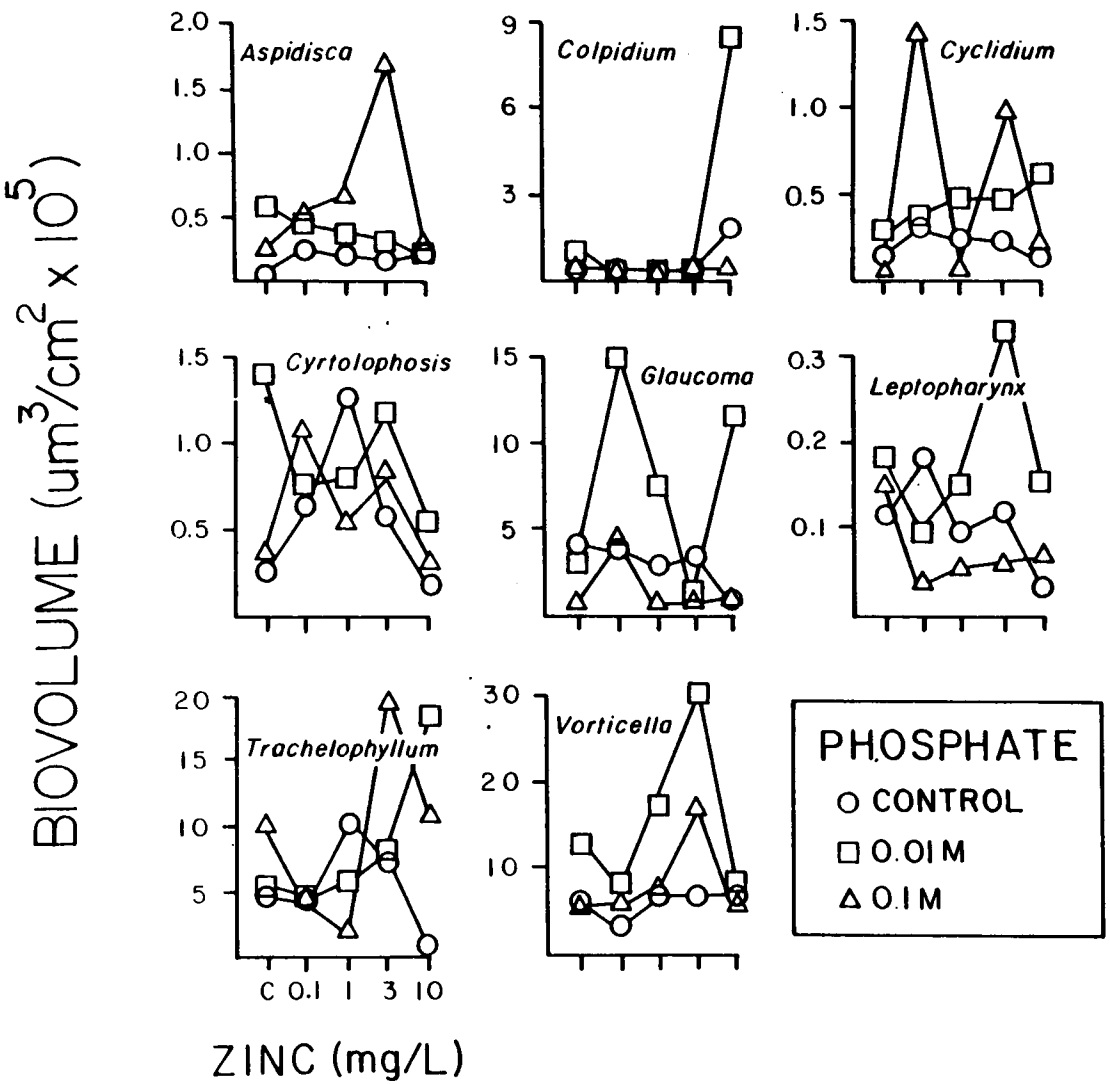
Five of eight protozoan taxa responded to P treatment (Table 3-1; Fig. 3-2). *Aspidisca* spp. had highest biovolume-density in 0.1 M P ($P = 0.016$), *Colpidium* spp. was most abundant in 0.01 M P ($P = 0.044$), and *Cyclidium* spp. was more abundant in both P treatments over the control ($P = 0.029$). Displaying moderately significant increased biovolume-density were *Leptopharynx* spp. ($P = 0.064$) and *Vorticella* spp. ($P = 0.103$).

3.3.2 Population Response to zinc

The filamentous portion of S. nanum increased abundance as Zn concentration increased ($P = 0.016$, Table 3-1, Fig. 3-1). This response to to Zn is independent of any interaction with P. Biovolume-density of *S. nanum* did not differ significantly between 10 and 3.0 mg $Zn \bullet l^{-1}$, but was more abundant in 10 mg $Zn \bullet l^{-1}$ than in other treatments (Table 3-1).

Four protozoans responded positively to Zn treatment (Table 3-1; Fig. 3-2). *Colpidium* was much more abundant in 10 mg $Zn \bullet l^{-1}$ than in other Zn treatments ($P = 0.004$). *Aspidisca* was most abundant in 3 mg $Zn \bullet l^{-1}$ ($P = 0.060$) and had similar abundances in other Zn treatments. *Cyclidium* was most abundant in 0.1 mg $Zn \bullet l^{-1}$ ($P = 0.076$), and *Vorticella* was most abundant in 3.0 mg $Zn \bullet l^{-1}$ ($P = 0.095$).

Figure 3-2. Biovolume-density ($\mu m^3\ cm^{-2}$) for eight dominant ciliate protozoan taxa. Shown are means (n = 3) for each Zn treatment (○ = control, □ = 0.01 M KH_2PO_4, △ = 0.1 M KH_2PO_4).


BIOVOLUME (um³/cm² x 10⁵)
Aspidisca
Colpidium
Cyclidium
Cyrtolophosis
Glaucoma
Leptopharynx
Trachelophyllum
Vorticella
C 0.1 1 3 10
ZINC (mg/L)
PHOSPHATE
O CONTROL
□ 0.01 M
△ 0.1 M

3.3.3 Population response to phosphate and zinc interaction

The increased biovolume-density of *S. nanum* filaments was affected by an interaction ($P = 0.001$) between Zn and P. Biovolume-density was 19 fold higher when high P (0.1 M) and high Zn (10 mg l^{-1}) were combined than for the sum of individual effects of these two treatments (Fig. 3-1). This type of response is referred to as synergism by Sokal & Rohlf (1981). *Nitzschia* displayed a moderate increase ($P = 0.090$) to combinations of P and Zn by being three times higher than additive effects of individual treatments (Fig. 3-1).

Three protozoan taxa responded to a significant interaction between Zn and P. *Aspidisca* was 14 times more abundant ($P = 0.020$) in 0.1 M P and 3 mg $Zn \bullet l^{-1}$ combined, *Colpidium* was 3.5 times more abundant ($P = 0.019$) in 0.01 P and 10 mg $Zn \bullet l^{-1}$ combined, and *Cyclicium* showed a strong non-linear response in 0.1 M P ($P = 0.028$, Fig. 3-2).

3.3.4 Other taxa

Three algal and two protozoan taxa were abundant but did not differ in biovolume-density between treatments. Algae were the diatom *Cymbella* spp., the coccoid blue-green alga *Microcystis incerta* Lemm., and the green alga *Chaetosphaeridium globosum* (Nordst.) Kleb.. Other algal taxa of 66 enumerated were considered to be of too low abundance for a reliable statistical analysis. The protozoans *Cyrtolophosis* and *Glaucoma* did not differ in biovolume-density among treatments.

3.3.5 Community response

Algal total biovolume-density, protozoan total biovolume-density, and number of protozoan species were used as community level parameters. Total algal biovolume-density ($\mu m^3\ cm^{-2}$)

Table 3-2. Total algal and ciliate-protozoan biovolume-density (x 10^6 $\mu m^3 cm^{-2}$), and the number of protozoan species on the flower pot substrates for the 15 treatments of phosphate and zinc ($\bar{X} \pm SE$)

Phosphate (M)	group	Zinc (mg l^{-1}) Control	0.1	1.0	3.0	10
Control	Algae	4.98± 0.126	5.26± 0.839	6.15± 2.35	4.56± 1.19	4.13± 0.763
	Protozoa	1.37± 0.311	1.18± 0.512	1.98± 0.312	1.67± 0.508	0.886± 0.426
	Protozoan species	26.0± 1.52	26.0± 2.08	22.0± 1.53	25.7± 4.33	19.7± 1.20
0.01	Algae	10.2± 2.84	5.54± 1.83	10.2± 5.03	9.09± 2.10	3.55± 0.434
	Protozoa	2.06± 0.586	3.02± 1.39	2.85± 0.796	4.11± 1.92	4.08± 0.829
	Protozoan species	29.3± 2.03	31.0± 2.65	25.3± 1.76	29.7± 5.21	22.3± 1.33
0.1	Algae	3.84± 0.419	6.65± 1.22	11.8± 1.75	10.1± 1.03	23.2± 12.2
	Protozoa	1.64± 0.350	1.73± 0.452	1.04± 0.190	4.13± 1.48	1.67± 0.706
	Protozoan species	26.7± 1.20	24.7± 0.667	30.7± 0.667	26.7± 1.86	30.7± 2.33

ranged from $5x10^6$ in the control to $23x10^6$ in 0.1 M P (Table 3-2). The 0.1 M P treatments had more total algal biovolume than the control ($P = 0.025$), and 0.01 M P did not differ significantly from control or 0.1 M P. There was a significant interaction between Zn and P ($P = 0.039$) so that algal total biovolume-density in treatments with Zn and P combined was much higher than in treatments with Zn and P alone. Total biovolume-density was not affected by Zn alone.

Biovolume-density was examined for 8 dominant ciliate taxa of a total of 17 ciliate genera. Protozoan total biovolume-density ranged from approximately $1x10^6$ to $4x10^6$ (Table 3-2). The 0.01 M P treatment had significantly more protozoan biovolume than control or 0.1 M P ($P = 0.010$), and 0.1 M P did not differ significantly from the control. There was no difference in protozoan biovolume attributable to Zn and no significant interaction between P and Zn.

Since the clay substrate used was homogeneous and generally without the complex interstitial structure of some natural substrates, it is possible to estimate areal standing crop of the limited array of protozoan species sampled. Biovolume-density may estimate biomass-density using a conversion factor of 0.416 pg μm^{-3} (Gates *et al*, 1982). Protozoan standing crop ranged from $1x10^6$ to $4x10^6$ μm^3 cm^{-2} which corresponds to approximately 4 to 16 g m^{-2} as wet weight. These values are an extreme underestimate of total protozoan community standing crop since they are based on only eight of the most common ciliate taxa. Natural protozoan communities commonly have from 40 to 80 total species. Gates and Lewg (1984) estimated lacustrine ciliate zooplankton biomass at 30-120 μg l^{-1}.

The number of protozoan species increased ($P = 0.023$) from approximately 24 in the control to approximately 28 in P treatments (Table 3-2). Although Zn alone did not significantly affect number of species, a moderately significant interaction between Zn and P ($P = 0.080$) suggests that the number of protozoan species was markedly lower in 10 mg $Zn \bullet l^{-1}$ (control P) (Table 3-2). The number of ciliate genera did not differ among treatments.

3.3.6 Zinc in microcosms

Zinc concentrations in microcosm water quickly decreased within the first four hours after substrates were transferred to the laboratory (Table 3-3). The 10 mg $Zn \bullet l^{-1}$ treatment was significantly higher in total Zn concentration ($P < 0.001$) than the other four treatments at the beginning and end of the experiment. The Zn concentration of all treatments decreased during the one week incubation in the laboratory. Although P treatments did not differ significantly in Zn concentration at the beginning of the experiment, high (0.1 M) P treatments had less Zn ($P < 0.001$) than control and 0.01 M P treatments by the end of the experiment (Table 3-3). A significant interaction ($P = 0.002$) between Zn and P treatments indicated that high P treatments lost Zn from water quicker than low P treatments.

3.4 Discussion

3.4.1 Phosphate

Decline in biovolume-density for certain taxa may be due to poisonous effects of excess P or excess potassium from KH_2PO_4 (Fairchild *et al.*, 1985). Fairchild *et al.* (1985) found *Cyclotella* spp. not to respond significantly to P enrichment, but a decrease was noted for this taxon. This discrepancy may be due to laboratory effects in this experiment or to annual differences in *Cyclotella* populations. Stoermer & Yang (1969) note that *C. michiganiana* shows inconsistent population densities from year to year in Lake Michigan. Our observation of *Cyclotella* decline with increased P is consistent with reports that *C. comensis* and *C. michiganiana* are more abundant in oligotrophic lakes (Lowe, 1974; Stevenson & Stoermer, 1979).

Table 3-3. Fisher's LSD test of mean zinc concentration (mg l^{-1}) measured in the microcosm water at the start and end of the experiment. Zinc treatment means include all phosphate treatment levels (n = 9), and phosphate treatment means include all zinc treatment levels (n = 15). Underlined means do not differ significantly at α = .05.

	Start					End				
Zinc (mg l^{-1})										
treatment	10	3.0	1.0	C	0.1	10	1.0	C	0.1	3.0
mean	1.7	.74	.72	.71	.51	.52	.14	.11	.11	.09
Phosphate (M)										
treatment		.01	C	0.1			C	.01	.10	
mean		1.05	.85	.72			.28	.27	.03	

S. nanum prostrate cells responded differently than filamentous cells to P. This suggests that growth form is closely related to P concentration. Drouet (1981) notes that cell morphology and maturity of blue-green algae are also governed by chemical and physical characteristics of the habitat.

The increased abundance of *Synedra* spp. in high P is consistent with the most abundant member of this taxon, *Fragilaria vaucheriae*, being characteristic of eutrophic habitats (Lowe, 1974). *Gomphonema tenellum* and naviculoid diatoms were stimulated by P, whereas Fairchild *et al.* (1985) found *G. tenellum* to increase only in nitrate treatments and naviculoid diatoms in nitrate plus P. Increased biovolume-density of these taxa and *Nitzschia* in this experiment may indicate that sufficient nitrate was available and that P was limiting.

Increased biovolume-density and number of protozoan species in 0.01 M P is especially interesting since biovolume-density was determined for only 8 protozoan taxa, all of which are generally considered bacterivorous (Pratt & Cairns, 1985). Protozoan communities respond positively to low levels of nutrient addition (Cairns *et al.*, 1979; Henebry *et al.*, 1984), although this response is generally attributed to changes in populations of photosynthetic species.

3.4.2 Zinc

Zinc concentrations measured at the beginning of the laboratory exposure were above 0.047 mg $Zn \bullet l^{-1}$ which is the criterion of the U.S. Environmental Protection Agency for the 24-hour average of total recoverable Zn (Environmental Criterion and Assessment Office, 1980). Zinc levels in 0.1 M P were below this level after the 1-week laboratory exposure. This suggests that all communities should have been stressed and that increased growth at high P may have been due to elimination of Zn stress by abundant P. However, none of these algal or protozoan taxa had reduced biovolume-density to high Zn, even though the total number of protozoan species decreased. Since the control had Zn levels as high as the four lowest Zn treatments, the background concentration of Zn may have been high or the flower pots may have leached a small amount of Zn.

High Zn levels increased biovolume-density of *Stigeoclonium nanum* filaments. *S. tenue*, closely related to *S. nanum*, was observed by Whitton (1970) to show intermediate resistance to Zn stress and potentially able to thrive when Zn levels (and copper or lead) are too high for more sensitive taxa.

Protozoan communities demonstrate dose responses analogous to those from single species tests (Ruthven & Cairns, 1973; Niederlehner *et al.*, 1985). The reduced number of species in 10 mg $Zn \bullet l^{-1}$ (control P) is consistent with results of Ruthven & Cairns (1973) who found protozoan communities to be adversely affected by 5-10 mg $Zn \bullet l^{-1}$ in 72 hrs, number of species after colonization to be reduced 80-85% in 6-9 days, and individual taxa to be sensitive to concentrations as low as 0.56 mg $Zn \bullet l^{-1}$ for acute exposures. Evaluation of only 8 major ciliate taxa may have restricted analyses to those species having broad tolerance to experimental conditions.

3.4.3 Phosphate and zinc interaction

Zinc and P interacted to increase biovolume-density of many taxa. Interpretation of interaction is complicated by many factors that could lead to it. For instance, (a) Zn and P could be needed simultaneously to stimulate growth; (b) one of them could have affected another taxon to produce or exude a vitamin (for example) that could stimulate growth; (c) competitive or predatory interactions may have lead to a decrease in one taxon which was compensated by an increase in another; and (d) Zn may have become bound to the clay flower pot, or excess phosphate may have precipitated Zn out of solution, to release organisms from Zn stress. Based on leaching rates measured by Fairchild *et al.* (1985), concentrations of P probably reached 2 mM in 0.1 M P and 0.2 mM in the 0.01 M P treatment. This implies that on a molar basis there was at least 10 times as much P as Zn in microcosms with P treated substrates.

Although *Stigeoclonium nanum* and *Nitzschia* spp. were the only algae to respond to combinations of Zn and P, the diatoms *Achnanthes minutissima*, *G. tenellum*, naviculoids, and *Synedra* spp. appear to have high biovolume-density only in high P-high Zn treatments (Fig. 3-1). In-

creased total algal biovolume-density suggests that these non-significant trends accumulated enough biovolume for a significant community response. Naviculoid diatoms and *S. tenue* were stimulated by the combination of PO_4 and NO_3 in Douglas Lake (Fairchild *et al.*, 1985) which suggests that Zn may also be able to stimulate growth of these taxa. The response of *A. minutissima*, *G. tenellum*, *Nitzschia*, and *Synedra* to combinations of P and Zn is not well known.

Although two protozoan taxa responded positively to Zn, it was clear that much potential Zn stress was ameliorated in P treatments. The total number of species increased in high Zn concentrations for 0.1 M P. This suggests that elevated P levels in the highest P treatment altered the expected dose-response relationship. The ability of communities from enriched ecosystems to resist poisonous effects has been previously demonstrated using acute tests of community response to much higher doses of poisonous material than used in this experiment (Hart & Cairns, 1984), although increased resistance to displacement is expected for systems with greater biomass. It seems obvious that the effective Zn concentration in experimental systems was much less than the apparent, dissolved concentration. Nevertheless, this experiment indicates that P levels can markedly affect the poisonous action of Zn, even at comparatively high Zn concentrations.

It seems clear that P and Zn significantly increased abundance of some taxa and that P and Zn interacted to increase biovolume-density of four taxa much higher than what would have been expected had individual effects been added.

4.0 Attached-algal abundance altered by individual and combined treatments of zinc and snail grazing

4.1 Introduction

Attached-algal communities are useful for identifying different levels of anthropogenic stress. For example, taxonomic composition can be used to identify different levels of zinc (Zn) stress (Chapter 2). and to differentiate between individual and combined treatments of phosphate and nitrate (Fairchild *et al.*, 1985), phosphate and Zn (Chapter 3), or Zn and pH (Chapter 5). In natural environments it is difficult to isolate effects of a poison, from effects that the poison has on the effects of grazing by primary consumers (snails, protozoa, aquatic insects), on taxonomic composition of attached algae. Hynes (1960:80-82) gives an example of how the recovery of algal communities may be influenced by decreasing pollutant concentration as well as recovery of the community of primary consumers. This example comes from samples collected at different sites along a river and, as a consequence of this, lacks experimental control for demonstrating cause-and-effect. Stream mesocosms provide a means for replication and control while maintaining a certain degree of environmental realism (Odum, 1984).

Attached-algal communities were employed to test individual and combined effects of Zn and snails in stream mesocosms continuously supplied with natural water from the New River, Virginia, USA. The experiment was designed to determine whether algal community composition and abundance would be altered by (a) 0.5 mg $Zn \bullet l^{-1}$, (b) 400 snails m^{-2}, or (c) the combination of 0.5 mg $Zn \bullet l^{-1}$ and 400 snails m^{-2}.

4.2 Materials and methods

4.2.1 Mesocosm operation

The experiment occurred along the New River at the Appalachian Power Company's Glen Lyn Plant in Giles County, Virginia, U.S.A.. Stream mesocosms were constructed from plywood and painted with a non-toxic chemical resistant paint (Farris, 1986). Plastic paddle wheels maintained a current of approximately 14 cm sec^{-1}. Natural river water was continuously supplied at 1.2 l min^{-1} to each mesocosm by a pump submersed in the New River. Peristaltic pumps delivered concentrated solutions of Zn (as $ZnSO_4$) from separate carboys for each mesocosm. Zn concentrations were calculated to deliver enough Zn to maintain 0.5 mg $Zn \bullet l^{-1}$. Snail (*Mudalia dilatata*) density of 400 snails m^{-2} was used based on the findings of Peters (1983) for the New River. Three replicates per treatment and four treatment combinations of snails (present, absent) and Zn (ambient, 0.5 mg $Zn \bullet l^{-1}$) resulted in a total of 12 stream mesocosms.

4.2.2 Sampling

Periphyton (heterotrophic and autotrophic microbial community) communities colonized stream mesocosms for 12 d before Zn and snail treatment. Sampling occurred when treatment began (day 0) and on days 2, 5, 10, 20, and 30. Vertical glass rods (5.38 cm^2) were used as an artificial substrate for periphyton. At least three randomly selected artificial substrates were pooled to form one sample per stream. Glass rods were scraped with a razor blade and rinsed with distilled water. The resulting sample was homogenized for 10 s in a blender to shorten algal filaments. An 18 ml sample was drawn from this slurry and placed in a vial containing 2 ml of 37% formaldehyde. Algae that were alive (containing chloroplasts) at the time of collection were enumerated using a Palmer-Maloney plankton counting chamber under 400x total magnification. Diatom identifications were confirmed by examining cleaned specimens at 1000x total magnification (Van Heurck, 1880-1881; Hustedt, 1930; Hustedt 1933; Patrick & Reimer, 1966, 1975; Lange-Bertalot, 1980). Counting proceeded by making one pass through the longest axis of the counting chamber and stopped when the 500th organism was counted. If less than 500 organisms were observed then another sample was prepared and counted in the same manner. Dense samples of algae were diluted (to 1/2, 1/4, or 1/8) by adding distilled water to 5 drops of sample.

