Hydrobiologia 153: 261–275 (1987)

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

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Received 14 April 1986; in revised form 20 March 1987; accepted 27 March 1987

Key words: algae, diatoms, zinc, stream, mesocosm, SIMI

Abstract

Three treatments of zinc (0.05, 0.5, 1.0 mg Zn 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 l^{-1} treatment could be 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 l^{-1} treatment could be identified by a filamentous green alga in fall. The 1.0 mg Zn l^{-1} treatment was dominated 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 l^{-1}. Total biovolume-density of all taxa responded slower than individual taxa in spring and failed to distinguish between Zn treatments in summer and fall. Zinc bound to periphyton was much better than total Zn in water for identifying Zn treatments. Zinc treatments as low as 0.05 mg Zn l^{-1} changed algal species composition despite 0.047 mg Zn l^{-1} being the Criterion of the US Environmental Protection Agency for the 24-h average of total recoverable Zn.

Introduction

Simulated habitats are reliable systems for testing safe concentrations of hazardous substances as long as these systems are environmentally realistic and include many interacting species (Cairns, 1981; National Research Council, 1981). Odum (1984) suggests that confined experimental setups that are continuous with the natural environment be referred to as mesocosms. Periphyton are 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 ~0.064 mg Zn l^{-1} with a maximum level of 1.183 mg Zn l^{-1} (Subcommittee on Zinc, 1979). This mean level is higher than the criterion of the US Environmental Protection Agency (USEPA) for the 24-h average of total recoverable Zn (0.047 mg Zn l^{-1}) which differs slightly depending on water hardness (Environmen-

tal Criteria and Assessment Office, 1980). The Zn concentrations used in this experiment were set near the USEPA criterion and near the maximum measured for US waterways.

Say *et al.* (1977) indicated that algae are 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 at 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 as an indicator of stress. Community level analyses used differences in total biovolume-density and a similarity index comparing overall species composition as indicators of 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.

Materials and methods

Sampling

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. 1) were constructed from plywood and painted with a nontoxic chemical resistant paint (Farris *et al.*, submitted). Plastic paddle wheels maintained a current of ~14 cm sec^{-1}. Natural river water was continuously supplied at 1.2 l min^{-1} for each stream by a pump submersed in the New River. Peristaltic pumps delivered concentrated Zn solutions (as $ZnSO_4$) from separate carboys for each stream. Alkalinity, hardness, pH, temperature, and total Zn were determined for each mesocosm on all sampling days (American Public Health Association [APHA], 1981). Samples were refrigerated and analyzed within 2 d. Total Zn was analyzed using the direct aspiration method for atomic absorption spectrometry (USEPA, 1979). Three replicate streams were used for each Zn treat-

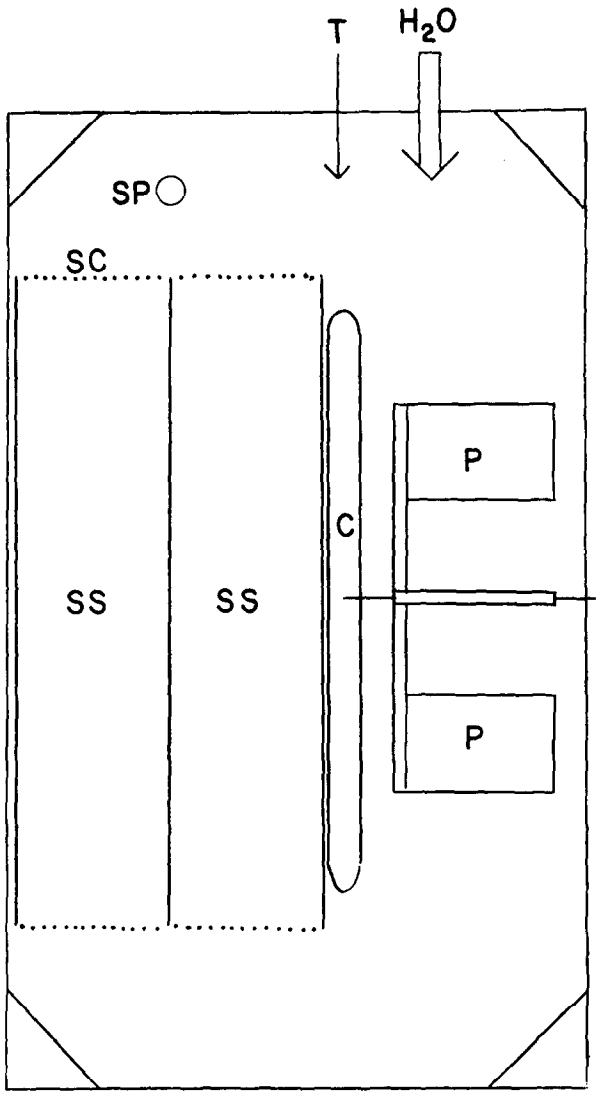


Fig. 1. Top view of one stream mesocosm with two sub-streams (ss), central longitudinal wall (c) that divides the stream into an oval course, paddle wheel (p) that causes the stream to flow clockwise in the picture, overflow stand pipe (sp), tubing from which Zn drips, and the faucet (H_2O) controls 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 × 49 cm, paddle wheel channel is 18.4 cm wide, and the stream is 76.2 cm long.

ment. Three treatments (control, 0.05, 1.0 mg Zn l^{-1}) were used in spring (28 April to 28 May) and summer (23 June to 23 July) of 1984 for a total of nine streams, and a 0.5 mg Zn l^{-1} treatment was added in fall (7 September to 7 October) of 1984 for a total of 12 streams.

