The maintenance of structural integrity in freshwater protozoan communities under stress

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Keywords: assimilative capacity, protozoans, communities, eutrophication, artificial substrates, colonization

Abstract

The structural assimilative capacity (ability to maintain biological integrity under stress) of protozoan communities from nine lakes in the area of the University of Michigan Biological Station, Pellston, Michigan, and six stations at Smith Mountain Lake, Virginia, were studied (1) to determine if communities from lakes of differing trophic state differ in their ability to assimilate various amounts of copper sulfate, and (2) to explore the possible influence of average density of individuals and/or qualitative differences in the types of species present on any observed differences in assimilative capacity.

In both the northern Michigan and Smith Mountain Lake studies, a trend in response was demonstrated along the eutrophic-oligotrophic gradient; eutrophic communities had a greater structural assimilative capacity than did oligotrophic communities. Both mean species density and community composition appear to be important factors in the ability to maintain structural integrity.

Introduction

The term *assimilative capacity* has been used in a restricted sense by engineers in reference to the assimilation of oxygen-demanding organic material as reflected by biological oxygen demand measurements (Velz, 1976) and also by others in conjunction with physical-chemical parameters such as temperature (Goubet, 1969). Engineering estimates of assimilative capacity generally fail to consider biological consequences of the proposed use or stress. Aquatic ecosystem assimilative capacity should be defined in a holistic sense as the ability of an ecosystem to assimilate a substance or stress without degrading or damaging its ecological integrity, i.e., the maintenance of structure and function characteristic of that locale (Cairns, 1977a). Since these characteristics may be expected to differ among ecosystems, care must be taken when making generalizations about the assimilative capacity of one receiving system based solely on information gathered from another.

A basic assumption for pollution control in industrialized countries is that three response zones (Cairns, 1977b) exist for most potentially deleterious materials: (1) one in which no adverse effects are noted and in which a change in concentration is not accompanied by a change in response, (2) one in which a change in concentration is accompanied by a change in response, usually increasing with increases in concentration, and (3) one in which the system has peaked out (i.e., organisms are all dead or incapable of further sublethal responses) and, therefore, changes in concentration produce no changes in response. Probably some substances exist for which humans and other organisms have zero tolerance, but society operates on the assumption that most potential toxicants have a threshold below which no significant adverse effects occur, e.g., food coloring and preservatives, automotive

Hydrobiologia 108, 171–180 (1984).

exhausts, etc. The assumption that individual organisms can assimilate certain amounts of these materials (i.e., ingest or inhale these materials and process them) without significant harm is used by government agencies to develop safe standards for exposure of humans and other organisms. This reasoning can be extended to communities through bioassays and toxicity tests utilizing the most sensitive species or stages in life cycle, or, as in the present investigation, through community bioassays.

As discussed by Cairns (1977b), natural systems have evolved a capacity to adjust to varied inputs of organic and inorganic substances from land runoff, falling leaves, and rain; it seems plausible that some communities are able to assimilate certain levels of society's wastes, particularly those similar to naturally occurring inputs. Some environmentalists, however, adhere to the zero discharge philosophy that calls for complete treatment of all wastes so that the discharge pipe can be connected to the intake pipe, and any discharge into the environment could be totally eliminated. Though obviously an ideal solution to human impact on the environment, the basic flaw in this reasoning is that, even when technologically feasible, such complete treatment takes comparatively enormous amounts of additional energy, chemicals, and facilities; the last 5% of a contaminant may be as expensive to remove as the first 95%. Since this energy and other components must be produced elsewhere, the ultimate result is a displacement of the environmental effects, rather than their elimination.

It is clear that society must find some way to optimize protection of ecological integrity while continuing industrialized production. Adjustment of life style, resulting in reduced consumption of material goods and energy, is an essential step toward this goal. Given that this change can be accomplished, dwindling fossil fuels and the displacement mentioned previously will nevertheless force us to forego complete treatment, to assume that assimilative capacity exists, and to make use of it to some degree. This study will demonstrate how protozoan communities may be used to compare the assimilative capacity of different systems. It will not, however, address the functional integrity component of assimilative capacity since this important topic requires a totally different approach.

