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POLLUTION RELATED STRUCTURAL AND FUNCTIONAL CHANGES IN AQUATIC COMMUNITIES WITH EMPHASIS ON FRESHWATER ALGAE AND PROTOZOA

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ABSTRACT. — This paper includes a discussion of certain select aspects of structural and functional changes in algal and protozoan communities and how they relate to the assessment and solution of pollution related problems. New methods and concepts developed by the authors are related to older methods and concepts. The various types of pollutional stress are discussed together with their effects on algal and protozoan communities.

INTRODUCTION

Despite abundant literature on the physiology of select algal and protozoan species and on the natural occurrence of numerous species, most algae and protozoans are still poorly understood in terms of general environmental requirements and operational prerequisites. These voids in our knowledge exist for at least two basic reasons: (1) Generally, laboratory culture studies involve simple, stable environments, lacking organismic interactions, succession, and other complex natural phenomena; and (2) Most field observations are limited to macroenvironmental conditions and recording of species without regard for ecotypes or ecophenes.

Of course, one cannot discount the value of limited information on the physiological ecology of individual species. However, even a relatively comprehensive understanding of individual species biology usually proves inadequate for accurately predicting the complex interactions, interlocking cause/ effect pathways, and operational characteristics of microbial communities. Consequently, one reasonable approach to an amelioration of the ever intensifying problems, in view of the insufficient time, personnel, and funds to acquire the necessary total knowledge for all species important to ecological systems, is to examine gross pollutional effects on aquatic communities as natural operating units. This approach is developed in this paper. In so doing, we shall discuss select aspects of structural and functional changes, especially in algal and protozoan communities and how they relate to the assessment and solution of pollution-related prob-No attempt at a comprehensive review of lems. literature was made. We shall discuss, however, some of the past and present practices and important ecological concepts which have led to or formed some basis for using structural and functional changes in algal and protozoan communities in pollution monitoring and assessment.

The motivation for this paper stems chiefly from our recognition of these points: (1) Increasing stress upon the environment, resulting from an expanding population and industrial base, necessitates wiser use and management of that environment than previously; (2) The environment must be managed more intensely and quite differently in the future; (3) To best achieve this goal, research will be required in parallel with the development of management practices in order to fill critical gaps in our knowledge of community structure and function.

PAST AND PRESENT APPROACHES

THE "TARGET SPECIES" APPROACH

Mount (1969) mentioned (but did not espouse) that we have two alternatives in protecting aquatic communities: (1) to protect diversified aquatic communities, and (2) to protect species of particular value to man. The protection of these "target species" alone (generally herbivores or carnivores such as trout, salmon, oysters, or bluegill sunfish) assumes that this protection would somehow preserve enough of the other components of the aquatic community to furnish food and other necessities to the target species. While many procedures employed to protect "target species" are relatively easily implemented, the assumption that protection of "target species" using current methods and procedures simultaneously preserves the necessary associated organisms is, in our opinion, unproven. For example, Patrick, Cairns and Scheier (1968) showed that diatoms, invertebrates, and fish varied in their sensitivities to various common constituents in industrial wastes. Furthermore, as "target species" rarely are primary producers or decomposers, they often exert less influence upon the aquatic ecosystem than do the species lower on the food chain which have greater biomasses and levels of metabolic activity.

Another inherent weakness in the "target species" approach stems from the idea that "target species" can be protected routinely by studying only one stage in their life cycles. This concept is not necessarily true. For example, response to some toxic materials varies with fish size (Cairns and Scheier, 1959). Also, the eggs of some species are more sensitive to some and less sensitive to other chemicals than are the adults of the same species (Cairns, Scheier, and Loos, 1965). At the level of the primary producer, the reproductive stages often are more temperature-sensitive than vegetative stages of their life cycles (Trainor, 1959; Parker and Bleck, 1966). It follows from the above examples that the protection of a "target species", while often relatively easily implemented, would probably not be successful because responses of other species associated with the "target species" have been ignored. Regulatory agencies probably favored the "target species" approach because, with the paucity of information and funds, the method seemed to be the only practical way to achieve results over a short time period. This justification for use of the "target species" approach today is, however, no longer tenable. Moreover, we feel that a continuous re-evaluation of both criteria and standards is essential, with the chief goal of an evolution of better control and maintenance of environmental quality aimed at a wide variety of uses over a long period.

The concept that a complex community with many interlocking, reciprocal cause-effect pathways cannot be preserved by protecting one or a few "target species" facilitates an appreciation of the value of determining effects of stress upon community structure and function. Our focus on responses of algal and protozoan communities to pollution, with lesser attention to other aquatic microorganisms, constitutes only one area of the community structure and function approach. These microorganisms nevertheless significantly influence higher aquatic organisms, and through their interactions affect entire aquatic communities.

Algae and protozoans also can be affected by higher aquatic organisms. For example, Brooks and Dodson (1965) compared two lakes and showed that one lake with alewives (Alosa pseudoharengus) had a different invertebrate community than another lake lacking this fish. Similarly, the phytoplankton communities were different in the two lakes. Where fish stocks were high, and Bosmina was common, the net phytoplankton (especially diatoms and Dinobryon) were much more abundant than when Daphnia dominated. Moreover, in both situations with Bosmina dominant, the standing crop of phytoplankton as determined by organic nitrogen and chlorophyll content was two to three times greater than in the presence of Daphnia. Parker and Wodehouse (1970) pointed out that digestibility may be one important factor in selective feeding by zooplankton. They postulated that nuisance blue-green algae are incompatible to most aquatic herbivores, because the latter organisms probably lack the necessary mucopeptidases (e.g., lysozymes) to dissolve the protective mucilaginous sheaths uniquely characteristic of this group of primary producers. While these papers are among the many which deal with communities of organisms belonging to particular taxonomic groups, they demonstrate the value in studying larger aquatic communities as operational units. Furthermore, virtual elimination of a vertebrate or invertebrate population may alter the structure of a microbial community even though there is no direct effect. It is within this context that we reject the "target species" approach and urge studies of the effects of pollution upon algal and protozoan "communities".

THE "SAPROBIAN SYSTEM" APPROACH

The "Saprobian System" which has been widely used in Europe, employs the concept of "indicator species," especially microorganisms, and stems from the work of Kolkwitz and Marsson (1908). The basic assumption of those using the "Saprobian system" is that the presence of certain species indicates a particular set of environmental conditions, this idea being a logical extrapolation of the niche concept (Hutchinson, 1957; Parker and Turner, 1961). That is, each organism has a particular set of environmental prerequisites essential to its survival. Users of the "Saprobian system" have extended their interpretation of the concept to suggest that the presence of certain species indicates pollution (i.e. that the organism is "pollution tolerant"). For example, Bodo minimus is listed as polysaprobic or "pollution tolerant" (Pascher and Lemmerman, 1914). B. minimus is indeed tolerant to certain types of organic pollution; however, one must also consider heavy metals, pesticides, and other toxic chemicals at concentrations sufficient to kill fish, and B. minimus apparently is not as tolerant to such pollutants as it is to sewage. Some advocates of the "Saprobian system" have claimed that a species tolerant or sensitive to one form of pollution is tolerant or sensitive to other forms of pollution without regard to the nature of the pollutants. This assumption has been shown to be false a number of times (Patrick, 1949; Hynes, 1960).

Refinements of the "Saprobian system" tend to correct this weakness by being more specific (Fjerdingstad, 1962). Despite these improvements, however, knowledge of the environmental requirements and tolerances of species to toxicants limits the "Saprobian system" to low predictive capability and requires constant readjustment in response to the appearance of information about new pollutants. The presence of species known to indicate certain conditions, such as, anaerobiosis, high salinity, high temperature, etc., can provide useful information if no assumptions are made regarding the characterization of the pollutional load by the presence of such The "Saprobian system" apindicator species. proach, therefore is useful but less comprehensive or precise for assessing pollutional load than by examining changes in the structure and function of algal and protozoan communities. If a vast body of appropriate information accumulates, this situation may be reversed but this is not likely to happen in the next thirty years when many critical decisions must be made.

USE OF ALGAL AND PROTOZOAN COMMUNITY STRUCTURE AND FUNCTION TO ASSAY POLLUTION

SPECIES DIVERSITY

Changes in species diversity are a reliable and generally useful means of presently assessing the biological effects of pollution. In essence, species diversity is one way to characterize community structure. The response pattern of a community to most forms of extreme pollutional stress is a reduction in the complexity of the community as evidenced by a reduction in the number of species present, as was pointed out by Patrick as early as 1949. This reduction in species numbers usually is accompanied, in the first stages of stress, by a widening of the disproportion in the population densities. That is, in a normal community unaffected by pollutional stress, the range in numbers of individuals per species is comparatively narrow. As the aquatic system becomes increasingly stressed, a number of species usually disappear or decline in population sizes concurrently with increases in abundance, or numbers of individuals, of a few species; thus the range of individuals per species widens. This feature of community structural change has been demonstrated in laboratory algal community

cultures (Bozniak, 1969). With still greater stress, more species eventually are affected and may either disappear or decline in density. An environment may have no algal or protozoan species, but this extreme stage would be uncommon for most situations of inorganic, organic, and thermal pollution because of the great variety of niches occupied by protozoans and algae. For example, the green alga Prasiola crispa thrives on bird guano where levels of ammonium, phosphate, and organics are toxic to most algae. Algae and protozoans also occur in thermal springs with temperatures much higher than those likely to be produced by steam electric power generating plants. However, nearly sterile conditions resulting from chemical toxicity, acid mine drainage streams bearing heavy metals, and several other kinds of toxic pollution have been observed. Invasion of a species particularly adapted to the new conditions can occur; however, as conditions become more extreme, fewer species can tolerate them and the basic response pattern will be main-Thus, the community often follows the tained. same general dose-response pattern as a population. That is, a population initially exhibits no detectable or a slight stimulatory response to low concentrations of toxicants or amounts of pollutional stress, then exhibits a graded inhibitory response to a range of concentrations, and finally reaches the point of maximum inhibition.

One way to view community structural and functional changes is to visualize a community as an information system for an environment. Then, a definition for pollution might be: any change in environment for which the species or community has inadequate information and is thus incapable of an appropriate response. This definition would include the introduction of substances which had no counterpart in nature (e.g., DDT) or a physical or biological change not previously experienced in the habitat such as temperature changes. The inadequate response to the environmental change could result from lack of previous exposure, occurrence at an inappropriate time or concentration, or exposure to a new combination of conditions.

Communities follow the same general dose-response pattern if one substitutes species for individuals, in the foregoing sequence. The first major use of this generalized response pattern is the widely quoted paper by Patrick, Hohn and Wallace (1954). Figures 1 and 2 are taken from that paper which employed the well-known diatometer technique, originally developed at the Philadelphia Academy of Natural Sciences. These figures clearly show both the reduction in species and the increased range of numbers of individuals per species in the community in response to pollutional stress. Patrick (1949) also showed earlier that pollution caused reduction in numbers of aquatic species present in the Cone-



FIGURE 1. — Graph of diatom population for November, 1951, from Ridley Creek, Chester County, Pennsylvania; a stream not adversely affected by pollution (from Patrick, Hohn, and Wallace, 1954).



FIGURE 2. — Graph of diatom population for November, 1951, from Lititz Creek, Lancaster County, Pennsylvania; a "polluted stream" (from Patrick, Hohn, and Wallace, 1954).

stoga River; one of us (Cairns) had the privilege of being a member of the team which provided the basic data for this paper. More specifically, this paper showed that various types of waste discharges ranging from sewage to effluents from industrial manufacturing plants induced a reduction in the number of species of aquatic organisms. The intermediate response, termed "semi-healthy" by Patrick, was characterized by a reduction in number of species of certain "higher" forms of aquatic organisms such as fish, accompanied by an increase in the number of species of the "lower" organisms (Figure 3). Ultimately, as seen in the "very polluted" histogram, virtually all species were eliminated. Cairns' (1965b) study of protozoans of the Conestoga Basin also indicated this pattern, after first exhibiting increased numbers of individuals and species in response to slight organic enrichment; this finding suggested that organic compounds might be utilized as nutrients, or in some other way, might stimulate protozoans.



FIGURE 3. — Histograms illustrating the response of an aquatic community to pollution (from Patrick, 1949).

Microbial community structural and functional changes as part of the aquatic ecosystem. — Before elaborating further the idea of microbial community changes in structure and function in pollution monitoring, we should stress the relationships between this approach and the ecosystem concept.

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Survey 1 Survey 6										
Station	1	3	5	6	Station	1	3	5	6	
1	100	78	55	55	1	100	40	50	40	
3	133	100	71	48	3	30	100	53	61	
5	20	58	100	50	5	33	47	100	53	
6	$\frac{20}{20}$	41	54	100	6	30	61	61	100	
Average	overlan — 48		51	100	Average	overlan —	46	• •		
Average	overlap 40					oronap				
Survey 2					Survey 7		_	-		
Station	1	3	5	6	Station	1	3	5	6	
1	100	62	88	88	1	100	60	40	40	
3	39	100	77	84	3	33	100	55	33	
5	50	71	100	71	5	25	62	100	50	
6	47	73	67	100	6	25	38	50	100	
Average	overlap — 68				Average	overlap	42			
0 1	-				Survey 9					
Survey 3		2		6	Survey o	1	2	5	6	
Station	1	5	4	0	Station	100	3	5	22	
1	100	- 28	/1	42	1	100	100	44	22	
3	27	100	33	40	3	50	100	50	50	
5	45	45	100	45	5	37	19	100	19	
6	38	75	62	100	6	33	33	33	100	
Average	overlap — 48				Average	overlap —	34			
Survey A					Survey 9					
Station	1	3	Δ	6	Station	1	3	4	6	
1	100	47	77	70	1	100	38	88	6Ž	
2	60	100	60	50	3	30	100	50	4 0	
5	60	100	100	67	5	41	30	100	48	
3	0/	40	71	100	6	50	40	80	100	
0	04	33	/1	100	Avarage	overlop	40	00	100	
Average	overlap - 39				Avelage	overlap —	47			
			Survey 5							
			Station	1	3 5	6				
			1	100		60				
			3							
			5							
			6	52		100				
			Average of	verlap 50	6					
				r						

TABLE 1. - Percentage overlap of species at various stations on a survey - Algae *

* From Patrick, Cairns, and Roback (1967).

Aquatic ecologists now recognize that political boundaries are artifacts of expedience, because such boundaries usually comprise only a part of or parts of several basins, each of which has a functional unity based on years of evolutionary development. It follows thus, as pointed out by Cairns (1967), that the natural system is a better base for developing water management practices than the political Besides geographical boundaries, biological one. taxa also are artifacts of expedience although few phycologists, protozoologists, bacteriologists, etc. have emphasized this point of view. Thus, if legislators must recognize that enlightened management of ecosystems necessitates the transcending of political boundaries, biologists also must recognize that the operating units of ecosystems transcend taxonomic boundaries. The overlap between algae and protozoa clearly demonstrates this latter point.

The evils of compartmentalization have become

especially obvious as a result of environmental pollution. We now know that even the drainage basins are not exclusively independent of each other. Airborne microorganisms travel great distances, often entering new drainage basins and even traversing oceans (Schlichting, 1969; Parker, 1971; Brown, 1971). Atmospheric pollutants similarly transcend watersheds, as well as political boundaries. The discovery of significant levels of soluble B vitamins (cobalamin, biotin, niacin) in rainwater falling upon ponds and the apparent pronounced effect on phytoplankton reproduction (Parker, 1968; Parker and Wodehouse, 1970; Parker and Wachtel, 1971) further exemplifies the open nature of aquatic ecosystems which must be considered in establishing pollution criteria.

Microbiologists, including phycologists and protozoologists, have long been aware of succession of organisms. Often, painstaking identification of spe-

Survey 1 Station 1 3 5 6	1 100 71 84 63	3 69 100 90 60	5 53 60 100 45	6 79 78 90 100	Survey 6 Station 1 3 5 6	1 100 78 77 70	3 70 100 72 64	5 74 78 100 67	6 87 67 83
Average	overlap — 70			100	Average	overlan	73	07	100
S	r					overlap	15		
Survey 2 Station 1 3 5 6 Average	1 100 78 73 78 overlap — 79	3 77 100 77 90	5 84 90 100 90	6 67 78 67 100	Survey 7 Station 1 3 5 6 Average	1 100 80 75 78 overlap —	3 80 100 79 78 76	5 74 79 100 90	6 64 65 77 100
Survey 3	-				Survey 8	F			
Station 1 3 5 6 Average	1 100 90 80 79 overlap — 71	3 52 100 63 50	5 61 83 100 69	6 72 80 82 100	Survey 8 Station 1 3 5 6 Average	1 100 81 71 78 overlap —	3 70 100 62 71 68	5 67 67 100 71	6 61 65 60 100
Survey 4		_			Survey 9				
Station 1 3 5 6 Average	1 100 69 92 75 overlap 74	3 79 100 87 73	5 74 61 100 71	6 72 61 84 100	Station 1 3 5 6 Average	1 100 75 68 60 overlap —	3 85 100 77 73 77	5 85 84 100 79	6 79 84 81 100
			Survey 5 Station 1 3 5 6 Average	1 100 88 overlap 80	3 5 	$\frac{\begin{array}{c} 6\\72\\\hline\\\hline\\100\end{array}$			

TABLE 2. - Percentage of overlap of species at various stations on a survey - Diatoms *

* From Patrick, Cairns, and Roback (1967).

cies in the field or laboratory on collection day proved useless for classes a day or so later because of their rapid change in species content. Little information exists on the rates of processes and other quantitative aspects of successional changes in natural aquatic systems and on the structure of laboratory microbial communities. We do not, of course, denigrate the limited research already accomplished in this area (e.g., Picken, 1937; Bozniak, 1969; Samsel and Parker, in press), but rather stress the need for more information about natural processes. Obviously, we must better understand the successional characteristics of algal, protozoan, and other microbial communities in order to distinguish between "natural" and pollutional changes.

Natural variations of microbial communities. — The viewpoint that free-living protozoan and algal successional patterns were essentially opportunistic

and without direction originally had at least some In studying the algal and protozoan foundation. communities of the Savannah River, Patrick, Cairns and Roback (1967) found unexplainable variations through both space and time. All sampling stations covering about 26 miles had similar environmental quality (i.e., gross chemical-physical characteristics). Despite this similarity in environmental quality, the percentages of species overlap between stations on a single survey sampled within a relatively short span of time were small. For example, the average overlap of algae at various stations was less than 50%. although on the second, fourth and fifth survey (out of nine) the average overlap increased to 68% (Table 1). Therefore, a comparison of any two stations on a single reach of river would have a high probability of containing less than half the species found at the other station. The nine surveys were

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Survey 1 Survey 6										
Station	1	3	5	6	Station	1	3	5	6	
1	100	10	21	27	1	100	42	42	30	
3	11	100	23	23	3	33	100	38	31	
5	$\bar{2}\bar{5}$	25	100	38	5	37	40	00	51	
6	22	19	28	100	6	22	30	45	100	
Average	overlap — 22				Average	overlap 3	6			
5 0	0.011-p				Survey 7	•				
Survey 2	1	2	5	6	Survey /	1	3	5	6	
Station	100	3	5	50	Station	100	40	37	34	
1	100	100	45	50	1	100	100	40	21	
3	50	100	4/	42	3	40	100	40	57	
.5	32	41	100	45	2	38	40	100	100	
6	29	30	35	100	0	27	24	42	100	
Average	overlap — 42				Average	overlap — 3	57			
Survey 3					Survey 8					
Station	1	3	5	6	Station	1	3	5	6	
1	100	17	33	45	1	100	21	25	25	
3	27	100	33	60	3	22	100	27	30	
š	29	18	100	53	5	22	22	100	29	
6	$\overline{2}\hat{2}$	19	30	100	6	24	28	31	100	
Average overlap $= 32$ Average overlap $= 25$										
Second 4	o o o o o o o o o o o o o o o o o o o				Sumor 0					
Survey 4	1	2	5	6	Survey 9	1	2	5	6	
Station	100	3	27	0	Station	100	40	13	34	
1	100	100	37	52	1	100	100	43	40	
3	40	100	33	33	5	20	100	100	40	
2	34	43	100	43	5	39	40	29	100	
6	30	33	23	100	0	29	34	30	100	
Average	overlap — 40)			Average	overlap — :	58			
			Survey 5							
			Station	1	3 5	6				
			1	100		25				
			3							
5										
			6	25		100				
Average overlap -25										
mondo oronge 20										

TABLE 3. — Percentage of overlap of species at various stations on a survey — Protozoa *

* From Patrick, Cairns, and Roback (1967).

carried out over a span of 14 years indicating the 50% overlap is fairly consistent from year to year. For example, the average species overlap in the first survey was 48.6%, while that in the ninth survey was 48.8%. Survey 5, one of the three surveys with an average overlap of over 50%, was a limited survey of two stations. Within the algae, the diatoms had a very high percentage overlap of species at various stations on a single survey (Table 2). The range for diatoms was between 58.8% for survey 8 and 80% for survey 5 (which had only two stations surveyed) but was almost as high for survey 2 (79.1%) in which four stations were surveyed. A very low percentage overlap was found for protozoan species at various stations on a single survey (Table 3). Percentage overlap of protozoan species ranged from 22.8% on survey 1 to 42.4% on survev 2. The percentage overlap of protozoan species at various stations on a single survey typically was substantially less than that found for the algae.

The above data might be viewed as variation in kinds of species through space; that is, the kinds of variations found at a particular point in time, in a reach of a fairly rapidly flowing river on which all stations in the survey had similar water quality.

As one might expect, the percentage overlap of species found at a single station through time is even less. For diatoms, the percentage overlap between surveys at a single station (Table 4) ranged from 42.8% at station 5 to 54.8% at station 1. In agreement with previous results, the percentage overlap of species of protozoans between surveys at a given station was still less, being 17.9% at station 3 to 20.9% at station 5. At a single station the diatoms had a higher percentage overlap of species through time and space than protozoans.