Seasonal sampling

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. At least three randomly selected artificial substrates were pooled to form one sample per stream. 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 ($\sim$0.25 cm) on the tiles made enumeration difficult. 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 stream (Fig. 1), and samples were collected from mesocosms on all sampling days. 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 stream was 80 in summer and 120 in fall.

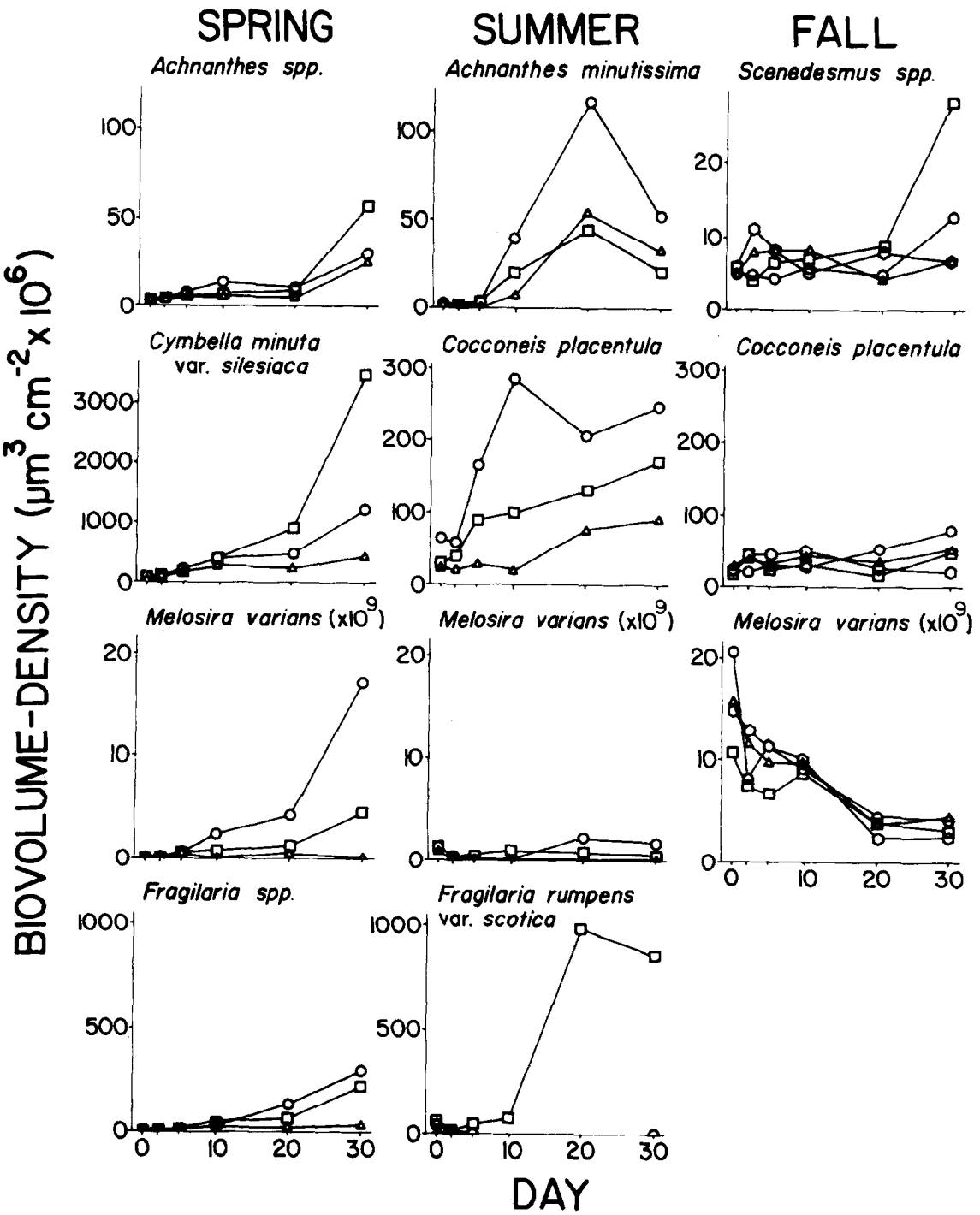
Artificial substrates were scraped with a razor blade and rinsed with distilled water. The resulting sample was homogenized for 10 s in a blender to shorten algal filaments. An 18-ml sample was drawn from this slurry and placed in a vial containing 2 ml of 37 percent formaldehyde. Algae that were alive (containing chloroplasts) at the time of collection were enumerated using a Palmer-Maloney plankton counting chamber under 400× total magnification. Diatom identifications were confirmed by examining cleaned specimens at 1000× 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 <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 five drops of sample.