4.2.3 Biovolume-density

Biovolume (μm^3) was estimated by taking direct measurements of dominant taxa and using mensuration formulas that approximate the geometric shape of each taxon (Beyer, 1981). Algal density ($cells \bullet cm^{-2}$) was estimated using the standard method (American Public Health Association [APHA], 1981). This resulting biovolume-density allows easy comparison of dominance between different taxa. Total biovolume-density was computed by summing dominant taxa observed in a sample.

4.2.4 Abiotic variables

Temperature, filtered Zn (0.45 μm pore size membrane filter), and total Zn were determined for each mesocosm on all sampling days. Zinc samples were acidified with 0.1 ml instra-analyzed nitric acid and digested using the following method.

Periphyton were processed for Zn analysis by obtaining the dry weight from 5.0 ml of slurry filtered on to a 0.45 μm pore-size metricel membrane filter (Valdes *et al.*, 1982). This sample and water Zn samples were placed overnight in a 20 ml vial with 3.0 ml instra-analyzed nitric acid and then heated for one-half hour at a temperature just below boiling. After cooling, 200 μl 30% hydrogen peroxide was added and the sample heated for 10 min. This solution was cooled and diluted to 20 ml with deionized distilled water. Zn concentration was measured using the direct aspiration method for atomic absorption spectrometry (United States Environmental Protection Agency [USEPA], 1979).

4.2.5 Statistics

Statistical analysis was performed on taxa with an average of more than five cells counted per treatment on days sampled. The experiment was analyzed statistically as a repeated measures or split-plot (without replication or blocking) design (Milliken & Johnson, 1984:323). The between subject or whole plot factors are Zn and snails and the within subject or subplot factor is day of sampling. This design provided testing for effects of Zn alone, snail grazing alone, and an interaction between Zn and snail grazing. Algal biovolume-density and Zn bound to periphyton was $\log_{10}$ transformed before statistical analysis because variances were correlated with means (Sokal & Rohlf, 1981). Differences are considered moderately significant if $0.01 < P < 0.06$, significant if $0.001 < P \leq 0.01$, and highly significant if $P \leq 0.001$.

4.3 Results

4.3.1 Algal response to snails

Biovolume-density of five diatom taxa was reduced by snail grazing (Fig. 4-1, Table 4-1). Snail grazing reduced biovolume-density of *Melosira varians* C.A. Ag. to 32% of control levels on day 5 ($P = 0.022$). Snail grazing reduced biovolume-density of *Navicula cryptocephala* var. *intermedia* Grun. to 24% on day 5 and to 5% on day 10 ($P = 0.001$), of naviculiod diatoms to 30% on day 5 and to 7% on day 10 ($P = 0.013$), of *Nitzschia palea* (Kutz.) W. Smith to 19% on day 5 and to 12% on day 10 ($P = 0.012$), and of *Nitzschia* sp. 2 to 23% on day 5 and to 14% on day 10 ($P = 0.015$). *Nitzschia* sp. 2 was composed of approximately 15% *N. palea* var. *debilis* (Kutz.) Grun. and 80% *N. romana* Grun.. On day 30 the biovolume-density of *N. cryptocephala* var. *intermedia* was reduced to 34% of control levels by snail treatment.

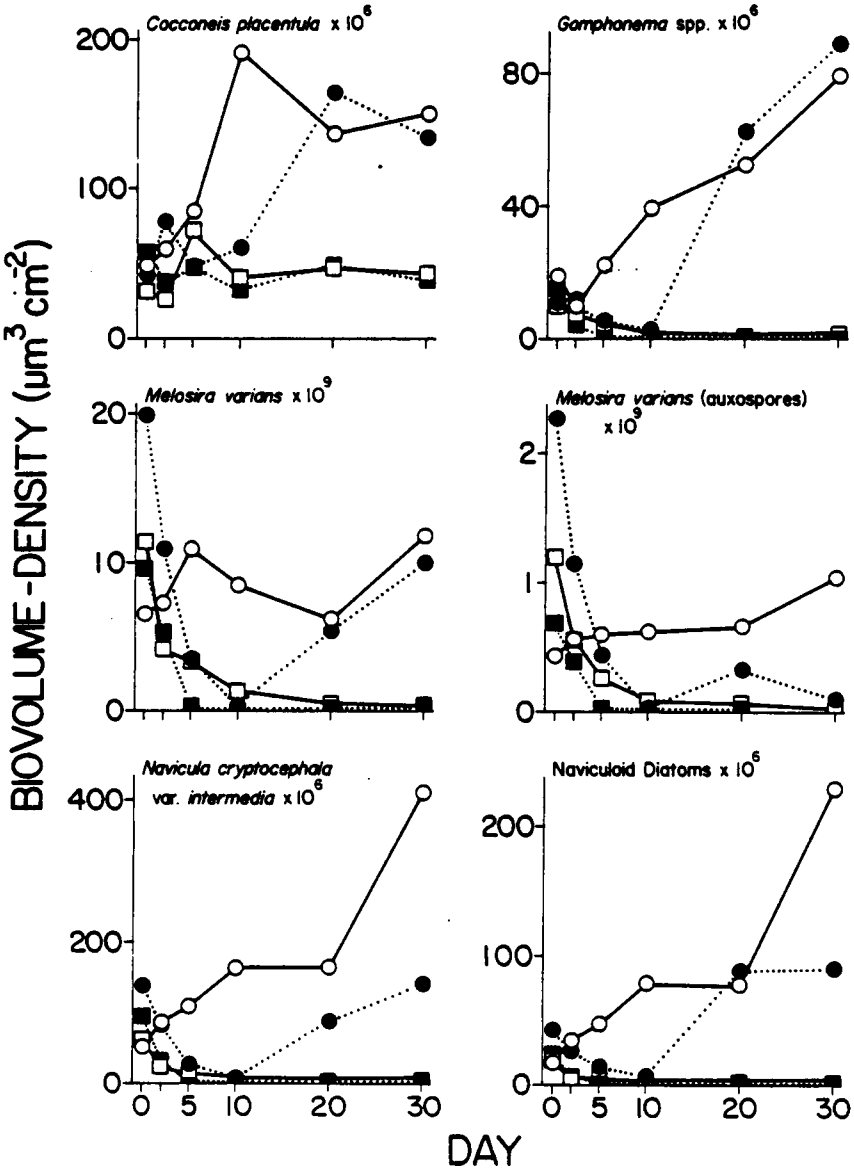
The attached-algal community recovered from snail grazing on day 20. *M. varians* and *Nitzschia* sp. 2 increased to control levels, but *Nitzschia palea* increased to be 2.5 times higher in snail treatment over control, and *N. cryptocephala* var. *intermedia* increased to only 44% and naviculoid diatoms increased to only 40% of control levels.

Snail grazing reduced algal total biovolume-density, relative to control levels to 32% on day 5 and to 5% on day 10 ($P = 0.019$). Total algal biovolume-density increased in snail treatment to control levels on day 20 and then decreased to 77% of control levels on day 30.

4.3.2 Algal response to zinc

Seven diatoms and a coccoid green-alga had biovolume-density reduced by 0.05 mg $Zn \bullet l^{-1}$(Fig. 4-1, Table 4-1). Reduced to less than 5% of control abundances by Zn from days

Figure 4-1. Mean (n = 3) biovolume-density ($\mu m^3\ cm^{-2}$) of 10 dominant algal taxa on days sampled, and Zn concentration of periphyton ($\mu g\ Zn \bullet g^{-1}$ dry weight periphyton). Symbols represent snail and Zn treatments: ○ = control, ● = 400 snails m^{-2}, □ = 0.5 mg $Zn \bullet l^{-1}$, ■ = 400 snails m^{-2} and 0.5 mg $Zn \bullet l^{-1}$. *Melosira varians* is graphed as the filamentous portion on the left and auxospores on the right.


Cocconeis placentula x 10^6
Gomphonema spp. x 10^6
Melosira varians x 10^9
Melosira varians (auxospores) x 10^9
Navicula cryptocephala var. intermedia x 10^6
Naviculoid Diatoms x 10^6
BIOVOLUME-DENSITY (µm^3 cm^-2)
DAY
0 5 10 20 30

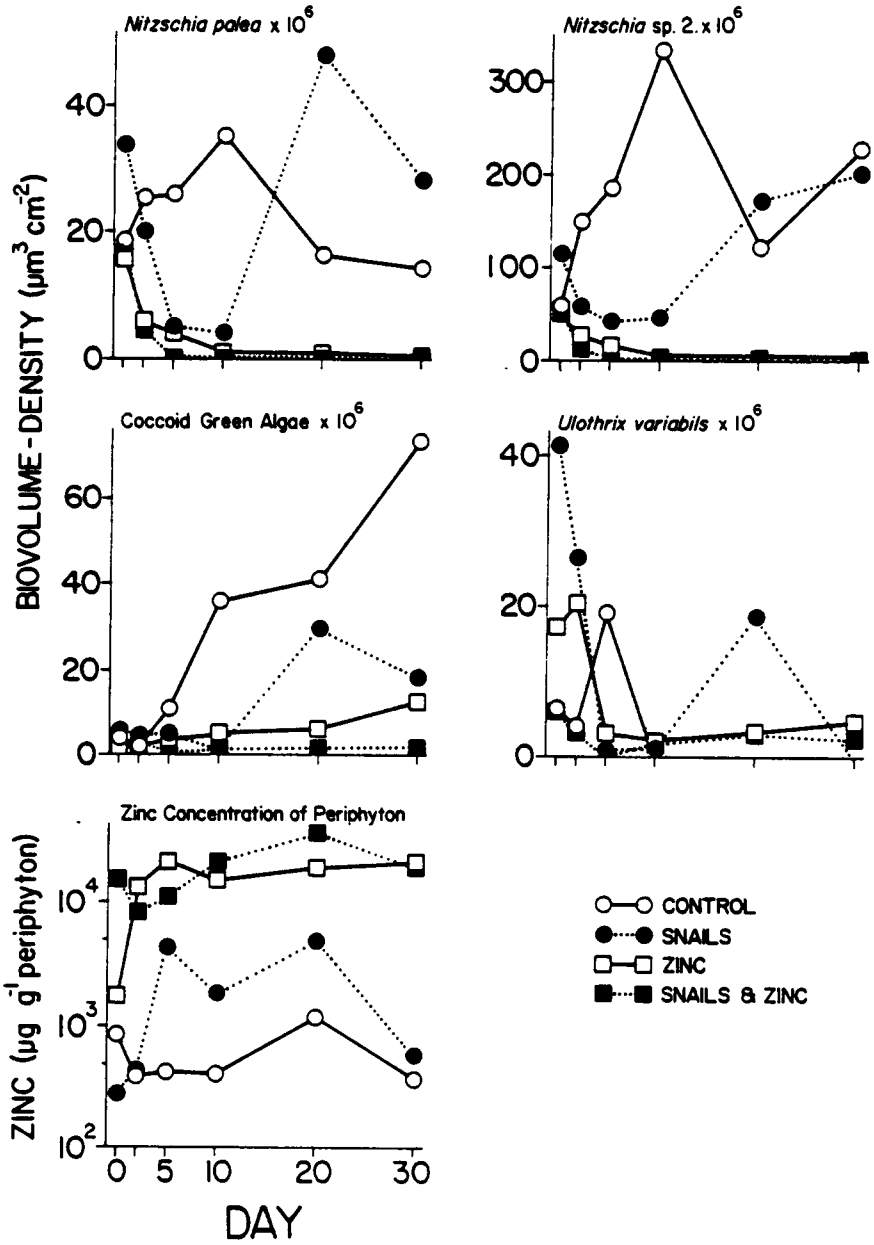

Nitzschia palea x 10^6
Nitzschia sp. 2. x 10^6
Coccoid Green Algae x 10^6
Ulothrix variabils x 10^6
BIOVOLUME-DENSITY (µm^3 cm^-2)
Zinc Concentration of Periphyton
ZINC (µg g^-1 periphyton)
DAY
CONTROL
SNAILS
ZINC
SNAILS & ZINC

Table 4-1. Differences[1] in mean biovolume-density within Zn (n = 6) and snail (n = 6) treatments[2] for dominant algal taxa. Asterisks indicate significant interaction between Zn and snail treatments.

Taxon	Day 0	2	5	10	20	30
Cocconeis placentula				C Z	C Z	C Z
Gomphonema spp.				C Z	C Z	C Z
Melosira varians			C Z N S		C Z	C Z
M. varians (auxospores)			C Z			
Navicula cryptocephala var. *intermedia*		C Z	C Z N S	*	C Z	C Z N S
naviculoid diatoms		C Z	C Z N S	C Z N S	C Z	C Z
Nitzschia palea		C Z	C Z N S	C Z N S	C Z	C Z
Nitzschia sp. 2		C Z	C Z N S	C Z N S	C Z	C Z
coccoid green algae					C Z	
total biovolume-density			C Z N S	N S	C Z	C Z

[1] larger means are to the left differ significantly at $\alpha = .01$
[2] C = ambient Zn, Z = 0.5 mg Zn•l^{-1}, S = snails, N = no snails

2 through 30 were *N. cryptocephala* var. *intermedia* ($P < 0.0001$), naviculoid diatoms ($P < 0.0001$), *N. palea* ($P < 0.0001$), and *Nitzschia* sp. 2 ($P < 0.0001$). Reduced to less than 5% of control abundances by Zn from days 5 through 30 was *M. varians* ($P < 0.0001$) with the auxospore abundance moderately significantly reduced on day 5 ($P = 0.061$). From days 10 through 30, *Cocconeis placentula* Ehr. was reduced by Zn to 30% of control levels ($P < 0.0001$), and *Gomphonema* spp. was reduced by Zn to less than 5% of control levels ($P = 0.0002$). *Gomphonema* spp. was composed of approximately 80% *G. parvulum* Kutz. and 20% *G. tenellum* Kutz.. Coccoid green algae showed a moderately significant reduction by Zn to 15% of control biovolume-density on day 20 ($P = 0.059$).

Algal total biovolume-density was reduced to less than 5% of control levels by Zn from days 5 through 30 ($P < 0.0001$).

4.3.3 Algal response to zinc and snail interaction

Biovolume-density of three diatoms was altered by a significant interaction. *Nitzschia palea* had a moderately significant interaction between snail and Zn treatments ($P = 0.056$) as a consequence of abundance on days 5 and 10 being lower in snail than in control treatments, and abundance on days 20 and 30 being higher in snail than in control treatments. The diatoms *Navicula cryptocephala* var. *intermedia* ($P = 0.009$) and naviculiods ($P = 0.024$) showed a significant response to a three-way interaction among snail, Zn, and sampling day. This was a consequence of biovolume being reduced on days 5 and 10 and then only partially recovering by day 30.

Algal total biovolume-density was moderately affected by a Zn, snail, day interaction ($P = 0.048$) due to the variable effect of snails over time.

4.3.4 Other taxa

The filamentous green alga *Ulothrix variabilis* Kutz. was abundant but did not differ significantly in biovolume-density between treatments (Fig. 4-1). Other taxa of the 65 enumerated were of too low abundance for reliable statistical analysis.

4.3.5 Abiotoc variables

Concentration of Zn in the water differed significantly ($P = 0.001$) between control and 0.5 mg $Zn \bullet l^{-1}$ treatments from days 2 through 30. Zinc concentrations did not differ significantly between filtered and unfiltered samples. Mean Zn concentration in the control treatment was 0.049 ± 0.015 (95% C.I.) from days 5 through 30. Mean Zn concentration in 0.5 mg $Zn \bullet l^{-1}$ treatment was 0.56 ± 0.10 for days 5 and 10, 48.4 on day 13, 6.9 on day 16, 1.46 on day 20, and 0.54 on day 30. High Zn concentrations on days 13, 16, and 20 were a consequence of a malfunction of the water supply system on day 13 which caused Zn concentrations to reach extremely high levels.

The Zn concentration of periphyton was 28 times higher in Zn treatment over control ($P < 0.0001$) (Fig. 4-1). Zinc concentration of periphyton increased to approximately 18,000 $\mu g\ Zn \bullet g^{-1}$ periphyton dry weight in 0.5 mg $Zn \bullet l^{-1}$ treatment, and it was approximately 640 $\mu g\ Zn \bullet g^{-1}$ periphyton dry weight in ambient Zn treatment.

Mean temperature was 19 C from day 0 to day 5 and steadily decreased to 14 C on day 10, 10 C on day 20, and 6 C on day 30.

4.4 *Discussion*

4.4.1 Algal response to snails

Reduced biovolume-density in snail treatments by day 10 is attributed to grazing by snails (Lamberti & Moore, 1984). Increased algal biovolume-density in snail treatment on days 20 and 30 suggest that snail grazing rate decreased. Cold temperatures during the last 20 days of the experiment would most likely reduce snail grazing by making snails sluggish from reduced metabolic rates or by reducing the need of food for maintenance. Alimov's (1975) data show that for bivalve molluscs, relative to metabolism at 20 C, metabolic rates will be reduced to 80% at 15 C, to 40% at 10 C, and to 25% at 5 C. Reduced biovolume-density of *Navicula cryptocephala* var. *intermedia* on day 30 may be due to reduced growth rate of this diatom or to selective feeding by the snail. Calow (1973) suggests that starved snails indiscriminately select food but that satiated snails will select particular algal taxa. We found no preference by snails for particular diatom taxa on days 5 and 10.

Although not statistically significant, the biovolume-density of *Cocconeis placentula* and *Gomphonema* appear to be reduced by snails on day 5. Hunter (1980) found that snails did not significantly reduce populations of *Cocconeis* in a shallow pond. The smooth surface of the glass rod substrate may have made it easier for the snail to remove *Cocconeis* in this experiment. Calow (1973) found snails to prefer diatoms, especially *Gomphonema*, in field and laboratory experiments.

4.4.2 Algal response to zinc

The 0.5 mg $Zn \bullet l^{-1}$ treatment was very poisonous to most algal taxa. In an experiment performed with these stream mesocosms one month earlier, we found *Ulothrix variabilis* and coccoid

green algae to have significantly higher populations in 0.5 mg $Zn \bullet l^{-1}$ over the control by day 10 (Chapter 2). However, no algae replaced diatoms eliminated in the present experiment by day 10. All of the diatom taxa in this experiment had reduced biovolume-density in 0.5 mg $Zn \bullet l^{-1}$ in the experiment performed one month earlier (Chapter 2). High Zn concentration resulting form system malfunction on day 13 may have delayed the colonization of algae in Zn treated mesocosms during later sampling days, or cold temperatures and low light levels may have provided a climate unsuitable for other taxa. These different responses by attached algae in contiguous months demonstrate the importance of including a seasonal component in bioassays.

Algal total biovolume-density often recovered to control levels from Zn stress in previous experiments with these mesocosms (Chapter 2). Lack of recovery in the present experiment is due to the inability of new algae to replace diatoms elimated by Zn.

4.4.3 Interaction between snail and zinc treatments

Significant ANOVA interaction terms indicate a changing relationship of algal biovolume-density between snail and Zn treatments. Because Zn stress resulted in low algal biovolume-density which remained constant throughout this experiment, the significant interaction term may be due to consistent differences between no-snail and snail treatments relative to Zn treatments (no-snail and snail). Significant interaction was evident for taxa that did not have biovolume-density in snail treatment equal to control levels on days 20 and 30. *Navicula cryptocephala* var. *intermedia* and naviculoid diatoms showed partial recovery in snail treatment, and biovolume-density of *Nitzschia palea* in snail treatment surpassed control levels. The other four diatom taxa did not show interaction and had very similar biovolume-density between control and snail treatments on days 20 and 30. These significant interaction terms provide evidence that *N. cryptocephala* var. *intermedia*, naviculoid diatoms, and *N. palea* had abundances in snail treatment that differed significantly from abundances in control treatment. It is unclear whether these differences are due to reduced growth

rates of the algae or to selective feeding by the snail. It is unclear why significant interactions were not evident for all diatoms on day 10.

In general, algal abundancae was reduced slightly (but not significant statistically) more by Zn than by snails. The combination of snails and Zn caused a greater reduction in biovolume-density than either variable alone.

4.4.4 Conclusions

The effect of snails on attached-algae can range from very intense to nonexistent depending on the time of year. Temperatures below 15 C may play an important role by inhibiting snail effect. During cold conditions, snail grazing may have its greatest effect on *Navicula cryptocephala var. intermedia* and naviculoid diatoms. *Nitzschia palea* may respond positively to temperatures below 15 C and snail grazing. Treatment of 0.5 mg $Zn \bullet l^{-1}$ was highly poisonous for the 30 day October-November, 1984, experiment. No taxa replaced diatoms lost, as was observed in previous experiments with these stream mesocosms.

5.0 Attached-algal abundance altered by individual and combined treatments of zinc and pH

5.1 Introduction

It is unclear whether elevated metal concentrations or altered pH is most responsible for a change in community composition. As an example, Charles (1985) found that euplanktonic diatoms were not found, or were very rare, in acidic lakes with total aluminum (Al) > 0.060 mg l^{-1} and suggested that Al may be an important pH-related variable that may have poisonous effects independent of pH stress. This was confirmed by Pillsbury & Kingston (personal communication). Similar processes may occur for zinc (Zn) and pH. Communities of attached-algae contain many taxa with individual tolerance to anthropogenic stress. Taxonomic composition of attached-algal communities is useful for identifying elevatated concentrations of Zn or altered pH. Concentrations of Zn could be identified by the algal taxa with higher relative abundance in stream mesocosms (Chapter 2). Mesocosms are confined experimental systems that are continuous with the natural environment (Odum, 1984). Algal communities are also useful for identifying acidic pH stress (Patrick *et al.*, 1968; Muller, 1980; Haines, 1981; Stokes, 1984; Dillon *et al.*, 1984). These

examples demonstrate that communities become altered from individual treatments of Zn or pH, but the effects when adverse pH and elevated Zn occur simultaneously are not well known. Schindler *et al.* (1980b) found unusually high concentrations of Zn in acidic lakes, and Say & Whitton (1977b) and Hargreaves & Whitton (1977) have shown Zn to be more poisonous at pH levels outside the natural range for the green filamentous alga *Hormidium rivulare*.