Station 1 Survey 1 2 3 4 6 7 8	1 100 77 78 77 64 63 52	2 71 100 74 69 64 62 48	3 63 100 90 59 65 41	4 40 38 57 100 38 44 39	5 58 61 64 65 100 60 47	6 59 61 74 80 62 100 51	7 45 44 45 45 45 49 100	8 40 43 44 60 41 49 62	10 43 47 40 47 50 44 40
9 5 Average	57 54 overlan —	58 54 54	51 40	43 30	53 55	64 51	79 43	100 41	49 100
Station 3 Survey 1 2 3 4 6 7 8 9 Average	1 100 70 80 72 64 60 53 57 overlap —	2 68 100 89 70 65 65 52 61 43	3 39 44 100 61 37 45 38 37	4 44 77 100 37 45 40 40	5 54 58 62 50 100 55 44 47	6 58 64 89 70 62 100 58 64	7 42 60 51 41 48 100 64	8 47 52 62 53 45 55 69 100	
Station 5 Survey 1 2 3 4 6 7 8 9 Average	1 100 50 78 55 51 45 35 40 overlap —	2 87 100 85 81 72 75 50 60 42	3 75 50 100 65 49 50 37 40	4 37 31 43 100 30 37 31 29	5 70 59 63 100 65 41 54	6 63 73 81 69 100 49 59	7 44 38 48 60 39 42 100 61	8 57 50 58 60 55 55 68 100	
Station 6 Survey 1 2 3 4 6 7 8 9 5 Average	1 100 83 80 73 69 79 60 60 60 00 vorlap —	2 54 100 57 67 51 67 50 52 59 52	3 49 52 100 67 44 60 50 42 43	4 29 40 43 100 25 43 34 29 28	5 60 70 65 58 100 78 59 60 68	6 50 63 60 69 54 100 55 49 53	7 33 43 44 49 37 50 100 59 43	8 44 60 52 53 50 58 78 100 59	10 32 49 40 38 41 47 42 42 100

TABLE 4. — Percentage of species overlap between surveys at a given station — Diatoms *

* From Patrick, Cairns, and Roback (1967).

In summary, species vary through both space and time, a generalization true of all groups, but appearing more marked on a short term basis in the microorganisms. The percentage overlap is higher for algae, such as the diatoms, than for protozoans. One explanation for this difference is that protozoans are more fragile than these algae and occur in lower densities more difficult to preserve and enumerate (Cairns, 1965a); thus, the sampling error is greater for protozoans than for these algae. A second possibility is that the successional rates for the protozoans may differ from those of the algae. Recent evidence generated in our laboratory indicates that when protozoans are well established on a series of identical substrates, the percentage overlap is comparable to that obtained for algae. Also, water from the same stream source flowing through comparable micro-ecosystems produced remarkably similar diatom communities (Patrick, 1968). This confirms that diatom community structure is not caused by fortuitous circumstances or opportunistic behavior as some of the early interpretations of suc-

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cessional patterns indicated. Yongue and Cairns (1972) also found that a core of protozoan species was remarkably stable over as long as five months. Perhaps conditions here were more favorable to the maintenance and continuation of protozoan populations than some of the rivers mentioned previously. One possible factor responsible for this finding is the ability of microbial communities to maintain a micro-environment different from that of the surrounding water (Yongue and Cairns, 1971). If this explanation holds, a certain community density must be necessary because Cairns, Yongue and Boatin (manuscript with editor) have found that protozoan communities cannot maintain their original structure under severe environmental change. An example of a functional change in an algal community is provided by the studies of Samsel and Parker (in press) using laboratory micro-ecosystems which simulated both an oligotrophic and eutrophic Antarctic freshwater lake. When the native algal communities from the two lakes were inoculated reciprocally into the simulated lake micro-ecosystems, their primary productivities approximated the values obtained in the control micro-ecosystems consisting of native algal communities inoculated in their simulated native lake media. These results strongly suggest that any means of assessing pollution-caused changes in algal and protozoan communities is doomed to failure unless normal patterns of variation are understood.

Present limits toward interpreting community structural changes. - The preceding discussion has suggested that certain mechanisms of normal community structural and functional changes must be understood in order to establish some useful criteria for monitoring water quality. Fortunately, knowledge toward this end is sufficiently developed that at least some practical approaches to monitoring water quality can be applied. Thus, if a lake or river is polluted, we can use changes in microbial community structure for detection. The evidence usually is based on species identification (e.g., Hohn, 1956; Hynes, 1960) or a method giving similar evidence without requiring formal identification (e.g., Cairns, et al., 1968; Cairns, et al., 1970). However, we don't entirely understand the interactions operative in maintaining the community structure or how these relate to the function of the entire community. Patrick (1967) has shown that one of the main results of a high invasion rate is to maintain a number of low density species in an algal community. She speculated that these species may function to preserve the species diversity of the community despite constant environmental change and that rare species may become common if appropriate environmental changes occur. Obviously, this phenomenon would be more important in streams than in lakes. Patrick (1967) also found that the size of the species pool which may potentially invade an area has a great effect on the number of species composing the community and that the size of the area to be invaded affects the community diversity. These species pool and invading area effects also were noted by Cairns and Ruthven (1970) for protozoan communities. Further to this point, Cairns et al. (1969) showed that the invasion of a previously uncolonized artificial substrate was by low density "pioneer" species followed by an increase in both numbers of species and individuals After a period of relative stability per species. there was a sharp increase in number of species generally with few individuals per species. This observation agrees with the concept of succession in other environments. The above examples also constitute evidence for environmentally mediated structural changes in aquatic communities. In addition, there is other evidence on the ways that algal and protozoan communities show environmentally mediated structure: (1) Patrick and colleagues found that diatom communities in unpolluted waters usually fit truncate normal curves and ellipses (Patrick, Roberts and Davis, 1968; Patrick and Strawbridge. 1963). (2) Stressed protozoan communities lose species with recovery to about the original number occurring when the stress is removed (Cairns, 1969a); recovery is delayed or incomplete if there is residual toxicity (Cairns and Dickson, 1970). (3) Diatom communities have similar structure in similar ecological conditions, and the number of protozoan and diatom species is relatively constant in a variety of fresh waters (Patrick, 1961). (4) The number of protozoan species in a mature community oscillates about a mean (Cairns, et al., 1971). (5) Literature exists on phytoplankton as indicators of lakes possessing different trophic states (Hutchinson, 1967).

While freshwater algal and protozoan communities have structure, it is probably flexible since the number and type of species involved differ in different areas (Patrick, 1961; Cairns, 1965a). The relatively constant number of species in communities of taxonomically different aquatic organisms (Patrick, 1961) suggests that a similar process is involved in all groups. The most likely hypothesis is that a rough balance between colonization rate and extinction rate is responsible, and that the oscillations in number of species in a particular habitat represent disequilibrium and adjustment in this process (Cairns, *et al.*, 1969). Since colonization depends upon invasion of new species, one would expect different invasion rates to produce communities with different structures, as has been demonstrated for diatoms (Patrick, 1967).

Several possible explanations exist for the relatively constant number of species found in algal and protozoan communities in fresh water, particularly flowing fresh water. First, colonization may be random, and there may be an equilibrium between colonization and extinction rates (Figure 4). That is, an invading species has an increased chance of colonizing an uncolonized habitat. As the number of species inhabiting the habitat increases, the chances of successful invasion decrease because the species face increased biological competition. However, successful colonizers are not immune from the effects of invading species, so that eventually each of the colonizing species may be displaced by the invaders; this latter point is especially true when the environment changes frequently, thus decreasing the competitive advantage of the established species. An alternative means of expressing the cause for relatively constant species numbers in mature aquatic communities is to view the environment as capable of supporting only a certain number of niches.

Not all interactions between species are competitive, particularly in the initial stages of the colonization process. In fact, a synergistic or favorable interaction in the early stages of community development often occurs between established protozoans and potential colonizers, which increases the probability of successful colonization. Thus, Cairns *et al.* (1969) showed that artificial substrates (polyurethane sponges) were initially colonized by a few species of protozoans with a low density of individuals per species (Figure 5). Subsequently, a rapid increase in number of species occurred followed by a relatively constant number for approximately three weeks, with the densities becoming higher than those in the initial invasion. This period of relative stability in numbers of species present, but with changes in densities of individuals per species, then was followed by another surge of low density spe-



FIGURE 4. — An estimate of the environmental suitability for the species distribution in Figure 1 (from Cairns, 1971).



FIGURE 5. — Protozoan colonization on artificial substrates (from Cairns, et al., 1969).

cies, after which, the numbers of species oscillated about a mean (Cairns, et al., 1971; Yongue and Cairns, 1972). Apparently, if the extinction rate increases and the number of species decreases, the likelihood of colonization by invaders increases. Thus, in theory an increase in colonization rate occurs after a brief lag period. When this increase in colonization rate results in a substantial increase in number of species present, the extinction rate apparently increases, causing the system to oscillate around a mean. Undoubtedly, the extinction rate consists of both internal interactions such as competition, and external interactions such as unfavorable environmental conditions. Under these circumstances occasional large scale extinctions could occur, and there could be occasional periods of very rapid colonization.

Apparently colonization rates vary enormously from one location to another (Patrick, 1967; Cairns, Yongue and Boatin, in press); however, regardless of the processes involved, all systems studied thus far reach a dynamic equilibrium, equatable with the microbial community maturation, after an initial period of adjustment. The time required to achieve this equilibrium will vary regionally and seasonally and may be affected by the past history of the area.

In understanding algal and protozoan community changes, it must be stressed that algae and protozoans are not always affected by pollutants directly. For example, pollutants may remove organisms preying upon algal and protozoan communities. Many predators are selective and therefore may reduce populations of one species more than another thereby favoring the population with the least predator pressure. If the predators are removed or reduced in number, the competitive advantage of the species with low predator pressure over that species with greater predator pressure is reduced and, consequently, other species may dominate. If one were not studying the entire system, these changes might be interpreted as direct effects of pollutional stress.

Evidence for the effect of presence or absence of a predator upon both invertebrates and microbial species was illustrated by Brooks and Dodson (1965). They showed that predation by *Alosa pseudoharengus* (the alewife) upon lake zooplankton eliminated the usual large size crustacean dominants (e.g., *Daphnia*, *Diaptomus*) which were replaced by small sized, basically littoral species, especially Bosmina longirostris. Evidence indicated that the antagonistic demands of competition and predation determined the body size and therefore the species present of the dominant herbivorous zooplankters. This, in turn, caused a shift in the types and ranges of smaller particles including certain algae and protozoans upon which these organisms probably grazed. This type of effect was shown by Pennington (1941) as well as Hrbacek, et al. (1961) and Hrbacek (1962). Pennington described the rapid replacement of rotifers by Daphnia in tub cultures of micro-algae. These examples illustrate that while it is generally recognized that changes in population structure and composition of algae and protozoans may affect the higher organisms, changes in population structure in higher organisms may similarly affect algal and protozoan populations.

The use of changes in algal and protozoan communities to assay pollution unfortunately has several drawbacks with regard to water quality control. While microbial communities usually respond relatively rapidly to changes in pollutional stress, there still is a significant time lag between the onset of toxic conditions and the determination of the responses which occur. Secondly, the method lacks precision because of natural fluctuations in species numbers and population densities. That is, the system oscillates around a mean number of species and individuals of each species (Cairns, Dickson and Yongue, 1971). Thus, to distinguish shifts in the structural characteristics of the community caused by pollution from natural cyclic changes, the degree of change must significantly exceed natural variation. This inherent property of microbial communities in nature reduces the precision of the method. That is, in a natural ecosystem, communities at two different locations rarely are identical qualitatively or quantitatively; even if the environments were nearly identical, it is doubtful that the communities would be oscillating in perfect phase with each other. A third problem is that sampling of microbial communities generally involves use of either artificial substrates (e.g., diatometers) or hand collections, and interpreting results from either method requires considerable ecological experience. For example, diatometer studies in the tidal area of the Neches River, Texas, indicated that the water quality, in an area in which fish kills were occurring, was quite comparable to that of the upstream control area with no fish kills.* Further investigation disclosed that the basic problem in the fish kill area was an oily waste floating on the surface of the The diatometer, of course, rose and fell water. with the tide, staying just under the surface of the water and therefore collected and supported a diatom community unaffected by the oil. However, as the tide rose and fell, the oil coated the exposed mud flat aquatic communities nearby, eventually affecting many of the organisms directly or indirectly associated with the mud flats, and these were ingested by fish, often with fatal results. When diatometers were positioned so that the oil slick contacted them, degradation of the diatom communities occurred. This case illustrates the need for careful interpretation of results even from well-established methods.

No artificial substrate presently available enables immediate assay, and the lag time before critical readings can be made remains undesirable, though a perfectly natural property of all artificial substrates. However, after the original two week period, slides may be introduced in a sequence to make daily removal possible. In cold weather the colonization process extends still further in certain streams (Patrick, personal communication). However, in streams where growth and other biological processes are depressed in cold weather this objection would apply equally to all artificial substrates and indicate the natural depressing effect of low temperature. Patrick, et al. (1954) have shown that leaving a substrate in for more than 3 weeks often reduces the numbers of species present as a result of competition and the better adapted species reproducing at a more rapid rate. This increase in population sizes of certain species is also characteristic of organically enriched water, but the shift is much more pronounced than seen in leaving a slide in for a longer period of time. A natural community when a slide is colonized for a long period of time does not extend over 12 intervals when the mode is in the third interval, whereas a light organic load is indicated by the curve extending 13 or more intervals. This pattern resembles at least superficially succession of an old field in a temperate, mesic environment; initially nearly every tree and shrub species from the surrounding forest establishes a few seedlings in the field, but as the density and biomass of woody species increase over some, a select few populations dominate. Despite these and other weaknesses, the diatometer remains a valuable tool in detecting and quantifying certain biological effects of pollution. Data can be obtained more frequently than every two to three weeks by using additional diatometers, and removing slides from separate diatometers at intervals. Slides can be stored without special treatment, a very marked advantage for pollution monitoring, and the investment required for sampling every second or third week is not excessive. Thus, the major cost in diatometer studies is identification time rather than placing and gathering the slides. We feel it highly improbable that large numbers of algae belonging to other major groups (e.g., the blue-green and green algae) will prove as useful at the species level for pollution monitoring. While such species are probably as sensitive to pollutional stresses, the increasing difficulty for their accurate species identification which necessitates axenic cultures (Archibald and Bold, 1970; Smith, 1971), all but eliminates these algae for field bioassays. However, their usefulness at the higher taxa level (genera, family, etc.) or in studies of community structural and functional changes will remain important.

Finally, changes in microbial community composition do not inevitably parallel short term pollutional changes in the macro-environment. For example, a protozoan community established on an artificial substrate for a number of weeks did not change substantially when the pH of the surrounding water changed qualitatively from 6.5 to 10.5 (Yongue and Cairns, 1971). However, the water associated with the polyurethane sponge substrate proved subsequently to have a pH of 6.8 despite the higher pH of the surrounding water. More recently T. W. Morgan (personal communication) found 5.6 ppm copper sulfate lethal to most of a population of Euglena within five minutes. A few individuals residing within a wheat husk, one component of the culture medium used, survived the entire three-day experimental period.

The ability of extracellular polypeptides of Ana-

^{*} The field work was carried out by one of us (Cairns) and Dr. Charles Reimer of the Limnology Department, Academy of Natural Sciences. The examination of the slides was carried out by the Limnology Department's diatom counting group under the supervision of Dr. Reimer and Dr. Ruth Patrick.

baena cylindrica to complex with copper ions (Fogg and Westlake, 1953) and the ease with which soil algae can be isolated from dry waste and slag from copper mines (Parker, unpublished) further documents the need for caution in any universal application of microbial community structure in monitoring macro-environments. In the latter instance, the copper ore was a cupric carbonate, essentially insoluble and biologically inert at the high pH's of the mine soil and slag. Other means of temporary protection from unfavorable conditions occur, for example, with respect to artificial substrates, but as seen from select examples above, such phenomena of partial microbial community insulations also can be natural.

SEQUENTIAL COMPARISON INDEX

The primary weakness of the species diversity method relates to the considerable amount of taxonomic training required for accurate data collection. Were these methods to be utilized universally even on a moderate scale, the paucity of properly trained people would render the approach ineffective. The development of the sequential comparison index (Cairns, et al., 1968 and Cairns and Dickson, 1971), a method depending on changes in the number of morphologically different macro-invertebrates, attempted to alleviate this problem. The method does not require formal species identification, but merely the ability to distinguish structurally different organisms. Admittedly, this method lacks the precision of the diatometer and related methods, but requires less time and virtually no experience. Thus, the problem of a sufficient labor force for employing this technique on a wide scale is resolved. In theory, the method should also have application to microorganisms.

The sequential comparison index has an operator error in that the level of discrimination of some operators is more precise than that of others, that is there are "splitters" and "lumpers." However, the same difficulties exist for biological assessment techniques involving formal systems of classification since "splitters" and "lumpers" are found at all educational levels.

Automation of pollution assessment techniques using microbial communities could greatly reduce the time and subjectivity involved. Cairns (1970) and Cairns, *et al.* (1970) suggested that the attainment of environmental quality control on a systems basis requires reduced biological data generation time (i.e., comparable to chemical and physical data). This suggestion, however, does not ignore the fact that biological information often provides additional value as an indicator of longer term, chronic doses of pollutants. For example, occasional toxic doses of some heavy metals or brief thermal pulses may be overlooked by an infrequent, widely spaced physical and chemical monitoring program, while the change in biological communities will more accurately reflect the impact of such transient pollutional stresses.

Of all aquatic life, microbial communities offer the best possible material for biological indicators: (1) One can study tremendous numbers of individuals and species without disturbing the natural community, whereas overcollecting fish, rooted plants, and other higher organisms could perturb most natural systems; (2) Individuals are small and easily collected and transported; (3) Microbial communities develop readily and rapidly in artificial media and on artificial substrates, assuring comparable habitats at all stations examined; and (4) Microbial species usually represent the major biomass in an aquatic system and are extremely important in determining its operational characteristics. The use of microorganisms also has drawbacks: (1) Some are fragile and not easily preserved or transported; (2) Microorganisms are less well understood by the average citizen who is more apt to judge pollution by the effects on sport fish or edible species, or recreational activities; and (3) Their identification is often more difficult.

Future Trends. — Ideally, all levels of the food chain should be studied in aquatic ecosystems. If species are to be described, they may be differentiated not only by form, but also by function. Even now function is used to classify bacteria as well as some other microbial species. However, we know relatively little about natural microbial species function and, therefore, must continue to describe microbial communities, and changes in them caused by pollution, in terms of the kinds of species present.

The use of species lists in describing a microbial community is essentially a description of the number of different kinds of cells present. By considering a diatom community as an aggregation of cells of different size and structure, one might generate a curve quite similar to that developed using traditional taxonomic methods without even knowing the Latin names or hierarchical positions of the species present. Coulter counters are not sufficiently discriminating to do this. However, the use of laser holography (Cairns, *et al.*, 1972) makes possible the evaluation of an array of living particles without using traditional taxonomic methods.

The use of function in pollution assessment (Lanza, Cairns and Dickson, 1970) might also be automated by using changes in the biochemical profile as an index of pollutional effects. This is a promising area for future exploration. Any approach to community function must take into consideration the interactions of populations comprising microbial communities and higher aquatic organisms. Most bioassays designed to evaluate the toxicity of industrial wastes with fish or insect larvae, using death as the chief criterion, do not provide information about the response of microbial species. Although more sensitive criteria have been introduced, most bioassays on industrial wastes today continue to emphasize survival rather than chronic changes relating to function, behavior, or other aspects of community interaction. The relatively short duration of microbial life cycles enables use of survival of the population for tests of extremely short duration. Even relatively short term exposures, such as five days, often involve several cell divisions, especially with organisms in their exponential growth phases, as recommended for many tests (ASTM, 1971). Thus, bioassays on the population, rather than the individual, constitute a logical extension of current bioassay methods. For example, Cairns, Scheier and Hess (1963) suggested that a 50% reduction in the growth rate of diatoms compared favorably with a 50% survival (or TL_m) for fish and snails. They based this idea on the assumption that a toxicant concentration producing a 50% reduction in division rate for a microbial population under otherwise optimal conditions would be approximately equivalent to lethal effects on a static population of fish or invertebrates. This assumption was adopted by Patrick, et al. (1968) when subsequently comparing the sensitivities of diatoms, snails, and fish to a series of common constituents in industrial wastes. This original assumption by Cairns, et al. (1963) was somewhat oversimplified, chiefly because of great differences in environmental requirements, life cycles, and other aspects of niches for aquatic test organisms ranging from microbial to vertebrate species. We now recognize that bioassays for pollution ideally should include both short term and long term effects, effects on the entire community relative to the interaction of populations, and effects on all phases of organism life cycles. Thus, an absolute minimal bioassay procedure for an aquatic system should include at least a representative microbial community (e.g., a primary producer, a decomposer and/or a detritus feeder), an invertebrate, and a fish. Even this test community may be inadequate because our ignorance of the total ecological roles (= niches)of these organisms may lead to poor choices. For example, an abundant and easily recognized phytoplankton species may constitute a less sensitive indicator of pollution-mediated change in an aquatic ecosystem than a species less abundant and more difficult to study. Nevertheless, bioassays on these organisms will contribute at least some information on relative sensitivities of members of each group. We know of no a priori way of determining this effect of a particular chemical and specific set of environmental conditions on an aquatic community other than some kind of direct community bioassay. In fact, even after the bioassays have been run and median response points for each group of organisms determined, one still must determine the maximum concentration which will not adversely affect the most sensitive species of importance. This task necessitates an accurate relating of the dose response curves or patterns of one species to those of other species involved in the test series. Comparing dose response curves for different taxonomic groups becomes even more problematic with the increased sophistication of tests, such as those based on performance and function. For example, a change in the EKG or heart rate of fish cannot easily be compared to a 50% survival in fish or a 50% reduction in algal or protozoan cell division. However, often due to time and cost limitations associated with environmental problems that urgently require attention, one must, without a comprehensive study, set an unnecessarily low permissible concentration of a particular waste to insure no harm to any major group of organisms. By doing so, we assume that a median response point for any type of bioassay represents a critical change in the population survival potential regardless of method or group of organisms involved. More specifically, a 50% reduction in cell increase is assumed to be essentially comparable to a 50% mortality. Finally, attempts to relate the results of laboratory bioassays to the responses of organisms in the receiving system are needed critically. Results obtained from both field and laboratory investigations are necessary if the consequence of introducing a change, such as a toxicant, into a complex ecosystem are to be realistically and accurately assessed.

TYPES OF POLLUTION AND THEIR POTENTIAL EFFECTS ON ALGAL AND PROTOZOAN COMMUNITY STRUCTURE AND FUNCTION

Applications of structural and functional changes in algal and protozoan communities for monitoring pollution relate directly to the types and intensities of different pollutants. We shall review briefly the types of pollution and select aspects of the physiology of these microorganisms which will be useful for implementing the community structural and functional change approach.