Materials and methods

The structural integrity of protozoan communities collected from nine inland lakes in the northern region of Michigan's lower peninsula (Fig. 1) was investigated during the summer of 1979. Detailed site descriptions of the three oligotrophic lakes (Charlevoix, Walloon, and Burt), mesotrophic lakes (Long, Douglas, and Crooked), and eutrophic lakes (Carp, Round, and Munro) are given in a technical report by Gannon & Paddock (1978).

A similar study was conducted during fall of 1979 with communities from six stations representing a trophic gradient at Smith Mountain Lake, Virginia (Fig. 2). Smith Mountain Lake is the storage reservoir of the Smith Mountain-Leesville Pumped-Storage Hydroelectric Development, located southeast of the city of Roanoke; Smith Mountain Dam impounds water of the Roanoke and Blackwater Rivers to form a 22 000 acre lake. Major municipal-industrial discharges from the Roanoke municipal area enter the Roanoke River and cause it to act as a point source discharge into the lake. Eutrophic tendencies in the upper reaches of the Roanoke arm have been demonstrated by Simmons & Neff (1969), Jennings *et al.* (1970), Benfield & Hendricks (1975), and Cairns *et al.* (1979).

To test the ability of protozoan communities to maintain structural integrity while under a heavy metal stress, the following experimental procedure was employed. Polyurethane foam (PF) units 5 × 7.5 × 6.5 cm in size were routinely used (Cairns & Ruthven, 1970) to assure collection of the majority of species present. Each of 10 PF units was tied firmly around the middle with monofilament line or string and secured to a circle of nylon rope that was then anchored in approximately 3 m of water so that the PF units floated just below the surface. This position allows colonization by most littoral, benthic, and other substrate-associated protozoan species. At least 2 wk are required for an asymptotic number of species to be reached in most lentic systems. In these studies, PF units were allowed to colonize for 2–3 wk. At the end of the colonization period, PF units were harvested by cutting the cord suspending them from the suspension line and placing them in individual screw-top jars with approximately 100 ml of surrounding surface water for transport to the laboratory.