Zinc is a common anthropogenic waste, so the U. S. Environmental Protection Agency (USEPA) has set a criterion level of 0.047 mg l^{-1} as a 24 h average of total recoverable Zn (Environmental Criteria and Assessment Office, 1980). Altered pH is often from anthropogenic sources with acidic pH transported by precipitation or snowmelt (Haines, 1980; Dillon *et al.*, 1984), and alkaline pH may be attributed to accidental spills (*e.g.*, Crossman & Cairns, 1975). The USEPA effluent standard for pH generally requires that discharge water fall in the range of pH 6 and 9 (Federal Register, 1982). These levels for Zn and pH are guidelines which may be overprotective or underprotective depending on the ability of the ecosystem to assimilate wastes (Cairns, 1977b).

Attached-algal communities were employed to test the USEPA guidelines for Zn and pH by using stream mesocosms continuously supplied with natural water from the New River, Virginia, USA. The experiment was designed to determine whether algal community composition and abundance would be altered by (a) pH 6 or 9, (b) 0.05 mg $Zn \bullet l^{-1}$, or (c) the combination of pH 6 or 9 and 0.05 mg $Zn \bullet l^{-1}$.

5.2 *Materials and methods*

5.2.1 Mesocosm operation

The experiment occurred along the New River at the Appalachian Power Company's Glen Lyn Plant in Giles County, Virginia, U.S.A.. Stream mesocosms were constructed from plywood

and painted with a non-toxic chemical resistant paint (Farris, 1986). Plastic paddle wheels maintained a current of approximately 14 cm sec^{-1}. A pump submersed in the New River continuously supplied natural river water at 1.2 l min^{-1} per mesocosm. Peristaltic pumps delivered concentrated solutions of Zn (as $ZnSO_4$) from separate 20-l carboys for each mesocosm. Zinc concentration in carboys was calculated to deliver enough Zn to maintain 0.05 mg $Zn \bullet l^{-1}$. Each mesocosm of a pH treatment had its own 20-l carboy of H_2SO_4 or NaOH delivered by a peristaltic pump activated by a Fischer model-650 pH meter/controller to continuously maintain a pH of 6 or 9. Two replicate mesocosms per treatment combination and six treatment combinations of pH (6, ambient, 9) and Zn (ambient, 0.05 mg $Zn \bullet l^{-1}$) resulted in a total of 12 stream mesocosms.

5.2.2 Sampling

Vertical glass rods (5.38 cm^2) were used as an artificial substrate for attached-algae. Algal communities colonized artificial substrates for 12 d before Zn and pH treatment began. Samples were collected shortly after treatment began on July 9, 1985 (day 0) and also on days 5, 10, 20, and 30 (August 8, 1985). Each mesocosm initially contained 65 snails and 85 clams, of which 6 of each were removed on days 0, 10, 20, and 30 for biochemical and bioaccumulation analysis; the result of this effort is reported in Farris (1986).

Three randomly selected artificial substrates were pooled to form one sample per mesocosm. Artificial substrates were scraped with a razor blade and rinsed with distilled water. The sample was then homogenized for 10 s in a blender to shorten algal filaments. An 18 ml sample was drawn from this slurry and placed in a vial containing 2 ml of 37% formaldehyde. Algae that were alive (containing chloroplasts) at the time of collection were enumerated using a Palmer-Maloney plankton counting chamber under 400x total magnification. Dense samples were diluted (to 1/2, 1/4, or 1/8) by adding distilled water to 5 drops of sample. Counts proceeded by scanning one transect through the longest axis of the counting chamber and stopped when the 500th organism was counted. If less than 500 organisms were counted then another sample was prepared and

counted in the same manner. Diatom identifications were confirmed by examining cleaned specimens at 1000x total magnification (Hustedt, 1930; Patrick & Reimer, 1966, 1975; Lange-Bertalot, 1980).

5.2.3 Biovolume-density

Biovolume (μm^3) was estimated using mensuration formulas that approximate the geometric shape of each taxon (Beyer, 1981). Direct measurements were made of dominant taxa. Algal density ($cells \bullet cm^{-2}$) was estimated using the standard method (American Public Health Association [APHA], 1981). The product of algal density and biovolume resulted in "biovolume-density" ($\mu m^3\ cm^{-1}$) to compare dominance between taxa. Total biovolume-density was computed by summing over dominant taxa.

5.2.4 Water chemistry

Alkalinity, ammonia, conductivity, hardness (as $CaCO_3$), and pH were measured in all mesocosms on day 0 (after dosing began) and on days 10, 20, and 30. Concentrations of these chemical variables were measured with the standard methods (APHA, 1981). Chloride, nitrate, phosphate, and sulfate concentrations were measured for each mesocosm on days 0 and 30 using ion exchange chromatography. Unfiltered samples and standards were measured on a Dionex System 10 Ion Chromatograph using a Dionex Ion Pac guard column, an HPIC AS3 separator column, and an ASC-2 supressor column. The eluent was 0.0024 M Na_2CO_3/0.0003 $NaHCO_3$ and separation was carried out using a flow rate of 3 ml min^{-1} with a 300 µl sample loop (USEPA, 1981; DIONEX, 1981). Samples were refrigerated and analyzed within 2 d. Total Zn was measured using the direct aspiration method for atomic absorption spectrometry (USEPA, 1979) on days 0, 10, 20, and 30. Concentration of the dissolved Zn ion was computed using chemical equilibrium

equations which included association constants for complexes of Zn-hydroxides, Zn-chlorides, and Zn-sulfates derived from Wagman *et al.* (1982).

5.2.5 Zinc concentration of periphyton

Periphyton (aufwuchs) was determined with a method modified from Valdes *et al.* (1982). The dry weight was measured from 5.0 ml of slurry filtered on to a 0.45 µm metricel membrane filter. This sample was placed overnight in a 20 ml vial with 3.0 ml instra-analyzed nitric acid and then heated for one-half hour at a temperature just below boiling. When the sample was cool, 200 µl 30% hydrogen peroxide was added and the sample heated for 10 min. This solution was cooled and diluted to 20 ml with deionized distilled water. Zinc concentration was measured using the direct aspiration method for atomic absorption spectrometry (USEPA, 1979).

5.2.6 Statistics

Statistical analysis was performed on taxa with an average of more than five cells counted per treatment on days sampled. The experiment was analyzed statistically as a repeated measures or split plot (without replication or blocking) design (Milliken & Johnson, 1984:323). The between subject or whole plot factors are Zn and pH and the within subject or subplot factor is day of sampling. This design provided a test for differences in algal biovolume-density due to Zn alone, pH alone, and an interaction between Zn and pH. Each individual day was analyzed separately and Duncan's New Multiple Range Test was used to determine if biovolume-density differed among treatments at a particular day. Biovolume-densities and Zn concentration of periphyton were $\log_{10}$ transformed before statistical analysis because variances were correlated with means (Sokal & Rohlf, 1981). Differences are considered significant if $P < 0.05$ and are considered moderately significant if $0.05 \leq P \leq 0.13$. More replications of mesocosms may have allowed more stringent

levels of significance. All 95% C.I. use the error mean square of the main factors (Lentner & Bishop, 1986).

5.3 Results

Zinc and pH exposures caused a change in algal community composition from diatoms and a filamentous blue-green alga to green algae, a coccoid blue-green alga, or new diatom taxa. Total algal biovolume-density was moderately increased by pH 6 treatment. Alkalinity, ammonia, and sulfate were affected by pH 6.

5.3.1 Algal response to pH

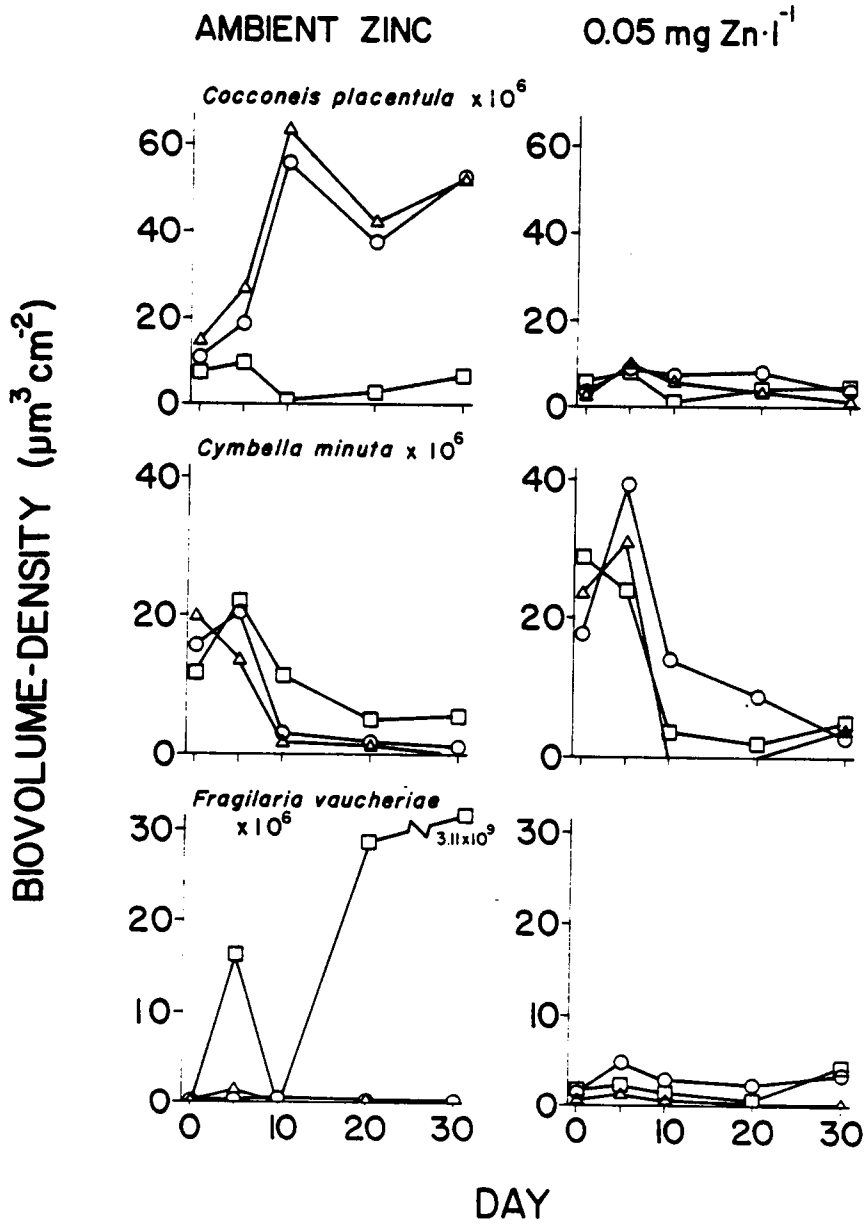
The abundance of four diatoms, one green alga, and one blue-green alga responded to pH treatments (Table 5-1, Fig. 5-1). Inhibited by pH 6 were the diatoms *Cocconeis placentula* Ehr. ($P = 0.057$) and *Nitzschia palea* var. *debilis* (Kutz.) Grun. ($P = 0.019$) and the filamentous blue-green alga *Phormidium* sp. ($P = 0.010$). The pH 6 treatment caused a moderate increase in biovolume-density of the diatoms *Cymbella minuta* Hilse *ex* Rabh. ($P = 0.063$) and *Gomphonema parvulum* Kutz. ($P = 0.130$) and the filamentous portion of the green alga *Stigeoclonium nanum* Kutz. ($P = 0.074$). The altered biovolume-density of these taxa by pH is independent of any Zn treatment effects.

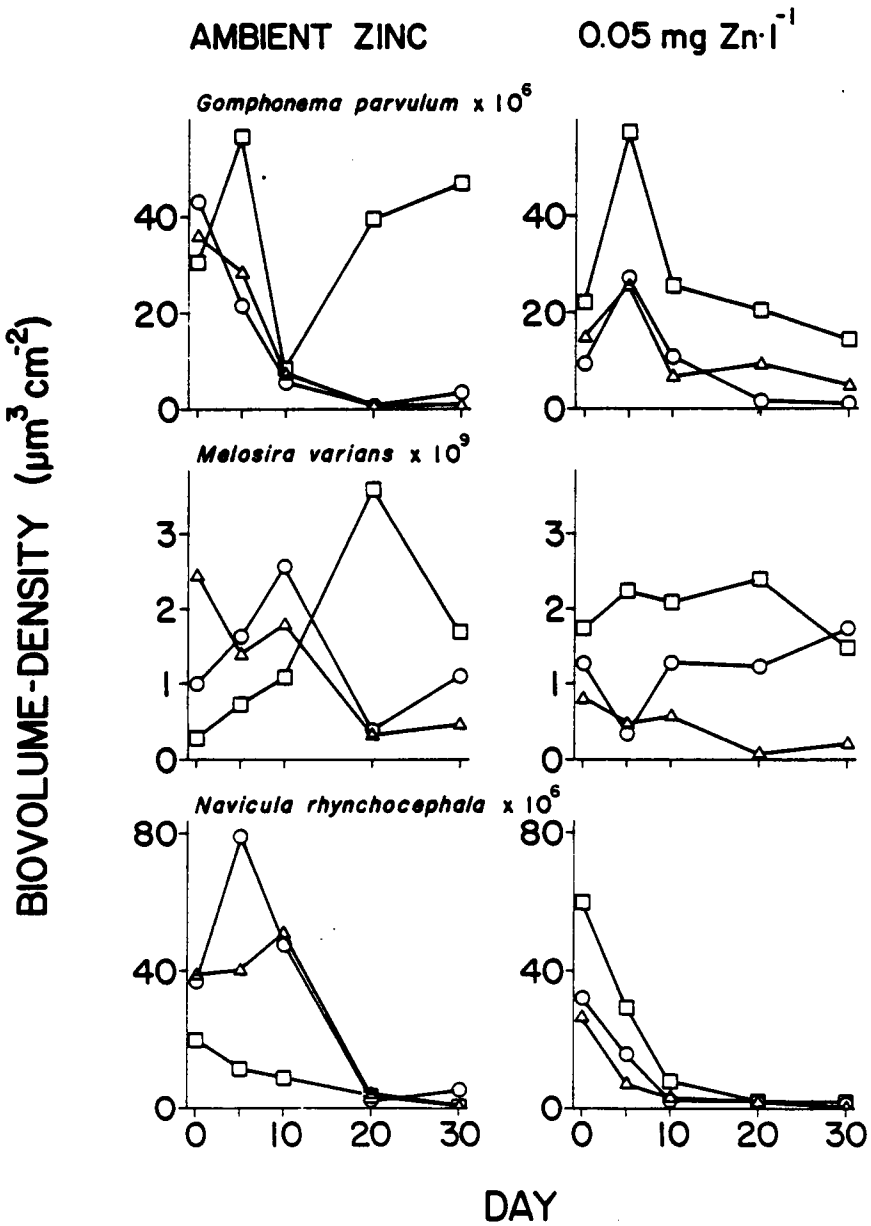
Table 5-1. Differences[1] in mean biovolume-density (μ^3 cm^{-1}) within Zn (n = 6) and pH (n = 4) treatments[2] for dominant algal taxa. Asterisks indicate significant interaction between Zn and pH treatments.

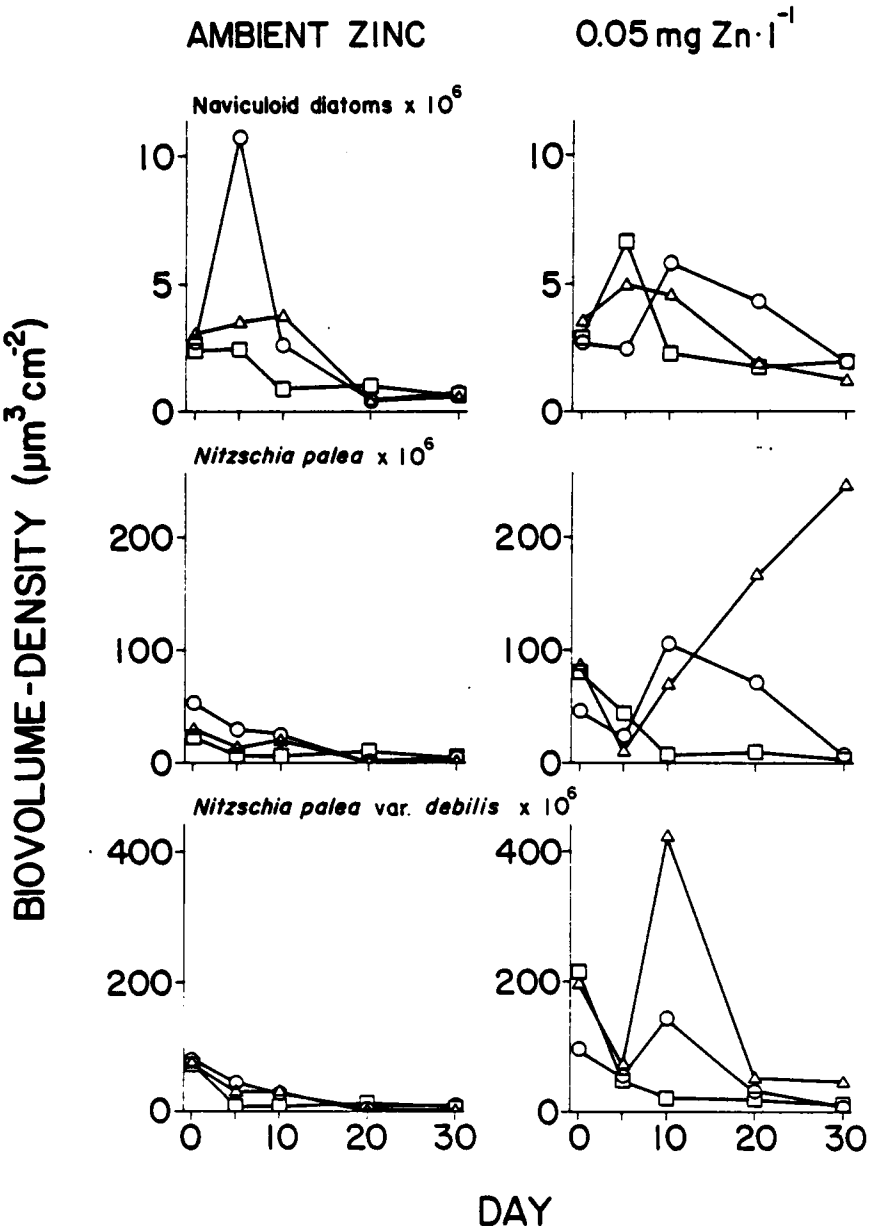
	Day				
Taxon	0	5	10	20	30
Cocconeis placentula					*
Cymbella minuta			6 A 9		
Gomphonema parvulum			6 9 A	*	6 A 9
Microcystis. incerta		6 A 9			
naviculoid diatoms			Z C	Z C	
Nitzschia palea				Z C	
Nitzschia palea var. *debilis*			9 A 6	*	
Phormidium sp.		A 9 6	A 9 6	Z C A 9 6	
Stigeoclonium nanum (prostrate)				Z C	
Stigeoclonium nanum (filament)		6 A 9		Z C 6 A 9	*
Ulothrix variabilis				*	

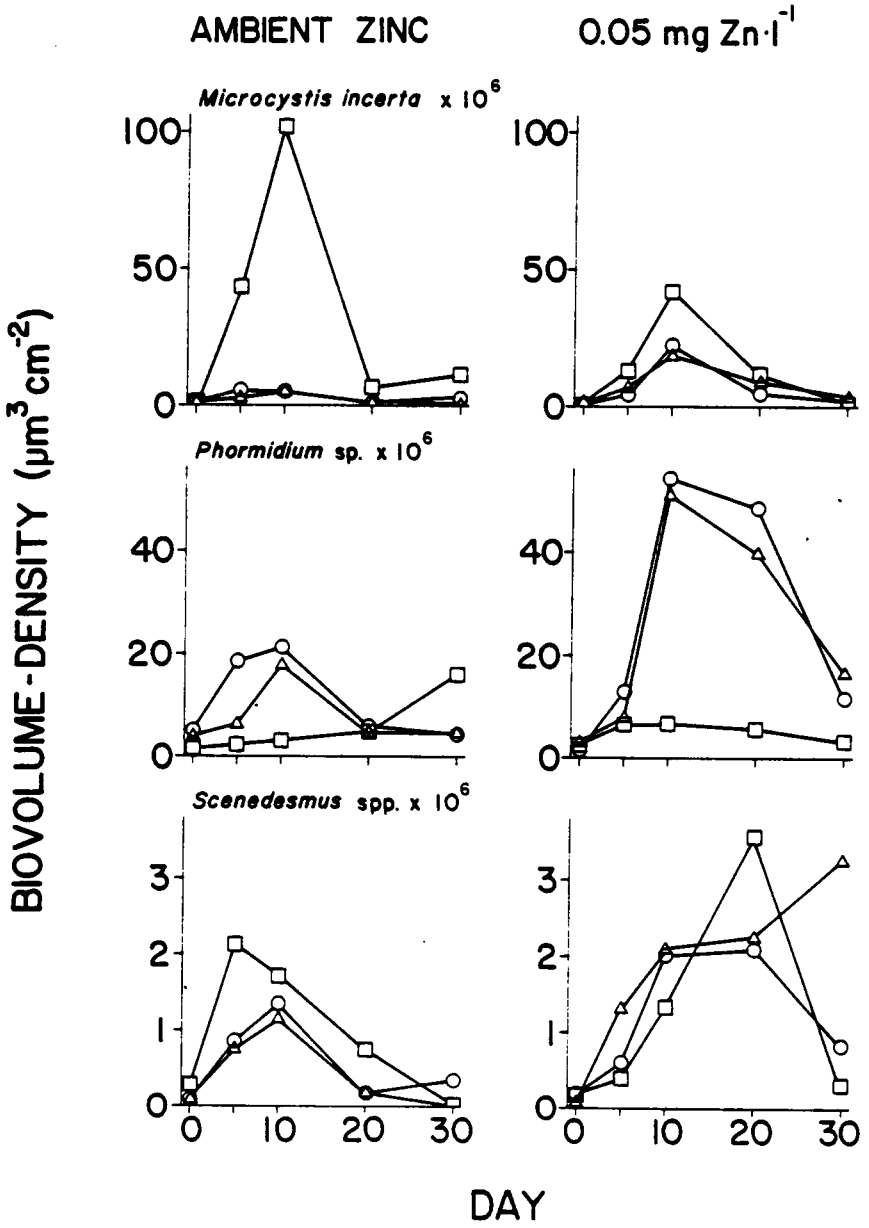
[1]larger means are to the left and are significantly different based on Duncan's Multiple Range Test and ANOVA at $\alpha = 0.05$.
[2]C = ambient Zn, Z = 0.05 mg $Zn \cdot l^{-1}$, 6 = pH 6, A = ambient pH, 9 = pH 9