INTRODUCTION

Without doubt changes in the biological, chemical, and physical properties of aquatic ecosystems may be induced by the addition of pollutants. Such pollution is most often manifest by: (1) excesses of organic substances, (2) excesses of inorganic substances, (3) specific toxic substances, (4) excesses of suspended solids, (5) thermal increases, or (6) radionuclides. A major goal of water quality control is the application of fundamental research on algal. protozoan, and other microbial communities directed toward preventing and reversing degradation or restoring the quality of the environment. Realization of this goal is, however, immensely complicated. It is complicated by the increasing human population densities and higher per capita living standards which tend to increase all forms of pollution and to introduce new forms from time to time. The goal is further complicated by interactions, such as that between temperature and organic pollution. A temperature increase results in (1) a decrease in dissolved oxygen from about 14 ppm at 0°C to about 9 ppm at 20°C, as saturation values; and (2) increases in metabolic rates up to a point in proportion to temperature, which in turn causes greater consumption of dissolved oxygen from the aquatic environment. The result of a reduced reserve and

increased rate of consumption of oxygen, thus, increases the probability of exhausting the oxygen supply in warmer habitats, especially those enriched with organic matter (Noland and Gojdics, 1967). This principle operates at some level in all aquatic ecosystems, whether in the highly eutrophic meltwater pools near penguin rookeries or elephant seal herds in Antarctica which are perpetually oxygensaturated despite excessive organic pollution or in the nearly anaerobic waste oxidation ponds in the tropics. In the former aquatic ecosystems at temperatures below 5°C, microbial decomposition rates do not overbalance oxygen input by diffusion and primary production (Samsel and Parker, 1971). Concurrently much of the autochthonous organic matter accumulates. A few of the many possible direct and indirect effects upon living organisms are illustrated by the occurrence of ammonia and its toxic potential under conditions of pH changes and hypoxia (Warren, 1962; Warren and Schenker, 1960), and the reaction of vital substances such as iron in the presence of increased organics and oxygen depletion (Reid, 1961).

In subsequent discussions, for convenience, we will often consider community components separately. However, the total community structure and its pattern of interaction must be kept in mind at all times. Algal and protozoan culture research and field studies are useful when carefully extrapolated data from one are utilized to complement our knowledge of the other. Pollution induced changes in cellular physiology which manifest themselves in both obvious and subtle changes on individual organisms, i.e., cell division, growth, production of intercellular and extracellular toxins (and other substances), also frequently affect the total community structure. Our discussion here will be limited to certain major facets involved, employing representative examples from the literature.

ORGANIC POLLUTION

The introduction of excess organic pollutants to an aquatic ecosystem can cause numerous changes. These changes stem from the fact that organic matter can function as (1) carbon substrates or energy sources, (2) carbon skeletons for biological synthesis, (3) metabolites or vitamins, (4) inhibitors, (5) chelators, etc. One or more of these activities involving organic matter can disrupt the balance in aquatic ecosystems, producing inevitable damage to the many vital functional roles of resident communities and populations. A major concern in any consideration of organic pollution is, thus, the changing of the critical nutrient balances induced by the organic substances. Two common criteria utilized by investigators attempting to classify bodies of water are (1) their nutrient content, and (2) the community structure of their component species. Thus, nutrient concentrations and nutrient effects on community structure are vital to aquatic ecosystem research and related disciplines.

Algal nutrient requirements have been defined by Ketchum (1954) as (1) an absolute requirement, consisting of a nutrient without which algae cannot grow, reproduce, or photosynthesize and for which no other nutrient will substitute; (2) a normal requirement, representing the quantity of each nutrient contained in cells produced during active growth of a population during non-limiting nutrient conditions; (3) a minimum requirement being the quantity of nutrient in the cell during periods when population growth is limited and when all other nutrients are present in adequate supply (determines the mass of cells which can be produced for a given amount of a nutrient, provided that some other environmental factor does not limit population growth); and (4) an optimum concentration, consisting of the concentration range which will permit maximum rates of growth, reproduction, or photosynthesis of algal populations. This classification of nutrient requirements appears applicable also to protozoa. However, in the light of more recent research, we must recognize several necessary additions to Ketchum's classification. A number of elements, ions, and compounds stimulate growth but are not required by organisms (e.g., manganese, vitamins, chelators, vanadium, strontium); others supplement essential elements (e.g., strontium for calcium, manganese for iron or magnesium, vitamin B_{12} for cobalt); others appear to be non-essential and without stimulation (e.g., aluminum, chloride); others are essential only when certain metabolic activities become mandatory in that particular environment or life cycle phase (e.g., molybdenum essential for N₂ fixation and NO₃ reductase systems, but not essential when adequate NH_4^+ or organic nitrogen sources are present); others are toxic at certain levels and may interfere with essential element functions (e.g., germanium interferes with silica uptake, tellurium inhibits many prokaryotes, chromium and mercury inhibit all microorganisms); and finally, some elements occur in algal cells but are non-essential and apparently have no other function (e.g., nickel, titanium, gold) (Meyer and Anderson, 1952; Arnon, 1958; Dixon and Webb, 1958; Livingston, 1963; Eyster, 1964).

As pointed out previously, the interrelationships of various organisms in the community, as well as other critical factors of community interaction, must be kept in mind during any examination of research data extracted from community components in culture or from field studies. For example, Wright and Hobbie (1966), studied uptake of ¹⁴C-labeled glucose and acetate by bacteria and algae in aquatic ecosystems. In this study, active uptake mechanisms were implicated at very low substrate concentrations for bacteria. In contrast, a simple diffusion mechanism, which appeared effective only at higher substrate concentrations, was traced to the algae. They concluded that bacteria effectively removed substrate from solution at low levels (unlike algae) and probably maintained the low substrate levels therefore possibly preventing heterotrophic algal growth in nature. That the latter conclusion is not universally applicable is borne out by the earlier studies of Parker (1961) which demonstrate rather conclusively the ability of a soil alga to grow in absolute darkness when joined with a bacterium isolated from the same soil sample. In the light of subsequent research, especially physiological and biochemical studies as discussed by Parker (1971), we now recognize the possibility that apparently obligate phototrophs (e.g., Chlorella vulgaris, Chlamydomonas eugametos) may function as heterotrophs or photoheterotrophs in nature. The situation may be similar ecologically in blue-green algae although the lightdependent biochemical pathways may be different (Smith, London, Stanier, 1967).

Of some interest relative to the impact of organic substances in aquatic ecosystems is the recent successful cultivation of synthetic phytoplankton communities in the laboratory (Bozniak, 1969) and their initial use, permitting more complex, controlled studies to be accomplished. These community cultures were composed of *Ankistrodesmus falcatus* var. *acicularis* (A. Braun) G. S. West, *Anabaena flosaquae* (Lyngbye) DeBrebisson, *Chlamydomonas dysosmos* Moewus, *Cosmarium botrytis* Meneghini, Euglena gracilus Klebs, Chlorella pyrenoidosa Chick, Navicula pelliculosa (DeBrebisson) Hilse, Kirchneriella lunaris (Kirchner) Moebius, Scenedesmus quadricauda (Turpin) DeBrebisson, Pediastrum sp., and Pandorina morum Bory. The effects of glycolic acid, humic acid, octadecanol, sodium tripolyphosphate, and a proteolytic detergent enzyme were examined utilizing this community culture and natural phytoplankton communities. Concentrations comparable to natural environments were used, and emphasis was placed on the succession of the synthetic algal community. While the glycolic acid, humic acid, and octadecanol caused some changes in the succession and population survival of the community, their effect was less pronounced than sodium tripolyphosphate and the proteolytic detergent enzyme. The adverse effect of sodium tripolyphosphate on the blue-green alga in both cultured and natural communities was of special interest in view of the generally held precept that blue-green algae flourish in the presence of high phosphate. Bozniak also found that non-dominant algal populations were superior indicators of the added substances. Samsel and Parker (in press) partially duplicated the phytoplankton algal community structures and densities of two natural Antarctic ponds by simulating nutrient, light, and temperature conditions in laboratory flasks. Once the communities were established, the rates of CO₂ uptake and chlorophyll concentrations also approximated values measured in the field.

Organic carbon occurs in dissolved and particulate form, and studies have been carried out as to its distribution and correlation with marine phytoplankton levels (Wangersky, 1965). Also, the role of organic substances as energy sources, carbon skeletons, growth promoters and inhibitors, chelators, feeding regulators, toxins, and the effects on animal movements as well as the use of such compounds as an aid in evaluating biological activity of marine water masses has been reported (Collier, 1953). Unfortunately considerably less attention has been paid to organic matter cycles in freshwater environments. As noted previously, organic substances such as sugars and organic acids can be utilized by many algae to maintain growth in the absence of light or as a sole or supplementary source of carbon in the presence of light (Fogg, 1966). However, concentrations of these substrates generally are many orders higher than those occurring in nature. Also, no truly planktonic forms have been grown in the dark, though the possibility of such growth cannot be ruled out (Fogg, 1966). Indeed, some studies on phytoplankton communities (Rodhe, 1955; Parsons and Strickland, 1962; Bernard, 1963; Lund, *et al.*, 1963) suggest heterotrophic phytoplankton growth.

Severe organic pollution in natural ecosystems causing massive decreases in critical oxygen levels inevitably leads to reductions or annihilations of algal species. Hynes (1960), in his comprehensive discussion of Butcher's (1947) research on glass slide communities of several rivers (Tame, Trent, Bristol, Avon) exposed to organic pollution, cites examples in fluctuations in the numbers of nonplanktonic algae as well as species changes in the community. Generally, in situations where some oxygen remained and a zone of Sphaerotilus was established below the outfall, algae exhibited an initial reduction in numbers which later increased downstream. Under conditions of milder organic pollution the initial decrease in numbers of algae was not noted and the population rose rapidly to a peak before declining. Downstream algal increases occurred apparently due to increases in available nutrient salts derived from organic matter, while the occurrence of different positions of the peaks observed probably resulted from different types of organic matter. Algal luxury consumption of nutrients, dilution with increase in river volume, and the lack of stability of large algal populations were three reasons proposed to explain why algal densities decreased for long distances downstream. Also, as one moved downstream into less polluted water, the population sizes of normal grazing invertebrates (which feed on algae) increased. With regard to changes in community structure, Butcher noted that many algal species typifying eutrophic conditions (e.g., species of Chamaesiphon, Cocconeis, and Ulvella) vanished. Resistant blue-green (Oscillatoria and Phormidium) and certain green algae (Ulothrix, Stigeoclonium tenue) remained. The diatoms, Gomphonema parvulum and Nitzschia palea also tolerated the organic pollution, and dominated even in the Sphaerotilus zone, sometimes accompanied by Navicula and Surirella ovata.

More detailed examination of Butcher's data revealed that normal communities of *Cocconeis*, *Chamaesiphon*, and *Ulvella* vanished for long distances below the pollution outfall except in one river receiving mild pollution (Avon). *Gomphonema*, Nitzschia, and Stigeoclonium tenue appeared as replacements to the original community in the mildly polluted Avon. A succession was observed further downstream and the reappearance of the normal community structure (accompanied by two other species of Stigeoclonium for awhile) occurred. Since the species in this genus lack complete definition, the possibility was noted that all three might be growth forms of a single species, with Stigeoclonium tenue being the one appearing in enriched water. Complete recovery leads to a return to more usual population numbers of the normal flora; however, the numbers could remain higher than they were above the outfall. The resistant types which dominated during pollutional stress returned to their normal levels of occurrence. Return to normal community structure required a long distance: The River Tame recovered after 43.5 miles downstream, the Trent approached its original state 35 miles downstream, and the Avon demonstrated the impact of mild pollution for 7.5 miles downstream. Of the larger algae, Cladophora glomerata can develop enormous growths in polluted waters causing great nuisance to the system, with a rich supply of nutrients probably being the responsible factor. However, peculiarly in the South River, Waynesboro, Virginia, Cladophora disappears as the pollution increases, failing to re-establish itself even 10 to 15 miles below this point; strikingly at the point of disappearance of Cladophora, Rhoziclonium made its first appearance and remained abundant thereafter (Parker, Obeng, and Samsel, in press).

IMPORTANCE OF AVAILABLE NITROGEN

Of the complex balance of nutrients required for the establishment, expansion, and continued maintenance of algal communities, the elements nitrogen and phosphorus are among the foremost in the essential category and, consequently, are vital controlling factors of algal community structure. Nitrogen is available in the ecosystem in the uncombined (N_2) state and as inorganic as well as organic nitrogen compounds. The major sources of nitrogen input into an ecosystem include: (1) rainfall, rivers, and streams (directly or indirectly in runoff or ground water); (2) input from agriculture and urbanization; (3) nitrogen from natural ecosystems; and (4) nitrogen from *in situ* fixation (Steward, 1969). The latter source of nitrogen involves many heterocystous blue-green algae. Losses of nitrogen usually depend upon the efficiency and demands of a given biotic community and generally occur through (1) outflow from the system, (2) loss to the sediments, or (3) the release of elemental nitrogen or ammonia through Nitrogen sometimes constitutes a dentrification. limiting factor in that addition in appropriate form stimulates growth. Nitrogen assimilation into protein, one obviously critical building block of living material, highlights its tremendous importance to the complex interactions of the aquatic community. Thus, in a given ecosystem, the concentration of nitrogen and the total volume of water bearing that concentration coupled with the utilization efficiency level of the total community, will ultimately determine its importance as a controlling factor.

Studies on algal populations have provided valuable information on nitrogen as a nutrient control of community components. Changes in one or more of the components of a community possess the potential to express themselves through the alteration of that community (i.e., the loss or gain of a species or changes within existing species).

Syrett (1962) has reviewed inorganic nitrogen sources available to algae and has discussed nitrogen Most chlorophyllous algae demonassimilation. strate the ability to utilize either ammonium or nitrate when available in adequate concentrations. In fact, several species of algae have the same maximum rate of growth in the presence of nitrate or ammonium (Bongers, 1956; Fogg, 1949; Noack and Pirson, 1939; Samejima and Myers, 1958); however, nitrate requires more energy for reduction and, in instances where there is limited energy, more growth occurs on ammonium. As a result, cell synthesis in Chorella pyrenoidosa demonstrates a glucose utilization efficiency level which is 16% greater in the presence of ammonium than with nitrate (Samejima and Myers, 1958). Preferential uptake of ammonium by Chlorella species is quite wellestablished (Ludwig, 1938; Pratt and Fong, 1940; Schuler, Diller, and Kerslen, 1953; Urban, 1932; Syrett, 1962). In contrast to this example, Haematococcus pluvialis (Proctor, 1957) and Botryococcus (Chu, 1942) prefer nitrate to ammonium, while still other algae utilize ammonium almost to the exclusion of nitrate (Pinervich, Versilin, and Maslov, 1961; Tomova, Erstingneyeva, and Kretovich, 1964; Samsel and Parker, in press). The interplay of light intensity and nitrogen assimilation are clear from the finding that light energy conversion efficiency in *Chlorella* is approximately 30% higher in the presence of ammonium than nitrate (Van Oorschot, 1955). Thus, ammonium produces a faster rate of cell synthesis than nitrate under conditions of limiting light intensity (Bongers, 1956).

Nitrogen also can produce harmful effects. One such effect which is indirect is caused by assimilation of ammonium which induces a drop in the pH of poorly buffered media (Pratt and Fong, 1940). Urea nitrogen does not produce this harmful effect in cultures, and nitrates have been considered best for use as a nitrogen source in large scale outdoor cultures where bacterial contamination occurred (Pinervich, Versilin, Maslov, 1961). Chlorella and Scenedesmus display good growth in 0.01 M solutions of nitrate or ammonium salts; however, these concentrations were found to be inhibitory to many planktonic algae (Chu, 1942; ZoBell, 1935). Growth of planktonic algae was inhibited by 0.001 M solutions of ammonium salts although somewhat higher nitrate salt levels were tolerated. Bozniak (1969) further has shown that freshwater phytoplankton thrive on relatively low nutrient salt concentrations, especially phosphates.

Nitrite can provide a nitrogen source for many algal species provided it exists in sufficiently low concentrations to prevent inhibitory effects (Fogg and Wolfe, 1954; Ludwig, 1938; ZoBell, 1935).

As for organic nitrogen, it is not known to what degree algae utilize such sources in nature. However, culture studies reveal that a number of algae can utilize organic nitrogen compounds, including amino acids and even complex molecules, such as, uric acid (Schofield, in press).

IMPORTANCE OF AVAILABLE PHOSPHORUS

Phosphorus exists in the ecosystem as soluble and insoluble inorganic and organic phosphate; some of the insoluble fraction consists of suspended particulate phosphate. In some aquatic environments phosphorus apparently is the nutritional factor most limiting to biological productivity (and maintenance of biogeochemical cycles) since it usually exists in small amounts while being involved as an essential element in cellular energy transfer. The phosphorus concentration in aquatic ecosystems depends upon such factors as (1) geochemistry; (2) geophysics as related to groundwater and soils, drainage and runoff, rainfall and waste water effluents; (3) loss to, and release from the sediments; and (4) organic reactions within aquatic ecosystems. Because phosphorus has no gaseous phase under standard conditions, it occurs only sparsely in the atmosphere and atmospheric transport is relatively unimportant for phosphorus which is confined primarily to soils, water and rocks. Like other nutrients, phosphorus concentrations depend upon the total quantity available in a given area at a given time, and the volume of water containing it. Phosphorus occurs in nutrient media usually as inorganic phosphate and the uptake and assimilation of phosphate from such media occurs above certain threshold levels, depending on the species. Rodhe (1948) proposed classifying algae on the basis of their ability to tolerate different concentrations of phosphate, i.e., low tolerance = $\langle 20 \text{ ug/liter} (e.g., Dinobryon divergens}),$ medium tolerance = ≈ 20 ug/liter (e.g., Asterionella formosa) and high tolerance = >20 ug/liter (e.g., Scenedesmus quadricauda). Interestingly, most planktonic algae thrive in habitats representing the < 20 ug/liter and ≈ 20 ug/liter categories wherein both deficiencies and excesses of phosphate often inhibit their growth. However, phosphate deficiencies often are ecologically offset by the ability of many algae to store phosphate in excess during periods of surplus phosphate in aquatic ecosystems; this mechanism of surplus storage of phosphate enables the algae to draw upon their reserves during subsequent periods of phosphate scarcity (Ketchum, 1939; Lund, 1950).

The above points on phosphate in algal nutrition reveal a wide variety of responses may be expected from an algal community, depending on its species composition. Rice (1953) found that when algae grew in culture media with abundant phosphate, they often accumulated phosphate in proportion to the quantity in the media, and at least in some cases, the excess phosphate in cells remained accumulated as inorganic salts. Contrastingly, the marine diatom, Asterionella japonica loses its surplus reserve of phosphate when washed with seawater (Goldberg, Walker and Whisenand, 1951). Losses of stored phosphate excesses may be less common in the bluegreen algae where ultrastructural studies have revealed apparent membrane-bound polyphosphate deposits (Parker and Wodehouse, 1970). Cultures of Euglena gracilis (streptomycin-bleached) indicated that growth depended in part on the availability of phosphorus, and that maximum cell density on defined medium was reached at inorganic phosphate levels of 400-500 ug/1; yet inorganic phosphate concentrations of 1 mg/ml inhibited growth (Buetow and Schmidt, 1968). Cultured populations of Tabellaria flocculosa displayed a lower tolerance than some of the other algae to higher levels of phosphate which would restrict this species from communities in many lakes. The optimum growth range of the organisms tested was 90 to 1800 ug/liter with phosphate acting in the capacity of a limiting factor at concentrations of <9 ug/liter. Phosphate concentrations in the range of 18 to 90 ug/liter resulted in suboptimal growth (Chu, 1943).

In addition to research on the individual effects of nitrogen and phosphorus on algal populations in culture, some laboratory investigations with both of these nutrients demonstrated that increases or decreases in the nitrogen/phosphorus ratio do not markedly affect the growth of certain planktonic algae provided that the concentrations of these elements stayed within the optimum range for the organisms investigated (Chu, 1943). In considering the ecological implications of this point, one should keep in mind that the optimum concentrations of N and P in natural environments is perhaps the exception more than the rule.

THE ROLES OF NITROGEN AND PHOSPHORUS IN EUTROPHICATION

Although we have stressed that a thorough review of the literature is not an objective of this paper, we would be remiss to omit mention of the close relationships between nitrogen and phosphorus enrichment, community structural and functional changes, and eutrophication.

First, we wish to emphasize several points on eutrophication which are sometimes omitted from discussions of this process:

- Eutrophication is a natural process, only one aspect of which involves man-induced eutrophication. (Figures 6 and 7)
- (2) Numerous factors in aquatic ecosystems can lead to eutrophication, only some of which are chemical, such as, enrichment with nitrogen and phosphorus. (Figure 7)
- (3) No satisfactory approach to the measurement of

the rate of eutrophication has been adopted and no satisfactory quantitative scale of trophic level has yet been proposed.

- (4) Time and space variability in nearly all parameters (e.g., nutrients, primary productivity, species diversity, etc.) signifying trophic levels precludes the value of seasonal or short-term studies for assessment of eutrophication rates.
- (5) Figure 8 further illustrates our concept of eutrophication, and reveals that we do not necessarily feel that eutrophication is an entirely reversible process.



FIGURE 6. — Biological productivity per unit area of lake surface (from Hasler, 1947).



FIGURE 7. — Hypothetical productivity curve of a hydrosere, developing from a deep lake to climax in a fertile, cold-temperate region (from Lindeman, 1942).

Returning to the specific area of the roles of nitrogen and phosphorus in eutrophication, consider the effects on community structure. Changes in the algal communities experiencing increases in nitrogen



FIGURE 8.— Hypothetical Scheme of Eutrophication in Lakes (after Parker, 1971). Arrows up indicate potential maximum induced eutrophic levels; arrows down represent theoretical maximum return to original, natural trophic level.

and phosphorus are no doubt due to: (1) tolerances of the various algae and their requirements for certain levels of these two nutrients, (2) differential efficiencies of algae with reference to their ability to concentrating these nutrients, and (3) competitive interactions of the different species of algae (Provasoli, 1969). Often, these changes in community structure alter the sequence of the normal food chain of a given ecosystem at the base level and different species interactions will ultimately result at this, and higher levels. Studies conducted on natural communities following changes in their ecological structure, due at least partly to nutrient changes, offer much useful information. For example, increased nutrients resulted in changes in the phytoplankton community structure in western Lake Erie (Verdiun, 1964). The dominant species prior to 1950 were Asterionella formosa, Tabellaria fenestra, and Melosira ambigua and in 1960-61 the community was dominated by Fragilaria capucina, Coscinodiscus radiatus and Melosira binderana. Data available from measurements taken between 1930 and 1958 in the western basin of Lake Erie indicate increases in ammonium $(5 \times)$ and total nitrogen $(\approx 3 \times)$. Phosphate levels measured between 1942 and 1958 also increased $(2 \times)$ (Beeton, 1969). Davis (1964), in studying Lake Erie phytoplankton records, attributed alterations in community structure to accelerated eutrophication. The phytoplankton bio-

mass had significantly increased $(3 \times between 1919)$ and 1963) with spring and fall maxima of higher magnitude and longer duration and different diatom genera assuming dominant roles. Spring growth pulses of algae generally shifted from a predominance of Asterionella to Melosira while fall growth pulses changed from Synedra to Melosira and then from Melosira to Fragillaria. An increased tendency, during autumnal pulses of later years, for a greater participation by green and blue-green algae (partially replacing previous dominance by diatoms) was also noted. A similar change of dominance occurred during the winter phytoplankton minimum; Stephanodiscus and Cyclotella were of the greatest importance in most years until 1944, then Melosira, Fragillaria, and Asterionella were most abundant from 1945 to 1949. From 1956, during a period when little or no winter minimum was noted, Melosira and Fragillaria maintained themselves as the most frequent dominants. Probably the phytoplankton from 1945 to 1949 can be considered transitional in the lake leading to the elimination of winter minima. In addition, blooms of blue-green algae were beginning to appear. In another study, Melosira binderana (not reported in the United States prior to 1961) became a prominent species, representing 99% of the phytoplankton community at certain times around Lake Erie islands (Hohn, 1966).