Upon arrival at the laboratory, the 10 PF units

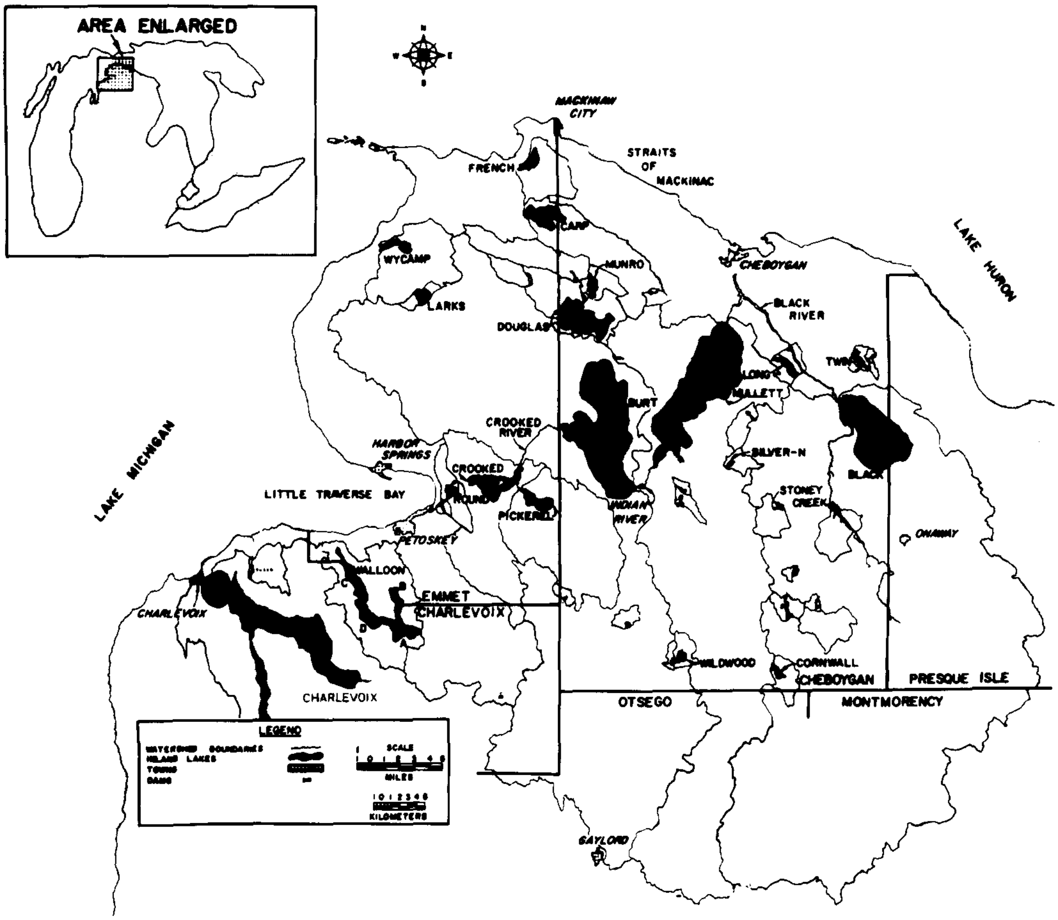

Fig. 1. Map of the northern region of lower Michigan (from Gannon & Paddock, 1978).

were distributed randomly among 5 battery jars (2 per jar) containing 5-l solutions of 5.0, 1.0, 0.5, 0.25, and 0 mg · l^{-1} $CuSO_4$. In Michigan, these solutions were made with pasteurized water (heated at 50 °C for 20 min) from Douglas Lake, and the experiments were carried out at the University of Michigan Biological Station Lakeside Laboratory located on its shore. Due to the high hardness and alkalinity of Douglas Lake water, some of the added copper sulfate may have been converted into other chemical compounds, such as copper carbonate, during the course of the experiment. The experiments on protozoan communities from Smith Mountain Lake were conducted with charcoal-filtered tap water to make the solutions and were carried out at Virginia Polytechnic Institute and State University. The PF units remained in the toxicant for approximately 24 h.

Examination of the substrate communities was done consistently in the order of decreasing concentration of copper sulfate. The PF unit was firmly squeezed over a glass jar where the sample was allowed to settle for at least 0.5 h; some species tend to settle and accumulate on the bottom, while others aggregate near the meniscus. Wet mounts were made of 3–4 drops from the bottom and were scanned on 100× power systematically from left to right and top to bottom over the entire coverslip (22 mm^2 No. 2). Identifications to species level were made wherever possible using standard keys to the Protozoa (e.g., Kahl, 1930–1935; Kudo, 1966; Leidy, 1879; Pascher, 1913–1927). Relative densities were estimated and recorded according to the system outlined in Table 1. Time did not permit