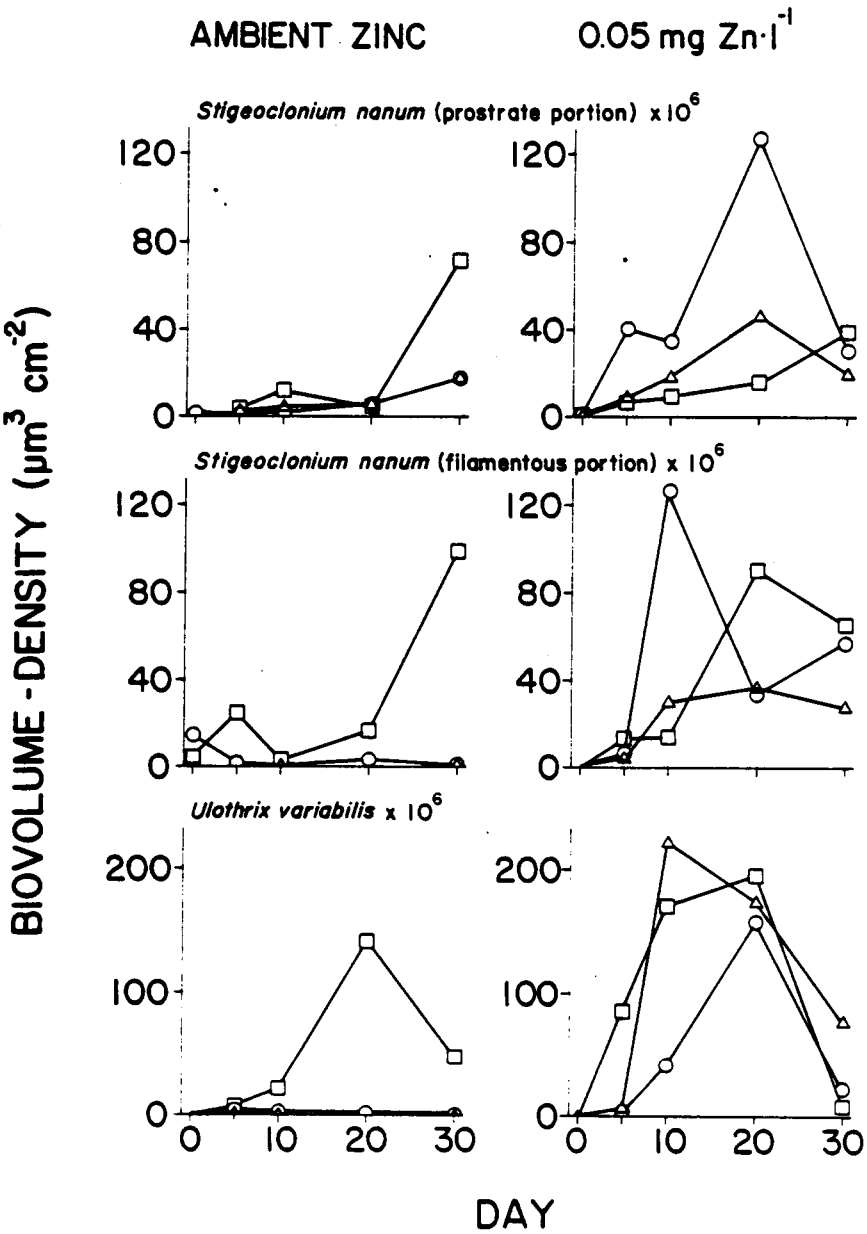
Figure 5-1. Mean (n = 2) biovolume-density ($\mu m^3\ cm^{-1}$) of algal taxa at each sampling day in ambient and 0.05 mg $Zn \bullet l^{-1}$ treated mesocosms for three pH treatments: □ = pH 6, ○ = ambient pH, and △ = pH 9.


AMBIENT ZINC
0.05 mg Zn·l⁻¹
Cocconeis placentula x 10⁶
Cymbella minuta x 10⁶
Fragilaria vaucheriae x 10⁶
3.11x10⁹
BIOVOLUME-DENSITY (µm³cm⁻²)
DAY
0
10
20
30
40
60


AMBIENT ZINC
0.05 mg Zn·l⁻¹
Gomphonema parvulum x 10⁶
Melosira varians x 10⁹
Navicula rhynchocephala x 10⁶
BIOVOLUME-DENSITY (µm³cm⁻²)
DAY
0
10
20
30
40
80
1
2
3


AMBIENT ZINC
0.05 mg Zn·l⁻¹
Naviculoid diatoms x 10⁶
Nitzschia palea x 10⁶
Nitzschia palea var. debilis x 10⁶
BIOVOLUME-DENSITY (µm³cm⁻²)
DAY
0
5
10
100
200
400
0
10
20
30


AMBIENT ZINC
0.05 mg Zn·l⁻¹
Microcystis incerta x 10⁶
Phormidium sp. x 10⁶
Scenedesmus spp. x 10⁶
BIOVOLUME-DENSITY (µm³cm⁻²)
DAY
100
50
0
40
20
3
2
1
0
10
20
30


AMBIENT ZINC
0.05 mg Zn·l-1
Stigeoclonium nanum (prostrate portion) x 10^6
Stigeoclonium nanum (filamentous portion) x 10^6
Ulothrix variabilis x 10^6
BIOVOLUME - DENSITY (µm3 cm-2)
120
80
40
0
200
100
0
10
20
30
DAY

5.3.2 Algal response to zinc

Of nine taxa that responded to Zn (Table 5-1, Fig. 5-1), 5 were diatoms, 3 were green algae, and 1 was a blue-green alga. Inhibited by 0.05 mg $Zn \bullet l^{-1}$ treatment were the diatoms *Cocconeis placentula* ($P = 0.089$) and *Navicula rhynchocephala* Kutz. ($P = 0.130$) to a moderate degree. Of the diatoms with increased biovolume-density due to 0.05 mg $Zn \bullet l^{-1}$ treatment, naviculoids were most significant on days 10 and 20 ($P = 0.009$), and *Nitzschia palea* (Kutz.) W. Smith ($P = 0.024$) and *N. palea* var. *debilis* ($P = 0.001$) differed most significantly on day 20. Of the green algae with increased biovolume-density due to added Zn, *Scenedesmus* spp. was most significant on day 20 ($P = 0.010$), the prostrate portion of *Stigeoclonium nanum* was most abundant on day 20 ($P = 0.059$), the filamentous portion of *S. nanum* was generally more abundant from days 5 through 30 ($P = 0.018$), and the filamentous alga *Ulothrix variabilis* Kutz. was generally more abundant from days 5 through 30 ($P = 0.031$). The biovolume-density of the filamentous blue-green alga *Phormidium* was most clearly increased by Zn treatment on day 20 ($P = 0.035$). These differences due to Zn are independent of any pH effects.

5.3.3 Algal response to pH and zinc interaction

Altered biovolume-density of four taxa (one diatom, two green algae, and one blue-green alga) was due to an interaction between pH and Zn. A significant interaction for *Cocconeis placentula* on day 30 resulted from biovolume-density being unchanged by Zn at pH 6 but being decreased by Zn at ambient pH and pH 9. Significant interaction for *Gomphonema parvulum* on day 20 resulted from biovolume-density being decreased by Zn at pH 6 and being increased by Zn at ambient pH and pH 9. Biovolume-density of the coccoid blue-green alga *Microcystis incerta* Lemm. increased in pH 6 treatment, but the 0.05 mg $Zn \bullet l^{-1}$ treatment inhibited this response ($P = 0.066$). A significant interaction for *M. incerta* on day 5 resulted from biovolume-density being slightly

decreased by Zn at pH 6 but slightly increased by Zn at ambient pH and pH 9. The biovolume-density of *N. palea* var. *debilis* was 2.5 times higher when pH 9 and 0.05 mg $Zn \bullet l^{-1}$ were combined than for the sum of individual effects of these two treatments on day 10 ($P = 0.077$). Significant interaction for *N. palea* var. *debilis* on day 20 resulted from biovolume-density being unchanged by Zn at pH 6 but being increased by Zn at ambient pH and pH 9. The increased abundance due to Zn was matched by increase due to pH 6 treatment on day 30 for the filamentous portion of *Stigeoclonium nanum* ($P = 0.069$) and *Ulothrix variabilis* ($P = 0.112$). Significant interaction for *S. nanum* on day 30 resulted from biovolume-density being unchanged by Zn at pH 6 but being increased by Zn at ambient pH and pH 9. Significant interaction for *U. variabilis* on day 20 resulted from biovolume-density being decreased by Zn at pH 6 but being increased by Zn at ambient pH and pH 9.

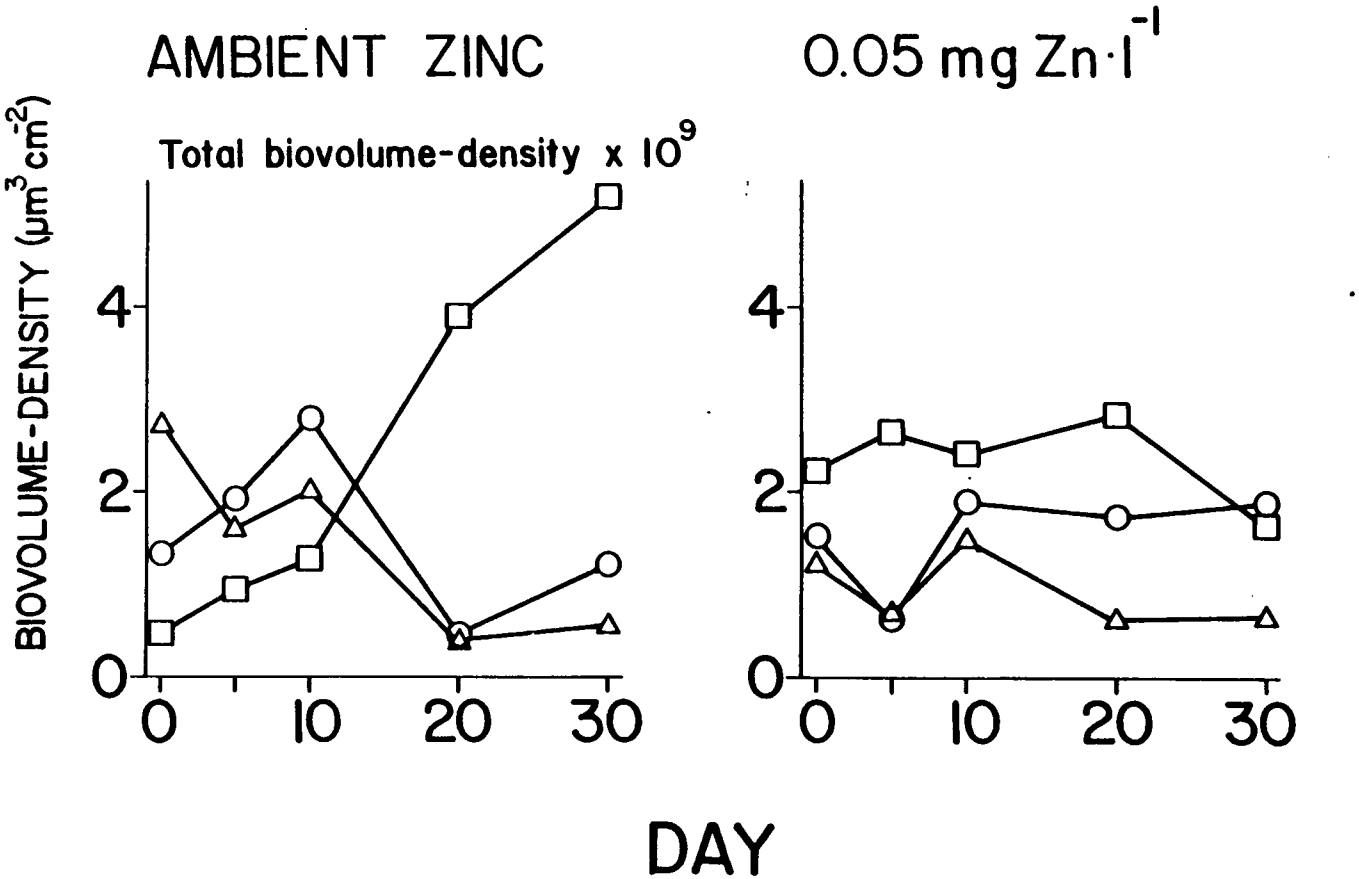
5.3.4 Other taxa

The diatoms *Fragilaria vaucheriae* (Kutz.) Peters. and *Melosira varians* C. A. Ag. were abundant but did not differ significantly in biovolume-density between treatments. Other taxa of the 61 enumerated were considered of too low abundance for reliable statistical analysis.

5.3.5 Total biovolume-density

The pH 6 treatments caused a moderate ($P = 0.103$) increase in total algal biovolume-density (Fig. 5-2). *Fragilaria vaucheriae* accounted for 60% of the biovolume on day 30 in pH 6 (ambient Zn) treatment. Total algal biovolume-density was not significantly altered by Zn or an interaction between Zn and pH.

Figure 5-2. Mean (n = 2) total biovolume-density ($\mu m^3\ cm^{-1}$) of algae.


AMBIENT ZINC
0.05 mg Zn·l-1
Total biovolume-density x 10^9
BIOVOLUME-DENSITY (µm3cm-2)
0
2
4
0
10
20
30
DAY

5.3.6 Water chemistry

Zinc and pH treatments did not significantly alter conductivity (139 ± 86.6 μohm), hardness (71.8 ± 8.10 mg $CaCO_3 \bullet l^{-1}$), nitrate (3.62 ± 2.63 mg l^{-1}), and chloride (4.48 ± 0.445 mg l^{-1}). Measured pH was lower ($P = 0.004$) in pH 6 treatments, but measured pH did not differ significantly between ambient and pH 9 treatments. The mean (± 1.94) measured pH from days 10 through 30 was 6.22 for the pH 6 treatment, 8.57 for the ambient pH treatment, and 9.01 for the pH 9 treatment. Phosphate concentrations were unable to be determined with the column and eluent used in the ion chromatograph.

Acidic pH altered alkalinity ($P = 0.005$), ammonia ($P = 0.040$), and sulfate ($P \leq 0.0001$). Mean alkalinity (± 195 mg $CaCO_3 \bullet l^{-1}$) from days 10 through 30 was 234 in pH 6 treatments, 427 in ambient pH, and 479 in pH 9 treatments. Ammonia (± 0.003 mg l^{-1}) was generally 0.0874 mg l^{-1} in all treatments, but ammonia increased to 0.299 in the pH 6 (ambient and 0.05 mg $Zn \bullet l^{-1}$) treatment on day 20 and returned to ambient levels by day 30. Sulfate was higher in pH 6 treatments than in ambient-pH and pH 9 treatments on days 0 and 30. Sulfate (± 1.79 mg l^{-1}) in pH 6 treatments was 23.9 on day 0 and 45.6 on day 30; ambient-pH and pH 9 treatments were 20.9 on day 0 and on 16.3 day 30. The only consistently measurable Zn concentrations were in the 0.05 mg $Zn \bullet l^{-1}$-pH 6 treaments. Concentrations were 0.034 mg $Zn \bullet l^{-1}$ (0.031 mg $Zn^{2+} \bullet l^{-1}$) on day 10, 0.04 (0.036 mg $Zn^{2+} \bullet l^{-1}$) on day 20, and 0.04 (0.036 mg $Zn^{2+} \bullet l^{-1}$) on day 30.

5.3.7 Zinc concentration of periphyton

The pH 6 treatment (Fig. 5-3) had substantially less Zn bound to periphyton than ambient or pH 9 treatments ($P \leq 0.0001$). Treatments of 0.05 mg $Zn \bullet l^{-1}$ (ambient-pH) had approximately six times as much Zn as ambient-Zn treatments from days 5 through 30 ($P \leq 0.0001$). A significant

Figure 5-3. Mean (n = 2) Zn concentration of periphyton (μg $Zn \bullet g^{-1}$ dry weight periphyton) in Zn and pH treatments:

□——□ = ambient Zn and pH 6,

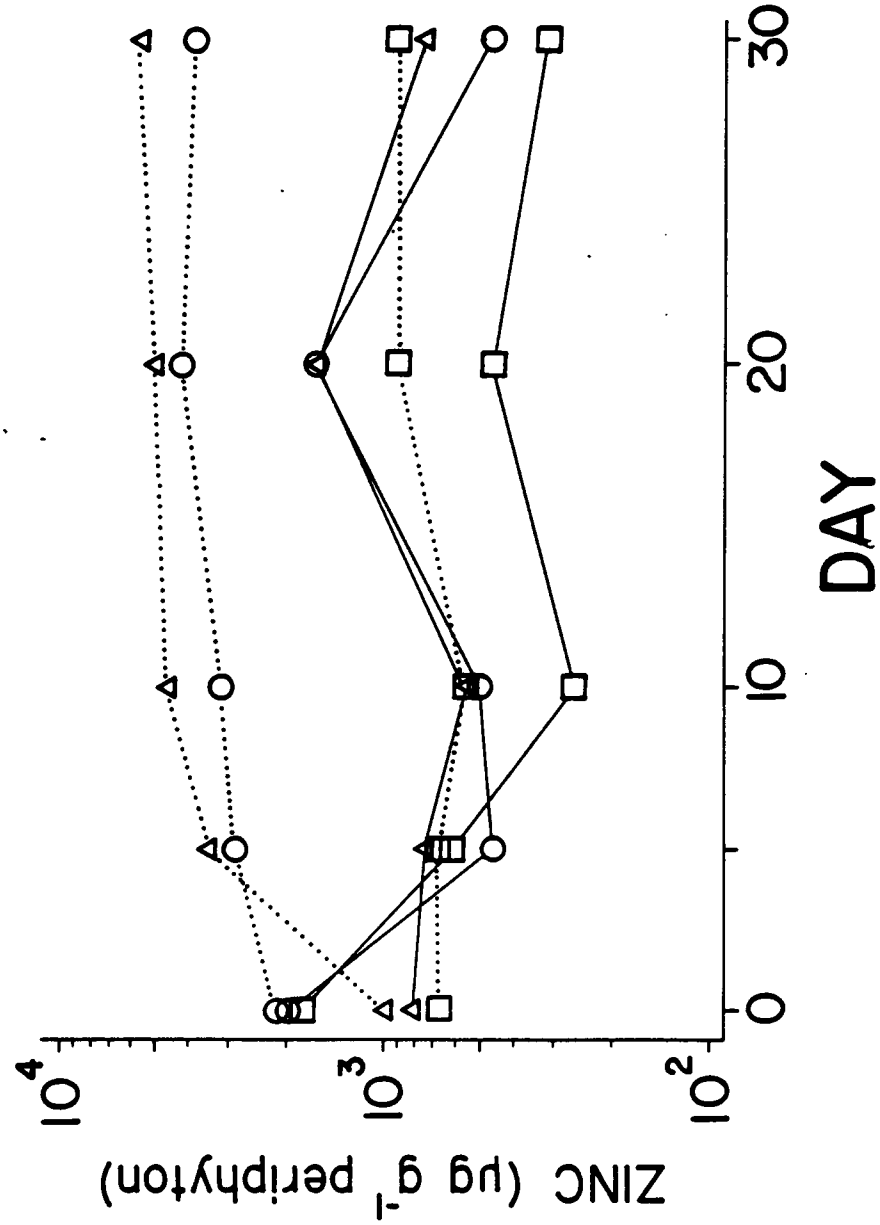
○——○ = ambient Zn and ambient pH,

△——△ = ambient Zn and pH 9,

□·····□ = 0.05 mg $Zn \bullet l^{-1}$ and pH 6,

○·····○ = 0.05 mg $Zn \bullet l^{-1}$ and ambient pH,

△·····△ = 0.05 mg $Zn \bullet l^{-1}$ and pH 9.


ZINC (µg g⁻¹ periphyton)
10⁴
10³
10²
0
10
20
30
DAY

interaction ($P = 0.004$) indicated that substantially less Zn adsorbed in 0.05 mg $Zn \bullet l^{-1}$ treatments at pH 6 than at either ambient-pH or pH 9. This is demonstrated by no significant difference occuring between pH 6-0.05 mg $Zn \bullet l^{-1}$ and ambient-Zn treatments (Fig. 5-3). The mean Zn concentration in periphyton ($\mu g\ Zn \bullet g^{-1}$) in ambient-Zn treatments was approximately 374 in pH 6, 608 in ambient-pH, and 765 in pH 9. The mean Zn concentration of periphyton in the 0.05 mg $Zn \bullet l^{-1}$ treatment was approximately 720 in pH 6, 3500 in ambient-pH, and 4630 in pH 9.