Lake Ontario also has exhibited a shift in dominant genera over the years as Asterionella has been replaced or has shared the dominant role with Melosira and Cyclotella in the community profile (Nalawajko, 1966). Similarly Lake Michigan reveals structural changes; the diatoms, (Stephanodiscus hantzschii and S. binderanus) which were not previously reported in its waters have been recorded in abundance (Vaughn, 1961). Other changes in the form of blue-green blooms of Aphanizomenon in Green Bay in 1938-39 and Schizothrix in 1952 (Surber and Cooley, 1952) and later in 1962-65 have occurred.

Research conducted on Lake Sebasticook in Maine indicated significant changes in its algal community over recent years. A 1966 survey of the lake showed a pronounced organic nutrient loading along with elevated nitrogen and phosphorus levels, leading to community population shifts dominated periodically by various blue-green algae (Gomphosphaeria, Anacystis, and Anabaena). Studies on Lake Washington revealed a good correlation between the most rapid growth of Oscillatoria and high concentrations of inorganic nutrients. Nearby Lake Sammamish also was examined and appeared to be responding to an increase in available nutrients (Edmonson, 1969).

Studies on the Madison Lakes after community changes in the form of blooms (the two worst being dominated by *Anabaena* and *Microcystis*) revealed that of the nutrients involved, inorganic nitrogen seemed to be a critical community controlling factor (Sawyer, Lackey and Lenz, 1943).

Short term, yearly seasonal changes in algal communities consist of a succession of species, and are often correlated with nutrient availability during periods of overturn in many temperate lakes and reservoirs. In Deer Creek Reservoir, Utah, for example, increases in types and numbers of green algae occurred in spring, while diatoms dominated the community in the winter. In all probability, the spring change in community structure can be attributed to increased availability of nitrogen and phosphorus due to overturn, and increased runoff from incoming streams (Gaufin and McDonald, 1955). Tributary streams not only are potential sources of enrichment, but can contribute to the abundance of certain types of plants in the community. Such plants include certain species of the angiosperm Potamogeton and such algae as Stigeoclonium, Cladophora, Ulothrix, Rhizoclonium, Oscillatoria, Phormidium, Gomphonema, Nitzschia, Navicula, and Surirella (Hynes, 1960).

Patrick (1966) studied the effects of nutrients on community structure under controlled semi-laboratory conditions (greenhouse laboratory). Slide colonies of diatom communities were maintained in experimental holding boxes through which test water from a natural creek reservoir passed. The creek selected (Darby Creek) as a water source was eutrophic with well developed (but at no time abnormal) algal growths. Additions of various amounts of ammonium, nitrate and phosphate and the ratio of nitrogen to phosphorus were monitored. Some experiments involved the addition of glucose. Changes in the structure of experimental communities resulting from the addition of nutrients were detected by (1) measures of variance from the control (an adaptation of the information theory); (2) structure of the diatom community as determined by

the truncated normal curve; (3) dry weights and volatile solids; and (4) analyses of shifts in species abundance. The measure of variance from the control, analyses of the truncated normal curves, and comparison of weights did not demonstrate any great shift from the control in the abundance of diatoms, or the community structure. The most pronounced alteration occurred in the shift of abundance of certain species, i.e., in a given series of experiments 50% or more of the species occurred in all experiments, but the frequent occurrence of certain species appeared to be definitely correlated with certain experimental conditions. Specifically, the abundance of Melosira varians correlated with increased nitrates but not ammonium. Synedra parasitica (var. subconstricta) demonstrated a marked increase in abundance while the reproduction rate of Synedra rumpens var. familiaris was stimulated by increases of nitrate, ammonium, and phosphate. The increases of Synedra parasitica induced by nitrate or ammonium and phosphate additions did not occur if glucose was also added. Furthermore, glucose with ammonium voided the reproductive stimulatory effect in Synedra rumpens. Increased abundance of Gomphonema parvulum, Nitzschia palea and Synedra rumpens var. meneghiniana usually correlated with the addition of nitrate, ammonium, phosphate, and glucose. Greatly increased diatom growth patterns which often result from sewage enriched water were not seen in this series of experiments. Many of the case histories cited above as examples of eutrophication have been intimately related to available nitrogen and phosphorus.

IMPORTANCE OF AVAILABLE CARBON

Inorganic carbon sources apparently play a very important role in determining algal community structure and function and also may be important in eutrophication. Carbonates occur abundantly in many natural aquatic ecosystems, and both carbon dioxide and bicarbonate have usually been considered available carbon sources for photosynthesis. Characteristic communities of algae occur in aquatic environments which contain carbon dioxide in various forms: (1) below pH 5.0, essentially only free CO_2 is important and in such acid ecosystems dissolved CO_2 must be the major carbon source in photosynthesis; (2) between pH 7-9, bicarbonate predominates and is readily assimilated directly or indirectly by most algae; (3) above pH 9.5, carbonate dominates but is not apparently utilized in that form by most algae. In fact, carbonate complexes with a number of nutrient ions (e.g., Ca^{++} , Mg^{++} , Fe^{+++} , Cu^{++}) to form relatively insoluble precipitates. The major cations remaining in such high pH, carbonate-rich waters are generally Na+, K+ and Mg⁺⁺. Certain hard water lakes contain large amounts of calcium bicarbonate and perhaps even some colloidal calcium carbonate (Round, 1965). Bound carbonate ions are not readily available to algal communities and actually may prove detrimental to their growth and development. Since algae often utilize free CO_2 and perhaps also HCO_3 in their photosynthetic processes, higher pH ranges in the system can induce a lower concentration of available inorganic carbon. Such shortages ultimately will cause decreases in community component populations and thus, offset the community balance. The reaction of specific component populations will, of course, differ and Scenedesmus, for example, is said to utilize both undissociated CO₂ and bicarbonate ions while Chlorella pyrenoidosa cannot (Osterlind, 1950). In laboratory studies, CO₂ usually is provided as a carbon source and must be kept below inhibitory levels in order to allow successful growth. Algae cultured in the presence of adequate light and nutrients had a cell ash-free dry weight of 51-56% carbon upon examination (Ketchum and Redfield, 1959), while Chlorella exposed to varying environmental conditions had 49.5-70.17% (Spoehr and Milner, 1949).

King (1970) has suggested recently that carbon, rather than phosphorus or nitrogen, is the chief limiting nutrient in production of algal blooms (King, 1970). The Lange-Kuentzel-Kerr thesis, for example, indicates the existence of an extremely efficient mutually supportive relationship between blue-green algae and bacteria. Bacteria degrade organic matter and produce CO₂ which the algae utilize in the photosynthesis of new organic matter. At the same time the algae liberate oxygen which the bacteria, in turn, utilize in the digestion of organic matter. Nitrogen, phosphorus and other necessary substances are cycled among the algae, bacteria and environment during the process (Legge and Dingeldein, 1970). On the basis of CO_2 enrichment studies, it has long been established that this nutrient stimulates primary productivity when other nutrients and physical factors are optimal for growth and metabolism (Burlew, 1953). King (1970) also has postulated that free CO_2 concentrations are instrumental in regulating algal community structure. At least in waste oxidation ponds, King has succeeded in accurately predicting the dominance of blue-green algae, *Chlorella*, or *Chlamydomonas* solely on the basis of the available free CO_2 . Application and elaboration of King's predictions to other aquatic ecosystems awaits future research.

In considering inorganic carbon, one always must keep in mind the reservoir of organic matter, dissolved and particulate, which on complete oxidation, contributes CO_2 to the ecosystem. Even in unpolluted aquatic ecosystems, the quantity of organic matter not found in living cells is generally at least ten times that in the living cells (Parker, 1967). This reservoir of essentially autochthonous organic matter, is undoubtedly of ecological importance.

OTHER NUTRIENTS

Numerous other nutrients besides nitrogen, phosphorus, and carbon are required by algae and protozoa, and therefore, are doubtless involved in regulating community structure and function. They include oxygen, sodium, potassium, calcium, magnesium, silica, iron, manganese, molybdenum, vanadium, cobalt, zinc, copper, and boron. Natural aquatic ecosystems with single nutrient differences are rarely detected if indeed they exist at all. Consequently, we are forced to rely heavily upon laboratory culture data.

Provasoli (1969) has suggested that increases of monovalent ions, such as Na^+ and K^+ , along with other factors favor the abundance of blue-green algae. Sodium and potassium are absolute requirements for this group.

An investigation of the requirements of several green (*Chlorella pyrenoidosa*, *Scenedesmus quadricauda*, *Draparnaldia plumosa*, and *Stigeoclonium tenue*) and blue-green (*Microcytis aeruginosa* and *Nostoc muscorum*) algae for calcium, magnesium, and potassium revealed critical cell concentrations of the three cations; in this case, "critical" represented the minimum cell content of the elements permitting maximum or near maximum total growth of the organism (i.e., total biomass). When compared to the requirements of angiosperm crop plants, the calcium requirements of the six tested species were extremely low (critical cell contents of 0.06% or less, oven dry basis); requirements for magnesium were equal to or slightly less than in higher plants (0.15-0.30%) with the exception of Scenedesmus quadricauda (0.05%); potassium varied from critical levels less than the average values for higher plants (0.25-0.50%) to values equal to or in excess of higher plant averages (0.80-2.40%). The nutrition of Scenedesmus quadricauda was of particular interest due to its extremely low requirements for all three cations tested (Gerloff and Fishbeck, 1969). Thus, algal requirements for sodium and magnesium vary considerably for different algae; this also is true for silica. Pediastrum boryanum grew better than certain other green algae in low concentrations of calcium, magnesium, and silica and higher concentrations of potassium (Chu, 1942). The low silica requirement for *Pediastrum* is of special interest since silica has been found in the cell walls of this genus (Parker, 1969). Chu (1942) found contrastingly high requirements for these three nutrients by diatoms. Tolerance to various nutrients also varied. For example, Botryococcus had a greater tolerance for high sodium concentrations than did Pediastrum. In some instances increases in one nutrient allowed organisms to increase their tolerance to other nutrients.

The silicon composition of diatom frustules suggests that environmental scarcities of dissolved silica might limit growth of diatoms while probably not affecting most other groups of algae. This has been verified a number of times. One such well documented case history is the study on Asterionella populations in several English lakes by Lund (1950), following up earlier studies by Pearsall (1932). Lund demonstrated that insufficient quantities of silica may act as a limiting factor to algal growth when sufficient nitrogen and phosphorus were present. Since Asterionella was the only dominant alga in the community, any inhibitory effect on growth as a result of extracellular products of other algal populations was considered unlikely. Furthermore, evidence for significant adverse effects of light, temperature, predation, or parasitism was not apparent. Pearsall (1932) had found that diatom populations were incapable of increasing substantially when dissolved silica fell below 0.5 mg/l in certain lakes. Silica was recognized as being only one of the factors involved in the observed population changes, however. In addition, diatom populations in a study on

Deer Creek Reservoir, Utah, showed good correlations with existing dissolved silica concentrations (Gaufin and McDonald, 1965). In Lake Windermere, *Asterionella* populations were limited when silica levels were sufficiently low (Mackereth, 1953). Finally, in Antarctic freshwater melt pools and lakes, Parker, Samsel and Prescott (in press) reported well-developed diatom communities in only one of 17 habitats; that habitat contained the highest dissolved silica.

The potential of iron as a growth controlling factor in algal community structure has also been examined (Gerloff and Skoog, 1957 a, b). It has been suggested that fluctuations in the population density of phytoplankton communities in the Gulf of Maine correlated to periodic increases in both the iron concentrations of inshore waters and humic compounds and other nutrients from shoreline soils (Gran, 1933). However, studies of alterations in community structure involving increases in population numbers ("blooms") of Microcystis aeruginosa in Wisconsin lakes in the presence of iron and manganese revealed that neither of these nutrients acted as limiting factors on these particular populations. Another study by the same researchers involving the evaluation of combinations of elements as limiting factors for the same organism indicated that iron, phosphorus, and especially nitrogen could assume roles as limiting factors. Parker (1957) also identified fixed nitrogen, phosphate, and iron as important nutrients limiting growth of algae in Lake Zoar, a eutrophic calcareous impoundment of the Housatonic River, Connecticut, manifesting nuisance summer blooms of Microcystis aeruginosa and Cladophora attached to Potomogeton sp. in the littoral zone. A more recent study on phytoplankton populations in the Sargasso Sea indicated that the addition of a trace metal mixture induced a several-fold increase in C¹⁴ uptake (Menzel and Ryther, 1961). They found that the stimulatory factor of the trace metal mixture was iron which alone enhanced C14 uptake for 24 hours. The addition of nitrogen and phosphorus was also found necessary in order to extend the increased C^{14} uptake rate for a three-day period, further documenting the wide-spread deficiencies of these nutrients.

INTERACTIONS OF IONS AND OTHER FACTORS

We have, to this point, cited numerous select ex-

amples of apparent nutrient control of algal and to a lesser degree, protozoan and other microbial communities, some cases suggesting interactions of more than one element or ion. The complexity of nutrient interactions in aquatic ecosystems is undoubtedly far greater than our examples can show.

Recently, the effect of temperature and manganese as determining factors in the dominance of algal communities was investigated (Patrick, Crum, and Coles, 1969). The temperature and chemical composition of stream water were altered and the system examined for shifts in algal populations normally dominated by diatoms. Temperature increases from 34°C to 38°C favored growth of blue-green algae which became dominant, and increases in the manganese concentration to a few ppb stimulated growth of green and blue-green algae commonly seen in the presence of organic pollution (Ankistrodesmus falcatum, Stigeoclonium lubricum, Schizothrix calcicola, and Microcoleus vaginatus). A dominance of diatoms occurred when manganese concentrations rose still further and averaged 0.02-0.43 mg/l in the natural stream to 0.04-0.28 mg/l in recycled water.

An excellent example of the complexity induced by organic and inorganic pollutants and the response of algal communities is that of Jackson's (1969) studies in Onondaga Lake relating to primary productivity. Onondaga Lake is a small, urban, brackish, grossly polluted environment receiving large quantities of partially treated sewage, CaCl₂, CaSO₄, grease, oil, and heavy metals. Jackson made monthly determinations of photosynthetic rates, respiration rates, and standing crop estimates via algal counts and ash-free dry weight from May to October of 1967. He took samples from two stations, one at the southern end which was organically polluted and the other at the northern end which was inorganically polluted. The organically polluted lake section possessed 96.3 total algal cells/m², while the inorganically polluted area had 61.5. The organically polluted section also had higher values of total nitrate and phosphate than the inorganic section, and possessed significant differences in metabolism. This habitat contained the highest dissolved silica which exceeded 1.0 ppm. Inhibition of diatom growth by dissolved silica deficiencies is, consequently, an important factor influencing algal community structure. Since diatoms constitute the only dominant group of freshwater algae requiring significant amounts of silica as a wall structural component, it is clear that this fundamental physiological difference will have considerable ecological relevance.

To our knowledge, no clear cut physiological, biochemical, or ecological explanation has been elaborated for the observation that algal cells tend to be larger in fresh waters containing high total dissolved solids than those in waters of low total dissolved solids. Parker, Samsel and Prescott (in press) have discussed this phenomenon with respect to Antarctic melt pools and lakes. They found that Chlamydomonas sp. occurred in nearly every pool near the U.S. Palmer Station, Anvers Island, Antarctica. As the total dissolved solids and perhaps also organic matter increased, an approximate equivalent increase in mean cell size of Chlamydomonas occurred. On the basis of current classification, each Chlamydomonas type is presumed a separate species. Regardless, however, it is clear that some correlation exists between dissolved solids and cell sizes of certain algae. We are tempted to propose that change in total dissolved solids directly influences the efficiency of solute uptake and perhaps water loss (i.e., osmoregulation) as correlated with the surface area/ That is, where low total disvolume of the cell. solved solids occur, the surface/volume ratio will be high, enabling greater cell surface area for uptake. In flagellates, contractile vacuoles should be well developed. Correspondingly, in waters of relatively high total dissolved solids, the surface/volume ratio will be lower commensurate with the reduced need for solute uptake. If the organisms are truly different species, then the community structure has been changed; if conversely the different types are merely ecophenes, then the difference in total dissolved solids has changed the community function.

Culture Studies on Nutrient Requirements and Metabolism of Protozoa

Many protozoans, which in the broadest sense include photosynthetic organisms also referred to the algae, have been discussed in the preceding pages. A few of these and the bulk of protozoa not previously mentioned employ the process of phagocytosis as one means of consuming food. Phagocytosis provides a new dimension to the niches of protozoa as the process enables exploitation of the additional nutrient reservoir of small particulate matter (seston, detritus, etc.). Colloidal calcium carbonates, iron phosphate precipitates, organic nitrogenous debris or colloidal protein aggregates of sulfated polysaccharides, iron and manganese-rich loricae of euglenoid flagellates, and whole cells of microorganisms are among the particulate nutrient sources available to protozoans; these sources probably are of little direct value to the algae. Also, while the particulate nutrient reserve constitutes yet another source for most protozoans, the chemical nature of this reserve to some degree reflects the community structure and ecology of the primary producer.

In a study concerned with the ecology of plankton in a small artificial pond, Bamforth (1958) reported that the amount of phosphate seemed to influence which phytoplankters appeared. When phosphate levels were above 0.02 ppm, chrysomonads were not evident while cryptomonads and diatoms increased. Euglenoids were also abundant at several times this concentration. Also, within each phosphate range, other factors (i.e., nitrogen sources and growth factors) led to more immediate effects and the numbers of euglenoids appeared to be influenced by the organic nutrient reserves. He emphasized further that the nature and amount of food appeared to influence ciliates and many plankters existed perilously close to their minimum survival conditions, whereby slight environmental changes could promote significant changes in populations of the communities.

In considering entire organisms (such as bacteria) as organic nutrients one must again consider community interaction. Offsetting the nutrient balance of a given ecosystem no doubt leads to changes in the bacterial flora so essential to ecosystem function. One of the many possibilities resulting from this interruption could be the numbers and specific types of organisms available as food to algal and protozoan food web relationships. For example, the bacterial content of drinking water increases greatly after the addition of phosphates (Bosset, 1965), and thus, such increases could result following influxes of organic pollutants. More important is the fact that in certain waters, increases in bacterial density are directly proportional to the phosphate content of the water and the increased addition of phosphates to an aquatic ecosystem could result in increases in (1) bacterial content; (2) oxygen demand; (3) production of growth factors for algae; and (4) algal growth (Thomas, 1969). Thus, organic pollutants and their breakdown lead to increased phosphates (and other factors). This basic sequence could alter bacterial community structure, which would induce changes in the community structure of protozoa as well as algae which depend upon these bacteria directly as food or indirectly for their metabolic products. Organics themselves also could introduce bacteria or alter existing populations by increases or decreases in species and number of individuals available. Much of this information on bacteria is speculative, since there is a paucity of information on the role of this important group.

Studies on protozoan cultures have highlighted the significance of food chain interactions. For example, Didinium nasutum was capable of maintaining optimal fission rates and encystment rates only if fed Paramecium aurelia which itself had been well nourished with one or a mixture of two species of bacteria. Progressive starvation of Paramecium prior to use as a nutrient source by Didinium caused (in the latter) decreases in fission rates, loss of encystment efficiency, and development of abnormal cells. The alterations described were, however, capable of reversal by feeding Didinium with properly nourished Paramecium (Butzel and Bolten, 1968). Another study involving the feeding of Amoeba proteus on Paramecium aurelia indicated that variations in the density of prey had little effect on reproduction and feeding rates of the predator. Increasing the predator density produced a slower rate of capture of Paramecium, and less were ingested before division even though division size did not change appreciably. These data suggested to Salt (1968) that amoebae of low density populations with constant food carry more nutritional reserves from one generation to another than do those amoebae in populations of higher density.

Culture studies of starved protozoan populations also are numerous. For example, *Tetrahymena pyriformis* displayed marked ultrastructural and biochemical alterations after a 25-hour starvation period, including degeneration of mitochondria, nucleoli formation, changes in lipid reserves, and a loss in cellular protein (Levy and Elliott, 1968). Bacterial interaction can also assume critical roles in protozoan community nutrition patterns. Sustained reproduction of four species of foraminfera required the presence of bacteria in a study of gnotobiotic cultures. *Quinqueloculina lata, Spiroloculina hyalina, Rosalina leei*, and *Allogromia laticollaris*

were studied with various species of algae, alone and in combination. None of the algae supported continuous reproduction in the absence of bacteria (Muller and Lee, 1969). In another study (Hairston and Kellerman, 1965), the effects of temperature and limited food were examined on interacting populations of two varieties of Paramecium aurelia. One variety surpassed the other in lab cultures at 5, 15, 20 and 25°C, even when the first was given an initial population excess. A four-fold difference in food did not change the rate at which the first variety surpassed the other, which suggested that food was severely limiting to populations at both levels. Different combinations of clones of the two varieties produced markedly different rates at which the first variety surpassed the second. The first variety also excluded the other from certain habitats.

Varying periods of dominance of Vorticella species in an activated sludge sewage disposal plant were correlated with activity patterns of their accompanying mixed microflora (Reid, 1969). This correlation was further investigated by studying clonal populations of Vorticella species and their accompanying mixed microflora as culture isolates in the laboratory. Reid found that log phase bacterial activity stimulated V. octava and stationary and declining phases stimulated V. convallaria. He concluded that V. octava was predominantly dependent on the by-products produced in the medium by the microflora. In addition, bacterial activity was stimulated by the predatory activity of V. octava.

In another study conducted over two years, natural populations of *Paramecium aurelia* were sampled and examined (Hairston, 1967). Over this time period, four major peaks in population density were noted, two closely correlating with added nutrients from leaf falls and the other two with rainfall. He concluded that the populations were limited primarily by the availability of food.

IMPORTANCE OF VITAMINS AND OTHER ORGANIC REQUIREMENTS

Polytoma, an auxotrophic protozoan (or alga) requires minute quantities of specific vitamins or metabolites from the environment (Hall, 1965). The existence of such organisms raises the question of the ecological significance of vitamins. Apparently some natural waters periodically contain limiting concentrations of some vitamins, which may

induce decreases in auxotroph populations and consequent alterations in community structure and function (Noland and Gojdics, 1967). Culture studies of auxotrophic algae and protozoa have established that the optimal, minimal, and maximal concentrations of vitamins differ among the species and strains. However, culture data may not be precisely applicable to natural aquatic ecosystems, because of vitamin analogs, sparing factors, vitamin antagonistic agents, differences in rates of production and destruction, etc.