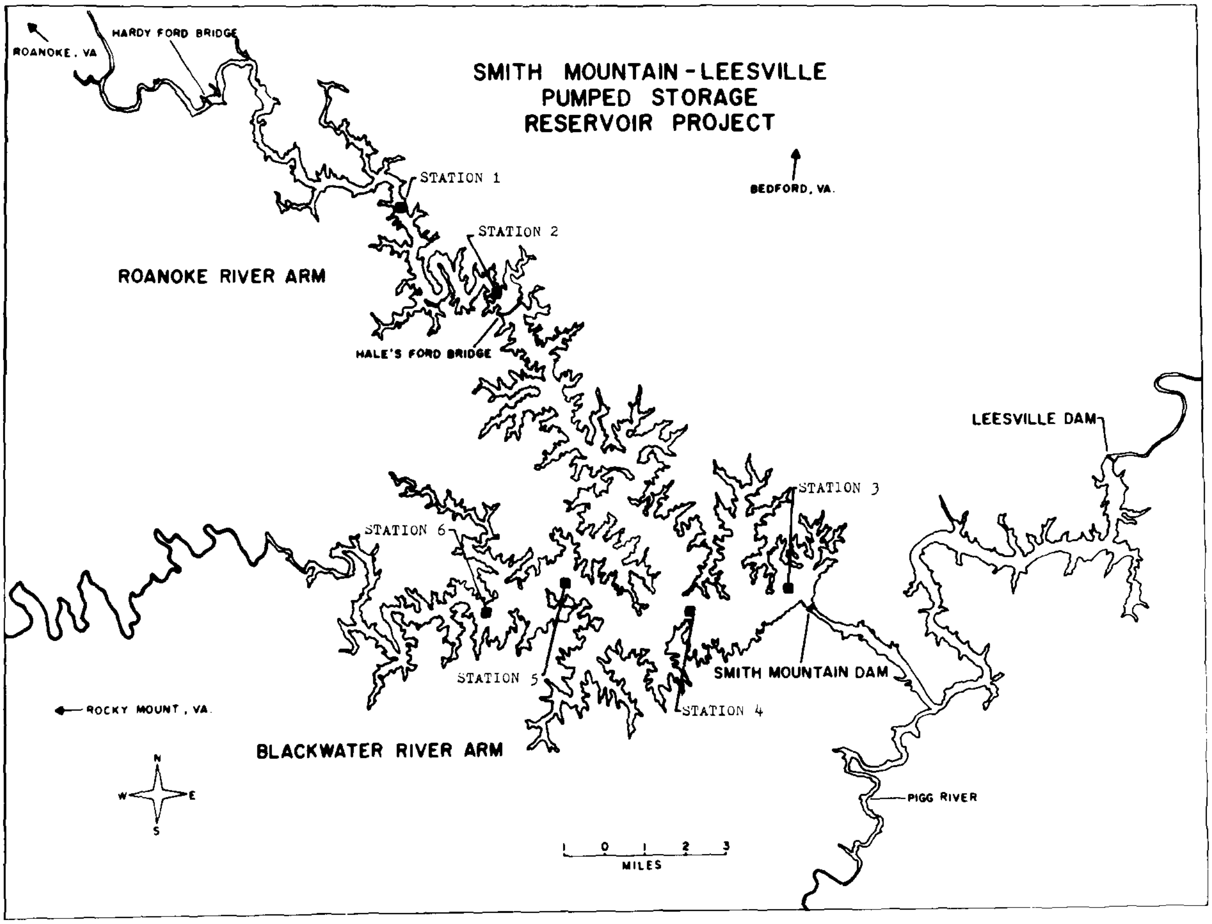

Fig. 2. Map of Smith Mountain Lake, Virginia, and selected stations (from Benfield & Hendricks, 1975).

examination of more than one slide from each of the 10 substrate samples since an average of 8 h was required to complete the analysis. Examination of living specimens at the end of the designated exposure time was necessary because many protozoan species are destroyed or distorted beyond recognition when fixed with conventional preservatives. and they often are difficult to separate from detrital material once fixed (Cairns, 1974).

Table 1. Rating system for estimation of species densities.

Density rating	No. individuals · slide^{-1}
I	1-2
II	3-10
III	11-25
IV	26-100
V	100+

Results and discussion

The communities tested that were exposed to identical levels of toxic stress demonstrated distinct differences in percent reduction of overall species numbers. A direct relationship between trophic state and ability to maintain structural integrity is clearly indicated by the Michigan lakes at 1.0 mg · l^{-1} (Table 2): the more eutrophic the lake, the smaller the percent reduction. This relationship is confirmed by the Smith Mountain Lake results (Table 3), though the variation among stations at each concentration was considerably smaller.