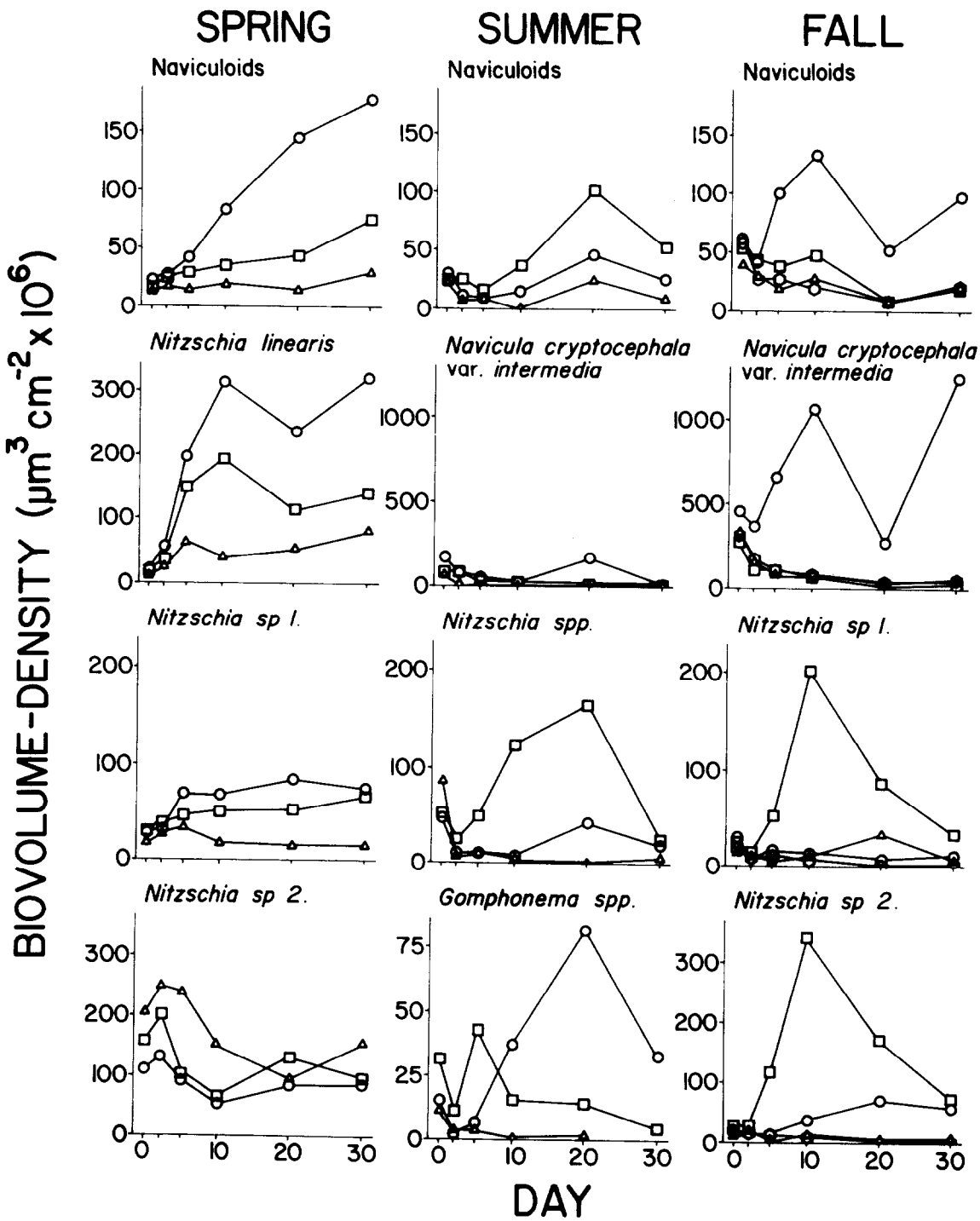
Biovolume was estimated using mensuration formulas that approximate the geometric shape of each taxon (Beyer, 1981). Direct measurements were made of dominant taxa, and median dimensions from the literature were used for less abundant taxa (Prescott, 1962; Patrick & Reimer, 1966, 1975). Algal density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

Periphyton samples were processed for Zn analysis by obtaining the dry weight from 5.0 ml of slurry filtered onto 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 percent 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).