Many photosynthetic algae require: cobalamin (Vitamin B_{12}), thiamin (Vitamin B_1), or biotin (Vitamin H), and the influence of these three substances upon the community structure is evident (Provasoli, 1969). Furthermore, vitamins appear to affect several algal groups differentially and could influence algal succession patterns as suggested by an examination of natural communities under controlled conditions (Provasoli, 1969). The diatom Cyclotella nana isolated from the Sargasso Sea was utilized in a bioassay in one study; data correlations from field observations and in vitro surveys indicated that the diatom blooms probably were influenced by levels of Vitamin B_{12} (Guillard and Ryther, 1962; Guillard and Cassie, 1963). In another instance, primary productivity in phytoplankton communities of the Sargasso Sea was shown not to be B_{12} -regulated although fluctuations in this vitamin apparently induced significant changes in plankton community structure (Menzel and Spaeth, 1962). With respect to fresh water, Parker (1968) found direct correlations with increases in cobalamins and blooms of Chlamydomonas sp. in a small goldfish pond. This and other vitamins entered the pond apparently solely in rainwater (Parker, 1968; Parker and Wachtel, 1971).

Organic sources of nitrogen, in the case of protozoa, range from individual amino acids to natural proteins and the different organisms prefer different organic sources for growth. Any changes in form or availability of organic substances, therefore, produce changes in the community.

Obviously changes in the nutrient balance as a result of pollution can render the aquatic environment especially favorable for the dominance of one or a few species of protozoa. Frequently massive numbers of a few species most benefited by the pollution-caused changes develop. Relatively high cobalamins (Vitamin B_{12}) have correlated closely with marine red tides, caused by blooms of dinoflagellates which are known to require an exogenous supply of this vitamin (Sweeney, 1951). Also, cobalamins have been found in relative excess during recurrent Euglena blooms, and this protozoan (alga) also requires this vitamin (Robbins, Hervey and Stevens, 1950). Sources of vitamin B_{12} are in situ production, especially bacteria and blue-green algae, by input from runoff, and direct precipitation (Parker, 1968). These sources, generally, should apply to any organic substance. Both freshwater and marine protozoan blooms are well documented occurrences, and a great abundance of literature on their existence is available. It has been reported, for example, that 509 blooms were reported in 16 fresh water lakes and three rivers, nearly half of which were due to protozoans (in the broadest definition which includes photosynthetic ones) (Lackey, 1944, 1949). The coast of Angola experienced a typical marine bloom in 1962 which persisted for 15-20 days and resulted in a high fish and crab mortality. The major organism was Exuviella baltica occurring with dinoflagellates and diatoms (Fogg, 1966). Numerous other examples of nutrient-controlled changes of protozoan communities could be cited.

TEMPERATURE EFFECTS ON COMMUNITY STRUCTURE

All living cells, including algae and protozoans, are exposed to some normal thermal variation in aquatic ecosystems. Optimum temperatures for one cellular function may not be optimum for another. In addition, a temperature optimum for a specific function during brief exposure of a cell may not be identical to that for longer exposure times. Living cells usually are active metabolically within a narrow range of temperatures ($\approx 10^{\circ}-45^{\circ}C$) referred to as the biokinetic range. This generalization of course excludes cryophilic and thermophilic organisms which tolerate temperatures at somewhat greater extremes. Populations of blue-green algae (and bacteria) existing naturally at temperatures of 60 and 90°C in hot springs exemplify thermophilic types (Giese, 1968; Brock and Brock, 1970). Many of the general effects of heat on the cellular components of organisms are well known; certain critical aspects of cellular physiology are damaged by the denaturization of proteinaceous enzymes, and derangements of cellular lipids (leading to membrane disruption). Also, it is believed that the release of coagulating enzymes is another possible source of cellular heat damage. Liberation of calcium from the outer portion of the cytoplasm in certain cells due to heat is believed to release a clotting enzyme which gels the cell (Heilbrunn, 1954). All of these direct effects, along with more subtle manifestations of temperature alterations in organisms influence community structure.

Elevated temperature as a stress factor in aquatic environments has received increased attention recently. The rapid growth of the human population and the increased demand for electric power, accompanied by the trend toward power grids as opposed to small self-contained electric systems, has led to larger generating facilities. Fossil fueled power plants are now being built to produce four or five times the electricity of those built 20 years ago. Nuclear power plants, not economically feasible in small sizes, are being built to produce even more electricity than the new conventional plants. Nuclear plants produce heat less efficiently and as a result require about 50% more cooling per BTU than conventional methods (Kolflat, 1968). The increased exposure of communities of algae and protozoans to various forms of thermal pollution can thus be anticipated when aquatic systems are used for cooling. Temperature alterations which exceed the tolerance ranges of individual populations will induce changes, directly and indirectly, on the structure of aquatic communities.

Research on the effects of temperature on algal and protozoan cultures and field communities will enhance our ability to predict the consequences of heated waste-water discharges into aquatic ecosystems. Marre (1962) has suggested that two useful approaches to these studies are: (1) effects upon species distribution studied primarily from the ecological viewpoint; and (2) effects upon the biochemical and biophysical mechanisms of heat and cold tolerance in select species in culture, especially those which flourish under seemingly adverse thermal stress. Studies on cultures of thermophilic algae and some common mesothermal species represent this second approach. Many thermophilic algae grow in culture at lower temperatures, though in the natural aquatic ecosystem they tend to be suppressed by mesothermal species (Marre, 1962). For example, blue-greens removed from a hot spring (65°C) had the capacity for active photosynthesis when maintained at temperature as low as 20°C (Inman, 1940). Heat tolerance studies of algae have also proven useful in providing data on survival time at various temperatures. For example, *Ceratium tenuissimum* survived for 300-400 minutes at 28°C, 75-85 minutes at 33°C and 7-10 minutes at 38°C (Ayres, 1916).

Cultured algae sometimes show morphological and physiological alterations when maintained at various temperatures. Scenedesmus obliquus cultures at 13.5°C and 23°C produced different cell sizes, smaller cells resulting in the higher temperature range. In addition, the water content of the larger cells was higher so that the actual dry weights of both types were not as different as the size suggested. The shorter and broader small cells also had higher rates of oxygen consumption (Margalef, 1954). Scenedesmus affords an excellent example of extreme morphological variation in response to environmental change to the extent that species and sometimes even genus identifications in field collections are highly questionable (Trainor, 1971).

In another study, populations of Navicula pelliculosa and N. seminulum cultured at 20° C were exposed to abrupt temperature increases of 20° C (to 40° C) for 24 hours (Lanza, Cairns and Dickson, 1970). Observations with reference to overall cell content and pigmentation and stored lipids were made. Light microscopic examination revealed varying degrees of cellular damage, while ultra-violet microscopic surveys, utilizing 3,4 benzpyrene fluorescent stain on lipids, demonstrated a decrease in cellular fluorescence.

High temperatures combined with high light intensity, inhibited spore formation in *Melosira nummuloides* (Bruckmeyer-Berkenbusch, 1955). The ecological implications of reproductive stage sensitivity are of crucial importance. Various developmental stages of a given organism are known to possess different specific tolerance ranges beyond which survival is difficult or impossible.

Another investigation indicated that the increase of temperature above 24° C, which provided the most abundant growth of the rhizoidal system and shoots of *Chara zeylandica*, caused the shoot growth to halt. A temperature of 32° C restricted the lateral

branches to small outgrowths (Anderson and Lommasson, 1958).

Since the largest number of algal species live at the intermediate temperature range, it is clear that species numbers will be less in microbial communities subjected to temperatures beyond this range. However, a qualitative shift may occur before the number of species changes. Seeded slides from the Sabine River, Texas, collected at temperatures of 18-20°C and exposed to a gradual increases of temperature to 40°C, produced pronounced group shifts (Cairns, 1956). At the beginning of the experiment, the algal community on the slides was dominated by diatoms along with a few green and bluegreen algae. Within the temperature range of 20-30°C diatoms remained the dominant group component, within the range of 30-35°C green algae dominated, and in the range above 35°C, the bluegreens, often abundant in other forms of pollution, dominated. Furthermore, when the temperature was gradually lowered, green algae and diatoms reappeared at their respective temperatures. Apparently some members in each population survived when their population optimum range was exceeded, although the species were incapable of sustaining their relative abundances with respect to the original community structure.

Wallace (1955) examined pure and mixed algal cultures under carefully controlled thermal conditions. The brackish water diatom, *Nitzschia filiformis* grew optimally at 26°C and ceased growing at 34°C. The sharp reduction in growth within a rather narrow range of 4°C (i.e., 30-34°C) appears fairly common in this group. On the other hand, the heat-tolerant freshwater diatom *Gomphonema parvulum* grew optimally at 22°C, but continued to grow well up to 34°C. *Nitzschia linearis* Sm. grew optimally at 22°C, and practically ceased growing at 30°C (Figure 9).

Patrick (1969) conducted detailed studies on diatom communities developed on glass slides mounted in plastic boxes through which natural stream water passed. Temperature increases above ambient caused an increase in biomass up to $29-30^{\circ}$ C. A decrease in biomass occurred at 30° C and 33.8° C, while the greatest diversity, measured in terms of numbers of species and similar sizes of populations, occurred at about 24° C. In the October-November experiments, at 18° C and 30° C the communities were



FIGURE 9.— Nitzschia linearis; effect of temperature on population density (from Wallace, 1955).

dominated by one or two extremely common species, and in the September-October experiments, at 33.8°C a lower degree of this dominance was noted; also, more species were very common. *Nitzschia palea*, the dominant species at 30°C and 33.8°C, is broadly tolerant of many ecologically variable factors. No marked reduction in the number of species was seen, and these conclusions were in agreement with laboratory experiments which indicate suppressed cell division resulting from high temperatures near the limits of tolerance.

Diatom movement patterns through the top few millimeters of mud and their relationship to temperature and light were studied in the Ouse estuary in Sussex, England (Hopkins, 1963). Temperature increases from 5° to 15°C demonstrated that smaller diatom species (*Navicula* sp., *Stauroneis salina*, and *Nitzschia closterium*) had a greater horizontal velocity increase than large diatom species (*Trophidoneis vitrae* and *Pleurosigma* sp.). At temperatures above 17.5°C the velocity showed no increase, while at 20°C a decrease was noted. *Nitzschia closterium* and *Stauroneis salina* were the only species moving at 0°C.

Studies of temperature related distributions within communities are useful in predicting changes induced by thermal pollution. Kullberg (1969) examined several thermal spring effluents in western Montana

for their algal diversity patterns along temperature gradients. Individual communities found at intervals along the effluents were recorded as to the number of individuals and volume for each species. Dominance-diversity curves for the simpler thermal communities were found to be steeply oblique due to the dominance of one or a few species. As the water cooled, more species found suitable habitats and the curve became, graphically, more oblique in its profile. Curves for some of the more complex communities containing more intermediate species tended toward a sigmoid shape. Generally, the macrohabitats were relatively uniform with reference to temperature and dissolved substances while the microhabitats from which the individual communities were sampled displayed considerable variability in certain streams or stream sections. As a result, large portions of gradient communities appeared uniform macroscopically or microscopically, but possessed pronounced differences in community structure when percent volume analysis was employed. In certain streams, communities of algae a few centimeters apart developed markedly different curves.

Of the limited available field research on the effects of heated discharge on the algae of different types of aquatic ecosystems, various results have been noted. A recent investigation of the offshore waters of four of Lake Michigan's 22 electric power generating facilities was conducted with reference to the effects of effluents on phytoplankton, zooplankton, and benthos (Krezoski, 1969). The phytoplankton consisted mainly of diatoms, along with some of the typically warmer water green and blue-green algae, and a slight decrease in numbers of organisms found in power plant outfalls was noted. No other adverse trends appeared in the data collected.

Another preliminary report on the environmental effects of condenser discharge water in southwest Lake Michigan indicated that, while no changes in plankton could be attributed to plant operations, attached algae were reduced in the area of the outfall (Beer and Pipes, 1968).

Trembley (1960) studied the effects of condenser discharge water from the Martin's Creek Plant of the Pennsylvania Power and Light Company on river aquatic life, including microorganisms. Blue-green algae and certain members of the diatom family Fragillariaceae (heat tolerant) increased in abundance with a corresponding loss or complete suppression of less heat tolerant forms, such as green algae and many species of protozoans in the heated water. The standing crop of organic material varied in that it was found to be higher, at times, in heated water than in water at normal temperatures, or at other times lower. Periphyton were markedly reduced in number of species when heated water was compared to normal water of the river.

TEMPERATURE EFFECTS ON COMMUNITY FUNCTION

Increases in temperature along with light and current velocity also have been evaluated as to their effects on the function of communities. By comparing artificial streams which were subjected to 20° C and 25.6° C an increase in gross productivity was noted with the increasing temperature (Kevern and Ball, 1965). However, since an increase in respiration also occurred, there was no significant net increase in productivity noted. It was felt that the differences between the streams would have been greater if the temperature differences had been greater.

The effects of thermal effluents from the Virginia Electric and Power Company's steam electric generating plant at Yorktown, Virginia were studied on a community of marine organisms, involving both laboratory and field observations (Warinner and Brehmer, 1966). Cooling water for the plant came via a canal from the York River. During the winter months temperature increases which were comparable to those resulting from passage through a power plant condenser, enhanced phytoplankton primary production. For example, when a temperature of 10°-15°C was reached by the river water, as a result of a 3°C rise in temperature on passing through the condenser, a significant increase in carbon assimilation occurred. However, temperature increases of 5.5°C caused a decrease in carbon assimilation. Heating of river water above 15°C always depressed net primary production significantly if the temperature increment exceeded 5.5°C. Furthermore, at the higher river temperatures noted, a 3.5°C increase drastically depressed primary production. Thus, the range of tolerance to temperature change narrowed towards the higher natural river temperatures. This is similar to the findings of Alabaster (1963) for fish.

The majority of protozoans, like algae, are capable of living within specific ranges of temperature variation, and certain species possess the ability to extend their survival capacity through the process of encystment. In addition, any one species may possess various temperature tolerances under different environmental conditions. This fact is clearly demonstrated by Ochromonas malhamensis which was successfully grown above its "maximum" tolerable temperature of 35°C in minimal medium by addition of extra Vitamin B₁₂, thiamin, metals such as Fe, Mg, Mn, Zn and several amino acids (Hutner et al., 1957). Thus, certain protozoans may be ecologically restricted, in terms of limits of temperature tolerance, due to a lack of available nutrients required at elevated temperatures. Also, a phospholipid requirement, along with morphological abnormalities (i.e., extreme variation in size and shape, fractures and displaced kineties, and abnormalities of karyokinesis) could be induced by growth of Tetrahymena pyriformis at supraoptimal temperatures (Rosenbaum, et al., 1966). These studies show that at supraoptimal temperatures, nutritional requirements of protozoa may be altered along with a variety of other metabolic responses and critical organism functions (i.e., reproductive rate, growth and development, regeneration, encystment, etc.).

Dallinger (1887) subjected cultures of *Tetramitus* rostratus, Dallingeria drysdali, and Monas dallingeri to a gradual increase in temperature. In this experiment the temperature in a water bath surrounding the cultures was elevated from about 22° C to 70° C over a period of more than five years. Less gradual temperature increases may allow for the natural selection for the tolerance of increased temperature, and this possibility can be considered as one mechanism for survival (Noland and Gojdics, 1967). An example in support of this is a thermal strain of *Tetrahymena*, which was found in a New Mexico hot spring environment, and was successfully cultured at 41.2°C while other strains succumbed at temperatures above 40° C (Phelps, 1961).

Kasturi Bai, *et al.* (1969) studied the effect of temperature increases upon the activity, structure, and reproductive rate of the ciliate, *Blepharisma intermedium*. Initially, increases in temperature resulted in an increase in the organisms's reproductive rate, i.e., at 26° C one division per 24 hours oc-

curred, at 27°C two divisions occurred, at 28°C the maximum of three divisions in 24 hours was attained. Temperature increases beyond 28°C induced a decline in the fission. Between 31°C and 36°C a leveling effect occurred, and at 37°C, fission again declined, halting completely at 38°C. Blepharisma also acclimated to its maximum reproductive temperature level of 28°C. Cytochemical studies revealed conditions optimal for cell division (i.e., glycogen, basic proteins, unsaturated lipids, enzymes, and alkaline and acid phosphates present in required quantities). At 38°C, alterations occurred in metabolism with a decrease in glycogen, protein, lipids, and activities of some enzymes; also changes in the free amino acid pattern and inhibition of cell division occurred. When returned to room temperature with the addition of fresh culture medium, Blepharisma resumed normal metabolism and reproduction by fission after a three-day lag phase. Studies by Thormar (1959) indicated that division in protozoa may be synchronized through heat-induced cellular alterations, and that such synchronization in Tetrahymena pyriformis appears related to thermal sensitivity which is dependent upon cell age. Thus, delays in the division of Tetrahymena result from heat shocks and the length of the delay increases with increasing cell age.

Gross and John (1962) studied Euglena and Chlamydomonas with reference to their cellular response to interacting thermal and photo stresses. Both organisms were cultured in an organic medium in the dark and at several light intensities and temperatures ranging from 20°C to 35°C. At temperatures below 32.5°C, Euglena growth became inversely light-dependent; that is, growth decreased with increasing illumination. Giant, multinucleated Euglena cells occurred at 35°C, with higher percentages of abnormal cells at the higher light inten-Chlamydomonas did not develop such absities. It was postulated that at elevated normalities. temperatures a dark-formed thermosensitive protein, essential for normal cellular division, is denatured in Euglena. Furthermore, light could enhance the harmful effect of heat on chlorophyll and the chloroplast.

Gross (1962) studied three apochlorotic *Euglena* substrains in light and dark cultures at 25°C and 33-35°C. Permanently chlorophyll-less with differ-

ent carotenoid distributions, the strains were derived originally from a single photosynthetic stock culture. Also employed were Astasia longa (a naturally pigmentless euglenoid) and the ciliate Tetrahymena. At 25°C, none of the organisms were light dependent, while at higher temperatures, only the three Euglena substrains demonstrated an effect of light on growth. One substrain (PBZ-G4) was inhibited as was the photosynthetic parent strain, while the other two (SM-L1, HB-G) were stimulated. At elevated temperatures, abnormal forms developed in all three substrains; two became multinucleate (SM-L1, HB-G), while the other (PBZ-G4) developed an enlarged nucleus. Multinucleate forms occurred in a small percentage of the Astasia cells, while almost all of the Tetrahymena cells became giants in high temperature culture. Neither Astasia nor Tetrahymena were affected by light, in contrast to the unexplained light-temperature interactions of the nonphotosynthetic substrains of Euglena. While the physiological explanation for these phenomena is lacking, it is clear that the major factor causing the abnormalities was thermal stress. Temperature induced developmental abnormalities even occur in the microtubular organelle of the ciliate Nassula, in the flagellum of Naegleria gruberi (Tucker, 1967; Dingle, 1967), and in other instances. No doubt such temperatureinduced abnormalities accompany functional changes.

The relationship of temperature range and cell size in protozoa has indicated that such adaptation to environmental change has considerable implications. The growth of *Tetrahymena pyriformis* at three different incubation temperatures, 10° , 20° , and 30° C resulted in three classes of cell size (James and Reed, 1957). Cell size increases at low growth temperatures as well as the highest temperature utilized in later research were shown by Thomar (1962).

Another temperature effect of ecological significance is the change in the regenerative rate of organisms. For example, Giese and McCaw (1963) studied the effects of temperature and other factors on the ability of *Blepharisma americanum*, *B. undulans*, and *B. japonicum* to regenerate removed hypostomes. These protozoa were cultured at various temperatures under continuous yellow light. At 30° C cells accelerated regeneration, while at 35° C they showed retarded regeneration. Brief exposures to a temperature of 40° C also resulted in a retardation of regeneration. Inhibited or retarded regeneration could be caused by enzyme denaturation at increased temperature. Temperatures below 25° C retarded regeneration down to 10° C where *Blepharisma* lost pigment in a "shock" reaction (many dying at 5° C). In all cases, nutrient-starved cells had a slower regeneration rate. No diurnal rhythms of the rate of regeneration were noted. High and low temperature limits for division were like those for regeneration. On the basis of preliminary evidence, Giese and McCaw noted *Blepharisma* adapted to some degree to temperatures higher and lower than those usually tolerated.

One protozoan response to adverse conditions is the mechanism of encystment, which temperature also influences. For example, *Oxytricha fallax* showed an accelerated rate of change into pre-cystic forms on exposure to temperature increases (Hashimoto, 1962). To what degree the encystment process has survival value to protozoan communities subjected to pollution, including thermal types, remains to be determined; knowledge in this area would prove extremely valuable in future community interaction analysis. Numerous algae also produce resistant cellular stages in response to environmental stimuli. These include cysts, hypnospores, akinetes, auxospores, and zygospores.

Cairns (1969a) studied the rate of species diversity restoration in freshwater protozoan communities following stress in the form of pH and temperature shocks. Freshwater protozoan communities inhabiting plastic troughs through which unfiltered Douglas Lake (Michigan) water flowed were investigated to ascertain both temperature effects and the time required to restore the community (by recolonization) to its original species diversity (i.e., number of species). Generally, the recovery rate was comparatively rapid. For example, a shock from 20° to 50°C for 7-8 minutes caused a decrease from 26 to 7 species, recovering to 18 species in 24 hours and to the original diversity of 26 species in 72 to 144 hours. In contrast, the thermal shock from 18° to 40°C for 7-8 minutes reduced the species from 31 to 22 with recovery to approximately control diversity in 72 hours. A thermal regime of slightly over 30°C for about 24 hours reduced the diversity from 34 to 21 species in the initial four-hour period, and then while this thermal regime was sustained, there was an increase of three species at 12 hours. This

latter variation is sufficiently small to constitute sampling error. However, the rapid restoration of initial species diversity after return to normal temperatures suggests that some temperature-tolerant species began to invade the sampling site while still under thermal stress. Two major conclusions of this preliminary study are that: (1) the magnitude or intensity of the shock is more important in reducing protozoan species numbers than its duration, and (2) restoration of protozoan species diversity takes only a few hours following mild thermal shock, but as much as five or six days following severe shocks. The latter statement is probably only true of temperature, pH, and other shocks where there is no test material or toxicant residual left in either substrate or organisms.

In spite of the above-cited extensive literature on thermal effects, knowledge of the actual effects of thermal effluents on aquatic ecosystems, including algae and protozoa, is sparse. The effects of the artificial temperature increase as a factor influencing the various different complex interactions of communities at various critical phases thus deserves an intensified research effort. The sequence of events, indirect and direct, leading to a temperature-induced decrease in community diversity and stability will, hopefully, be better explained through such future research efforts.