That the variance in percent reduction follows an approximately linear trend, especially between oligotrophic and mesotrophic lakes, indicates that differences in community structure characteristically associated with various stages of eutrophication

Table 2. Observed species numbers and mean percent reduction for replicate communities of nine northern Michigan lakes.

Lake	Control	$CuSo_4$ concentration ($\text{mg} \cdot \text{l}^{-1}$)							
		0.25		0.5		1.0		5.0	
		No. sp.	% Red.	No. sp.	% Red.	No. sp.	% Red.	No. sp.	% Red.
Charlevoix	46,48	28,25	43.6	18,21	58.5	9,11	78.7	1,3	95.7
Walloon	39,36	29,25	28.0	20,22	44.0	8,10	76.0	3,4	90.7
Burt	37,39	31,34	14.5	24,29	30.3	10,7	77.6	3,4	90.8
Long	31,34	29,31	7.7	30,28	10.8	18,14	50.8	2,2	93.8
Douglas (mature)	41,42	43,43	0	44,40	0	18,16	59.0	6,6	85.5
Crooked	32,36	44,42	0	40,42	0	16,12	65.0	3,3	92.5
Carp	32,36	30,27	16.2	28,26	20.6	22,24	32.4	9,9	73.5
Round	40,37	42,40	0	39,40	0	29,34	17.1	9,12	72.4
Munro	47,49	40,44	12.5	29,33	35.4	32,38	27.1	22,24	52.1
Douglas (immature)	20,23	11,10	51.2	6,9	65.1	5,3	81.4	3,0	93.0

Table 3. Observed species numbers and mean percent reduction for replicate communities from six stations of Smith Mountain Lake, Virginia.

Station	Control	$CuSo_4$ concentration ($\text{mg} \cdot \text{l}^{-1}$)							
		0.25		0.5		1.0		5.0	
		No. sp.	% Red.	No. sp.	% Red.	No. sp.	% Red.	No. sp.	% Red.
1	36,40	37,35	5.3	37,39	0	24,19	43.4	6,7	82.9
2	37,40	37,35	6.5	32,34	14.3	29,31	22.1	7,7	81.9
3	30,32	24,22	25.8	25,24	22.6	9,12	67.7	4,6	83.9
4	30,28	34,30	0	20,24	24.1	12,16	51.7	3,2	91.4
5	30,32	31,34	0	24,28	16.1	17,17	45.2	3,5	87.1
6	42,37	34,32	16.5	31,27	26.6	25,23	39.2	8,10	77.2

Table 4. Duncan's multiple range test on mean species numbers of northern Michigan Lake communities.

Lake	Control	$CuSO_4$ concentration			
		0.25 $\text{mg} \cdot \text{l}^{-1}$	0.5 $\text{mg} \cdot \text{l}^{-1}$	1.0 $\text{mg} \cdot \text{l}^{-1}$	5.0 $\text{mg} \cdot \text{l}^{-1}$
Charlevoix	47.0	25.6	19.5	10.0	2.0
Walloon	37.5	27.0	21.0	9.0	3.5
Burt	38.0	32.5	26.5	8.5	3.5
Douglas	41.5	43.0	42.0	17.0	6.0
Long	32.5	30.0	29.0	16.0	2.0
Crooked	40.0	43.0	41.0	14.0	3.0
Munro	48.0	42.0	31.0	35.0	23.0
Carp	34.0	28.5	27.0	23.0	9.0
Round	38.5	41.0	39.5	31.5	10.5

have an influence on structural assimilative capacity (i.e., ability to resist displacement). This conclusion is supported by the results of Duncan's Multiple Range test of each lake/station (Tables 4 and 5); the two most oligotrophic lakes (Charlevoix and Walloon) and the least polluted Smith Mountain Lake site (station 3) were significantly decreased in species numbers at 0.25 $\text{mg} \cdot \text{l}^{-1}$ whereas the remainder of the communities were not significantly affected at this concentration. Further confirmation is given by Duncan's test of the percent reduction values at 1.0 $\text{mg} \cdot \text{l}^{-1}$ for both northern Michigan (Table 6) and Smith Mountain Lake communities (Table 7).