Statistics

Statistical analysis was performed on taxa with average relative abundance >1 percent 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 Multiple Range Test determined if biovolume-density


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 10^6)
DAY

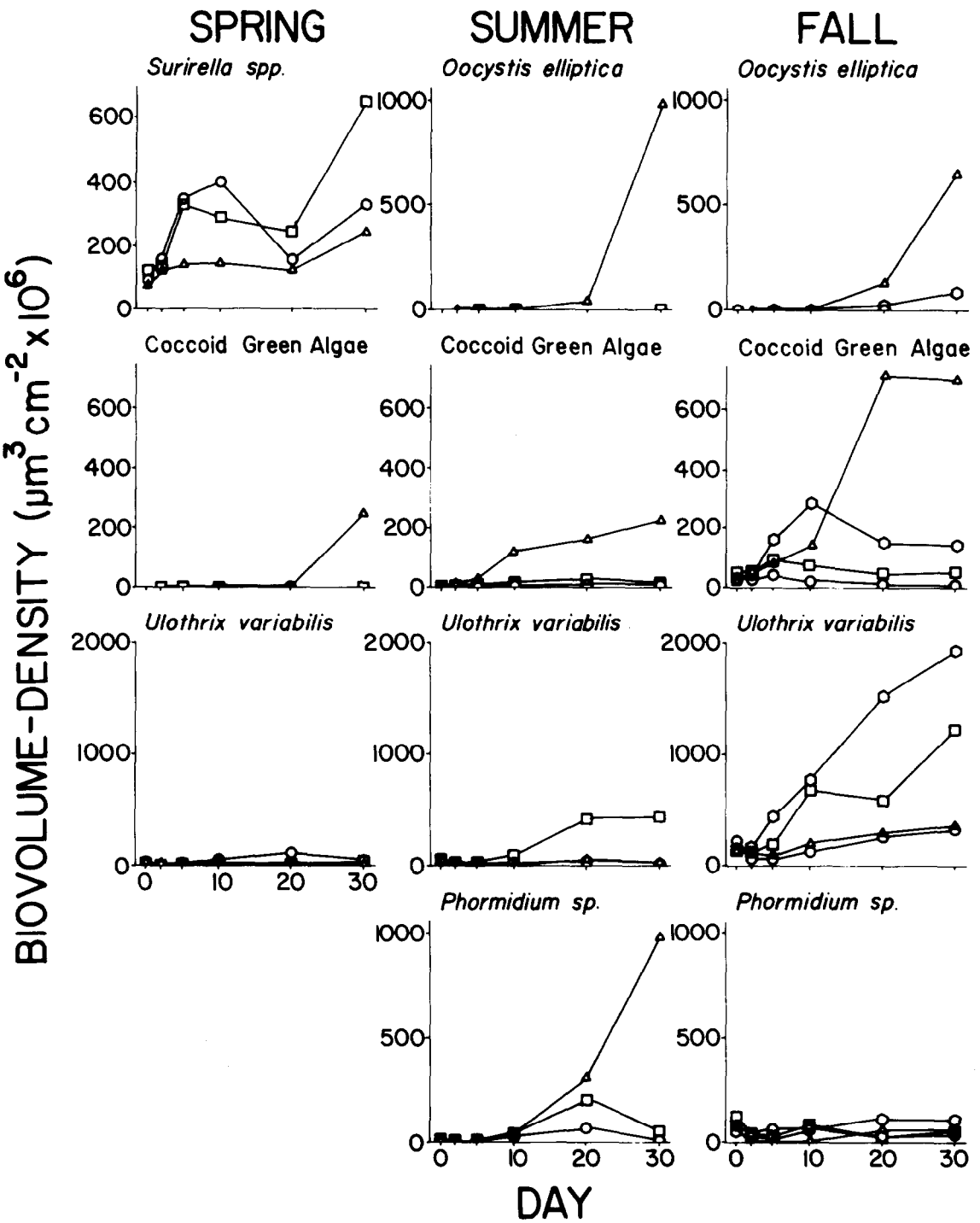


Fig. 2. Mean biovolume-density (n = 3) of dominant algae on sampling days in three seasons. Spring data are the mean of snail and nonsnail treatments. All taxa have biovolume-density × 10^6 except for *Melosira varians* that is × 10^9. Zinc treatments are Control ○, 0.05 mg Zn l^{-1} □, 0.5 mg Zn l^{-1}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

Table 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.