TOXIC SUBSTANCES

Toxic substances can occur as heavy metals, halogens, pesticides, surface-active agents, oils, inorganic reducing agents (sulfides, sulfites) and a variety of other materials. The introduction of toxic material to aquatic ecosystems produces a variety of complex responses governed by several basic factors: (1) nature of the toxicant; (2) toxicant concentration; (3) exposure time; (4) environmental characteristics of the receiving system; (5) age, condition, etc. of exposed organisms; and (6) the presence of other toxicants. Antagonistic additives or synergistic interactions with other substances, changes in dissolved oxygen concentration, redox potential, pH, nutrient and salt balance, etc. alter the response of aquatic organisms to toxic substances. Most laboratory bioassays are carried out for short time periods in relatively simple, stable conditions while in nature, the toxicant may be altered and react differently. Thus, a given toxicant can react quite differently in a natural situation than in a laboratory assay, e.g., biological magnification is rare in laboratory bioassays but relatively common in nature. Thus, results of the type discussed here should be used as guides, not standards.

Heavy Metals. — Excess heavy metals are often introduced into aquatic ecosystems as by-products of industrial processes, and acid mine drainage residues. Recently, the significance of airborne non-ferrous metals and their accumulations in plants and soils have received increasing attention (Goodman and Roberts, 1971). In addition, research on the effects of certain metals (Zn, Cu, Cd, Li, Cr, Hg, Ni, Pb) on algae and protozoa has also received increased impetus. Patrick (1968b) examined temperature relationships to zinc toxicity in diatoms in two synthetic water types (soft and hard) in the laboratory. Nitzschia linearis showed a 50% reduction in growth as compared to soft water controls (44 mg/l Ca-Mg as CaCO₃) at 72°F and 4.29 mg/l zinc; 82°F and 1.59 mg/l zinc, and at 86°F and 1.32 mg/l zinc. N. seminulum had a 50% reduction relative to hard water controls (170 mg/l Ca-Mg as CaCO₃) at 72°F and 4.05 mg/l zinc, 82°F and 2.31 mg/l zinc, and at 86°F and 3.22 mg/l.

Exposure of the giant kelp, *Macrocystis pyrifera* to zinc sulfate for a four-day period (with 1.31 mg/l as zinc) had no appreciable effect on photosynthesis but 10 mg/l caused a 50% inactivation of bottom kelp fronds (Clendenning and North, 1960; North and Clendenning, 1958).

Bringmann and Kuhn (1959a and b) evaluated threshold effects of zinc added to the water containing algae and protozoa during a two to four-day exposure. *Scenedesmus* had a median threshold response at 1.0-1.4 mg/l zinc while the protozoan, *Microregma*, was more sensitive with a median threshold response at 0.33 mg/l.

Williams and Mount (1965) observed, for 14 weeks, glass slide colonies of periphyton communities in four outdoor canals in Ohio exposed to four concentrations of zinc. Zinc was added continuously as zinc sulfate to three of the four artificial canals with the intention of producing concentrations of 1, 3, and 9 mg/l respectively. However, due to the precipitation and absorption of zinc on the sides and bottom of the canals and some distribution problems somewhat smaller quantities than desired occurred periodically. The number of dominant species in the community declined on exposure to <1 mg/l of zinc. A geometric regression of the average number of dominant algal species occurred, commencing with the control (no added zinc) to that with the highest zinc concentration. Relatively high zinc concentrations induced comparatively low biotic diversity. Community restructuring in the form of an increase in the standing crop of fungi and slime forming bacteria occurred in the highest zinc concentration, apparently resulting from the digestion of zinc killed phytoplankton used as their food supply. Thus, in general, zinc increases caused marked decreases in sensitive species and an increase in tolerant species.

Sporadic fish kills in the Upper Sacramento River below Keswick Reservoir in California prompted an investigation by Benoit, Cairns, and Reimer (1968). The results of a biological survey, including diatom communities, indicated that Spring Creek, one of the lower tributaries of Keswick Reservoir and two of its branches, were severely degraded by acid mine drainage. Chemical tests on the waters and delta muds of Spring Creek confirmed the presence of high concentrations of iron, copper, and zinc along with a low pH which, undoubtedly, were the main contributors to the existing biological degradation.

The extensive research on the various toxic effects of copper on algae and protozoa will be briefly surveyed. In addition to changes caused by death, copper-induced metabolic alterations may also affect community structure. Copper (as copper sulfate) at a threshold concentration of 0.15 mg/l produced noticeable effects upon Scenedesmus (Bringmann and Kuhn, 1959a, b). At 0.5 mg/l of copper different organism sensitivities resulted: Diatoms (six species tested) were most sensitive and were annihilated; Cyanophyta (17 species) were next in sensitivity with 57% death, while Chlorophyta (17 species) were least sensitive with 35% death (Mahoney and Palmer, 1956). Copper (as copper sulfate) at 0.2 mg/l was also found to kill 100% of Microcystis aeruginosa (Fitzgerald, Gerloff, Borg, 1952), while 0.05 mg/l proved fatal to Microcystis in other experiments (Crance, 1963). In assays for copper toxicity to algae (i.e., Scenedesmus) and protozoa (i.e., Microregma) in water from their natural habitats, threshold levels of copper detected were 0.15 mg Cu/l and 0.05 mg Cu/l respectively (Bringmann and Kuhn, 1959a, b). Photosynthesis in the giant kelp, *Macrocystis pyrifera* following exposure to copper chloride and copper sulfate revealed that 0.1 mg/l of copper caused a 50% decrease in net photosynthesis in two to five days and a 70% decrease in seven to nine days. Morphological injury appeared within a ten-day period. Copper was slightly less toxic than mercury, but more toxic than nickel, lead, chromium, or zinc (Clendenning and North, 1958, 1960; North and Clendenning, 1958, 1959).

The long history of copper use as an algicide has contributed to our knowledge of the effects of this heavy metal on community structure. Some recommended levels for control of some species in ponds are: Anabaena, 0.09 mg/l; Beggiatoa, 5.0 mg/l; Chara, 0.2-5.0 mg/l; Cladophora, 1.0 mg/l; Cladothrix, 0.2 mg/l; Conferva (a form genus), 0.4 mg/l; Navicula, 0.07 mg/l; Oscillatoria, 0.1-0.4 mg/l; Scenedesmus, 5.0-10.0 mg/l; Spirogyra, 0.05-0.3 mg/l; Ulothrix, 0.2 mg/l; and Volvox, 0.25 mg/l (Anon, 1954). Such values are, of course, very generalized because of the extensive modification of copper toxicity by many environmental factors (e.g., pH). Because of the formation of relatively insoluble copper carbonate at high pH's, recommended "control" concentrations of copper may often be inadequate. For example, Parker (unpublished) found numerous blue-green algae grew in intimate association with copper carbonate ore from mines in the Mohave Desert.

Hynes (1960) discussed copper pollution in the Churnet River, a tributary of the Dove River. The Churnet River, while recovering from organic pollution, received an effluent raising copper levels to 1.0 mg/l, and sometimes above. Serious effects upon the algal community structure resulted. Species above the outfall in the recovery area were sampled by using glass slides for three-week periods and found to support Stigeoclonium, Nitzschia palea, Gomphonema parvulum, Chamaesiphon, and Cocconeis (in combined quantities of approximately $1000/\text{mm}^2$ on the slides). Below the outfall, the number decreased abruptly to 150-200/mm² on the slides with Chlorococcum and the diatom Achnanthes affinis persisted dominating the flora. This community structure persisted for at least three miles. Five miles below the outfall, the cell numbers increased steeply to over 33,000/mm², and more than 30 miles downstream and below the confluence

with the Dove, the numbers exceeded 50,000/mm². The latter community included not only *Chlorococcum* and *Achnanthes*, but also the original algal community above the outfall, with the exception of *Cocconeis*.

To our knowledge, no available evidence explains microbial differences in sensitivity to copper at the biochemical level. Such information would aid prediction and possible diminution of the effects of copper toxicity. 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 metalloflavo-proteins (Dixon and Webb, 1958), these and other cellular processes may involve copper toxicity mechanisms in the different algae and protozoa.

Information on cadmium, lithium, and chromium toxicities to aquatic algae and protozoans is less extensive than that for copper. Scenedesmus was tested in the river water from which is was collected and the threshold concentration of cadmium was found to be 0.1 mg/l (Bringmann and Kuhn, 1959a, b). The effect of lithium on morphogenesis of protozoa has also been investigated. Naegleria amoeba stages displayed characteristic "tadpole" forms when subjected to lithium (Willmer, 1956), while abnormal, delayed regeneration occurred in Condylostoma (Suhama, 1961). Various effects, including a broadening of cells with a great increase in the number of lateral stripes and kineties leading to conversion into doublets in Stentor also have been attributed to lithium exposure (Tartar, 1957). Hervey (1949) investigated chromium toxicity to algae and protozoans. Chlorococcales were inhibited in the 3.2-6.4 mg/l range, diatoms at 0.032-0.32 mg/l, and euglenoids at 0.32-1.6 mg/l. He also found a stimulating effect of chromium on algae at certain sub-inhibitory concentrations. The relationship of temperature to chromium toxicity in diatoms has also been investigated (Patrick, 1968). In soft water (44 mg/l Ca-Mg as CaCO₃), Nitzschia linearis responded with a 50% reduction in population growth at 22°C and 0.208 mg/l chromium; 28°C and 0.261 mg/l chromium; and at 30°C and 0.272 mg/l chromium. In hard water (170 mg/l Ca-Mg as CaCO₃), Navicula seminulum var. hustedtii responded with a 50% reduction in population growth at 22°C and 0.254 mg/l chromium; 28°C and 0.343

mg/l chromium; and 30°C and 0.433 mg/l chromium. Bringmann and Kuhn (1959a, b) reported chromium toxicity thresholds to be 5.0 mg/l for *Scenedesmus* and 37 mg/l for *Microregma*. 1.0 mg/l of hexavalent chromium added as $K_2Cr_2O_7$ for two days induced no immediate decrease in photosynthesis in *Macrocystis pyrifera*, although a 10-20% reduction occurred after five days and a 20-30% reduction after seven to nine days. The chromium concentration required to produce a 50% photosynthetic reduction in a four-day exposure period was estimated at 5.0 mg/l (Clendenning and North, 1958, 1960; North and Clendenning, 1958, 1959).

Nickel and lead have also received attention. Bringmann and Kuhn (1959a,b) reported the threshold concentration of nickel as 1.5 mg/l for Scenedesmus and 0.05 mg/l for Microregma. Threshold concentrations of lead (four days at pH 7.5 and 24°C) were 2.5 mg/l for Scenedesmus and 1.25 mg/l for Microregma. In contrast, lead nitrate did not inhibit photosynthesis in Macrocystis pyrifera during a four-day exposure at 4.1 mg/l (North and Clendenning, 1958). Much remains to be learned about heavy metals in ecosystems. One excellent example of the potential sublethal effects of lead in biological systems is provided by the recent research of Hemphill et al. Exposure to subclinical doses of lead nitrate decreased resistance of mice to bacterial infection.

The Halogens. --- While extremely useful in controlling certain human health hazards in water, chlorine has been implicated in situations where the biota has been degraded, both when present in thermal effluents and alone. The possibility that chlorination of heated water at the point of discharge can reduce algal populations is suggested in the data collected by Trembley (1960) for the Delaware River. Furthermore, protozoan cysts (including useful nonpathogenic types) can be damaged or annihilated by chlorination. Kott and Kott (1967) found that exposure to 8 mg/l of chlorine at 10°C for one hour killed cysts of Endamoeba histolytica. While the destruction of this human parasite is desirable, the concurrent destruction of free-living forms representing constituent parts of aquatic ecosystems also must be carefully evaluated.

The combination of halogens with other substances could conceivably result from increasing use of our aquatic ecosystems. Such combinations as both antagonists or synergists could influence a multitude of factors essential in normal ecosystem function. Research has demonstrated the potential of halogens in influencing algal populations under different ecological conditions. Kott (1968) in a study of the addition of chlorine and bromine in various, or equal concentrations to suspensions of Chlorella sorokiniana noted increasing mortality. Combined halogens did not accelerate mortality. Furthermore, in the presence of light, bromine when in measurable concentration, either free or combined, caused a decrease in the numbers of Chlorella, while in the dark, no death occurred. Chlorine, which was algistatic in the light, became algicidic in the dark. Clendenning and North (1958, 1960) studied the photosynthetic capacity of the bottom fronds of Macrocystis pyrifera, after exposure to chlorine. Concentrations of 1.0 mg/l for a period of five days had no effect; however, at concentrations of 5 to 10 mg/l there was a 10-15% reduction in photosynthetic activity after a two-day exposure and a 50-70% reduction after exposure for five to seven days. Of further interest is the relationship between metals and chlorine and their ecological implications as indicated by Burton's (1969) study of the Vellar Estuary in India. Here, molybdenum levels correlated directly with the chlorinity of the water.

Pesticides. --- Information on the effects of pesticides upon algae and protozoa is more limited than that for heavy metals and halogens. We know that submerged plants concentrate pesticides to severalfold their original levels (Hoffman and Drooz, 1953), and further magnification is possible. Also important are the toxic effects of insecticides on organisms throughout their life cycles. Both laboratory and field studies help to better understand the potential effects of pesticide residues in aquatic eco-For example, the effects of dieldrin on systems. populations of cultured Navicula seminulum var. Hustedtii, a moderately sensitive freshwater diatom common to many unpolluted streams, was examined by Cairns (1968a). Concentrations of dieldrin at 1.8 ppm resulted in only a 10.6% reduction in cell number, while dieldrin at 12.8 ppm resulted in a 50% reduction compared with the controls at the end of a five-day growth period. Cell division was arrested in this diatom at concentrations of 32 ppm. Although this study concerned only effects on growth, there is a possibility of accumulation of dieldrin in the cells and its further concentration and influence at other levels of the aquatic food chain. Indeed, recent studies dealing with the biological concentration of pesticides and their effects on metabolism have demonstrated that while certain Cyanophyta (Microcystis aeruginosa and Anabaena cylindrica) and Chlorophyta (Scenedesmus quadricauda and Oedogonium sp.) are generally quite resistant to dieldrin, endrin, aldrin, and p,p'-DDT at concentrations of 5, 10 and 20 ug/ml (M. aeruginosa, however, was killed by less than 5 ug/l of dieldrin, aldrin, or endrin), accumulation did occur. The concentrations of these pesticides in the algae reached at least 120 times that initially occurring in the natural waters, and the concentration effect by algae was evident only 30 minutes after exposure. In terms of function, none of the pesticides examined, at a concentration of 1 ppm, produced any significant effect upon the respiration of the freshwater algae tested (Vance and Drummond, 1969). However, even lower concentrations under different conditions might induce subtle damage resulting in functional changes with ultimate effects upon community structure. A few ppb of DDT in seawater, for example, has the potential of drastically reducing the rate of photosynthesis of certain components of the phytoplankton community (Wurster, 1968; Menzel, Anderson and Randtke, 1970). Numerous other pesticides have the potential of inflicting severe damage on aquatic ecosystems and must be used carefully. Many pesticides are translocated throughout the biosphere and, hence, may have widespread deleterious effects. The occurrence of pesticides and their derivatives in several levels of food chains in remote Antarctica where no pesticide use has even been reported is now well documented (Parker, 1971).

Surfactants. — Paramecium aurelia lost its ability to respond to a difference in electrical potential when treated with anionics. Treatment with cationics and non-ionics did not affect the protozoan's normal movement towards the cathode (Butzel, Brown and Martin, 1960). A non-ionic surface-active agent at concentrations of 1.0, 5.0, and 10.0 mg/l stimulated the growth of cultures of Ankistrodesmus convolutus for the first few days, but later resulted in a retardation. Initial growth retardation resulted at concentrations of 50 mg/l. A cationic surface-active agent

applied at 5.0 mg/l hindered growth of Ankistrodesmus, while an anionic surface-active agent applied at 10.0 mg/l proved slightly toxic for 25 days, at which time growth commenced (Wurtz-Arlet, Growth inhibition occurred in the blue-1961). green alga, Oscillatoria at concentrations of 5.0 mg/l of either of two ammonium chloride cationic complexes (Arquad C and Arquad D) (Williams, Groniger and Albritton, 1952). Exposure of kelp to sodium dodecyl sulfate at concentrations of 10 mg/l and zephiran chloride at concentrations of 1.0 mg/l produced marked inhibiting effects (Pearson, Pomeroy and McKee, 1960). Macrocystis pyrifera had 50% loss of photosynthesis in the bottom fronds after four days' exposure to concentrations less than 1.0 mg/l of zephiran chloride or 5-10 mg/l of sodium dodecyl sulfate. Alkyl benzene sulphonate resulted in complete inactivation in 48 and 96 hours at 2 mg/l and 5 mg/l, but 0.5 mg/l produced only slightly detrimental effects in 96 hours and no effect in 48 hours (Clendenning and North, 1960; North and Clendenning, 1958; North, Clendenning and Scotten, 1959). Other studies involved toxicity tests of several quarternary ammonium compounds on Cylindrospermum, Microcystis (sometimes substituted with Gloeocapsa, Scenedesmus, Chlorella, Gomphonema, and Nitzschia and various degrees of toxicity, or lack of toxicity were determined in preliminary screenings (Palmer and Maloney, 1955).

Oils. — Oil can form films on the surface and thus interfere with gaseous exchange, photosynthesis, and a multitude of other phenomena. Settleable oily substances may coat bottom surfaces, substrate surfaces, or algae and protozoans themselves and, thus lead to indirect or direct death in species. Certain oils also contain toxic substances (i.e., napthenic acids, phenol, etc.) which intensify the danger of these types of pollutants to aquatic ecosystems.

Suspended Solids. — Suspended solids often pollute aquatic ecosystems even though the range of variation of suspended solids concentrations in natural systems is enormous. For example, McCarthy and Keighton (1964) reported sediment concentrations for the Delaware River at Trenton, New Jersey ranging from one part per million to 4100 ppm. The daily loads of suspended sediment in the Delaware River at Trenton ranged from half a ton per day to as much as one million tons per day (on August 20, 1955). In the six years from October, 1949, to September, 1955, a total of 6.7 million tons of suspended sediments was carried by the Delaware past Trenton; twenty-six percent of this accompanied the flood caused by Hurricane Diane.

Cairns (1968b) listed such ecological effects of suspended solids as: (1) mechanical or abrasive action (i.e., clogging or irritational); (2) blanketing action or sedimentation; (3) loss of light penetration; (4) availability as a surface for growth of microorganisms; (5) adsorption and/or absorption of various chemicals; and (6) change in temperature fluctuations. While offsetting the balance of suspended solids in the system can produce any or all of these ecological effects, certain of them are more likely to endanger the integrity of the community structure of algae and protozoa. Any reduction in the penetration of visible radiation into aquatic ecosystems due to increases in suspended solids would restrict or prohibit the growth of photosynthetic organisms. Predator-prey relationships (i.e., zooplankton grazing on phytoplankton) might change leading to abnormal increases or decreases of individuals, thus offsetting the system's population balance and stability. King and Ball (1964) studied the influence of highway construction on a stream, and noted that the sediments from the area of highway construction drastically reduced production at all levels of the biotic community. During the period of heavy sedimentation there was an apparent reduction of 61% in the number of primary producers and a 58% reduction in the energy required by the heterotrophic community members (because they were reduced in numbers).

The surfaces of particulate matter may act as substrates for bacteria, fungi, and other microorganisms, while the particle itself also may contribute to microbial nutrition. When the presence of particulate matter enables the environment to support substantially increased populations of these organisms, the dissolved oxygen concentration, pH, and other characteristics of the water are usually altered. Furthermore, if this type of activity increases on the bed of the stream or lake, anaerobic conditions are likely to develop, thus giving rise to an accumulation of noxious gases (e.g., H_2S) which are harmful to other organisms.

Adsorption, absorption and precipitation of chemicals is particularly important if they may lead to build-up of toxic substances in a limited area with

the possibility of sudden release. One such example has been reported and briefly mentioned previously in our discussion of zinc toxicity (Benoit, Cairns and Reimer, 1968). The acid waters of Spring Creek flowed into Keswick Reservoir, a regulatory reservoir just below Shasta Dam, California, on the headwaters of the Sacramento River, bearing iron, zinc, and copper in solution. Typically, the Spring Creek waters were neutralized by the alkaline waters in Keswick and the heavy metals were precipitated in association with other types of particulate matter to the bed of Keswick Reservoir. However, the reserve alkalinity of Keswick waters was not enough to neutralize the increased volume of acid Spring Creek water which occurred during spring freshets. On these occasions some of the heavy metals went back into solution, and together with those already dissolved, caused fish kills. This situation has apparently been corrected.

Finally, Brungs and Bailey (1967) report that the availability of endrin in an aquatic environment is influenced by the suspended solids load.

Reducing Agents. — Inorganic reducing agents such as sulfides and sulfites are often components of various industrial and sometimes sewage wastes. In certain natural waters they may result from the anaerobic decomposition of organic matter. Soluble sulfide salts such as Na₂S disassociate into sulfide ions which then react with existing hydrogen ions in water thus forming HS⁻ or H₂S. At pH 9.0, approximately 90% of the sulfide is in the form of HS-, at pH 7.0 it is about equally divided between HSand H₂S, while at pH 5.0 less than 1% occurs as HS⁻, and as a result H₂S escapes into the atmosphere. Only at higher pH values of 10 or above are appreciable sulfide ions available. Sulfide in aquatic systems is thus usually available as HS^- or H_2S . Certain types of bacteria can oxidize sulfides and H_2S to colloidal sulfur in which case these organisms function as auxiliary polluters. Paper pulp waste effluent, i.e., sulfite waste liquors (SWL) or spent sulfite liquors (SSL), are known to contain in solution or finely divided suspension approximately 50% of the weight of the wood used for pulping. This waste comprises fiber-binding substances, such as, lignin, pectin, hemicelluloses, sulfur dioxide, sulfites, polythionates, organic acids, calcium, magnesium, etc. Such effluents exert a tremendous B.O.D. on the system and cause surface foaming in addition to

the introduction of direct organic increases (McKee and Wolf, 1963a). While the specific effects of these substances with reference to algae and protozoans deserves much research, limited investigations have been conducted with regards to H_2S toxicity which revealed interesting effects on community structure.