Table 5. Duncan's multiple range test on mean species numbers from Smith Mountain Lake communities.

Station	Control	$CuSO_4$ concentration (mg · l^{-1})			
		0.25	0.5	1.0	5.0
1	38.0	36.0	38.0	21.5	6.5
2	38.5	36.0	33.0	30.0	7.0
3	31.0	23.0	24.0	10.0	5.0
4	29.0	32.0	22.0	14.0	2.5
5	31.0	32.5	26.0	17.0	4.0
6	39.5	33.0	29.0	24.0	9.0

Table 6. One-way ANOVA and Duncan's multiple range test of mean control species numbers and percent reduction values at 1.0 mg · l^{-1} for northern Michigan communities.

ANOVA	F	PR > F
Control	28.31	0.0001
1.0 mg · l^{-1}	27.93	0.0001

Duncan's multiple range test

Control		1.0 mg · l^{-1}	
Lake	Mean	Lake	Mean
Munro	48.0	Douglas (immature)	81.5
Charlevoix	47.0	Charlevoix	79.0
Douglas (mature)	41.5	Burt	78.0
Crooked	40.0	Walloon	76.0
Round	38.5	Crooked	65.0
Burt	38.0	Douglas (mature)	59.5
Walloon	37.5	Long	51.0
Carp	34.0	Carp	32.5
Long	32.5	Munro	27.0
Douglas (immature)	21.5	Round	17.5

Table 7. One-way ANOVA and Duncan's multiple range test of mean control species numbers and percent reduction values at 1.0 mg · l^{-1} for Smith Mountain Lake communities.

ANOVA	F	PR > F
Control	8.36	0.0112
1.0 mg · l^{-1}	9.20	0.0088

Duncan's multiple range test

Control						
Station	6	2	1	3	5	4
Mean	39.5	38.5	38.0	31.0	31.0	29.0
1.0 mg · l^{-1}						
Station	3	4	5	1	6	2
Mean	67.0	52.0	45.0	43.5	39.5	22.5

Table 8. Jonckheere's test on lake/station ordering.

Classification of lakes/stations		
Oligotrophic	*Mesotrophic*	*Eutrophic*
Charlevoix	Long	Carp
Walloon	Douglas	Round
Burt	Crooked	Munro
	SML 3	SML 1
	SML 4	SML 2
		SML 5
		SML 6
Test results		
J	*α (J)*	*Description*
71.0	0.0036	Significant trend with trophic state

To confirm the apparent relationship between structural assimilative capacity (i.e., the ability to maintain, under stress, the community structure characteristic of that locale) and lake trophic state, Jonckheere's test for ordered alternatives (Hollander & Wolfe, 1973) was used. Lakes and stations were categorized and the groups ordered according to trophic state; the mean percent reduction at 1.0 mg · l^{-1} was the variable tested. The results presented in Table 8 indicate a significant trend ($p \leqslant 0.0036$), i.e., the more eutrophic the conditions of the habitat from which the community is sampled, the greater will be its ability to maintain structural integrity when exposed to copper sulfate.

Having established that differences exist among the communities with regard to structural assimilative capacity, qualitative differences in community structure and composition were evaluated by measuring the percent overlap between species lists, as described by Sorensen (1948). Examination of species overlap values revealed no distinct patterns in species composition with regard to trophic state, though each comparison between the mesotrophic lakes (Long, Douglas, and Crooked) resulted in greater than 60% species overlap. Greater overlap was seen among the Smith Mountain Lake communities which might be expected when comparing assemblages from different sites within a single system. Percent overlap among species considered to be dominant (≥25 individuals) was generally lower in both studies than those based on complete species lists. This may reflect the importance of both time and space as factors that determine ultimate community structure, since most free-living protozoan species are considered to be cosmopolitan in distribution (Cairns, 1974; Cairns & Ruthven, 1972; Corliss, 1973; Lackey, 1938).