5.4 Discussion

5.4.1 Algal response to pH

To facilitate comparison of pH ranges for algal taxa, the pH spectrum outlined by Lowe (1974) and VanLandingham (1982) will be used. "Acidophilous" refers to taxa that occur at pH 7 with best development below pH 7, "indifferent" refers to taxa with best development around pH 7, and "alkaliphilous" refers to taxa that occur at pH 7 with best development over pH 7. Descriptors for more extreme pH ranges exist but were considered not appropriate for taxa observed in this experiment.

Four diatom taxa and a filamentous blue-green alga were inhibited by pH 6, while this treatment moderately increased the abundance of two diatoms and a coccoid blue-green alga. The decline of *Cocconeis placentula* at pH 6 is consistent with this taxon being alkalophilous (Lowe, 1974) with an optimum pH of about 8.0 (Bahls *et al.*, 1984). Growth of *Nitzschia palea* var. *debilis* was inhibited by pH 6 and stimulated by pH 9 which indicated that it was alkaliphilous in our experiment even though the nominate variety, *N. palea*, is pH-indifferent (Lowe, 1974) with an optimum near pH 8.3 (Bahls *et al.*, 1984). Our taxon of *Phormidium* most closely resembles *P. luridum* of VanLandingham (1982) which has insufficient information to generalize about a pH range. Our

data suggest that this taxon is alkaliphilous which is supported by its low abundance in pH 6 treatments. *Gomphonema parvulum* is generally pH-indifferent (Lowe, 1974) but showed a marginal response as an acidophilous taxon in this experiment. The moderate increase of *Cymbella minuta* to pH 6 appears to agree with this taxon's classification as pH-indifferent (Patrick & Reimer, 1975). Although *Navicula rhynchocephala* is alkalophilous (Lowe, 1974) with an optimum pH near 8.2 (Bahls *et al.*, 1984), this taxon did not respond significantly to pH 6. The decline of this taxon may be due to its preference for spring and fall conditions (Lowe, 1974). The response of attached-algal communities to alkaline pH stress is not well known. The taxon stimulated by pH 9 will be discussed in the pH and Zn interaction section below.

5.4.2 Algal response to zinc

Two diatoms were inhibited by 0.05 mg $Zn \bullet l^{-1}$ while three diatoms and three green-algae were stimulated by this Zn treatment. The results of this experiment will be repeatedly compared to a study that was done in 1984 (Chapter 2). The seasons referred to from this paper will be spring (April 28 to May 28), summer (June 23 to July 23), and fall (September 7 to October 7). The summer dates most closely coincide between these two experiments.

Two diatoms were significantly inhibited by the 0.05 mg $Zn \bullet l^{-1}$ treatment. *Navicula rhynchocephala* may be closely related to *N. cryptocephala* var. *intermedia* which was found to be inhibited by 0.05 mg $Zn \bullet l^{-1}$ treatment during summer in stream mesocosms (Chapter 2). *Cocconeis placentula* has been observed to occur in the low end of Zn gradients in natural streams (Rushforth *et al.*, 1981) and to be inhibited by 0.05 mg $Zn \bullet l^{-1}$ in steam mesocosms in summer (Chapter 2).

The increased biovolume-density of naviculoid diatoms, *Nitzschia palea*, and *N. palea* var. *debilis* in 0.05 mg $Zn \bullet l^{-1}$ is consistent with results of previous experiments performed with these mesocosms (Chapter 2). Naviculoid diatoms were stimulated by Zn in both summer experiments but inhibited by 0.05 mg $Zn \bullet l^{-1}$ in spring, 1984; this seasonal response was attributed to

taxonomic or biochemical changes at the species level (Chapter 2). Rushforth *et al.* (1981) found *Nitzschia palea* to be positively correlated with Zn in natural streams and considered this taxon useful for identifying elevated heavy metal concentrations. A summer population of *Nitzschia palea* was found to have increased biovolume-density due to 0.05 mg $Zn \bullet l^{-1}$ in stream-mesocosms (Chapter 2).

Three green-algal taxa responded positively to the 0.05 mg $Zn \bullet l^{-1}$ treatment. Patrick (1978) notes that more severe pollution stress may cause a shift from sensitive algae (often diatoms) to green or even blue-green algae. The filamentous and prostrate portions of *Stigeoclonium nanum* were abundant in this experiment but were not an important component in 1984 (Chapter 2). The biovolume-density of *Ulothrix variabilis* and *Scenedesmus* increased in 0.05 mg $Zn \bullet l^{-1}$ treatments in summer and fall of the 1984 experiment. Acute exposures of *Scenedesmus* to Zn stress (Bringman & Kuhn, 1959; Shehata & Badr, 1980) suggest that this alga is more resistant than diatoms (Rizet *et al.*, 1978; Rachlin *et al.*, 1983) and *Selenastrum capricornutum* (USEPA, 1980; Bartlett *et al.*, 1974).

We noted *Cymbella minuta* var. *silesiaca* to increase biovolume-density in spring in 0.05 mg $Zn \bullet l^{-1}$ treatment (Chapter 2). The non-significant response by *C. minuta* in this experiment may be due to the summer season or the different taxonomic variety of this species. Although *Gomphonema* spp. was inhibited by Zn in our previous experiment, *G. parvulum* did not show a significant response and even appears to have done slightly (but not significantly) better in 0.05 mg $Zn \bullet l^{-1}$ treatment.

5.4.3 Algal response to pH and zinc interaction

One diatom, two filamentous green algae, and a coccoid blue-green alga had abundances influenced by an interaction between Zn and pH. Significant interaction indicates that the response by these taxa to pH differed between ambient Zn and 0.05 mg $Zn \bullet l^{-1}$ treatments. The pH preference of *Microcystis incerta* is not well known (VanLandingham, 1982), but our data suggest that

it is acidophilous. The increase by *Microcystis* in pH 6 was inhibited in the 0.05 mg $Zn \bullet l^{-1}$ treatment. Zn had a very strong effect on the response of *Nitzschia palea* var. *debilis* to pH. Increased biovolume-density of this taxon in pH 9, and decreased abundance in pH 6, only occurred with added Zn. Variability may have been too high for a similar interaction to be significant for the nominate variety. The increased biovolume-density of the filamentous green-algae *Stigeoclonium* and *Ulothrix* in pH 6 treatments on day 30 correspond with reports from the literature that show other green algae to replace diatoms of the attached-algal flora in lakes of pH 6 and below (Muller, 1980; Haines, 1981; Stokes, 1984; Dillon *et al.*, 1984). Zinc and phosphate also interact to increase the biovolume-density of *Stigeoclonium nanum* (Chapter 3). Since phosphate was below detection in our experiment, it is not clear whether phosphate was important or not. These results indicate that the individual responses of algae are not soley affected by Zn or pH alone. Other variables may have interacted with Zn or altered pH to change algal species composition. Zinc or pH stress may have an indirect effect on algal community composition by altering snail grazing, microbial activity, availability of nutrients, or successional processes in the algal community (Hall *et al.*, 1980; Haines, 1981; Dillon *et al.*, 1984).

5.4.4 Other taxa

The non-significant response by *Fragilaria vaucheriae* is because it became abundant in only one pH 6 (ambient Zn) mesocosm. Lowe (1974) classifies this taxon as alkaliphilous and Bahls *et al.* (1984) give 8.38 as an optimum pH. Although sulfate and pH levels of this mesocosm were no different than other pH 6 mesocosms on day 20, on day 30 the sulfate concentration of this mesocosm was the same as non-pH-6 treatments, and the measured pH was about 7. This suggests that the increased abundance of this taxon was useful to identify a dosing malfunction which reduced pH stress by day 30. If this is true, then the community which recovers from pH 6 stress may at least initially be very different from control communities (ambient-Zn and ambient-pH). Although *Melosira varians* is generally considered a summer alga that is alkaliphilous (Lowe, 1974)

with a pH optimum of 8.1 (Bahls *et al.*, 1984), this taxon did not respond significantly to pH or Zn treatments in this experiment. We found it to be inhibited by 0.05 mg $Zn \bullet l^{-1}$ in spring and summer of 1984 (Chapter 2).

5.4.5 Total biovolume-density

The marginally significant increase in total biovolume-density from pH 6 treatments corresponds with some reports, but the literature on acidic pH stress shows a decrease in abundance for periphytic biomass in a stream (Hall *et al.*, 1980), and planktonic communities either increased, decreased, or were unchanged by acidic pH (Stokes & Hutchinson, 1976; Muller, 1980; Haines, 1981; Dillon *et al.*, 1984). The response is dependent on many factors that differ among ecosystems. The non-significant effect of Zn on total biovolume-density is due to the replacement of diatoms by green algae. We observed the same process in the summer experiment of 1984 (Chapter 2).

5.4.6 Water chemistry

The decrease in alkalinity and increase in sulfate are directly attributed to the addition of the strong acid. Low alkalinity also occurs in lakes of low pH (Schindler *et al.*, 1980) and the sulfate concentration is in the upper part of the range observed in lakes (Hutchinson, 1957). The increased ammonia concentration on day 20 in pH 6 (ambient Zn and 0.05 mg $Zn \bullet l^{-1}$) treatment may be due to increased ammonification from the heterotrophic microbial community (Rheinheimer, 1980) on organic matter from clams and snails which were dying by this day. Farris (1986) reports that snails were inhibited in movement by day 5, started to die by day 10, and were all dead by day 20 in this treatment.

The unmeasurable amount of Zn in 0.05 mg $Zn \bullet l^{-1}$ treatments suggests that added Zn was rapidly adsorbed by periphyton or mesocosm structures. The measurable amount of Zn in the water and the decreased Zn concentration of periphyton in pH 6-0.05 mg $Zn \bullet l^{-1}$ treatment corresponds to the increased solubility of Zn below pH 6. A pH of 6 is near a threshold below which a majority of Zn is released from artificial and natural sediments (Baccini, 1984; Adams & Sanders, 1984).

5.4.7 Conclusion

This experiment tested the USEPA recommendations for Zn and pH. Although pH 9 showed no significant effect, pH 6 and 0.05 mg $Zn \bullet l^{-1}$ reduced the biovolume-density of certain taxa. The USEPA bases its criterion levels on the most sensitive taxa to a pollutant that has been reported in the literature. Certain constraints must be met, such as measuring the Zn concentration in the water. The criterion of the USEPA for Zn is based on the most sensitive species tested which were the flagfish, *Jordanella floridae*, and the cladoceran, *Daphnia magna*. No algae were used because water Zn concentrations were not measured, or because the algae tested were more resistant than other organisms to Zn. None of the diatoms inhibited by Zn or pH 6 in this experiment were considered by the USEPA. Since diatoms are sensitive to Zn and pH, and provide an important food source for higher-level consumers, it is recommended that further tests include diatom taxa.

6.0 Summary

The results demonstrate that abundance of dominant algal taxa are useful for identifying different levels of Zn stress in stream mesocosms. Attached algae can be used to distinguish Zn stress from increased levels of phosphate, snails, and pH.

The criterion of the USEPA for Zn is based on the most sensitive species tested which were the flagfish, *Jordanella floridae*, and the cladoceran, *Daphnia magna*. No algae were used because water Zn concentrations were not measured, or because the algae tested were more resistant than other organisms to Zn. A scientifically justifiable criterion of the USEPA has not been determined for pH, hence, the standard of the USEPA was used. None of the diatoms inhibited by Zn or pH 6 in these experiments were considered by the USEPA. Since diatoms are sensitive to Zn and pH, and provide an important food source for higher-level consumers, it is recommended that further tests include diatom taxa.

6.1 Chapter 2

1) Zinc treatments as low as 0.05 mg l^{-1} had communities with relatively more green and blue-green algae despite this being near the safe concentration set by the USEPA. In general, diatoms were more sensitive than a filamentous green alga which was more sensitive than unicellular green algae and a filamentous blue-green alga.

2) Five diatoms were inhibited by Zn stress and are recommended for laboratory and field tests that determine safe concentrations of Zn and other poisons for aquatic habitats. These taxa are *Cocconeis placentula*, *Gomphonema* spp., *Melosira varians*, naviculoid diatoms, *Navicula cryptocephala* var. *intermedia*, and *Nitzschia linearis*.

3) Snail grazing did not alter algal abundance in spring, 1984.

4) Total algal abundance (biovolume-density) differed between control and Zn treatments in spring but not in summer and fall. This suggests that information about individual taxa is important to include with total biomass estimates.

5) Treatments differed in all seasons according to the SIMI similarity index, but lack of a significant difference between treatments for the most dominant taxa may reduce the usefulness of this index for identifying treatments.

6.2 Chapter 3

1) The abundance of one diatom was reduced by the KH_2PO_4 treatment. This may be from the phosphate or the potassium.

2) Different portions of the same taxon may respond differently to phosphate treatment. This was observed for the prostrate and filamentous portions of the green alga *Stigeoclonium nanum*.

3) Phosphate and Zn interacted synergistically to increase the abundance of *Stigeoclonium nanum*, and *Nitzschia* spp.. Although not statistically significant, the diatoms *Achnanthes minutissima*, *Gomphonema tenellum*, naviculoids, and *Synedra* spp. appear to follow a similar trend.

6.3 Chapter 4

1) Temperature may play a very important role in determining the effect of snail grazing on attached algal communities. Cold temperatures (< 15 C) may have inhibited snail grazing to the extent that abundance of many taxa increased to levels found in non-snail treatments. However, *Nitzschia palea* was more than twice as abundant in snail treatment over non-snail treatment -- apparently stimulated by the presence of snails during cold conditions; and *Navicula cryptocephala* var. *intermedia* and naviculoid diatoms remained at low abundance in snail treatment despite rapid growth in non-snail treatment -- apparently inhibited or selected as a food source by snails during cold conditions.

2) No algal taxa replaced the diatoms inhibited by 0.5 mg $Zn \bullet l^{-1}$ in this October-November, 1984, experiment by day 10. This is in contrast to an experiment performed one month earlier, September-October, in which a community characteristic of this treatment developed by day 5.

6.4 Chapter 5

1) The abundance of the diatom *Nitzschia palea* and the filamentous blue-green alga *Phormidium* spp. was reduced by pH 6 despite this being the standard of the USEPA for pH.

Treatment of pH 9 did not significantly affect algal abundance possibly because this level was only slightly above ambient pH levels near 8.5.

2) The abundance of the diatoms *Cocconeis placentula* and *Navicula rhynchocephala* was reduced by treatment of 0.05 mg $Zn \bullet l^{-1}$ despite this being near the criterion of the USEPA for Zn.

3) Individual and combined treatments of Zn and pH 6 lead to different communities with relatively more green algae than in control (and pH 9) treatments.

6.5 General conclusions

General conclusions from this dissertation are as follows.

1) The abundance of attached-algae are useful for identifying anthropogenic stress and grazing by snails in freshwater steam mesocosms.

2) Snail grazing, pH, and phosphate exert important effects independent of and in combination with Zn. These and other variables should be considered in environmental impact analysis.

3) Certain diatoms were more sensitive than green algae to Zn and pH. This is important because diatoms comprised a major proportion of total algal abundance, and they were important food to primary consumers.

4) Analysis of individual algal taxa was more useful than the total algal abundance estimate (biovolume-density) and the community similarity index for differentiating treatments.

5) Environmental realism and experiments performed in different seasons are important components of experiments investigating anthropogenic stress or disturbance by snail grazing.

6.6 *Future research*

Future experiments could investigate the following points.

1) The experiment that investigated the change in abundance of attached algae to Zn stress in three seasons could be replicated with different years, different locations along the New River, and rivers in other locations to test the general applicability of these results.

2) Abundance of rare taxa could be included. This would be beneficial to find the most sensitive species, as well as to include taxa of large biovolume that are not numerically abundant.

3) Many variables not tested here will influence the response of the algal community to Zn. These could be measured in a similar fashion for a fuller understanding of algal ecology.

4) Other metallic and non-metallic chemicals could be tested to determine how specific the response of algae is to stress.

5) Laboratory single-species and community tests could be performed to determine if the sensitivities of these taxa are the same between laboratory and field.

6) Field surveys of sites impacted by Zn waste could be investigated to observe whether correlations between Zn loading and species composition correspond to these results.

Appendix A. A randomization procedure

A procedure was adopted for testing differences in similarities associated with some treatment. Standard statistical procedures (ANOVA, Kruskal-Wallis test) are not appropriate because of dependence between similarity scores. We assume k treatment levels and n_i replicate samples for each level. Data are then a vector of abundances for S species $X_{ij} = (X_{1ij}, X_{2ij}, \ldots, X_{Sij})$ where i indicates the treatment level and j the replica number. An example of data is given in Table A-1.

The first step in the analysis is to summarize data using a similarity measure. One such measure is Stander's (1970) measure, which is also referred to as a cosine measure.

$$SIMI_{(ab)(cd)} = \frac{\sum_l X_{lab} X_{lcd}}{\sqrt{\sum X_{lab}^2 \sum X_{lcd}^2}}$$

where $SIMI_{(ab)(cd)}$ is the similarity between replicate b of treatment level a and replicate d of treatment level c. Similarity values are computed for each possible pairwise combination of data vectors, resulting in an N by N matrix of similarities, where $N = \Sigma n_i$ is the total number of observations (see example, Table A-1).

The randomization procedure consists of several steps carried out on this matrix. A test statistic is chosen that is useful for measuring changes in similarity. Some possibilities include $\overline{B}$, the mean between similarity; $\overline{W}$, the mean within similarity; or, analogous with ANOVA, $\overline{B}$ /$\overline{W}$

(Good, 1982). This test statistic, say $\overline{B}$(data), is the value of the mean between similarity computed for the observed data. The null hypothesis is that the treatment has no effect. In this case, differences are expected in the data due solely to random variations. Hence, $\overline{B}$(data) should be relatively large. If some data are switched from one treatment with some data from a second treatment, the new mean between similarity, say $\overline{B}$ (permute), differs only slightly from $\overline{B}$(data). However, if the null hypothesis is false, $\overline{B}$(permute) is likely to be quite different from $\overline{B}$(data).

The second step in the randomization procedure is to carry out a number of data switches or permutations of data. For each permutation, $\overline{B}$(permute) is computed. This step is performed a great number of times (say 1000). The final step of the procedure is to compare $\overline{B}$(data) with the 1000 $\overline{B}$(permute) values. If $\overline{B}$(data) is smaller than most (95%) of the $\overline{B}$(permute) values, then the null hypothesis of no treatment effect can be rejected.

There are several things to note at this point. First, there are $N!/(n_1!n_2! \ldots n_k!)$ possible permutations. If the n_i are small, this number may be small and all possible permutations may be computed. In general, this number is large and a computer is needed to carry out the test based on a random sampling of permutations. Second, recomputing similarities at each step of the test procedure is not necessary as these values do not change. Hence, the test may be carried out on the similarity matrix. Third, statistical significance does not imply biological significance. In some cases, the test may reject when the similarities are close. If, for example, the similarities in one group are only 0.01 less than those in the second, the test is likely to reject although that difference is not considered to be relevant. It is important to look at the similarities as well as carry out the tests.

An example of the way the randomization procedure works is shown in the two scenarios in Table A-1. In scenario 1, periphyton communities do not differ significantly between control and Zn treatments. If there are no differences between control and treatment groups, SIMI scores should be of the same magnitude among control samples, among treatment samples, and for scores that compare control to treatment samples. The randomization procedure works by switching some of the control SIMI scores with some of the treatment scores and recomputing the mean within (control and treatment combined) and mean between scores. Since there is no difference

between control and treatment scores in this scenario, the mean between and mean within scores do not change by more than a relatively small amount. Table A-1 part A provides data and SIMI scores for scenario 1. The mean within similarity for the control and treatment combined is 0.868, and the mean between similarity is 0.889. When the first row of the control SIMI scores is switched with the first row of the treatment SIMI scores, the mean within similarity is 0.879 and the mean between similarity is 0.878 – only a slight change.

In scenario 2, there is a difference between periphyton communities due to Zn treatment. When control SIMI scores are switched with treatment SIMI scores, the mean between scores will usually increase and the mean within scores will usually decrease. Table A-1 part B provides SIMI scores for scenario 2. The mean within similarity (0.863) is larger than the mean between similarity (0.651). When the first row of each group is switched, the mean within similarity (0.713) decreases considerably while the mean between similarity (0.757) increases considerably. After randomly switching SIMI scores a great number of times, most of the means between values are >0.651, which suggests a difference between communities due to treatments.

In summary, the switching of SIMI scores causes the similarity within communities to decrease (or between to increase) when there are differences due to treatments, but the switching causes only slight changes of similarity within or between communities when there are no differences. This randomization procedure is well suited for testing a null hypothesis resulting from similarity data.

Table A-1. Example data and similarity matrices for cases where a treatment does and does not have an effect on a replicate of a community of algae.