Taxon*	Day					
	0	2	5	10	20	30
Spring						
Achnanth						
Fragilar						
Navicula			C 0.05 1.0	C 0.05 1.0	C 0.05 1.0	C 0.05 1.0
Niline				C 0.05 1.0	C 0.05 1.0	C 0.05 1.0
NISP1				C 0.05 1.0	C 0.05 1.0	C 0.05 1.0
Surirell				C 0.05 1.0		
NISP2				1.0 0.05 C		
Ulvari					C 0.05 1.0	
Mevari					C 0.05 1.0	C 0.05 1.0
Cyminusi					0.05 C 1.0	0.05 C 1.0
Coccoid						1.0 0.05 C
Total biovolume					C 0.05 1.0	C 0.05 1.0
Summer						
Achnanth						
Frrumpsc						
Phormidi		0.05 C 1.0				1.0 0.05 C
Coplac	C 0.05 1.0		C 0.05 1.0	C 0.05 1.0		
Nacrypin			C 0.05 1.0		C 0.05 1.0	
Ulvari			0.05 C 1.0		0.05 1.0 C	0.05 1.0 C
Coccoid			1.0 0.05 C	1.0 0.05 C	1.0 0.05 C	
Navicula				0.05 C 1.0	0.05 C 1.0	0.05 C 1.0
Gomphone					C 0.05 1.0	
Nitzschi					0.05 C 1.0	0.05 C 1.0
Mevari						C 0.05 1.0
Ooelli						1.0 0.05 C
Total biovolume						
Fall						
Coplac					C 1.0 0.5 0.05	C 1.0 0.05 0.5
Mevari	C 1.0 0.5 0.05					
Nacrypin		C 0.5 1.0 0.05	C 0.5 0.05 1.0	C 1.0 0.05 0.5	C 1.0 0.5 0.05	C 1.0 0.5 0.05
Navicula			C 0.05 0.5 1.0	C 0.05 1.0 0.5	C 0.5 0.05 1.0	C 1.0 0.05 0.5
NISP1	C 0.05 0.5 1.0		0.05 C 0.5 1.0	0.05 C 1.0 0.5	0.05 C 1.0 0.5	0.05 C 1.0 0.5
NISP2			0.05 C 0.5 1.0	0.05 C 1.0 0.5	0.05 C 1.0 0.5	0.05 C 1.0 0.5
Ooelli		1.0 0.05 0.5 C			1.0 0.5 C 0.05	1.0 0.5 0.05 C
Coccoid	0.05 C 0.5 1.0		0.5 0.05 1.0 C	0.5 1.0 0.05 C	1.0 0.5 0.05 C	1.0 0.5 0.05 C
Scenedes		0.5 1.0 C 0.05				
Phormidi	0.05 C 1.0 0.5	0.05 C 0.5 1.0	C 0.05 0.5 1.0	0.05 C 0.5 1.0	0.5 1.0 C 0.05	
Ulvari			0.5 0.05 1.0 C	0.5 0.05 1.0 C	0.5 0.05 1.0 C	0.5 0.05 1.0 C
Total biovolume	C 1.0 0.5 0.05					

* ACHNANTH = *Achnanthes* sp., 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 = naviculoids, 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 = *Surirell* spp., PHORMIDI = *Phormidium* sp., ULVARI = *Ulothrix variabilis.*

differed among treatments. The Zn bound to periphyton was log 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, we have included this method in an appendix to this discussion.

Results

Treatments as low as 0.05 mg Zn l^{-1} significantly changed species composition in mesocosms for each season (Fig. 2; Table 1). Diatoms dominated the control treatment in all three seasons. Filamentous green algae dominated 0.05 and 0.5 mg Zn l^{-1} treatments in summer and fall, and diatoms different than those diatoms in the control dominated 0.05 mg Zn l^{-1} in spring and summer. Unicellular green algae dominated 1.0 mg Zn l^{-1} in all seasons, and a filamentous blue-green alga dominated 1.0 mg Zn 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).

Spring

Zinc treatments led to communities dominated by green algae or a different diatom taxa that prevailed over diatoms characteristic of control treatments (Table 1, Fig. 2). The control could be identified by the diatoms *Melosira varians* C. A. Ag., naviculoids, *Nitzschia linearis* W. Smith, and the filamentous green alga *Ulothrix variabilis* Kuetz.

Table 2. Means (± 1 SE, n = 15) for selected water chemistry variables 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	8.39	71.1	49.8
			(±1.8)	(±0.30)	(±13.5)	(±7.1)
	0.05	0.043	-	8.31	70.7	49.5
		(±0.060)		(±0.38)	(±14.6)	(±5.9)
	1.0	0.819	-	8.06	72.3	49.8
		(±1.014)		(±0.26)	(±10.6)	(±6.1)
Summer	control	0.028	25.5	8.44	70.2	47.6
		(±0.016)	(±0.8)	(±0.10)	(±3.65)	(±1.5)
	0.05	0.035	-	8.42	70.5	48.5
		(±0.012)		(±0.09)	(±4.2)	(±1.3)
	1.0	1.101	-	8.08	70.8	48.4
		(±0.955)		(±0.08)	(±2.7)	(±1.4)
Fall	control	0.094	20.6	8.31	88.8	56.2
		(±0.228)	(±1.9)	(±0.12)	(±2.3)	(±0.7)
	0.05	0.087	-	8.27	88.3	55.5
		(±0.109)		(±0.09)	(±2.6)	(±0.8)
	0.50	0.504	-	8.20	87.1	59.7
		(±0.286)		(±0.05)	(±2.6)	(±1.1)
	1.0	0.975	-	8.14	88.3	56.1
		(±0.299)		(±0.02)	(±2.3)	(±0.7)

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

on day 20. These taxa had reduced biovolume-density from 0.05 mg Zn l^{-1}. The 0.05 mg Zn 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 l^{-1} over control and 1.0 mg Zn l^{-1} treatments. The diatoms *Nitschia* sp. 1 (~30 percent *N. palea* [Kuetz.] W. Smith and 70 percent *N. dissipata* [Kuetz.] Grun.) and *Surirella* spp. were equally abundant in control and 0.05 mg Zn l^{-1} but were reduced in abundance by 1.0 mg Zn l^{-1}. The 1.0 mg Zn l^{-1} treatment could be identified by the presence of a coccoid green alga, similar in morphology to *Chlorella*, and by *Nitzschia* sp. 2 (~90 percent small forms of *N. palea*) that had significantly higher biovolume-density in 1.0 mg Zn l^{-1} on day 10. The diatoms *Achnanthes minutissima* Kuetz. and *Fragilaria* spp. 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 in Zn-treated streams on days 20 and 30 (Table 1). Differences in community similarity between treatments occurred from day 10 to 30 ($p < 0.05$). Figure 3 indicates 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) was ~270 in the control, ~1200 in 0.05 mg Zn l^{-1}, and ~6200 in 1.0 mg Zn l^{-1} (Fig. 4).