Bick (1958) demonstrated that in systems with anaerobic bottom conditions and with H₂S concentrations and limited oxygen conditions above, protozoans capable of tolerating low oxygen but intolerant to H_2S occurred only in the limited oxygen layer. Examples of this type are Spirostomum ambiguum, Halteria grandinella, and Paramecium caudatum. In another study, Rylov (1923) noted that both H_2S and oxygen influenced the movement of Loxodes rostrum in a pond. The protozoan moved out of the H_2S containing bottom layer, also avoiding the zone of high oxygen content, and as a result of such restriction occurred in a narrow stratum in sufficient numbers to be visible as a white layer with the naked eye. In general, with the exception of sapropelic species, H_2S is more toxic to protozoa than other gases such as H₂, CO₂ or CH₄ (Nikitinsky and Mudrezowa-Wyss, 1930).

Radioactive Wastes. - Radioactive materials have received increasing attention with the growth in plans to utilize atomic power plants as energy sources, and due to increased use of isotopes in hospitals, industry, and research. Algae, as well as other lower forms of life and higher aquatic vegetation, often accumulate radioactive materials by both adsorption and absorption. Generally, lower organisms are more resistant to ionizing radiations than higher forms; for example, the acute dosage in roentgens causing 50% mortality in algae ranged between 8,000-100,000 while the range seen to induce 100% mortality was between 25,000-600,000 roentgens. Protozoan susceptibility to radiation was noted as a range of 10,000-300,000 roentgens to induce 50% mortality and 18,000-1,250,000 roentgens to cause 100% mortality (Donaldson and Foster, 1957). Such direct effects of radiation no doubt offer limited threat to organisms in aquatic systems although the ranges of response in terms of mortality among different algae and protozoa as groups are somewhat more important.

Of greater ecological importance is the concentration and translocation of radionuclides in aquatic communities, which is dependent upon many interrelated phenomena of the component organisms' metabolic pathways and the complexity of the aquatic ecosystem. Organisms generally accept all isotopes of a given element, so the accumulation of a radionuclide depends chiefly upon the ratio of the radionuclide to the non-radioactive form of the same element and, in some cases, closely related elements (McKee and Wolf, 1963b). As mentioned previously, certain elements not essential for growth are taken up by microorganisms. These include strontium, cesium, lithium, ruthenium, etc., all of which have radioactive forms (O'Kelley and Herndon, 1959).

Blinks (1952) examined reef communities of marine algae one year after the atomic tests in the Bikini Islands. He noted that surviving organisms had a considerable increase in catalase activity. Other physiological functions investigated, including genetic alterations or damage, revealed no abnormalities although the possibility that some occurred and went overlooked exists.

Cushing and Watson (1968) studied the uptake and accumulation of radioactive materials by both living and formal in-fixed phytoplankton. Accumulation of 65Zn by living plankton was significantly lower, while accumulation of ³²P by living plankton was higher than that of dead plankton. The ability of living algae to accumulate surplus phosphate may be responsible for the latter observation. Killing algae with formalin no doubt would have destroyed the capability of algal for luxury consumption of phosphate. Of further ecological significance are the relationships between the amount of ³²P and ⁶⁵Zn accumulated by the living cultured phytoplankton and the naturally occurring phytoplankton. Mean concentrations of ³²P and ⁶⁵Zn after the same approximate exposure time were much higher in the living natural populations. Also, these studies indicated that periphyton generally accumulated more of both of these radionuclides than net plankton. However, the higher radioactivity of naturally occurring plankton could be an artifact due to inclusion of more detached periphyton from the river bed within the reactor areas. Dominant organisms in the plankton were free floating algal species although many periphyton forms were also found. Another explanation of the higher concentrations in the natural populations may be caused by differences between laboratory and field methods. In the field algal communities were retained in plankton nets, as opposed to culture vessels in the laboratory. Another difference might be the type of illumination.

McKee and Wolf (1963b) point out the following generalizations offered by Krumholz and Foster (1957):

- 1. Radioactive materials are taken into the body of an organism either through physiological processes and incorporated directly into the tissues, or they are adsorbed on the surfaces of the organisms. In general, adsorption and absorption are governing mechanisms for the lower forms of life, while ingestion is the principal route for predators.
- 2. The direct concentration of certain radioelements reaches a higher level in the lower plant and animal forms, such as bacteria, protozoa, and phytoplankton, than in higher forms such as vertebrates. In such instances there is an inverse correlation between the complexity of body structure and the direct concentration of the radioelement in question. (This generalization, of course, does not apply to accumulation of radioactivity through the food chain, as with ¹⁴C.).
- 3. Certain plants and animals have a predilection for concentrating specific radionuclides in certain tissues or organs. Iodine, for example, is concentrated in the thyroid, silicon in the frustules of diatoms, calcium in the shells of mussels, calcium and phosphorus in the bony skeletons of vertebrates, strontium in bones, and cesium in soft tissues.
- 4. Although certain radioelements may occur in acceptable concentrations for drinking water, many freshwater organisms can concentrate them to levels that might be harmful.

CONCLUSIONS

The past and present tendency to ignore or minimize the importance of aquatic microbial communities when assessing the effects of pollution is clearly irrational. Microbial communities are not haphazard aggregations of species thrown together by the whims of nature but rather structured communities with numerous interlocking cause-effect pathways. It is evident that the requirements of microbial species and communities are as complex or nearly as complex as those of taxonomically higher organisms and that disruption of these communities by pollution can affect the entire aquatic food web. We urge that all environmental impact studies of aquatic ecosystems include an evaluation of the effects of pollution upon these communities and that standards be developed to protect them as well as fish and other organisms.

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LITERATURE CITED

- ALABASTER, J. S. 1963. Effects of heated effluents on fish. Journal of Air and Water Pollution, 7(6-7): 541-563.
- AMERICAN SOCIETY FOR TESTING AND MATERIALS (ASTM). 1971. Standard method of test for evaluating inhibitory toxicity of industrial waste waters. — 1971 annual book of ASTM standards. Designation D2037-68: 552-559.
- ANDERSON, R. G. AND R. LOMMASSON. 1958. Some effects of temperature on the growth of *Chara zeylanica* Willd. — *Butler University Botanical Studies*, 13(2): 113-120.
- ANON. 1954. The destruction of algae in farm ponds and other bodies of water. — United States Department of Agriculture, Bureau of Plant Industry, Soils and Agricultural Engineering, Bulletin 77CC: 2.
- ANON. 1968. Standard Method of Test for evaluating inhibitory toxicity of Industrial Waste Waters. — American Society for Testing and Materials Standards, pt. 23: 657-665.
- ARCHIBALD, P. A. AND H. C. BOLD. 1970. Phycological Studies XI. The genus Chlorococcum Meneghini. University of Texas, Publication No. 7015: 115 pages.
- ARNON, D. I. 1958. The role of micronutrients in plant nutrition with special reference to photosynthesis and nitrogen assimilation. In: C. A. Lamb, O. Bentley, and J. Beattie (eds.); Trace elements. — Academic Press, New York, 32 pages.

- AYRES, A. H. 1916. The temperature coefficient of the duration of life of Ceramium tenuissimum. — Botanical Gazette, 62: 65-69.
- BAMFORTH, S. S. 1958. Ecological studies on the planktonic protozoa of a small artificial pond. — Limnology and Oceanography, 3(4): 398-412.
- BEER, L. P. AND W. O. PIPES. 1968. A practical approach: environmental effects of condenser water discharge in Southwest Lake Michigan. — Staff Report, Commonwealth Edison Company, 106 pages.
- BEETON, A. M. 1969. Changes in the environment and biota of the Great Lakes, pp. 150-187. *In:* Eutrophication: causes, consequences, correctives. — National Academy of Sciences, Washington, D.C.
- BENOIT, R. J., J. CAIRNS, JR., AND C. W. REIMER. 1968. A limnological reconnaissance of an impoundment receiving heavy metals, with emphasis on diatoms and fish. — Reservoir Fishery Resources Symposium, American Fisheries Society, 69-99.
- BERNARD, F. 1963. Density of flagellates and Myxophyceae in the heterotrophic layers related to environment, pp. 215-228. In: Symposium on marine Microbiology. --- C. H. Oppenheimer (ed.).
- BICK, H. 1958. Okologische Untersurchungen an ciliaten fallaubreicher kleingewasser. — Archiv für Hydrobiologie, 54: 506-542.
- BLINKS, L. R. 1952. Effects of radiation on marine algae. — Journal of Cellular and Comparative Physiology, 39(Supplement 2): 11.
- BONGERS, L. H. J. 1956. Aspects of nitrogen assimilation by cultures of green algae (Chlorella vulgaris strain A and Scenedesmus). — Mededelingen van de Landbouwhoogeschool te Wageningen, 56: 1-52.
- BOSSET, E. 1965. Indicences hygieniques de la vaccination des eaux de boisson au moyen de polyphosphates. — Monatsbulletin Schweizerischer Verein von Gas Wasserfachmannern, 45: 146-148.
- BOZNIAK, E. G. 1969. Laboratory and field studies of phytoplankton communities. Ph.D. Thesis. — Washington University, St. Louis, Missouri.
- BRINGMANN, G. AND R. KUHN. 1959a. The toxic effects of waste water on aquatic bacteria, algae, and small crustaceans. — Gesundheits-Ingenieur, 80: 115.
- . 1959b. Water toxicology studies with protozoans as test organisms. — Gesundheits-Ingenieur, 80: 239.
- BROCK, T. D. AND M. L. BROCK. 1971. The Algae of Waimanger Cauldron (New Zealand): Distribution in relation of pH. — Journal of Phycology, 6(4): 371-375.
- BROOKS, J. L. AND S. I. DODSON. 1965. Predation, body size, and composition of plankton. — Science, 150: 28-35.
- BROWN, R. M., JR. (In press). Studies of Hawaiian freshwater and soil algae. I. The atmospheric dispersal of algae and fern spores across the island of Oahu, Hawaii, 200 pp. In: Contributions in Phycology, B. C. Parker and R. M. Brown, Jr. (eds.). — The Allen Press, Lawrence, Kansas.

- BRUCKMEYER-BERKENBUSCH, H. 1955. Die beeinflussung der aurosporenbildung von Melosira nummuloides durch aussenfaktoren. — Archiv für Protistenkunde, 100: 183-211.
- BRUNGS, W. A. AND G. W. BAILEY. 1967. Influence of suspended solids on the acute toxicity of endrin to fathead minnows. Purdue University Engineering Bulletin No. 121(1): 3-12.
- BUETOS, D. E. AND K. E. SCHUIT. 1968. Phosphorus and the growth of Euglena gracilis. — Journal of Protozoology, 15(4): 770-773.
- BURLEW, J. S. 1953. Algal culture from laboratory to pilot plant. — Carnegie Institution of Washington, Publication No. 600: 357 pp.
- BURTON, J. D. 1969. Molybdenum in the Vellar Estuary, Southern India. — Journal Conseil du Conseil International pour l'exploration de la Mer, Copenhague, 33(1): 103-104.
- BUTCHER, R. W. 1947. The algae of organically enriched waters. Journal of Ecology, 35: 186-191.
- BUTZEL, H. M., JR. AND A. B. BOLTEN. 1968. The relationship of the nutritive state of the prey organism *Paramecium aurelia* to the growth and encystment of *Didinium nasutum.*— Journal of Protozoology, 15(2): 256-258.
- , L. H. BROWN, AND W. B. MARTIN, JR. 1960. Effects of detergents upon electromigration of Paramecium aurelia. — Physiological Zoology, 33: 39.
- CAIRNS, J., JR. 1956. Effects of increased temperatures on aquatic organisms. — Industrial Wastes, 1(4): 150-152.
- . 1965a. The environmental requirements of protozoa. In: Biological problems in water pollution, Third Seminar, 1962, Environmental Health Service Publication No. 999-WP-25: 48-52.
- . 1965b. The protozoa of the Conestoga Basin.
 . Notulae Naturae, Academy of Natural Sciences of Philadelphia, No. 375: 14 pp.
- . 1967. The use of quality-control techniques in the management of aquatic ecosystems. — Water Resources Bulletin, 3(4): 47-53.
- Mosquito News, 28(2): 177-179.
 - . 1968b. Suspended solids standards for the protection of aquatic organisms. Purdue University Engineering Bulletin, 129(1): 16-27.
 - -------. 1969. Rate of species diversity restoration following stress in freshwater protozoan communities. ----The University of Kansas Science Bulletin, 48(6): 209-224.
- ———. 1970. New concepts for managing aquatic life systems. — Journal of the Water Pollution Control Federation, 42(1): 77-82.
- D. W. ALBAUGH, F. BUSEY, AND M. D. CHANEY.
 1968. The sequential comparison index a simplified method for non-biologists to estimate relative differences in biological diversity in stream pollution studies.
 Journal of the Water Pollution Control Federation, 40(9): 1607-1613.

——, M. L. DAHLBERG, K. L. DICKSON, N. SMITH, AND W. T. WALLER. 1969. The relationship of freshwater protozoan communities to the MacArthur-Wilson Equilibrium Model. — *American Naturalist*, 103(933): 439-454.

- AND K. L. DICKSON. 1970. Reduction and restoration of the number of fresh-water protozoan species following acute exposure to copper and zinc. — *Transactions of the Kansas Academy of Sciences*, 73(1): 1-10.
- ------, AND K. L. DICKSON. 1971. A simple method for the biological assessment of the effects of waste discharges on aquatic bottom dwelling organisms. ---Journal of the Water Pollution Control Federation, 43(5): 755-772.
- , KENNETH L. DICKSON, G. R. LANZA, S. P. ALMEIDA, AND D. DEL BALZO. 1972. Coherent optical spatial filtering of diatoms in water pollution monitoring. Archiv für Mikrobiology, 82: 141-146.
- , K. L. DICKSON, R. E. SPARKS, AND W. T. WALLER. 1970. A preliminary report on rapid biological information systems for water pollution control. Journal of the Water Pollution Control Federation, 42(5): 685-703.
 - ——, K. L. DICKSON, AND W. H. YONGUE. 1971. The consequences of non-selective periodic removal of portions of freshwater protozoan communities.— *Transactions of the American Microscopical Society*, 90(1): 71-80.
 - ——, AND GUY R. LANZA. 1972. Effects of heated waste water upon some microorganisms. — Water Resources Research Center, Virginia Polytechnic Institute and State University Bulletin No. 48: 101 p.
- AND J. RUTHVEN. 1970. Artificial microhabitat size and the number of colonizing species. — *Transactions of the American Microscopical Society*, 89(1): 100-109.
- AND A. SCHEIER. 1959. The relationship of bluegill sunfish body size to tolerance for some common chemicals. — Proceedings of the 13th Industrial Waste Conference, Engineering Bulletin, Purdue University, No. 96: 243-252.
- ——, A. SCHEIER, AND N. E. HESS. 1963. The effects of alkyl benzene sulfonate on aquatic organisms. — Proceedings of the Fourth Annual Symposium on Industrial Waste Control. The Johns Hopkins University and the State of Maryland: 43-54.
- ———, A. SCHEIER, AND J. J. LOOS. 1965. A comparison of the sensitivity to certain chemicals of adult zebra danios, *Brachydanio rerio* (Hamilton-Buchanan) and zebra danio eggs with that of adult bluegill sunfish, *Lepomis* macrochirus Raf. — *Notulae Naturae*, *Academy of Natural Sciences of Philadelphia*, No. 381: 9 p.
- , W. H. YONGUE, JR., AND H. BOATIN, JR. (In Press.) The protozoan colonization of artificial substrates anchored in the benthos. — *Transactions of the American Microscopical Society*.

- CHOLNOKY, B. J. 1968. Die okologie der diatomeen in binnengewassern. — J. Cramer, Germany. 699 p.
- CHU, S. P. 1942. The influence of the mineral composition of the medium on the growth of planktonic algae. I. Methods and culture media. — Journal of Ecology, 31: 109-148.
- . 1943. The influence of the mineral composition of the medium on the growth of planktonic algae. II. The influence of the concentration of inorganic nitrogen and phosphate phosphorus. Journal of Ecology, 31: 109-138.
- CLENDENNING, K. A. AND W. J. NORTH. 1958. The effects of discharged waste on kelp. — Quarterly Progress Report Institute of Marine Resources, University of California Institute, La Jolla, IMR Ref. 68-6.
- AND W. J. NORTH. 1960. Effects of wastes upon the giant kelp, *Macrocystis pyrifera*, p. 82. *In*: Proceedings of the 1st International Conference on Waste Disposal in the Marine Environment. — Pergamon Press, New York.
- COLLIER, A. 1953. The significance of organic compounds in sea water. — North American Wildlife Conference Transactions, 18: 463-470.
- CRANCE, J. H. 1963. The effects of copper sulphate on Microcystis and zooplankton in ponds.— Progressive Fish Culturist, 25(4): 198-202.
- CUSHING, C. E. AND D. G. WATSON. 1968. Accumulation of ³²P and ⁶⁵Zn by living and killed plankton. — *Oikos*, 19: 143-149.
- DALLINGER, W. H. 1887. "The President's address". Journal of the Royal Microscopical Society, Ser. 2, 7: 185-199.
- DAVIS, C. C. 1964. Evidence for the eutrophication of Lake Erie from phytoplankton records. — *Limnology* and Oceanography, 9(3): 275-283.
- DINGLE, A. D. 1967. Evidence for a temperature-sensitive control over flagellum development in transforming cells of Naegleria gruberi. — Journal of Protozoology, 14(Supplement): 12.
- DIXON, M. AND E. C. WEBB. 1958. Enzymes. Longman, Green and Co. Ltd., London. 782 p.
- DONALDSON, L. R. AND R. F. FOSTER. 1957. Effects of radiation on aquatic organisms. — National Academy of Science, National Research Council, Publication 551: 96.
- EDMONDSON, W. T. 1969. Eutrophication in North America, pp. 124-149. In: Eutrophication, causes, consequences, correctives. — National Academy of Sciences, Washington, D. C.
- EYSTER, C. 1964. Micronutrient requirements for green plants, especially the algae. *In:* Algae and Man, D. F. Jackson (ed.). — Plenum Press, New York, 86-119.
- FITZGERALD, G. P., G. C. GERLOFF, AND F. S. BORG. 1952. Studies on chemicals with selective toxicity to bluegreen algae. — Sewage and Industrial Wastes, 24(7): 888-897.
- Fogg, G. E. 1949. Growth and heterocyst production in Anabaena cylindrica Lemm. II. In relation to carbon

and nitrogen metabolism. — Annals of Botany (London), 13: 241-260.

——. 1966. Algal cultures and phytoplankton ecology. — University of Wisconsin Press, Madison. 126 p.

- AND D. F. WESTLAKE. 1953. The importance of extracellular products of algae in fresh water. — *Verhandlungen Internationale Vereinigung Limnologie*, 12: 219-232.
- AND M. WOLFE. 1954. The nitrogen metabolism of the blue-green algae (Myxophyceae). — Symposium of the Society for General Microbiology, No. 4: 99-125.
- GAUFIN, A. R. AND D. B. MCDONALD. 1965. Factors influencing algal productivity in Deer Creek Reservoir, Utah. — Transactions of the American Microscopical Society, 84(2): 269-279.
- GERLOFF, G. C. AND K. A. FISHBECK. 1969. Quantitative cation requirements of several green and blue-green algae. Journal of Phycology, 5(2): 109-114.
- AND F. SKOOG. 1957a. Availability of iron and manganese in southern Wisconsin lakes for the growth of *Microcystis aeruginosa.* — *Ecology*, 38: 556-561.
 - AND F. SKOOG. 1957b. Nitrogen as a limiting factor for the growth of *Microcystis aeruginosa* in southern Wisconsin lakes. — *Ecology*, 38: 556-561.
- GIESE, A. C. 1963. Regeneration rate of *Blepharisma* with special reference to the effect of temperature. — *Journal of Protozoology*, 10(2): 173-182.
- GOLDBERG, E. D., T. J. WALKER, AND A. WHISENAND. 1951. Phosphate utilization by diatoms. — Biological Bulletin, 101(3): 274-284.
- GOODMAN, G. T. AND T. M. ROBERTS. 1971. Plants and soils as indicators of metals in the air. — *Nature*, 231 (5301): 287-292.
- GRAN, H. H. 1933. Studies on the biology and chemistry of the Gulf of Maine. II. Distribution of the phytoplankton in August, 1932. — *Biological Bulletin*, 64: 159-181.
- GROSS, J. A. 1962. Cellular responses to thermal and photo stress. II. Chlorotic Euglenoids and Tetrahymena. — Journal of Protozoology, 9(4): 415-418.
- AND T. L. JOHN. 1962. Cellular responses to thermal and photo stress. Euglena and Chlamydomonas. — Journal of Protozoology, 9(3): 340-346.
- GUILLARD, R. R. L. AND V. CASSIE. 1963. Minimum cyanocobalamin requirements of some marine centric diatoms. Limnology and Oceanography, 8: 161-165.
 AND J. H. RYTHER. 1962. Studies of marine planktonic diatoms. I. Cyclotella nana Hustedt, and Detonula confervacea (Cleve) Gran. Canadian Journal of Microbiology, 8: 229-239.
- HAIRSTON, N. G. 1967. Studies on the limitation of a natural population of *Paramecium aurelia*. — *Ecology*, 48: 904-910.

- AND S. L. KELLERMAN. 1965. Competition between varieties 2 and 3 of *Paramecium aurelia*; The influence of temperature in a food-limited system. — *Ecology*, 46(1,2): 134-139.
- HALL, R. P. 1965. Protozoan Nutrition. Blaisdell Publishing Company, New York. 91 p.
- HASHIMOTO, K. 1962. Relationships between feeding organelles and encystment in Oxytricha fallax Stein. — Journal of Protozoology, 9(2): 161-169.
- HASLER, A. D. 1947. Eutrophication of lakes by domestic drainage. *Ecology*, 28: 383-395.
- HEILBRUNN, L. V. 1954. Heat death. Scientific American, 190: 70-75.
- HEMPHILL, F. E., M. L. KAEBERLE, AND W. B. BUCK. 1971. Lead suppression of mouse resistance to Salmonella typhimurium. — Science, 172: 1031-1032.
- HERVEY, R. K. 1949. Effect of chromium on the growth of unicellular chlorophyceae and diatoms. — Botanic Gazette, 111(1): 1-11.
- HOFFMAN, C. H. AND A. T. DROOZ. 1953. Effect of a C-47 application of DDT on fish food organisms in two Pennsylvania watersheds. — American Midland Naturalist, 50(1): 172-188.
- HOHN, M. H. 1956. The diatometer a continuous record method for indicating water quality. — Western Petroleum Refineries Association, PC 56-6. 5p.
- . 1966. Analysis of plankton ingested by Stizostedium vitreum vitreum (Mitchell) fry and concurrent vertical plankton tows from southwestern Lake Erie, May, 1961 and May, 1962. — Ohio Journal of Science, 66(2): 193-197.
- HOPKINS, J. T. 1963. A study of the diatoms of the Ouse Estuary, Sussex. I. The movement of the mud-flat diatoms in response to some chemical and physical changes. — Journal of the Marine Biological Association of the United Kingdom, 43(3): 653-663.
- HRBACEK, J. 1962. Species composition and the amount of zooplankton in relation to the fish stock. — Rozpravy Ceskoslovenske Akademie Ved Rada Matematickych A Prirodnich Ved., 72(10): 1-116.
- M. DVORAKOVA, V. KORINEK, AND L. PROCHAZ-KOVA. 1961. Demonstration of the effect of the fish stock on the species composition of zooplankton and the intensity of metabolism of the whole plankton association. — Verhandlungen Internationale Vereinigung Limnologie, 14: 192-195.
- HUTCHINSON, G. E. 1957. Concluding remarks Cold Spring Harbor Symposium. — Cold Spring Harbor Symposium, Quantative Biology, 22: 415-427.
- John Wiley and Sons, Inc., New York. 1115 p.
- HUTNER, S. H., S. BAKER, S. AARONSON, H. A. NATHAN, E. RODRIGUEZ, S. LOCKWOOD, M. SANDERS, AND R. A. PETERSON. 1957. Growing Ochromonas malhamensis above 35°C. — Journal of Protozoology, 4: 259-269.
- HYNES, H. B. N. 1960. The biology of polluted waters. Liverpool University Press, Liverpool. 202 p.
- INMAN, O. L. 1940. Studies on the chlorophylls and the

photosynthesis of thermal algae from Yellowstone Park, California and Nevada. — Journal of General Physiology, 23: 661-666.