A) No treatment effect

Community	Rep	Species 1	2	3	4	5
1	1	20	50	20	5	5
	2	40	20	15	15	10
	3	30	25	25	10	10
	4	20	30	10	30	10
2	1	30	40	20	5	5
	2	25	40	10	15	10
	3	10	40	30	15	5
	4	35	25	15	10	5

Community	Rep	SIMI Scores							
1	1	1.00	0.76	0.87	0.81	0.97	0.94	0.93	0.83
	2		1.00	0.95	0.84	0.88	0.87	0.71	0.98
	3			1.00	0.84	0.95	0.90	0.86	0.96
	4				1.00	0.82	0.93	0.84	0.85
2	1					1.00	0.95	0.89	0.93
	2						1.00	0.88	0.92
	3							1.00	0.77
	4								1.00

B) Treatment has an effect

Community	Rep	Species 1	2	3	4	5
1	1	20	50	20	5	5
	2	10	40	30	15	5
	3	30	25	25	10	10
	4	20	30	10	30	10
2	1	10	10	20	20	30
	2	15	0	35	20	30
	3	5	15	20	25	35
	4	15	20	5	40	20

Community	Rep	SIMI Scores							
1	1	1.00	0.93	0.87	0.81	0.49	0.41	0.53	0.57
	2		1.00	0.86	0.84	0.65	0.58	0.67	0.65
	3			1.00	0.84	0.69	0.71	0.67	0.67
	4				1.00	0.79	0.60	0.75	0.93
2	1					1.00	0.91	0.98	0.89
	2						1.00	0.88	0.66
	3							1.00	0.84
	4								1.00

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Appendix C. Data

APPENDIX B-1. BIOVOLUME-DENSITIES OF ALGAE FOR GLEN LYN SPRING, 1984, EXPERIMENT IN TREATMENTS WITHOUT ADDED SNAILS. EACH ESTIMATE TAKES UP SIX PRINT POSITIONS. MULTIPLY THESE ESTIMATES BY 1,000,000 TO GET ACTUAL AMOUNT. TREATMENTS: K=CONTROL, L=0.05 MG ZN/L, N=1.0 MG ZN/L. REPLICATES: A, B, C.

TAXON	DAY	KA	KB	KC	LA	LB	LC	NA	NB	NC
ACHNANTH	0	2.89992.	38561.	66225.	12192.	21634.	46751.	2928.	459162.	0316
CYMINUSI	0	.07601.	08471.	06656.	12204.	03631.	07156.	04437.	07020.	04236
FRAGILAR	0	3.35781.	28319.	8371.	010782.	5662.	000002.	13851.	0633	4.277
MEVARI	0	.12191.	03106.	05694.	01305.	000008.	3471.	04659.	00000.	00000
NAVICULA	0	.01362.	012597.	1446.	018015.	44358.	50395.	2734	4.6525.	2734
NILINE	0	.037686.	3548.	02153.	02343.	00000	5.5069.	7691.	023119.	2952
NISP1	0	.02911.	02408.	02769.	04036.	02276.	03670.	02348.	02793	6.232
NISP2	0	.17190.	09667.	05515.	23643.	11795.	20652.	12414.	27217.	10886
SURIRELL	0	.16530.	07049.	07615.	260694.	7023.	16829.	05077.	07945.	05264
GRCOCCOI	0	.00000.	00000.	00000.	00000.	00000.	00000.	00000.	00000.	00000
ULVARI	0	2.27232.	8943	3.3059.	7291.	01109.	036565.	7885.	014395.	7885
ACHNANTH	2	4.43257.	01827.	01826.	64881.	84693.	69383.	32445.	18582.	5856
CYMINUSI	2	.06454.	08875.	08471.	21784.	07665.	12909.	12909.	12035.	06051
FRAGILAR	2	3.42162.	56621.	7108	8.554.	85540.	012753.	4216.	011195.	1324
MEVARI	2	.15528.	07247.	04141.	00000.	00000.	24846.	00000.	00000.	00000
NAVICULA	2	.02450.	02143.	01871.	01735.	01055.	01463.	01429.	014937.	1446
NILINE	2	.12425.	07247.	05577.	07929.	01764.	02940.	02447.	09621.	01271
NISP1	2	.05735.	03367.	05644.	06800.	04050.	04208.	03895.	03322.	01682
NISP2	2	.21358.	20780.	14666.	32719.	22474.	20945.	18301.	36684.	18921
SURIRELL	2	.39672.	17677.	13157.	31016.	03570.	24816.	09587.	17818.	09779
GRCOCCOI	2	.00000.	00000.	00000.	00000.	00000.	00000.	00000.	00000.	00000
ULVARI	2	.05306.	01254.	06464.	01930.	015446.	7533.	00000.	04233.	03280
ACHNANTH	5	.014465.	2895.	053527.	17864.	9117.	027139.	06772.	38034.	5996
CYMINUSI	5	.51900.	121721.	8224.	15267.	17536.	74060.	20631.	18485.	10988
FRAGILAR	5	9.5698.	01090.	076235.	2497.	01400.	03533.	012256.	12472.	6629
MEVARI	5	6.2541.	148251.	58832.	1284.	026472.	61331.	5883.	14825.	00000
NAVICULA	5	.10657.	01201.	09570.	01566.	01322.	06402.	011836.	08996.	0899
NILINE	5	1.7582.	11680.	88283.	23069.	12582.	24638.	08382.	13908.	03720
NISP1	5	.21952.	04065.	72405.	04237.	03087.	13638.	07644.	01519.	01378
NISP2	5	.33970.	05536.	62328.	12973.	04289.	46455.	47412.	15145.	16237
SURIRELL	5	2.2819.	17786	3.029.	49992.	091341.	4755.	28073.	13866.	09216
GRCOCCOI	5	.00000.	00000.	00000.	00000.	00000.	00000.	00000.	00000.	00000
ULVARI	5	.10254.	047868.	22343.	9472.	01974.	01328.	01776.	04628.	02102
ACHNANTH	10	5.66738.	31219.	80576.	98443.	09935.	25947.	55645.	2895.	01154
CYMINUSI	10	.25995.	59417.	19888.	36613.	31591.	30087.	31772.	23107.	37824
FRAGILAR	10	.02068.	031508.	4344	7.5488.	9717.	04803.	088857.	87469.	7217
MEVARI	10	2.11781.	1648.	36119.	09135.	81072.	29481.	50827.	01059.	13236
NAVICULA	10	.02784.	10649.	03561.	01758.	01974.	01615.	02401.	01009.	02320
NILINE	10	.21983.	64379.	21624.	32735.	12414.	16951.	08188.	01300.	05686
NISP1	10	.04308.	13740.	04532.	11065.	03801.	02853.	01138.	02311.	02856
NISP2	10	.03761.	08620.	02569.	09061.	04622.	02213.	17410.	18339.	19367
SURIRELL	10	.21339.	52279.	16108.	31016.	21291.	16442.	17502.	04613.	22972
GRCOCCOI	10	.00000.	00000.	00000.	00000.	00000.	42840.	00000.	80784.	00000
ULVARI	10	.010851.	9736	5.854.	015811.	3492.	01832.	00000.	05723.	00000
ACHNANTH	20	1.1335.	02755.	011727.	77879.	7954.	027969.	06779.	96311.	5113
CYMINUSI	20	.27233.	63183.	52236.	814121.	00961.	4854.	33010.	35115.	10109
FRAGILAR	20	.04900.	535627.	6016.	05254.	02114.	31117.	020687.	81794.	2952
MEVARI	20	2.07418.	87692.	89131.	3625.	291691.	2283.	04236.	03808.	02647
NAVICULA	20	.03167.	10530.	02289.	02090.	01522.	05150.	01357.	016274.	1759
NILINE	20	.20954.	32398.	19818.	15945.	07765.	10982.	14086.	12931.	02348
NISP1	20	.01518.	16549.	10404.	06110.	06215.	07180.	03981.	024124.	3151
NISP2	20	.05789.	16176.	05717.	23127.	25334.	07522.	12888.	13625.	07691
SURIRELL	20	.06341.	15424.	07395.	19788.	06537.	64980.	08834.	17742.	07499
GRCOCCOI	20	.00000.	00000.	60158.	00000.	00000.	00000	1.1313.	0986.	16157
ULVARI	20	.00000.	03598.	00000.	00000.	019199.	8681.	029601.	1828.	00000
ACHNANTH	30	.01965.	04232.	03627.	02265.	04120.	11400.	071446.	83995.	4719
CYMINUSI	30	.627181.	26321.	05632.	09274.	00815.	0463.	99598.	23655.	11454
FRAGILAR	30	.14095.	06533.	06872.	11187.	10107.	40415.	129763.	51999.	5038
MEVARI	30	9.9934	28.7	26.53.	909371.	5845	10.65.	48989.	13845.	00000
NAVICULA	30	.07377.	15590.	09688.	03665.	03266.	10360.	07070.	011206.	5099
NILINE	30	.24563.	19413.	19496.	04213.	00000.	24900.	12450.	041177.	2599
NISP1	30	.05346.	14239.	05841.	06173.	05099.	11180.	03018.	000002.	8844
NISP2	30	.03296.	15145.	02704.	05957.	10074.	11050.	22610.	02465.	06961
SURIRELL	30	.04613.	02858.	04303.	16493.	190201.	3071.	62254.	06960.	07309
GRCOCCOI	30	.00000.	00000.	00000.	00000.	00000.	00000.	61229.	24911.	08892
ULVARI	30	.00000.	05895.	00000.	02558.	00000.	000005.	9549.	00000.	00000

APPENDIX B-2. BIOVOLUME-DENSITIES OF ALGAE FOR GLEN LYN SPRING, 1984, EXPERIMENT IN TREATMENTS WITH ADDED SNAILS. EACH ESTIMATE TAKES UP SIX PRINT POSITIONS. MULTIPLY THESE ESTIMATES BY 1,000,000 TO GET ACTUAL AMOUNT. TREATMENTS: K=CONTROL, L=0.05 MG ZN/L, N=1.0 MG ZN/L. REPLICATES: A, B, C.

TAXON	DAY	KA	KB	KC	LA	LB	LC	NA	NB	NC
ACHNANTH	0	.734654.	76538.	09621.	67151.	66224.	7682	2.2049.	0162	2.543
CYMINUSI	0	38.1191.	07499.	47156.	79348.	408111.	0930.	08749.	23330.	858
FRAGILAR	0	3.4026.	689722.	34361.	72045.	9489	5.1532.	9773.	00000.	65435
MEVARI	0	61.76916.	694.0000078.	079.0000017.	818.0000042.	11523.	757			
NAVICULA	0	4.5674	20.319.	5757.	86892.	381512.	2972.	368311.	7656.	7665
NILINE	0	8.772326.	00233.	9563.	433511.	76259.	7493.	395311.	962.	00000
NISP1	0	14.22934.	18637.	40513.	51314.	792	57.31	11.7	19.9913.	725
NISP2	0	74.565174.	55117.	71105.	31158.	43182.	74	84.63	468.9183.	29
SURIRELL	0	51.43878.	847140.	8459.	5436.	5735237.	84	6.537218.	0458.	947
GRCOCCOI	0	.00000.	00000.	00000.	00000.	00000.	00000.	00000.	00000.	00000
ULVARI	0	16.78933.	4496.	167642.	688	3.85917.	4352.	8782	9.81259.	778
ACHNANTH	2	1.47755.	54079.	60384.	80194.	80195.	17135.	54076.	39864.	6893
CYMINUSI	2	40.34145.	22181.	5372.	612129.	0984.	714104.	88145.	58133.	15
FRAGILAR	2	.855401.	710830.	483.	000002.	56622.	56623.	18838.	64372.	1719
MEVARI	2	20.705465.	85186.	3410.	35220.	705.	0000010.	352.	00000131.	42
NAVICULA	2	13.60926.	53725.	51610.	887	16.3311.	90813.	60911.	7878.	2062
NILINE	2	57.66796.	93724.	47123.	524	31.341.	16611.	762.	0000014.	932
NISP1	2	15.05758.	69543.	169	14.5237.	72526.	53436.	09221.	802	22.12
NISP2	2	144.59200.	7897.	496161.	12150.	79136.	33228.	87325.	61320.	97
SURIRELL	2	178.59	171.1300.	85133.	499.	404678.	88290.	206119.	41183.	71
GRCOCCOI	2	.00000.	00000.	00000.	00000.	00000.	94775.	00000.	00000.	00000
ULVARI	2	5.7885.	000004.	823829.	907.	0000019.	295	20.2612.	53458.	789
ACHNANTH	5	9.6344	2.07879.	893	28.05	16.7221.	3293.	123711.	88710.	957
CYMINUSI	5	241.3876.	3351353.	91094.	1390.	09355.	2271.	071308.	3190.	777
FRAGILAR	5	18.3742.	624951.	03927.	83978.	7189.	878510.	3586.	88176.	9996
MEVARI	5	1937.863.	53477.	211449.	23575.	09283.	94.	000001374.	2.	00000
NAVICULA	5	25.57712.	876126.	8777.	50835.	69936.	343	6.95314.	3696.	6119
NILINE	5	187.13120.	781462.	82014.	7293.	49200.	1728.	20666.	764	39
NISP1	5	65.592	25.18490.	25613.	21123.	39	77.4523.	28758.	44522.	416
NISP2	5	151.4932.	115597.	751313.	1216.	7587.	665103.	94477.	76138.	18
SURIRELL	5	325.98224.	931907.	44607.	7282.	34393.	4291.	434192.	81132.	61
GRCOCCOI	5	.00000.	00000.	00000.	00000.	000005.	0164.	00000.	00000.	00000
ULVARI	5	54.76811.	84250.	36831.	3987.	9398118.	562.	719623.	28440.	459
ACHNANTH	10	446.314.	357	44.635.	77234.	533915.	49110.	201	2.8057.	0527
CYMINUSI	10	7040.	3528.	15	1372395.	43404.	37499.	27191.	87406.	99245.	51
FRAGILAR	10	459.	3539.	61277.	602	51.3720.	99956.	87237.	4645.	567912.	249
MEVARI	10	847784087.	3463.	271382.	4518.	861651.	926.	472202.	1561.	769
NAVICULA	10	1065.	7102.	31170.	5228.	99934.	103	24.3610.	96210.	70319.	082
NILINE	10	6510.	7257.	661056.	8255.	19121.	85157.	7422.	99244.	44846.	631
NISP1	10	963.	5194.	172226.	21	43.6340.	462	40.67	11.5318.	07223.	652
NISP2	10	1053.	855.	778199.	66	120.	973.	94933.	805	57.68158.	65178.	96
SURIRELL	10	10345307.	631960.	4462.	02180.	71386.	5558.	649181.	56224.	34
GRCOCCOI	10	.00000.	00000.	00000.	00000.	00000.	00000.	00000.	00000.	00000
ULVARI	10	1511.	174.	997.	00000.	000001.	9736	68.09	46.3811.	5132.	3026
ACHNANTH	20	6.35769.	823311.	33514.	3572.	26699.	0677	4.15614.	35730.	054
CYMINUSI	20	270.	78660.	19445.	63907.	771126.	5779.	85171.	24	676.	71033.	9
FRAGILAR	20	14.	72355.	04358.	54283.	99611.	53338.	89613.	641	72.	86114.	84
MEVARI	20	2893.	83377.	24890.	81037.	7794.	17	321921.	178.	00000	1095	
NAVICULA	20	47.	43170.	29531.	31932.	71116.	00824.	0128.	699822.	27277.	508
NILINE	20	278.	8183.	817	337.	6179.	6695.	847108.	8513.	969	104432.	92
NISP1	20	44.	62878.	48294.	843	58.946.	17451.	05610.	69836.	912106.	87
NISP2	20	53.328	97.1968.	455142.	8378.	17446.	48227.	255305.	94379.	83
SURIRELL	20	323.	5773.	029223.	01520.	09192.	19149.	9943.	238392.	44810.	47
GRCOCCOI	20	.00000.	00000.	00000.	00000.	00000.	000001.	6157	10.34.	64260
ULVARI	20	288.93.	00000324.	53.	000007.	89456.	9077.	00000.	0000015.	699
ACHNANTH	30	18.89121.	15845.	94395.	758	15.254.	54825.	83957.	75969.	919
CYMINUSI	30	561.16	19411742.	94266.	11718.	13628.	5340.	29888.	08995.	98
FRAGILAR	30	47.	248232.	5233.	598408.	3153.	919174.	1433.	439141.	9231.	679
MEVARI	30	6596.3	10374	193143791.	33322.	76059.	563.	899	21.342.	599
NAVICULA	30	75.	863200.	72202.	67	12641.	29941.	10733.	59942.	69955.	999
NILINE	30	263.	88453.	09581.	13280.	99	176.8	89.	1333.	174	104.	6317.	69
NISP1	30	42.	72463.	27981.	673104.	48	23.8246.	09819.	36736.	66950.	849
NISP2	30	79.	442132.	5283.	837	215.	932.	29956.	57189.	248	198.	9586.	49
SURIRELL	30	238.	33473.	831119.	51786.	5309.	19161.	56	147	201.	2858.	48
GRCOCCOI	30	.00000.	00000.	00000.	00000	1.95.	00000358.	14959.	38261.	29
ULVARI	30	.00000.	00000.	0000099.	24881.	383.	00000	1.985	23.8271.	459

APPENDIX B-3. BIOVOLUME-DENSITIES OF ALGAE FOR GLEN LYN SUMMER, 1984, EXPERIMENT. EACH ESTIMATE TAKES UP SIX PRINT POSITIONS. MULTIPLY THESE ESTIMATES BY 1,000,000 TO GET ACTUAL AMOUNT. TREATMENTS: K=CONTROL, L=0.05 MG ZN/L, N=1.0 MG ZN/L. REPLICATES: A, B, C.

TAXON	DAY	KA	KB	KC	LA	LB	LC	NA	NB	NC
ACHNANTH	0	3.84042	.54862	.5518	.980221	.3723	.784182	.0181	.490114	.6723
COPLAC	0	59.1155	.91273	.16253	.24931	.949	.7841835	.23813	.312	18.13
FRAGILAR	0	.0000029	.25779	.707149	.69	34.7	.784184	.0023	.000001	.8532
GOMPHONE	0	14.93614	.20214	.49815	.34175	.8041	.568419	.8466	.13657	.1978
MEVARI	0	1128.	61293.	91106.	42986.	9963.	36.784181	486.	2576.	13511.
NACRYP	0	207.74133	.91140	.8577	.099144	.66.7841844	.15915	.626129	.31	
NAVICULA	0	23.035	36.4331	.41422	.282	50.941	.568439	.54811	.91418	.461
NITZSCHI	0	32.651	.37755	.27956	.70993	.2461	.5684189	.0539	.61224	.513
OOELLI	0	.00000	.00000	.00000	.00000	.00000	.00000	.00000	.00000	.00000
GRCOCCOI	0	1.2181	.5842	5.6545	.47777	.39061	.56847	.17367	.6205	.63507
ULVARI	0	21.44621	.2195	.337194	.56242	.0691	.568410	.5821	.47611	.0051
PHORMIDI	0	17.1614	.36772	.10577	.279621	.847	.784182	.19622	.93067	.8349
ACHNANTH	2	.78598	.98022	.980221	.3741	.784182	.9407	.84952	.58813	.49011
COPLAC	2	49.92139	.93785	.198	8.65325	.29379	.87424	.40612	.64717	.972
FRAGILAR	2	.000002	.38146	.463710	.2066	.4637	35.72	.00000	.00000	.68039
GOMPHONE	2	1.12442	.57023	.14821	.011916	.239	15.13	.07382	.49783	.3014
MEVARI	-2	127.64505	.29488	.76286	.88129	.86	.0000067	.68736	.952139	.31
NACRYP	2	44.02791	.70782	.374	16.0810	.145204	.923	.95693	.246322	.115
NAVICULA	2	5.957712	.90116	.61810	.36720	.30145	.926	5.1146	.65046	.7779
NITZSCHI	2	3.113310	.62214	.256	5.60612	.53555	.0838	.5964	10.265	.7339
OOELLI	2	.00000	.00000	.00000	.00000	.00000	.00000	.08193	.00000	.00000
GRCOCCOI	.2	.986352	.20481	.85671	.79866	.96256	.96257	.078525	.0077	.6007
ULVARI	2	.369024	.42834	.98181	.19936	.273449	.818	.79955	.78418	.00000
PHORMIDI	2	1.96552	.32951	.89272	.76623	.63982	.1839	.24265	.50937	.45551
ACHNANTH	5	3.9695	4.5092	.84264	.39615	.23343	.4308	.68615	.68111	.88220
COPLAC	5	159.8154	.42185	.0452	.61963	.177150	.1335	.94325	.43817	.306
FRAGILAR	5	.000001	.0206	.6803911	.63746	.41660	.857	.00000	.00000	.00000
GOMPHONE	5	6.3545	.28538	.20869	.1351	111.85	.71663	.71113	.85132	.4419
MEVARI	5	42.68785	.002160	.56145	.38560	.28440	.7530	.695393	.7656	.668
NACRYP	5	36.09368	.17138	.955	8.94515	.64729	.9832	.43471	.40986	.4925
NAVICULA	5	4.90147	.144112	.6546	.400227	.24413	.6752	.04123	.102620	.034
NITZSCHI	5	5.30019	.322913	.2145	.854584	.78654	.9331	.830716	.5712	.1968
OOELLI	5	.00000	.00000	.00000	.00000	.00000	.47790	.49155	.00000	.49155
GRCOCCOI	5	1.0441	.35211	.49273	.492114	.1577	.206316	.88644	.19925	.066
ULVARI	5	8.00579	.04119	.133412	.41224	.62849	.5117	.7495	.00000	.27677
PHORMIDI	5	8.25273	.80411	.34781	.8814	15.178	.52932	.62063	.2879	.00000
ACHNANTH	10	52.27855	.19113	.274	47.88	.67556	.003922	.501	.74582	.72995
COPLAC	10	284.73268	.42299	.5362	.82274	.977157	.2524	.20410	.12919	.827
FRAGILAR	10	.00000	.00000	.00000	.0000088	.959133	.95	.00000	.00000	.00000
GOMPHONE	10	71.52413	.292	28.212	.305837	.4255	.97551	.2417	.57033	.00000
MEVARI	10	13.11826	.948108	.22	51.071633	.8	115742	.93165	.873	.00000
NACRYP	10	24.798	20.8411	.8353	.989432	.64912	.427	.000001	.02923	.0218
NAVICULA	10	24.09712	.6199	.868311	.53960	.334	39.7	.579884	.5298	.00000
NITZSCHI	10	8.12257	.66116	.11598	.6776242	.33117	.36	1.6796	.50035	.5498
OOELLI	10	.00000	.000001	.0241	.96653	.00000	.00000	.00000	.62334	.00000
GRCOCCOI	10	3.76794	.5709	7.6469	.142417	.2524	.578664	.64670	.149214	.71
ULVARI	10	23.064	12.46	40.1742	.81146	.393183	.6529	.7743	.04182	.7481
PHORMIDI	10	25.47913	.88318	.19917	.64763	.93631	.55896	.95136	.2824	.3368
ACHNANTH	20	169.25125	.3357	.85295	.08129	.4799	.1487154	.384	.93644	.9011
COPLAC	20	132.01199	.68283	.21146	.4326	.625217	.43125	.4426	.81666	.561
FRAGILAR	20	.00000	.00000	.0000081	.647714	.412151	.2	.00000	.00000	.00000
GOMPHONE	20	98.29469	.06877	.2764	.497526	.35110	.494	.000004	.52981	.1244
MEVARI	20	578.491264	.94715	.4118	.061038	.91542	.631	.78547	.56347	.224
NACRYP	20	208.3	188.480	.7594	.057812	.238	.00000	.000002	.04354	.5732
NAVICULA	20	19.34954	.674	63.09113	.7196	.95592	.66450	.02623	.2951	.2757
NITZSCHI	20	34.21139	.32252	.812135	.67112	.86240	.57	.000001	.8439	.00000
OOELLI	20	.00000	.00000	.00000	.00000	.00000	.0000016	.54389	.11619	.662
GRCOCCOI	20	10.7763	.66553	.981832	.50618	.206	24.94120	.86147	.51216	.19
ULVARI	20	11.30129	.65443	.686100	.56	474.2667	.32131	.641	.39389	.6869
PHORMIDI	20	39.94285	.30685	.473	215.5225	.97156	.47	625.258	.939227	.12
ACHNANTH	30	86.01866	.1653	.890644	.82112	.3735	.082689	.5812	.01819	.4642
COPLAC	30	283.61359	.4393	.66682	.665	84.02345	.1369	.027137	.0464	.266
FRAGILAR	30	.00000	.000001	.841324	.966130	.78	2399.	00000.	00000.	00000
GOMPHONE	30	67.78921	.604	8.6941	.542111	.707	.00000	.00000	.00000	.00000
MEVARI	30	2167.82526	.5216	.83253	.26379	.34183	.65306	.08	.00000	.00000
NACRYP	30	8.23338	.11572	.92836	.8721	.00000	.00000	.00000	.00000	19.59
NAVICULA	30	22.00251	.0295	.063576	.33840	.99139	.689	.0000026	.265	.00000
NITZSCHI	30	24.14414	.64611	.90231	.18721	.19419	.153	.000007	.53828	.8379
OOELLI	30	.00000	.00000	.000004	.1623	.00000	.00000547	.991619	.2783	.09
GRCOCCOI	30	3.3163	.9821	.2938711	.08310	.024	.0000092	.552108	.96477	.19
ULVARI	30	18.71910	.1486	.1583	185.4	277.9	820.	469.	363.	.00000
PHORMIDI	30	9.790719	.676	.7880060	.38829	.66871	.3441317	.3467	.611156	.2

APPENDIX B-4. BIOVOLUME-DENSITIES OF ALGAE FOR GLEN LYN FALL, 1984, EXPERIMENT. EACH ESTIMATE TAKES UP SIX PRINT POSITIONS. MULTIPLY THESE ESTIMATES BY 1,000,000 TO GET ACTUAL AMOUNT. TREATMENTS: K=CONTROL, L=0.05 MG ZN/L, M=0.5 MG ZN/L, N=1.0 MG ZN/L. REPLICATES: A, B, C.