Though many protozoans are considered to be cosmopolitan in distribution, certain structural patterns have been demonstrated for protozoan communities. Patrick (1961) found that while species composition of a protozoan community in a freshwater stream is constantly changing, the diversity of species remains fairly constant. This constancy may indicate that a given species is replaced by another species with comparable function but slightly different environmental requirements. Various other studies lend support to the concept of structural stability within protozoan communities. Such studies include Cairns (1974), Cairns & Yongue (1973), Cairns *et al.* (1969), as well as earlier studies by Picken (1937) and Noland (1925).

Several studies have considered the nature of ecologic factors affecting the distribution of Protozoa. An early study by Lackey (1938) found that despite the cosmopolitan nature of protozoan species a given habitat may be prohibitive to many species because of local environmental characteristics. The quantity and quality of available nutrients have long been recognized as important factors governing the density and kinds of protozoans to be found in freshwater (Noland, 1927; Sandon, 1932); quality of decomposed organic material (Bick, 1966) and food supply in general (Noland, 1925) are considered to be of major importance in determining the occurrence of ciliates in a particular habitat.

The purpose of discussing relationships between environmental factors and species distribution is to emphasize that protozoan communities from lakes differing in trophic state are probably composed of different types of species that vary in sensitivity to copper sulfate. Ruthven & Cairns (1973) reported considerable differences from one species to another in response to toxic materials (including copper), as well as differences within a species in the response to various toxicants. Cairns (1974) noted that no available evidence explains protozoan differences in sensitivity to copper at the biochemical level; however, because copper is a constituent of certain oxidizing-reducing enzymes (e.g., tyrosinase and ascorbic acid oxidase), an enzyme activator to catalyze transfer of some amino acids, and a component of metalloflavoproteins (Dixon & Webb, 1958), these and other cellular processes may involve copper toxicity mechanisms in the different protozoans. This difference in sensitivity among species, therefore, is a primary factor in determining the structural assimilative capacity of freshwater protozoan communities. Communities from aquatic systems differing in water quality or trophic state may be expected to differ in composition, and thus to differ also in overall community response (e.g., percent reduction in species numbers) to a toxicant.

Species abundance or density (number of individuals per species) is another type of information used to quantify structural integrity. Several studies relate species abundance with levels of organic material, bacteria, and nutrients. Pollutional stress, such as organic enrichment of an aquatic environment, results in an increased range of species abundance (Patrick, 1949), i.e., some species become much more abundant under pollutional stress. Cairns (1965) found that organic enrichment alone usually results in an increase both in the density of individuals and the diversity of species. In a study of the population dynamics of Protozoa associated with the decay of organic materials in freshwater, Bick (1973) found that the high intensity of decomposition in heavily polluted systems favors high individual counts of certain ciliate species, in spite of the lack of dissolved oxygen and the presence of ammonia and other products of microbial decomposition. After conducting continuous culture experiments with ciliates, Straskrabova & Legner (1969) reported that: (1) all organisms studied showed a direct relationship between their quantity and the concentration of decomposable organic substances present, (2) the production of ciliates was directly related to bacterial numbers, and (3) the relationship between production of ciliates and bacterial numbers was different for different species. Mean density values of both northern Michigan and Smith Mountain Lake communities generally agree with these studies; the mesotrophic and eutrophic communities were higher in mean species abundance than oligotrophic communities. For the species with individual counts, the range of sensitivity or tolerance to a toxicant is probably increased (more individuals to make up the range), so that the probability of at least one individual surviving the toxic stress is greater than species lower in abundance.

Table 9. Kendall's test of concordance.