Summer

Zinc treatments lead to communities dominated by green algae that prevailed over diatoms characteristic of control treatments (Table 1, Fig. 2). The control could be identified by the diatoms *Cocconeis placentula* Ehr., *Navicula cryptocephala* var. *intermedia* Grun., *Gomphonema* spp., and *M. varians*, which were reduced in biovolume-density by 0.05 mg Zn l^{-1}. The 0.05 mg Zn l^{-1} treatment could be identified by the filamentous green alga *U. variabilis* and the diatoms *Nitzschia* spp. and naviculoids; which had lower density in control and

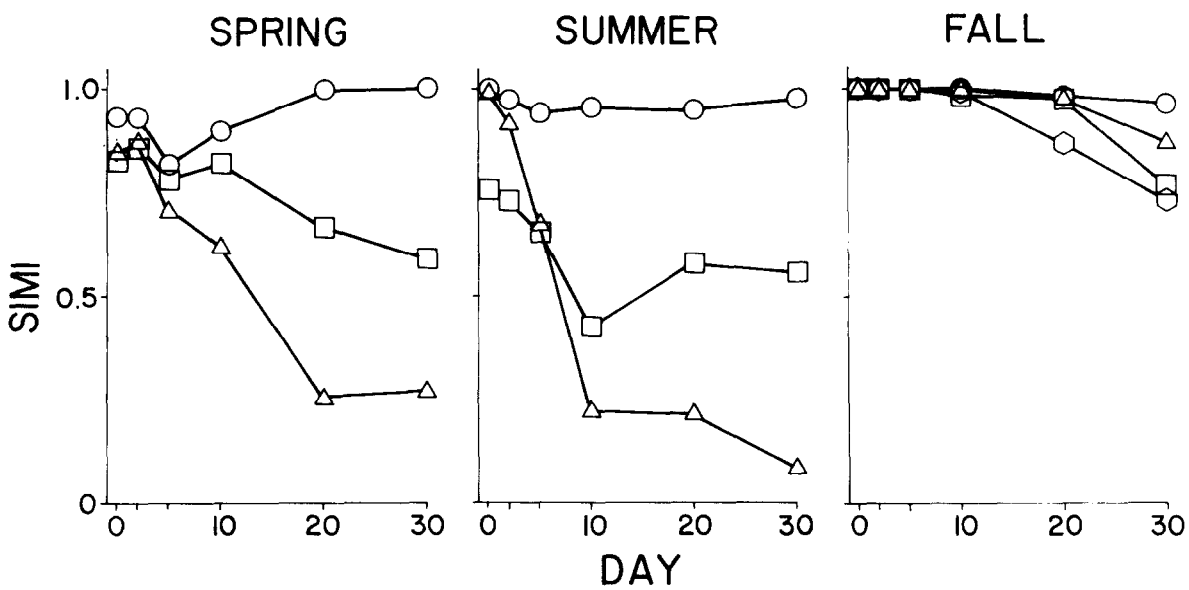


Fig. 3. Similarity (SIMI) scores for algal periphyton communities in three seasons. o = mean SIMI among control samples (n = 3); □ = mean SIMI between control and 0.05 mg Zn l^{-1} samples (n = 9); ◇ = mean SIMI between control and 0.5 mg Zn l^{-1} samples (n = 9); △ = mean SIMI between control and 1.0 mg Zn l^{-1} samples (n = 9).