TAXON	DAY	KA	KB	KC	LA	LB	LC	MA	MB	MC	NA	NB	NC
COPLAC	0	28.51514.	15916.	9767.	6678	13.	1822.	81212.	51420.	77233.	79514.	08127.	57440.801
MEVARI	0	22209	16620	222549028.4		11229	11178	10227	22005	11534	17115	11011	18306
NACRYP	0	409.29356.44		582172.	71340.	53302.	24240.	41359.	74276.	12307.	84131.	53551.41	
NAVICULA	0	48.75547.	86886.	08939.	33259.	92459.	83648.	14289.	25134.	80147.	27725.	07445.	663
NISP1	0	25.14724.	64535.	45817.	08423.	859		2711.	32618.	59430.	58811.	76517.	66311.931
NISP2	0	18.4396.	867125.	82330.	42727.	89431.	17911.	03536.	89513.	90714.	90114.	5899.	7145
OOELLI	0	.000001.	7035.	00000.	00000.	00000.	00000.	00000.	00000.	00000.	00000.	00000.	00000
GRCOCCOI	0	39.88311.	48643.	45248.	90540.	55658.	06927.	65520.	91835.	92418.	90730.	854	17.12
SCENEDES	0	.328497.	58476.	6003	6.625	6.3771.	57676.	4872	8.9222.	33598.	17575.	14611.	6921
ULZONA	0	159.39194.	86269.	21	121.6142.	95	128.978.	125	264.598.	049157.	85	172.9105.	98
PHORMIDI	0	86.28391.	19774.	852137.	89121.	93	98.6161.	66852.	33540.	41865.	00953.	878113.	23
COPLAC	2	23.46313.	766	22.1610.	428	26.0714.	00743.	54930.	73952.	89715.	33657.	35535.	578
MEVARI	2	138715889.	44447.	29523.	66692.	26102.4		161529051.4		13111	10157	8351	15680
NACRYP	2	554.4390.	83142.	49119.	17113.	9991.	469243.	17138.	26	134.7186.	6890.	673187.	17
NAVICULA	2	47.41350.	207	23.2553.	79540.	11832.	19231.	91229.	98919.	65630.	16924.	16234.	401
NISP1	2	3.267213.	9665.	445417.	4256.	5345	18.399.	52957.	43436.	9054	8.0085.	445416.	611
NISP2	2	24.13911.	9657.	241757.	93411.	03520.	11612.	069	14.9818.	36724.	34214.	82822.	699
OOELLI	2	.00000.	00000.	00000.	00000.	00000.	00000.	00000.	00000.	00000.	00000.	000001.	88193. 0214
GRCOCCOI	2	18.81643.	238	14.4435.	00680.	73240.	97439.	33255.	39536.	62345.	044	45.0777.	629
SCENEDES	2	2.43275.	3011	6.352.	810896.	62234.	356816.	126	7.04510.	1128.	14878.	10896.	5095
ULZONA	2	106.2175.	2767.	1282141.	14	87.32	89.18161.	03	214.1119.	7795.	91655.	243204.	85
PHORMIDI	2	52.74251.	51527.	04739.	21833.	13376.	81224.	742	33.1512.	8629.	446617.	58110.	313
COPLAC	5	31.96826.	40225.	06134.	76115.	701	13.3431.	86440.	96457.	93443.	01628.	10116.	946
MEVARI	5	173099528.	96804.	46005.	83961.6		10049	11344	10336	117355662.	77362.	2	15944
NACRYP	5	717.04514.	23728.	44138.	1744.	579	141.486.	35586.	501152.	15.	00000124.	84147.	67
NAVICULA	5	72.67395.	109132.	5737.	48444.	71531.	10431.	40625.	07123.	15613.	67720.	234	25.53
NISP1	5	17.1715.	44114.	39565.	3455.	622185.	4496.	05053.	208615.	5584.	35634.	14329.	8017
NISP2	5	7.8539.	0801	19.8975.	09936.	197236.	4513.	7948.	804913.	684	4.48313.	5566.	2072
OOELLI	5	.00000.	00000.	00000.	00000.	00000.	00000	2.091.	00000.	00000.	000002.	3865.	94096
GRCOCCOI	5	27.21165.	14223.	66145.	216	79.06150.	02209.	55110.	01	160.148.	352125.	9667.	605
SCENEDES	5	4.49822.	46375.	8463.	300337.	441811.	0659.	00997.	96337.	29389.	190111.	4833.	9193
ULZONA	5	34.96364.	24840.	257246.	3285.	247249.	24156.	82658.	02476.	9163.	084159.	0947.	402
PHORMIDI	5	51.52658.	20777.	18918.	78322.	68933.	447	9.76713.	28119.	31910.	14317.	1491.	0143
COPLAC	10	35.578	12.528.	67765.	1765.	126218.	24960.	54132.	58852.	14141.	71367.	78315.	642
MEVARI	10	4522.7	14877	7690	10296	124843031.5		20448	28176164.7		9352	115548222.3	
NACRYP	10	794.431515.	4867.	5890.	67361.	12841.	451	69.6631.	08880.	598	139.956.	99572.	539
NAVICULA	10	109.06177.	05113.	6727.	35393.	22720.	05935.	6619.	573710.	13131.	00132.	824	15.5
NISP1	10	7.868415.	66514.	642386.	62132.	77127.	423.	99334.	35639.	68076.	534519.	6034.	3563
NISP2	10	6.643890.	93412.	644422.	43366.	15240.	0119.	3883.	79338.	429518.	62111.	7258.	2762
OOELLI	10	.00000.	00000.	00000.	00000.	00000.	000002.	3001.	0000014.	6373.	76385.	6458.	94096
GRCOCCOI	10	11.94329.	371	13.37106.	1159.	367	59.51487.	21143.	09228.	51136.	96170.	65131.	27
SCENEDES	10	4.99067.	12774.	95553.	378715.	4132.	16245.	94661.	75697.	20798.	64959.	1901	6.352
ULZONA	10	88.693182.	8483.	637858.	94459.	73724.	22924.	37	283.71092.	2233.	09	216.7142.	56
PHORMIDI	10	52.652111.	8564.	462130.	1698.	38826.	37196.	69451.	052	45.8312.	84710.	8194.	0571
COPLAC	20	68.34952.	57530.	416	17.387.	241819.	49743.	45123.	17411.	58731.	86438.	237	26.07
MEVARI	20	7466.73984.	81684.	27435.	81032.	83065.	73622.	61652.	42065.	53687.	74080.	23400.	1
NACRYP	20	498.08.	00000305.	1211.	5148.	635512.	91634.	54217.	27123.	02850.	66241.	45128.	785
NAVICULA	20	55.77756.	27744.	0698.	10477.	59829.	091812.	157	5.5727.	091611.	70110.	9412.	0262
NISP1	20	3.56918.	65217.	865691.	96722.	387142.	531.	2101.	60505.	00000	7.3212.	9042.	00000
NISP2	20	75.3461.	535	72.8198.	48	113.8195.	14.	766321.	91581.	53264.	21472.	75875.	3642
OOELLI	20	.00000.	000001.	0455.	00000.	00000.	0000046.	0031.	045512.	546195.	51158.	0852.	276
GRCOCCOI	20	13.3848.	824415.	072	57.3727.	956	60.54335.	9652.	509	65.15553.	53917.	15656.	36
SCENEDES	20	1.18117.	59845.	70639.	00998.	10898.	422813.	215	6.307	4.5054.	12968.	2891.	60066
ULZONA	20	244.5114.	13385.	32598.	77	397.2756.	991379.	71011.	82144.	8108.	03612.	07119.	59
PHORMIDI	20	38.4124.	79330.	42849.	58716.	15321.	913153.	2760.	105109.	699.	9173100.	073.	7566
COPLAC	30	84.488108.	2842.	77261.	55531.	36643.	45134.	07914.	48413.	03544.	60934.	368	66.29
MEVARI	30	9082.91565.	71915.	93773.5		4779428.99		6075953.	311255.	23390.	64052.	95988.	7
NACRYP	30	2026.2	787.	4927.	9828.	78520.	7798.	635559.	26428.	78514.	393	69.6625.	61461. 999
NAVICULA	30	133.9573.	15486.	01417.	72928.	03411.	65134.	2667.	09164.	558925.	63113.	522	21.82
NISP1	30	9.411818.	504	2.97831.	76542.	22122.	3873.	55911.	2101.	605053.	99333.	58936.	5159
NISP2	30	90.89429.	94650.	91899.	62163.	616	59.392.	2539.	766321.	14958.	4295	2.2735.	1579
OOELLI	30	.00000.	00000.	00000.	00000.	00000.	00000190.	6532.	41146.	003936.	15499.	28	512.3
GRCOCCOI	30	.000005.	369317.	34989.	94521.	05832.	089279.	56	119.641.	569720.	39389.	36981.	73
SCENEDES	30	18.6872.	5514	16.26	22.955.	6464.	805311.	0424.	95553.	75416.	27694.	008710.	107
ULZONA	30	149.38567.	87250.	461308.	81252.	11092.	22563.	62036.	71144.	5445.	19382.	92158.	86
PHORMIDI	30	43.826	21.746.	22362.	92265.	08231.	555		158123.59	51.8433.	058148.	1921.	239

APPENDIX B-5. BIOVOLUME-DENSITIES OF ALGAE FOR ZINC AND PHOSPHATE TREATMENTS AT UNIVERSITY OF MICHIGAN BIOLOGICAL STATION. EACH ESTIMATE TAKES UP SIX PRINT POSITIONS. MULTIPLY THESE ESTIMATES BY 1,000,000 TO GET ACTUAL AMOUNT. TREATMENTS: L=CONTROL, M=0.1 M KH2PO4, N=0.1 M KH2PO4. REPLICATES: A, B, C.

TAXON	ZINC	LA	LB	LC	MA	MB	MC	NA	NB	NC
ACMINU	C	1.1262.	61214.	33718.	64403.	69820.	36004.	21275.	62959.	38911
CYCLOTEL	C	1.4257.	523191.	11031.	4816.	18250.	63349.	40535.	26819.	35444
CYMBELLA	C	.75384.	808971.	27023.	6346	5.646.	76751.	41804.	433741.	3648
GOTENE	C	.35253.	33373.	34547.	33727.	61448.	20015.	27617.	45465.	58039
NAVICULA	C	.58448.	41382.	595831.	0153.	42229.	33623.	23953.	51679.	36246
NITZSCHI	C	.08924.	38503.	36025.	27601.	47948.	23955.	35005.	36234.	26769
SYNEDRA	C	.20688.	04502.	05760.	19907.	05415.	06576.	15175.	11593.	04811
GRCOCCOI	C	.12556.	09427.	01223.	02330.	38152.	12572.	07598.	60837.	10511
CTOSPHAE	C	.00000.	04830.	17507.	16780.	06971.	19536.	04884.	14659.	09032
SCENEDES	C	.29072.	20658.	050821.	5382.	29279.	25989.	23974.	18329.	05458
STIGEOCB	C	.00000.	98692.	84167.	000005.	53872.	1067.	33264.	30253.	00000
STIGEOCF	C	.00000.	26768.	00000.	146531.	1074.	45111.	41502.	55141.	12394
MICROCYS	C	.00000.	05297.	05534.	00000.	01338.	00536.	00357.	03653.	00000
ACMINU	0.1	1.4619.	76416.	50510.	38868.	84634.	69318.	376441.	3853.	37016
CYCLOTEL	0.1	1.4641.	68677.	733281.	3161.	57151.	27377.	62756.	31827.	30642
CYMBELLA	0.1	1.2885.	707842.	0416.	75671.	84698.	419832.	2159.	26368.	76506
GOTENE	0.1	.60400.	20435.	28796.	19782.	56701.	43083.	10171.	84619.	25056
NAVICULA	0.1	.80851.	29512.	35530.	36227.	06258.	26813.	35214.	79911.	29444
NITZSCHI	0.1	.34194.	17349.	15816.	84823.	23058.	07036.	87447.	57629.	31205
SYNEDRA	0.1	.10456.	04875.	12469.	20233.	03488.	06343.	17453.	25545.	08009
GRCOCCOI	0.1	.16182.	01109.	04207.	00000.	07303.	01732.	21132.	04651.	21144
CTOSPHAE	0.1	.11217.	13075.	23606.	15263.	03742.	04375.	03745.	41108.	07733
SCENEDES	0.1	.11862.	12279.	21135.	16033.	30366.	20835.	91343.	11592.	23340
STIGEOCB	0.1	.00000.	47492.	32155.	508204.	8423.	13243.	000002.	23981.	0143
STIGEOCF	0.1	.00000.	02898.	63666.	00000.	23502.	10776.	228281.	7212.	89517
MICROCYS	0.1	.00769.	00000.	00518.	01451.	32418.	01599.	00821.	00301.	03580
ACMINU	1.0	.12316.	22415.	20697.	280311.	0132.	328461.	30554.	67392.	0205
CYCLOTEL	1.0	.666662.	2953.	971761.	4647.	66806.	43292.	62045.	30579.	31411
CYMBELLA	1.0	1.19723.	97621.	54952.	32921.	7061.	766676.	5136.	22416.	40280
GOTENE	1.0	.09221.	19173.	15320.	11588.	68299.	16822.	447001.	3921	1.593
NAVICULA	1.0	.14214.	52544.	82447.	56961.	38319.	392811.	13181.	4778.	96756
NITZSCHI	1.0	.12666.	27797.	53292.	36669.	35193.	133071.	47941.	6648.	56996
SYNEDRA	1.0	.03644.	06467.	07320.	10936.	09681.	00914.	36024.	27869.	09529
GRCOCCOI	1.0	.08086.	02355.	03058.	008151.	7149.	36072.	27280.	06524.	07808
CTOSPHAE	1.0	.03665.	07631.	06929.	16800.	26708.	09561.	28986.	25628.	11501
SCENEDES	1.0	.08406.	16561.	22888.	08117.	18828.	20785.	19980.	13550.	69669
STIGEOCB	1.0	.000002.	6774.	34608.	000009.	09511.	4359.	837522.	0363.	00000
STIGEOCF	1.0	.00000.	05126.	11264.	053204.	1114.	38041.	21418.	710121.	3881
MICROCYS	1.0	.08722.	08635.	03875.	06711.	00000.	09073.	19072.	00000.	08408
ACMINU	3.0	1.75811.	0218.	330871.	0905.	81503.	37849.	982033.	22812.	2091
CYCLOTEL	3.0	1.1802.	62561.	28487.	90612.	64020.	63797.	17394.	34780.	06145
CYMBELLA	3.0	1.542.	43617.	556182.	02546.	2147.	891401.	2473.	24324.	54572
GOTENE	3.0	.43931.	39072.	44506.	47700.	53560.	21056.	99561.	81275.	52548
NAVICULA	3.0	.45517.	63261.	35234.	47058.	60091.	19777.	47227.	80561.	46080
NITZSCHI	3.0	.00000.	11888.	15609.	737131.	0102.	25410.	444141.	11821.	1138
SYNEDRA	3.0	.01860.	06124.	05136.	08044.	01149.	10270.	20385.	25922.	37244
GRCOCCOI	3.0	.12700.	05018.	14554.	03295.	59613.	34249.	43683.	21238.	11528
CTOSPHAE	3.0	.05987.	43364.	06199.	23732.	09244.	70458.	00000.	37079.	11987
SCENEDES	3.0	.20070.	00000.	00728.	07233.	29020.	190231.	1211.	15539.	24662
STIGEOCB	3.0	1.0873.	00000.	234561.	4694.	33576.	98738.	687461.	0522.	00000
STIGEOCF	3.0	.00000.	00000.	35882.	701411.	91241.	0027	4.7362.	08912.	3324
MICROCYS	3.0	.03611.	02594.	00906.	01183.	00000.	00095.	01107.	02033.	00000
ACMINU	10	.53564.	48400.	13028.	15869.	23985.	22542.	4513323.	4652.	0032
CYCLOTEL	10	1.3149.	77427.	70698.	43017.	41996.	31524.	65435.	11021.	68373
CYMBELLA	10	1.67382.	9571.	976801.	2092.	37399.	24759.	194061.	95781.	0734
GOTENE	10	.30248.	19272.	09767.	09948.	34579.	30456.	452883.	1083.	86773
NAVICULA	10	.53055.	24339.	25790.	36980.	21303.	39720.	325811.	8702.	42003
NITZSCHI	10	.23845.	07905.	17499.	10515.	08004.	12072.	577902.	7001.	80031
SYNEDRA	10	.01440.	01745.	01651.	01789.	00895.	01750.	140621.	5896.	11316
GRCOCCOI	10	.04718.	00477.	00902.	03482.	10630.	03942.	55046.	37988.	63047
CTOSPHAE	10	.06950.	11235.	04935.	12959.	10079.	83768.	17600.	63953.	10926
SCENEDES	10	.00668.	03960.	08489.	12059.	12062.	17019.	16189	1.072.	12210
STIGEOCB	10	.10519.	00000.	00000.	37591.	719141.	2785.	74206.	00000.	00000
STIGEOCF	10	.00000.	00000.	04488.	01330.	39897.	457721.	75579.	9236	9.742
MICROCYS	10	.01143.	02464.	05162.	03967.	00553.	00849.	00000.	00000.	03595

APPENDIX B-6. BIOVOLUME-DENSITIES OF PROTOZOA FOR ZINC-PHOSPHATE EXPERIMENT AT UNIVERSITY OF MICHIGAN BIOLOGICAL STATION. EACH ESTIMATE TAKES UP SIX PRINT POSITIONS. MULTIPLY THESE ESTIMATES BY 1,000 TO GET ACTUAL AMOUNT. TREATMENTS: L=CONTROL PHOSPHATE, M=0.01 M KH2PO4, N=0.1 M KH2PO4. REPLICATES: A, B, C. ZINC AS MG/L.