Lake/Station	S	α (S)
Charlevoix	22.89	<0.001
Walloon	25.36	<0.001
Burt	17.26	0.0048
Long	33.42	<0.001
Douglas (mature)	20.72	<0.001
Crooked	16.84	0.0048
Carp	19.16	0.0019
Round	32.08	<0.001
Munro	27.81	<0.001
Douglas (immature)	12.28	0.033
SML 1	27.01	<0.001
SML 2	33.06	<0.001
SML 3	25.62	<0.001
SML 4	28.28	<0.001
SML 5	24.47	<0.001
SML 6	28.83	<0.001

It may be hypothesized that density is an important factor in the maintenance of structural integrity. If this were the case, taxa represented by many individuals in a control community would be expected to be present in larger numbers relative to other taxa in a replicate community exposed to stress. The number of individuals per taxonomic order was estimated based on density ratings assigned to each species. Kendall's test of concordance (Sokal & Rohlf, 1969) was applied to these values to test this hypothesis. The variates (number of individuals per taxon) were ranked within each block (concentration of copper sulfate), ranks were summed for each treatment (taxonomic order), and a chi-squared statistic was then computed. Table 9 gives test statistics and significance levels for communities from all northern Michigan lakes and Smith Mountain Lake stations. The results indicate a strong rejection of the null hypothesis that assumes non-uniformity between ranks of each block. Thus, it may be concluded that the number of individuals representing a taxon in a community is a factor in determining its relative quantitative position after exposure to copper sulfate, i.e., that species density is also an important factor in the maintenance of structural integrity.

Summary

The results of this investigation of structural assimilative capacity may appear on first examination to contradict the traditional ecological concepts of diversity and stability. The following reasons illustrate that this is not the case.

(1) Stability is usually considered in terms of resistance to natural perturbations, such as introduction or removal of a predator, resource depletion, or a shift in nutrient ratios, rather than in terms of toxic stress that may have quite different effects on community structure.

(2) In the case of a natural perturbation, a diverse community with a complex food web and many energetic pathways can better withstand the elimination of some of these pathways, probably resulting in less variability of population numbers. This may be true for diverse communities under toxic stress, depending on which and how many pathways are affected, but also may be true for less diverse communities where the dominant and/or functionally important species are resistent to the particular toxicant under consideration. This capacity to assimilate a toxicant does not presuppose stability with regard to natural perturbation, and vice versa. In the present study, the eutrophic communities appear to have a greater proportion of species that are tolerant of low concentrations of copper, and, thus, are more capable of assimilating this heavy metal with regard to structural integrity. Again, this does not *ipso facto* assume greater stability under conditions of natural perturbation.

(3) As previously mentioned, eutrophic communities usually have a greater proportion of species present in high numbers (resulting in reduced diversity); therefore, a greater chance exists that some individuals of these species would survive a toxic stress and preserve species richness.

Consequently, stability in the traditional sense (e.g., Watt, 1964) and assimilative capacity as defined by Cairns (1977b) should not be considered interchangeable terms.

Acknowledgments

This research was partially funded by a grant from the Exxon Educational Foundation. Much of the field and laboratory work was carried out at the University of Michigan Biological Station, Pellston, Michigan.

We are indebted to Duncan J. Cairns, Michael S. Henebry, James L. Plafkin, and David L. Kuhn for assistance in collecting samples and identifying protozoans in the Michigan portion of the study and to Michael S. Henebry, Robert Honig, Richard A. Lechleitner, and J. Clark Miller for help in collecting samples for the Smith Mountain Lake study. Judy Alls was most helpful with regard to water chemical analyses. Kraus Hinkelmann and Jerry Mann of the Virginia Polytechnic Institute and State University Statistical Consulting Center provided much advice on data analysis. Darla Donald, Editorial Assistant, helped prepare this manuscript for publication and Betty Higginbotham typed the final draft.

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Received 4 October 1982; in revised form 4 March 1983; accepted 13 April 1983.