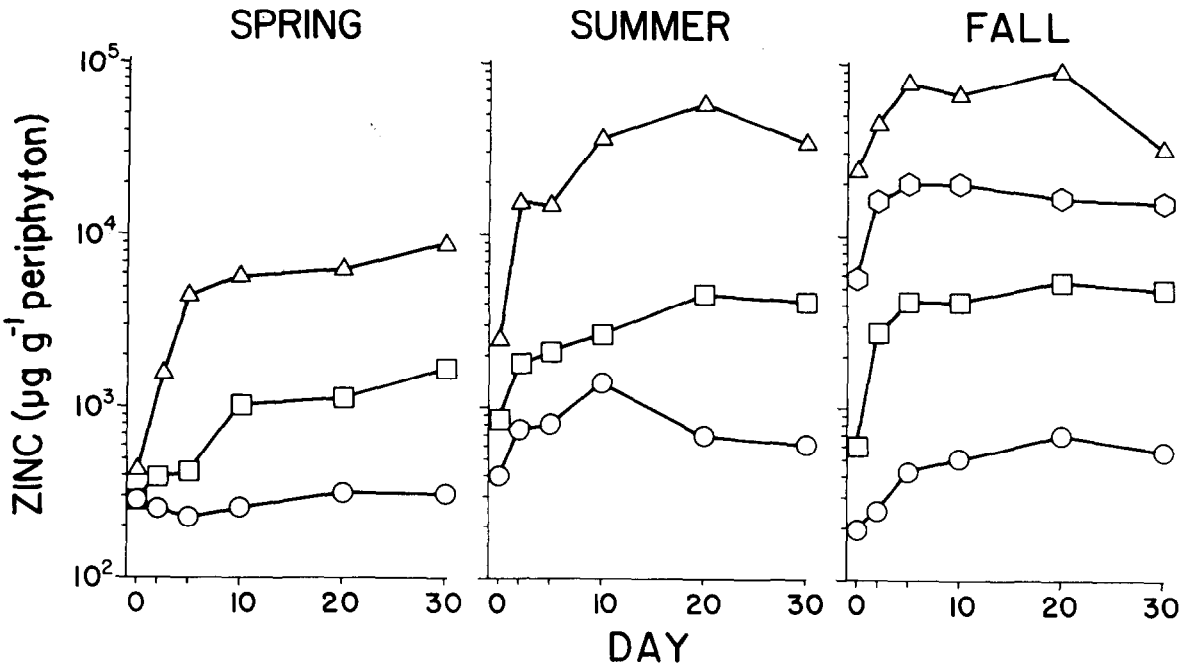


Fig. 4. Mean (n = 3) Zn concentration (µg Zn g^{-1} periphyton dry weight) on artificial substrates for three seasons. ○ = control, □ = 0.05 mg Zn l^{-1}, ◇ = 0.5 mg Zn l^{-1}, and △ = 1.0 mg Zn l^{-1}.

1.0 mg Zn l^{-1}. The 1.0 mg Zn l^{-1} treatment could be identified by the unicellular green alga *Oocystis elliptica* W. West and a coccoid green alga similar in morphology to *Chlorella*. *Phormidium* sp., a filamentous blue-green alga, identified 1.0 mg Zn l^{-1} in the middle of the experiment. The diatoms *Achnanthes minutissima* and *Fragilaria rumpens* var. *scotica* (Grun.) A. Cl. did not respond significantly to any Zn treatment, but absence of *F. rumpens* var. *scotica* in treatments other than 0.05 mg Zn 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 differed among treatments (ANOVA, $p < 0.05$), but Duncan's Multiple Range Test did not indicate which treatments differed (Table 1). Differences in community similarity between treatments occurred from day 5 to day 30 ($p < 0.05$). Figure 3 shows that Zn-treated communities became less similar to the control as Zn concentration increased from 0.05 mg Zn l^{-1}. The maximum Zn concentration in periphyton (µg Zn g^{-1} dry wt periphyton) was ~830 in the control, ~3600 in 0.05 mg Zn l^{-1}, and ~42000 in 1.0 mg Zn l^{-1} (Fig. 4).

Fall

Zinc treatments resulted in communities dominated by green or blue-green algae that prevailed over diatoms characteristic of control treatments (Table 1, Fig. 2). Control treatments could be identified by the diatoms *N. cryptocephala* var. *intermedia* and naviculoids that were reduced in biovolume-density by 0.05 mg Zn l^{-1}. The 0.05 mg Zn l^{-1} treatment could be identified by *Nitzschia* sp. 1 (primarily large forms of *N. palea*) and *Nitzschia* sp. 2 (primarily small forms of *N. palea*), which were reduced in biovolume-density by 0.5 and 1.0 mg Zn l^{-1}. *Ulothrix variabilis* identified both 0.05 and 0.5 mg Zn l^{-1} and was reduced in biovolume-density by 1.0 mg Zn l^{-1}. The filamentous blue-green alga *Phormidium* sp. shifted dominance repeatedly among treatments in fall. *Scenedesmus* spp., a green alga, was most abundant in 0.5 mg Zn l^{-1} on day 2. The 1.0 mg Zn l^{-1} treatment was dominated by *O. elliptica* and a coccoid green alga similar in morphology to *Chlorella*. *Melosira varians* did not respond significantly to any Zn treatment. *Cymbella placentula* did not differ in biovolume-density between control and

1.0 mg Zn l^{-1} but did have reduced abundance on different days for 0.05 and 0.5 mg Zn l^{-1}. Four taxa had significantly different biovolume-density between treatments on day 0.

Total biovolume-density differed among treatments on day 0, but significant differences were not observed thereafter (Table 1). Differences in community similarity between treatments occurred from day 2 to day 30 ($p < 0.05$). Figure 3 indicates 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) was ~530 in the control, ~4600 in 0.05 mg Zn l^{-1}, ~17000 in 0.5 mg Zn l^{-1}; and ~61000 in 1.0 mg Zn l^{-1} (Fig. 4).