TAXON	ZINC	LA	LB	LC	MA	MB	MC	NA	NB	NC
ASPIDISC	C	.00000	.000005	.618143	.31174	.364	.0000039	.32727	.06910	.215
COLPIDI	C	.00000	.00000	.00000	.00000126	.22	.00000	.00000	.00000	.00000
CYCLID	C	15.36511	.197	11.6228	.502	38.72	.000002	11.279	.161412	.484
CYRTOLO	C	11.95810	.29951	.245174	.55104	.08	.00000	24.3942	.51649	.825
GLAUCOMA	C	117.25162	.53	704.4181	.6540	.406	.00000	.00000	.00000144	.31
LEPTOPH	C	3.438311	.317	21.15	.562827	.767	.0000016	.72120	.14110	.315
TRACHEL	C	413.51290	.06575	.26747	.72224	.75	.000001658	.9747	.72535	.13
VORTICEL	C	265.28906	.07534	.98296	.822013	.8	.00000	567.4	281	.2722
ASPIDISC	0.1	.0000037	.18229	.6235	.1074	27.5888	.86976	.61147	.805	18.08
COLPIDI	0.1	.00000	.00000	.0000032	.199	.00000	.00000	.00000	.00000	.00000
CYCLID	0.1	20.57	.9987357	.92659	.53912	.44632	.305193	.024	.4943176	.77
CYRTOLO	0.1	57.1328	.759121	.37	100.934	.439	107.5124	.077	.9023181	.97
GLAUCOMA	0.1	68.185	37.52952	.075267	.219	.482	.00000252	.54	23.45	1192
LEPTOPH	0.1	1.14016	.7753	49.961	.628710	.16318	.4732	.89541	.31743	.4166
TRACHEL	0.1	597.64278	.27345	.64249	.32499	.13	536.1948	.63246	.6421	.527
VORTICEL	0.1	.00000214	.58649	.64	.00000509	.331453	.1471	.61	505.8695	.62
ASPIDISC	1.0	.0000043	.311	.00000	19.0000046	.579	20.4393	.15961	.289	
COLPIDI	1.0	.0000051	.197	.00000	.00000	.00000	.00000	.00000	.00000	.00000
CYCLID	1.0	.0000026	.46612	.907	103.626	.88910	.94811	.52417	.5162	.3048
CYRTOLO	1.0	27.902154	.48251	.1256	.22326	.657133	.77	39.8640	.045103	.44
GLAUCOMA	1.0	131.32334	.61191	.931688	.8270	.5841	.1289	.019220	.56443	.292
LEPTOPH	1.0	8.9179	.0000020	.67333	.8847	.41951	.65041	.99063	.30081	.0858
TRACHEL	1.0	701.5	10701062	.5	279681	.07	901.2370	.9441	.594	270
VORTICEL	1.0	1716.6109	.3516	.5061717	.82343	.3151	.21943	.21772	.84265	.28
ASPIDISC	3.0	.00000	.00000	.0000025	.74129	.11211	.032753	.65189	.59168	.54
COLPIDI	3.0	.00000	.00000	.00000	.0000018	.354	.00000	.00000	.00000	16.1
CYCLID	3.0	6.991110	.9488	.143549	.36824	.085	14.52142	.8920	.05178	.746
CYRTOLO	3.0	3.368278	.10168	.659	191.1109	.3455	.96453	.134182	.3551	.071
GLAUCOMA	3.0	525.28390	.71239	.01	.0000010	.282301	.96	.00000	.00000180	.38
LEPTOPH	3.0	.0000013	.61623	.7866	.384464	.159	39.874	.48795	.8777	5.067
TRACHEL	3.0	822.15	450.6483	.44116	.46762	.561352	.93770	.2564	.321313	.5
VORTICEL	3.0	153.271680	.162	.488460	.526485	.22196	.52357	.4	1607928	.47
ASPIDISC	10	.00000	.000002	.9623	.00000	.00000	.0000030	.6443	.3198	23.29
COLPIDI	10	.0000040	.249410	.871204	.3991	.7451	.197	.00000	20.93	.00000
CYCLID	10	3.84132	.88096	.126894	.01541	.19745	.8076	.72223	.745226	.274
CYRTOLO	10	7.7229	4.85813	.149140	.867	.9471	.0000028	.151	10.0464	.469
GLAUCOMA	10	13.52918	.038266	.791865	.2	1002124	.29243	.5211	.72520	.564
LEPTOPH	10	4.07171	.17633	.043839	.6131	.19446	.33017	.60055	.76376	.8079
TRACHEL	10	106.4282	.09395	.228876	.27193	.98	35711459	.4154	.15	1643
VORTICEL	10	1488.5	.0000085	.479324	.23226	.961421	.6707	.4157	.477487	.23

APPENDIX B-7. BIOVOLUME-DENSITIES OF ALGAE FOR GLEN LYN SNAIL-ZINC EXPERIMENT IN FALL, 1984. EACH ESTIMATE TAKES UP SIX PRINT POSITIONS. MULTIPLY THESE ESTIMATES BY 1,000,000 TO GET ACTUAL AMOUNT. TREATMENTS: X=CONTROL ZINC, Z=0.5 MG ZN/L; S=SNAILS PRESENT, N=SNAILS ABSENT. REPLICATES: A, B, C.

TAXON	DAY	XSA	XSB	XSC	XNA	XNB	XNC	ZSA	ZSB	ZSC	ZNA	ZNB	ZNC
COPLAC	0	61.77843	608 18	1726	34649	05968	81963	595 54	51 54	5150	87625	438 18	17
GOMPHONE	0	13.4696	734611	2242	244919	08134	51517	9592	806123	571 7	85721	326	00000
MEVARI	0	22442	10987	262905352	7 106273557	4 116903922	7 13159	14316	111278861	2			
MEVARIAU	0	3503	51313	81946	4474	43729	8982	1131094	8291	96656	911678	81094	8802 88
NACRYP	0	202	0693	052118	1627	91699	69925	922127	6146	526	10954	50265	13763 807
NAVICULA	0	58	65925	53443	32314	83727	2598	669427	60413	28430	70915	52715	87221 048
NIPALE	0	54	13314	03533	41614	53634	0847	142626	0646	014818	04422	054 12	0313 032
NISP2	0	125	6744	048173	4644	69584	20943	23859	59422	02482	91356	35554	41239 513
ULVARI	0	.00000	17	8105	87	0000014	659	4	3197	32934	71176	28224	1881 0000047 117
COPLAC	2	51	784144	31 36	3470	86339	06567	22929	07244	27437	24828	163 29	9819 078
GOMPHONE	2	5	612225	6863	367310	1026	173412	3476	73461	06013	9285	8418319	0811 6837
MEVARI	2	4922	9 17314	102059611	44305	67923	5 11627761	623477	31656	6 88302070	7		
MEVARIAU	2	620	411768	61021	9729	89948	86	00000875	8755	148218	97164	231240	8255 46
NACRYP	2	37	886146	7463	807 97	04 78	4383	74769	1248	537215	61915	61937	22116 949
NAVICULA	2	12	93945	98518	28839	68125	016 37	6114	1471	49912	76043	19176	38342 7604
NIPALE	2	7	518540	484 12	0343	10616	03917	04210	0251	13612	00495	01239	5234 3 258
NISP2	2	27	206105	5140	809143	15 128	9174	25 25	912	56946	477617	16644	69513 927
ULVARI	2	.0000063	42615	706	000003	14118	3763	00000	000009	4233	0000057	0634	4499
COPLAC	5	51	78466	77426	574 96	5765	412 90	8548	78652	87441	973104	4868	137 43 88
GOMPHONE	5	6	73463	78825	4719 43	6513	4698	9795	16837	00000	33673 7	857 5	051 50510
MEVARI	5	4313	41687	94260	7 125007673	5 12346	143112	52644	675555	93379	6907 22		
MEVARIAU	5	912	37 191	6 191	61040	8291	96437	9443	79410	94810	948474	43255	4632 845
NACRYP	5	49	18515	45314	456149	92 78	4395	711 1	9941	19642	1934 29	9113	2934 9849
NAVICULA	5	23	8086	599111	25761	72632	43546	237	72461	87988	879887	24614	22692 2256
NIPALE	5	5	01233	38336	7666 24	6917	04236	089	15037	00000	15037 6	5163	00741 6541
NISP2	5	48	58236	67941	052212	44130	85212	46	38866	97164	7773125	58612	1456 4128
ULVARI	5	.00000	000002	3558	0000057	587	00000	00000	00000	00000	00000 1	0477	6957
COPLAC	10	45	78879	03955	055144	53 353	373	379 29	9826	43738	97439	24737	61243 063
GOMPHONE	10	3	7042	35711	6837 43	65 49	4724	725	00000	33673	16837	673462	0204 33673
MEVARI	10	349	29248	49370	396823	68427	7 10270210	98419	6239	852846	273000	625	787
MEVARIAU	10	32	845	0000043	7941419	2	00000446	6110	94832	845	0000010	948240	87 00000
NACRYP	10	6	38077	975910	767155	09155	09178	951	1964 1	9941	19642	39285	58311 1964
NAVICULA	10	6	10745	58985	900476	24296	61463	762	46582	51758	51758	931643	1055 15527
NIPALE	10	3	45854	21044	511121	26446	78236	804	15037	15037	30074	751852	1052 30074
NISP2	10	86	47618	26730	898296	56475	26227	51	48582	58298	485824	37246	6071 29149
ULVARI	10	.00000	000003	1411	00000	00000	000001	57061	72761	5706	471175	9681	15706
COPLAC	20	76	314125	37290	72105	99161	71141	58	40	6156	14548	51455	87338 97446 606
GOMPHONE	20	14	592	50	51121	2287	30142	65325	808	00000	33673	673461	3469 50510 33673
MEVARI	20	2477	14016	59814	65301	14219	68943	5194	5732	819506	36433	69511	05602 47
MEVARIAU	20	255	46145	98583	92946	16291	96745	8810	948	0000076	63910	948142	3332 845
NACRYP	20	37	88671	783	154	2246	9971	783	169	8	99699	398801	79464 3867 3 9881 7946
NAVICULA	20	25	01655	208182	1998	40346	92785	948	87988	25879	672852	58791	3975 82812
NIPALE	20	18	54617	042108	2728	15614	0356	4026	00000	15037	30074 1	203	60148 45111
NISP2	20	59	59497	812354	97156	74117	8988	5351	3603	000001	55462	91492	0404 87447
ULVARI	20	.0000042	92812	564	00000	00000	000007	69571	0994	00000	314118	16691	2564
COPLAC	30	127	19105	45169	36135	31141	32174	43	51	3333	38731	34358	59832 02438 838
GOMPHONE	30	92	0449	107125	97102	1687	30147	142	28061	14030	140301	1224	42091 84183
MEVARI	30	15316	4521	10018	18686	104726157	5291	08246	15125	03453	22238	33183 63	
MEVARIAU	30	.00000	00000277	681509	8873	38729	89	00000	00000	0000018	24718	247 00000	
NACRYP	30	249	9146	289126	43494	96282	34451	971	66161	49551	16324	6526 3	9881 8278
NAVICULA	30	146	341	899 82	7388	28176	51120	081	5959	690101	07832	76041	5959 99202
NIPALE	30	66	1637	160511	44129	0315	99778	0197	12531	12531	00000	75185	37592 00000
NISP2	30	259	1188	54157	72348	38213	15 116	6	89067	16194	242911	61941	53841 4575
ULVARI	30	.00000	00000	00000	00000	00000	00000	000004	97342	3558	261768	50724	9734

APPENDIX B-8. BIOVOLUME-DENSITIES OF ALGAE FOR GLEN LYN ZINC AND PH EXPERIMENT DURING SUMMER, 1985. EACH ESTIMATE TAKES UP SIX PRINT POSITIONS. MULTIPLY THESE ESTIMATES BY 1,000,000 TO GET ACTUAL AMOUNT. TREATMENTS: X=AMBIENT ZINC AND Z=0.05 MG ZN/L; 6=PH 6, M=AMBIENT PH, AND 9=PH 9. REPLICATES: A AND B.

TAXON	DAY	X6A	X6B	XMA	XMB	X9A	X9B	Z6A	Z6B	ZMA	ZMB	Z9A	Z9B
COPLAC	0	1.152913.	80814.	899 6.	438 18.	8310.	1768.	42713.	07434.	61152.	11364.	6115.	51530
CYMBELLA	0	4.8606	20.3314.	04620.	23115.	27629.	03341.	331 22.	22 22.	2216.	665 22.	2229.	795
FRAGILAR	0	.00000.	00000.	00000.	26433.	36290.	000001.	13692.	90323.	2661.	362901.	4516.	00000
GOMPHONE	0	9.565951.	567 69.	416.	75835.	84236.	11433.	76110.	65614.	0464.	722415.	25714.	288
MEVARI	0	65.	86445.	351110.	2 899.	81950.	92927.	31890.	41580.	62267.	7 258.	1 320.	41284. 1
NARHYN	0	10.40339.	34764.	607 26.	7238.	32658.	75761.	74987.	60371.	1778.	760322.	44843.	316
NAVICULA	0	.944473.	81414.	06261.	32842.	01924.	08513.	57092.	08434.	03841.	23762.	01925.	0658
NIPALE	0	17.53928.	00987.	70918.	31122.	21538.	295 75.	0986.	52375.	41616.	66257.	877113.	67
NIPALEDE	0	31.187121.	21141.	7735.	85761.	569103.	38280.	28196.	38	167 47.	35163.	65272.	67
SCENEDES	0	.00000.	55183.	00000.	17486.	04365.	12166.	00000.	34918.	39283.	00000.	13094.	00000
STIGEOCP	0	.00000.	00000.	00000.	36746.	00000.	00000.	000002.	0179.	00000.	00000.	000001.	0147
STIGEOCF	0	8.1274.	0000028.	567.	00000.	00000.	00000.	00000.	00000.	00000.	00000.	00000.	00000
ULVARI	0	.00000.	00000.	00000.	00000.	00000.	00000.	00000.	00000.	00000.	00000.	00000.	56394
MICROCYS	0	.742031.	16291.	4963.	66677.	679621.	6044.	08690.	97088.	52705.	27046.	66575 1.	525
PHORMIDI	0	.97026 2.	0667.	52372.	54421.	94056.	2204.	911873.	49291.	55241.	84352.	32863.	1225
COPLAC	5	.7685818.	20224.	59412.	709 43.	0410.	568 5.	38 10.	7610.	7756.	830415.	7561.	2542
CYMBELLA	5	.0000048.	46941.	6633.	402219.	44310.	41627.	77524.	99882.	748 4.	11415.	27652.	877
FRAGILAR	5	15.96824.	427.	00000.	444522.	9032.	36290.	725805.	080610.	812 1.	075.	000003.	1583
GOMPHONE	5	2.9061110.	4832.	9369.	789252.	3094.	601385.	97229.	06147.	109 7.	17425.	18625.	819
MEVARI	5	1317.	2124.	252456.	4802.	372591.	7 195.	8170.	88	4272511.	58145.	01224.	28708. 73
NARHYN	5	17.321	10.92148.	9347.	61788.	69812.	0456.	022766.	79729.	7459.	73164.	927712.	308
NAVICULA	5	1.69353.	247614.	8516.	54246.	1227.	846763.	71279.	50982.	73962.	12252.	80087.	0859
NIPALE	5	7.01545.	829739.	75418.	61922.	2154.	38466.	430883.	01630.	73616.	88510.	52310.	176
NIPALEDE	5	8.04827.	691162.	50734.	17655.	532 11.	6712.	74394.	96879.	454 42.	1281.	55579.	966
SCENEDES	5	4.0592.	21762.	698361.	01581.	2221.	30553.	15277.	61106.	91789.	274761.	8768.	75973
STIGEOCP	5	6.0538.	00000.	000001.	54492.	5224.	882848.	57624.	540337.	13243.	339.	7567215.	367
STIGEOCF	5	12.45136.	212.	000002.	5418.	00000.	0000024.	5551.	3834 6.	6675.	1226.	000008.	2773
ULVARI	5	1.4019.	00000.	56075.	34344.	56075.	00000.	42057171.	591.	47414.	3605.	0000011.	133
MICROCYS	5	46.71338.	9336.	93494.	09443.	9113 1.	28316.	7699.	0153 2.	1396.	5123 6.	1727.	8309
PHORMIDI	5	1.55242.	902622.	12214.	97510.	8671.	84353.	104810.	0919.	181916.	09611.	4493.	7999
COPLAC	10	.000001.	537276.	858 34.	0177.	95448.	2222.	0204.	0000012.	2971.	563711.	727.	00000
CYMBELLA	10	8.332516.	665.	000007.	8035.	000003.	66884.	86752.	777513.	88816.	952.	00000.	00000
FRAGILAR	10	.00000.	00000.	000001.	0196.	00000.	00000.	00000 3.	629 7.	258.	00000.	000001.	4516
GOMPHONE	10	5.327810.	6568.	23392.	72169.	15095.	438120.	37130.	51420.	827.	000009.	60763.	3904
MEVARI	10	2150.	2.	000002513.	42595.	52683.	3869.	946.	23884158.	12242.	8318.	68510.	61626. 56
NARHYN	10	17.	5214.	380278.	84339.	99559.	88967.	259.	0000019.	711 1.	0954.	45576.	6835. 00000
NAVICULA	10	1.1724.	521082.	34492.	83654.	01573.	52751.	82642.	73577.	42554.	24056.	36082.	7357
NIPALE	10	12.	862.	0000026.	89221.	35232.	5558.	49442.	049112.	86249.	108161.	7698.	12542. 092
NIPALEDE	10	10.7311.	073134.	33924.	87336.	01326.	223.	7052144.	533 86.	92233.	05631.	21308.	25
SCENEDES	10	3.	099.	34918.	029301.	90071.	04171.	26841.	0326 1.	6151.	87682.	13122.	79721. 3967
STIGEOCP	10	.0000023.	2062.	0179 1.	0638.	0263.	66637.	00000 19.	1754.	48414.	3696.	927930.	269
STIGEOCF	10	6.2253.	00000.	00000.	00000.	00000.	91366 27.	88.	0000035.	968216.	7217.	94242.	885
ULVARI	10	24.	95418.	5053.	9253.	98466.	00000.	555529.	8271331.	131.	6823 80.	436.	4173435. 71
MICROCYS	10	37.753	166.15.	60344.	2474 6.	7583.	810777.	2466.	435628.	87715.	40724.	76112.	594
PHORMIDI	10	2.71673.	104813.	972 28.	35 10.	42 25.	121.	700411.	64339.	19969.	48488.	82913.	584
COPLAC	20	1.53723.	842937.	08437.	08439.	77444.	193.	000007.	685813.	0663.	07431.	5372 5.	38
CYMBELLA	20	11.	11.	00000.	694382.	7775.	694382.	0831.	000004.	16632.	777516.	665.	00000. 00000
FRAGILAR	20	.7258070.	403.	00000.	54435.	36290.	36290.	000001.	45165.	8064.	00000.	00000.	00000
GOMPHONE	20	25.	18653.	763.	48435.	48435.	72652.	1210924.	21716.	468 1.	4531.	937412.	1096. 2965
MEVARI	20	6272.	7911.	36299.	04450.	34 596.	3 53.	4 925.	63944.	51708.	8726.	24 21.	36128. 16
NARHYN	20	1.	0957.	6653.	273764.	92771.	64268.	76032.	19012.	7376 1.	0954.	38022.	19013. 2851
NAVICULA	20	1.3027.	65136.	29311.	48852.	65136.	162841.	56331.	82383.	7779 4.	821.	69351.	9541
NIPALE	20	2.	338517.	539.	87693.	584621.	4615.	0000016.	3692.	923135.	077 106.	4176.	55155. 51
NIPALEDE	20	6.	438522.	5353.	75582.	88393.	48752.	816928.	43710.	19434.	33931.	65677.	53138. 631
SCENEDES	20	.78565.	69836.	04365.	29462.	34918.	000002.	3133 4.	8232.	7061 1.	4841.	83322.	6625
STIGEOCP	20	4.	54033.	53145.	80154.	79262.	39638.	702312.	61218.	918 77.	69176.	0668.	60923. 711
STIGEOCF	20	3.	458529.	7436.	0524.	00000.	00000.	0000068.	478111.	718.	992156.	71920.	05951. 877
ULVARI	20	103.	74182.	81.	280382.	3832.	00000.	00000390.	29.	0000012.	337301.	1353.	552294. 68
MICROCYS	20	1.	664411.	7341.	09571.	2136 1.	602.	1317622.	719.	873794.	21644.	71579.	23728. 0999
PHORMIDI	20	4.	26914.	65721.	94059.	50856.	79182.	61975.	82165.	2394 45.	0251.	61826.	77952. 782
COPLAC	30	.7685812.	29758.	796 45.	5 53.	849.	5734.	61153.	9197 3.	7553.	4586.	960721.	0306
CYMBELLA	30	12.	036.	000001.	3888.	97874.	00000.	00000 11.	11.	000006.	0311.	000001.	38887. 4488
FRAGILAR	30	11.	6136218.	7.	36290.	00000.	00000.	000009.	43541.	23397.	09211.	4516.	36290. 00000
GOMPHONE	30	10.	17183.	3085.	08561.	3654 1.	453.	1210926.	1552.	11731.	0517.	72652.	968698. 4431
MEVARI	30	2368.	61110.	7615.	881575.	6879.	32 35.	62883.	669.	1661256.	22214.	3 28.	48381. 89
NARHYN	30	.73003.	00000.	5475212.	348.	00000.	547524.	3802.	000001.	18891.	6426.	82128.	00000
NAVICULA	30	.82505.	52108.	455951.	0099.	97703.	22797 3.	387.	411281.	41442.	4752.	553651.	7468
NIPALE	30	.779499.	35394.	09233.	70815.	8462.	000007.	0154.	851877.	61677.	015427.	477465.	65
NIPALEDE	30	2.	235612.	8777.	377511.	8176.	43851.	877917.	9745.	79859.	75734.	426514.	55485. 616
SCENEDES	30	.04365.	00000.	39283.	30761.	00000.	00000.	61106.	00000 1.	161.	501951.	34225.	1504
STIGEOCP	30	2.	3542141.	2527.	2427.	466313.	369 21.	4454.	988 21.	9332.	86327.	49430.	647 8. 794
STIGEOCF	30	36.	891160.	47.	69170.	974961.	3834.	8646217.	984112.	3894.	62419.	0227.	4357 48. 23
ULVARI	30	23.	27171.	777.	00000.	39520.	28038.	4906615.	982.	3404624.	96221.	3097.	2898146. 62
MICROCYS	30	1.	460920.	4162.	64912.	46331.	2899.	034671.	5812.	323361.	12941.	33151.	36625. 4306
PHORMIDI	30	2.	328629.	4962.	13466.	15426.	40372.	61974.	65722.	073616.	8556.	40373.	433527. 582

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