Discussion

Population response to Zn stress and snail grazing

Zinc treatments as low as 0.05 mg Zn l^{-1} had communities dominated by green and blue-green algae in all three seasons, despite this being near 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 were indicated by the diatoms *Cocconeis placentula, Gomphonema, M. 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 US 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 l^{-1} treatment could be identified by *Cymbella minuta* var. *silesiaca*, naviculoids (summer), *Nitzschia*, and *U. variabilis*. Rushforth *et al.* (1981) found *N. palea* to have relative abundance positively correlated with Zn in natural streams. The 0.5 mg Zn 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 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 is complicated by interactions between Zn and other chemicals (natural or anthropogenic) in water (Cairns, 1977; Hart & Cairns, 1984) or the potential ability of algae to adapt to changes in stress (Say *et al.*, 1977).

Diatoms were more sensitive than a filamentous green alga that was more sensitive than unicellular green algae and a filamentous blue-green alga (Table 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 literature values 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 that snails reduce algal abundance except for the most tightly attached diatoms (Lamberti & Moore, 1984). The nonsignificant 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.

Community response to Zn stress

Zinc clearly determined what taxa would dominate, 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 treatments as low as 0.05 mg Zn 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. Zinc 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 a poor indicator of 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 (Lambert & Moore, 1984), which would then be food stressed by lower food quality at higher Zn concentrations when green algae dominate. Taxonomic changes were indicated by SIMI because it is derived from this data, but changes in total biovolume-density did not correspond to taxonomic changes.

There was a discrepency 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 atributed to high variability in the early stages of algal colonization rather than Zn treatment. Although 0.05 mg Zn l^{-1} appears to have reduced SIMI on day 0 in summer (Fig. 3), there were no taxa with significantly different biovolume-density on this day.

Zn in stream mesocosms

Zinc bound to periphyton (bound Zn; Fig. 4) was more reliable than total Zn in water (water Zn; Table 2) for indicating 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 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 l^{-1} treatments (Table 2), yet bound Zn differed significantly between all treatments by day 2. Bound Zn is intimately associated with periphyton and may better indicate what algae experience, 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) show a bound Zn concentration of 350×10^3 μg Zn g^{-1} lake sediment that is comparable to that found on periphyton of the control treatment. Algal taxon abundance was consistent with the bound-Zn concentration among replicates despite high variability in water Zn.

Conclusions

Treatments as low as 0.05 mg l^{-1} Zn significantly changed algal community composition from diatoms to green or blue-green algae. Water concentrations of this treatment ranged above and below the criterion level of 0.047 mg l^{-1} Zn set by the 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.

Acknowledgements

This research was aided by grants from the Ameri-

can Electric Power Service Corporation, Canton, Ohio 44701; E. I. DuPont de Nemours Educational Foundation; and a grand-in-aid of research from Sigma Xi, the Scientific Research Society. The authors thank R. L. Lowe, B. C. Parker, and G. W. Prescott for taxonomic advice. Technical assistance was provided by J. Grudzien and L. Decker; Darla Donald edited the manuscript for publication; and Angie Miller typed the final draft.

Appendix

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 replicate number. An example of data is given in Table A1.

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.

$$SIMI_{(ab)(cd)} = \frac{\sum_i X_{iab} X_{icd}}{\sqrt{\Sigma X^2_{iab} \Sigma X^2_{icd}}}$$

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 for example, Table A1).

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 $\bar{B}$, the mean between similarity; $\bar{W}$, the mean within similarity; or, analogous with ANOVA, $\bar{B}/\bar{W}$ (Good, 1982). This test statistic, say $\bar{B}$(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, $\bar{B}$(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 $\bar{B}$(permute), differs only slightly from $\bar{B}$(data). However, if the null hypothesis is false, $\bar{B}$(permute) is likely to be quite different from $\bar{B}$(data).

The second step in the randomization procedure is to carry out a number of data switches or permutations of data. For each permutation, $\bar{B}$(permute) is computed. This step is performed a great number of times (say 1000). The final step of the procedure is to compare $\bar{B}$(data) with the 1000 $\bar{B}$(permute) values. If $\bar{B}$(data) is smaller than most (95%) of the $\bar{B}$(permute) values,

Table A1. Example data and similarity matrices for cases where a treatment does and does not have an effect.

A. No treatment effect

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

Community	Rep	SIMI Scores							
	1	1.00	0.76	0.87	0.81	0.97	0.94	0.93	0.83
1	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
	1					1.00	0.95	0.89	0.93
2	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	20	50	20	5	5
1	2	10	40	30	15	5
	3	30	25	25	10	10
	4	20	30	10	30	10
	1	10	10	20	20	30
2	2	15	0	35	20	30
	3	5	15	20	25	35
	4	15	20	5	40	20

Community	Rep	SIMI Scores							
	1	1.00	0.93	0.87	0.81	0.49	0.41	0.53	0.57
1	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
	1					1.00	0.91	0.98	0.89
2	2						1.00	0.88	0.66
	3							1.00	0.84
	4								1.00

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 A1. In scenario 1, periphyton communities do not differ significantly between control and zinc 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 A1 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 zinc 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 A1 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 null hypothesis resulting from similarity data.

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