- JACKSON, D. F. 1969. Primary productivity studies in Ononataga Lake, New York. — Verhandlungen Internationale Vereinigung Limnologie, 17: 86-94.
- JAMES, T. W. AND C. P. READ. 1957. The effect of incubation temperature on the cell size of *Tetrahymena pyri*formis. — Experimental Cellular Research, 13(3): 510-516.
- JOENSUU, O. I. 1971. Fossil fuels as a source of mercury pollution. Science, 172: 1027-1028.
- KASTURI BAI, A. R., R. SRIHARI, M. SHADAKSHARAS-WAMY, AND P. S. JYOTHY. 1969. The effects of temperature on Blepharisma intermedium. — Journal of Protozoology, 16(4): 738-743.
- KETCHUM, B. H. 1939. The development and restoration of deficiencies in the phosphorus and nitrogen composition of unicellular plants. — Journal of Cellular and Comparative Physiology, 13: 373-381.
- . 1954. Mineral nutrition of phytoplankton. — Annual Review of Plant Physiology, 5: 55-74.
- AND A. C. REDFIELD. 1949. Some physical and chemical characteristics of algae grown in mass culture. — Journal of Cellular and Comparative Physiology, 33: 281.
- KEVERN, N. R. AND R. C. BALL. 1965. Primary productivity and energy relationships in artificial streams. — Limnology and Oceanography, 10: 74-87.
- KING, D. L. 1970. The role of carbon in eutrophication. — Journal of the Water Pollution Control Federation, 42(12): 2035-2051.
 - AND R. C. BALL. 1964. The influence of highway construction on a stream. — Research Report No. 19, Michigan State University Agriculture Experiment Station: 4 p.
- KOLFLAT, T. 1968. Thermal pollution-1968. Hearings before the subcommittee on air and water pollution of the committee on public works, United States Senate, Ninetieth Congress, Second Session, February, 1968. Washington, D. C.: U. S. Government Printing Office: 63.
- KOLKWITZ, R. AND M. MARSSON. 1908. Okologie der pflanzlichen Saprobien. — Berichte der Deutschen Botanischen Gesselschaft, 26: 509-519.
- KREZOSKI, J. R. 1969. Benton Harbor power plant limnological studies. Part III. Some effects of power plant waste heat discharge on the ecology of Lake Michigan. — Special Report No. 44 of the Great Lakes Research Division, The University of Michigan, Ann Arbor. 47 p.
- KRUMHOLZ, L. A. AND R. F. FOSTER. 1957. Accumulation and retention of radio-activity from fission products and other radiomaterials by fresh-water organisms. — National Academy of Science, National Research Council Publication 551: 88.
- KULLBERG, R. G. 1969. Algal diversity in several spring effluents. *Ecology*, 49(4): 751-755.

- LACKEY, J. B. 1944. Stream microbiology, pp. 227-263. In: E. B. Phelps, Stream Sanitation. — Wiley, New York.
- . 1949. Plankton and nuisance conditions in surface waters, pp. 55-63. In: F. R. Moulton and Florence Hitzel, (eds.); Limnological Aspects of Water Supply and Waste Disposal. — American Association for the Advancement of Science, Washington, D. C.
- LANZA, G. R., J. CAIRNS, JR., AND K. L. DICKSON. 1970. The use of a simple staining technique on diatom populations as a possible indicator of thermal pollution (Abstract). — ASB (Association of Southeastern Biologists) Bulletin, 17(2): 52.
- LEGGE, R. F. AND D. DINGELDEIN. 1970. We hung phosphates without a fair trial. — Canadian Research and Development, March 1970, Maclean-Hunter Ltd., Ontario.
- LEVY, M. R. AND A. M. ELLIOTT. 1968. Biochemical and ultrastructural changes in *Tetrahymena pyriformis* during starvation. — *Journal of Protozoology*, 15(1): 208-222.
- LINDEMAN, R. L. 1942. The trophic-dynamic aspect of ecology. *Ecology*, 23(4): 399-417.
- LIVINGSTON, D. A. 1963. Data of geochemistry, Chapter G: chemical composition of rivers and lakes. — United States Geological Survey Professional Paper 440G, United States Department of Interior, Washington, D.C. 64 p.
- LUDWIG, C. A. 1938. The availability of different forms of nitrogen to a green alga (*Chlorella*). — *American Journal of Botany*, 25(6): 448-458.
- LUND, J. W. G. 1950. Studies on Asterionella formosa Hassall. II. Nutrient depletion and the spring minimum. — Journal of Ecology, 38: 1-14, 15-35.
- F. J. H. MACKERETH, AND C. H. MORTIMER. 1963. Changes in depth and time of certain chemical and physical conditions and of the standing crop of Asterionella formosa Hassall in the North basin of Windmere in 1947. — Philosophical Transactions of the Royal Society, Ser. B, 246: 255-290.
- MACKERETH, F. J. 1953. Phosphorus utilization by Asterionella formosa Hassall. — Journal of Experimental Botany, 4: 296-313.
- MALONEY, T. E. AND C. M. PALMER. 1956. Toxicity of six chemical compounds to 30 cultures of algae. — *Water and Sewage Works*, 103: 509-513.
- MARGALEF, R. 1954. Modifications induced by different temperatures on the cells of *Scenedesmus obiquus* (Chlorophyceae). — *Hydrobiologia*, 5(1-2): 83-94.
- MARRE, E. 1962. Temperature, pp. 541-550. In: Physiology and biochemistry of algae, R. A. Lewin (ed.). Academic Press, New York.
- MCCARTHY, L. T., JR. AND W. B. KEIGHTON. 1964. Quality of Delaware River water at Trenton, New Jersey. Geological Survey Water-Supply Paper 1779-X. — United States Government Printing Office, Washington, D.C.: 36-37.
- MCKEE, J. E. AND H. W. WOLF. 1963a. Potential pol-

lutants, pp. 123-298. *In:* Water quality criteria, J. E. McKee and H. W. Wolf (eds.). — The Resources Agency of California. State Water Quality Control Board, Publication 3-A.

- AND H. W. WOLF. 1963b. Radioactivity, pp. 343-354. In: Water quality criteria, J. E. McKee and H. W. Wolf (eds.). — The Resources Agency of California. State Water Quality Control Board, Publication 3-A.
- MENZEL, D. W., J. A. ANDERSON, AND A. RANDTKE. 1970. Marine phytoplankton vary in their response to chlorinated hydrocarbons. — Science, 167: 1724-1727.
 - AND J. H. RYTHER. 1961. Nutrients limiting the production of phytoplankton in the Sargasso Sea off Bermuda. — *Deep-Sea Research*, 7(4): 276-281.
- AND J. P. SPAETH. 1962. Occurrence of vitamin B₁₂ in the Sargasso Sea. — *Limnology and Oceanography*, 6: 151-154.
- MEYER, B. S. AND D. B. ANDERSON. 1952. Plant Physiology. Second Edition. — D. Van Nostrand Co., Inc., New York. 784 p.
- MOUNT, DONALD I. 1969. Developing thermal requirements for freshwater fishes, pp. 140-147. *In:* Biological Aspects of Thermal Pollution, P. A. Krenkel and F. L. Parker (eds.). — Vanderbilt University Press.
- MULLER, W. A. AND J. J. LEE. 1969. Apparent indispensability of bacteria in foraminiferan nutrition. — Journal of Protozoology, 16(3): 471-478.
- NALEWAJKO, C. 1966. Composition of phytoplankton in surface waters of Lake Ontario. — Journal of the Fisheries Research Board of Canada, 23: 1715-1725.
- NIKITINSKY, J. AND F. K. MUDREZOWA-WYSS. 1930. Uber die wirkung der kohlensaure, des schwefelwasserstoffs, des methans und der abwesenheit des sauerstoffs auf wasserorganismen. — Centralblat fur Bakteriologie, Abt. 2, 82: 167-198.
- NOACK, D. AND A. PIRSON. 1939. Die Wirkung von Eisen und Mangan auf die Stickstoff assimilation von Chlorella. — Berliner deutsches botanischer Gesellschaft, 57: 442-452.
- NOLAND, L. E. AND M. GOJDICS. 1967. Ecology of freeliving protozoa, pp. 215-266. In: Research in Protozoology, T. Chen (ed.). — Permagon Press, New York.
 — AND K. A. CLENDENNING. 1958. The effects of waste discharges on kelp. — Annals Progress Report, Institute of Marine Resources, University of California, La Jolla, IMR Ref. 58-111.
 - AND K. A. CLENDENNING. 1959. The effects of discharged wastes on kelp. — Annual Report, Institute of Marine Resources, University of California, La Jolla,
 - ——, K. A. CLENDENNING, AND H. L. SCOTTEN. 1959. The effects of waste discharges upon kelp. — Quarterly Progress Report, Institute of Marine Resources, University of California, La Jolla, IMR Reference 60-110.
- O'KELLY, J. C. AND W. R. HERNDON. 1959. Effect of strontium replacement for calcium on production of motile cells in Protosiphon. — Science, 130: 718.

- PALMER, C. M. AND T. E. MALONEY. 1955. Preliminary screening for potential algicides. — Ohio Journal of Science, 55: 1.
- PARKER, B. C. 1957. The ecology of Lake Zoar. M. A. Thesis, Yale University. 135 p.
- . 1967. Influence of method for removal of seston on the dissolved organic matter. — Journal of Phycology, 3: 166-173.

- . 1971. The case for conservation in Antarctica. — Antarctic Journal of the United States, VI (3): 50-53.
- AND J. BLECK. 1956. A contribution to the ecology of Pelagophycus. Annals of the Missouri Botanic Garden, 53: 1-16.
- E. K. OBENG-ASAMOA, AND G. L. SAMSEL, JR. 1971. Effects of detergent protease enzymes on sewage oxidation pond phytoplankton. — *Bioscience*, 21: 1035-1038 and 1042.
- , G. SAMSEL, AND G. W. PRESCOTT. (in press). Freshwater algae of the Antarctica Peninsula. I. Systematics and Ecology in United States Palmer Station Area. — Antarctic Research Series, National Academy of Sciences, Washington, D.C.
- AND M. WACHTEL. 1971. Seasonal distribution of cobalamins, biotin, and niacin in rainwater, pp. 195-207. *In:* The structure and function of fresh-water microbial communities, J. Cairns, Jr. (ed.). —
- AND E. B. WODEHOUSE. 1970. Ecology and water quality criteria. — 15th Water for Texas Conference, 15:
- PARSONS, T. R. AND J. D. H. STRICKLAND. 1962. On the production of particulate organic carbon by heterotrophic processes in sea water. — Deep-Sea Research, 8: 211-222.
- PASCHER, A. AND E. LEMMERMANN. 1914. Flagellaten I. Pantostomatinae, Protomastiginae, Distomatinae. In: Pascher, Die Susswasserflora Deutschlands, Osterreichs und der Schweiz. — Heft 1-Jessa: Gustav Fischer. 138 p.
- PATRICK, R. 1949. A proposed biological measure of stream conditions, based on a survey of The Conestoga Basin, Lancaster County, Pennsylvania. — Proceedings of the Academy of Natural Sciences of Philadelphia, 101: 277-341.
- . 1961. A study of the number and kinds of species found in rivers in the eastern United States. —

Proceedings of the Academy of Natural Sciences of Philadelphia, 113(10): 215-258.

------. 1966. The effect of varying amounts and ratios of nitrate and phosphate on algae blooms. ---Purdue University Engineering Bulletin, No. 121 (1): 41-51.

———. 1967. The effect of invasion rate, species pool, and size of an area on the structure of the diatom community. — *Proceedings of the National Academy of Sciences*, 58(4): 1335-1342.

. 1968a. The structure of diatom communities in similar ecological conditions. — American Naturalist, 102(924): 173-183.

. 1968b. In: Water Quality Criteria. Report of the National Technical Advisory Committee to the Secretary of the Interior. Section III. Fish, other aquatic life, wildlife. 234 p.

, J. CAIRNS, JR., AND S. S. ROBACK. 1967. An ecosystematic study of the fauna and flora of the Savannah River. — *Proceedings of the Academy of Natural Sciences of Philadelphia*, 118(5): 109-407.

, J. CAIRNS, JR., AND A. SCHEIER. 1968. The relative sensitivity of diatoms, snails, and fish to twenty common constituents of industrial wastes. — *Progressive Fish-Culturist*, 30(3): 137-140.

....., B. CRUM AND J. COLES. 1969. Temperature and manganese as determining factors in the presence of diatom or blue-green algal floras in streams..... *Proceedings of the National Academy of Sciences*, 64(2): 472-478.

-----, M. H. HOHN, AND J. H. WALLACE. 1954. A new method for determining the pattern of the diatom flora. — Notulae Naturae, Academy of Natural Sciences of Philadelphia, No. 259: 12 p.

, N. A. ROBERTS, AND B. DAVIS. 1968. The effect of changes in pH on the structure of diatom communities. — Notulae Naturae, Academy of Natural Sciences of Philadelphia, No. 416: 16 p.

AND D. STRAWBRIDGE. 1963. Methods of studying diatom populations. — Journal of Water Pollution Control Federation, 35(2): 151-16.

- PEARSALL, W. H. 1932. Phytoplankton in the English lakes II. The composition of the phytoplankton in relation to dissolved substances. — Journal of Ecology, 20: 241, 262.
- PEARSON, E. A., R. D. POMEROY, AND J. E. MCKEE. 1960. Summary of marine waste disposal research program in California. — State Water Pollution Control Board Publication No. 22:
- PENNINGTON, W. 1941. The control of the numbers of fresh-water phytoplankton by small invertebrate animals. — Journal of Ecology, 29(2): 204-211.
- PHELPS, A. 1961. Studies on factors influencing heat survival of a ciliate, a mite, and an ostracod obtained from a thermal stream. — American Zoologist, 1: 467.
- PICKEN, L. E. R. 1937. The structure of some protozoan communities. Journal of Ecology, 25(2): 324-368.
- PINERVICH, V. V., N. N. VERSILIN, AND Y. I. MASLOV. 1961. Effect of different forms of nitrogen on the

growth and the accumulation of biomass by Chlorella pyrenoidosa. — Vestnik Leningrad, University, No. 9(2): 16-25.

- PRATT, R. AND J. FONG. 1940. Studies on Chlorella vulgaris. III. Growth of Chlorella and changes in the hydrogen-ion and ammonium-ion concentrations in solution containing nitrate and ammonium nitrogen. — American Journal of Botany, 27: 735-743.
- PROCTOR, V. W. 1957. Preferential assimilation of nitrate ion by Haematococcus pluvialis. — Ecology, 38: 457-462.
- PROVASOLI, LUIGI. 1969. Algal nutrition and eutrophication, pp. 574-593. In: Eutrophication: Causes, Consequences, Correctives. — National Academy of Sciences, Washington, D.C.
- REID, G. K. 1961. Ecology of inland waters and estuaries. — Van Nostrand Reinhold Co., New York. 375 p.
- REID, R. 1969. Fluctuations in populations of 3 Vorticella species from an activated sludge sewerage plant. — Journal of Protozoology, 16(1): 103-111.
- RICE, T. R. 1953. Phosphorus exchange in marine phytoplankton. — Fish and Wildlife Service, Fishery Bulletin, No. 80: 77-89.
- ROBBINS, W. J., A. HERVEY, AND M. E. STEVENS. 1950. Studies on Euglena and vitamin B₁₂.—Bulletin of the Torrey Botanic Club, 77: 423-441.
- RODHE, W. 1948. Environmental requirements of freshwater plankton algae. Experimental studies in the ecology of phytoplankton. — Symbollas botanica upsaliensis, 10(1): 1-149.
- ROSENBAUM, N., J. ERWIN, D. BEACH, AND G. G. HOLZ, JR. 1966. The induction of a phospholipid requirement and morphological abnormalities in *Tetrahymena pyriformis* by growth at supraoptimal temperatures. — *Journal of Protozoology*, 13(4): 535-546.
- ROUND, F. E. 1965. The Biology of the Algae. Edward Arnold Ltd., London. 269 p.
- RYLOV, V. W. 1923. Über den Einfluss des im wasser gelosten sauerstoffs und schwefelwasser-stoffs auf den lebenszyklus und die vertkkale verteilung des infusors Loxodes rostrum. — International Revue der Gesamten Hydrobiologie, 11: 179-192.
- SALT, G. W. 1968. The feeding of Amoeba proteus on Paramecium aurelia. — Journal of Protozoology, 15(2): 275-280.
- SAMEJIMA, H. AND J. MYERS. 1958. On the heterotrophic growth of Chlorella pyrenoidosa. — Journal of General Microbiology, 18: 107-117.
- SAMSEL, G. L. AND B. C. PARKER. (in press). Nutrient factors limiting primary productivity in simulated antarctic microecosystems. — Virginia Academy of Science,
- SAWYER, C. N., J. B. LACKEY, AND A. T. LENZ. 1943. Investigation of the odor nuisances in the Madison Lakes, particularly Lakes Monona, Waubesa, and Kegonsa from July 1942 to July 1943. — Report to

Governor's Committee, Madison, Wisconsin. 79 p.

- SCHLICHTING, H. E., JR. 1969. The importance of airborne algae and protozoa. — Air Pollution Control Association Journal, 19: 946-951.
- SCHOFIELD, E. (in press). Preserving the scientific value of cold desert ecosystems: Past and present practices and a rationale for the future. *In*: Proceedings of the Colloquium on Conservation Problems in Antarctica, B. C. Parker (ed.). — The Allen Press, Lawrence, Kansas.
- SCHULER, J. F., V. M. DILLER, AND H. J. KERSLEN. 1953. Preferential assimilation of ammonium ion by Chlorella vulgaris. — Plant Physiology, 28: 299-303.
- SMITH, A. J., J. LONDON, AND R. Y. STANIER. 1967. Biochemical basis of obligate autotrophy in blue-green algae and thiobacilli. — Journal of Bacteriology, 94: 972-983.
- SMITH, R. L. 1971. Nutritional attributes as taxonomic tools in phycology, pp. 31-46. In: Contributions in Phycology, B. C. Parker and R. M. Brown (eds.). — The Allen Press, Lawrence, Kansas.
- SPOEHR, H. A. AND H. W. MILNER. 1949. The chemical composition of *Chlorella*, effect environmental conditions. *Plant Physiology*, 24: 120-149.
- STEWART, W. D. P. 1969. Nitrogen input into aquatic ecosystems, pp. 53-72. In: Algae, Man, and the Environment, Daniel F. Jackson (ed.). — Syracuse University Press, New York.
- SUHAMA, M. 1961. Experimental studies on the morphogenesis of Condylostoma spatiosum Ozaki and Yagiu. — Journal of Science, Hiroshima University, (B) 20: 33-91.
- SURBER, E. W. AND H. L. COOLEY. 1952. Bottom fauna studies of Green Bay, Wisconsin, in relation to pollution. — United States Public Health Service and Wisconsin Commission on Water Pollution. 7 p.
- SWEENEY, B. G. 1951. Culture of the marine dinoflagellate Gymnodinium with soil extract. — American Journal of Botany, 38: 669-677.
- SYRETT, P. J. 1962. Nitrogen assimilation, pp. 161-170. In: Physiology and biochemistry of algae, R. A. Lewin (ed.). — Academic Press, New York.
- ———. 1962. Nitrogen assimilation, pp. 171-188. In: Physiology and biochemistry of algae, R. A. Lewin (ed.). — Academic Press, New York.
- TARTAR, V. 1956. Reactions of Stentor coeruleus to certain substances added to the medium. — Experimental Cell Research, 13: 317-332.
- THOMAR, H. 1959. Delayed division in Tetrahymena pyriformis induced by temperature changes. — Comptes Rendues Travailles Laboratories Carlsberg, 32: 207-225.
 - . 1962. Cell size of *Tetrahymena pyriformis* incubated at various temperatures. — *Experimental Cell Research*, 27: 585-586.
- THOMAS, E. A. 1969. The process of eutrophication in Central European lakes, pp. 29-49. In: Eutrophication; Causes, Consequences, Correctives. — National Academy of Sciences, Washington, D. C.

- TOMOVA, N. G., Z. G. EVSTIGNEYEVA, AND W. L. KRETOVICH. 1964. Assimilation of nitrate and ammonium nitrogen by Chlorella pyrenoidosa. — Physiologia Rasteniy, 11 (6): 988-997.
- TRAINOR, F. R. 1959. A comparative study of sexual reproduction in four species of Chlamydomonas. — American Journal of Botany, 46: 65-70.
- ——, H. R. ROWLAND, J. C. LYLIS, P. A. WINTER, AND P. L. BANANOMI. 1971. Some examples of polymorphism in algae. — *Phycologia*, 10: 113-119.
- TREMBLEY, F. J. 1960. Research project on effects of condenser discharge water on aquatic life. Progress Report 1956 to 1959. — Lehigh University Institute of Research, Bethlehem, Pa., Water Pollution Abstract, 34(11): 2157.
- TUCKER, J. B. 1967. Abnormal development of a microtubular organelle induced by heat treatment of the ciliate Nassula. (Abstract). — Journal of Protozoology, 14 (supplement): 28.
- VANCE, B. D. AND W. DRUMMOND. 1969. Biological concentration of pesticides by algae. — Journal of the American Water Works Association, 61: 360-362.
- VAN OORSCHOT, J. L. P. 1955. Conversion of light energy in algal culture. — Mededelingen van de Landbouwhogeschool Te Wageningen, 55: 225-276.
- VAUGHN, J. C. 1961. Coagulation difficulties at the South District filtration plant. — Pure Water, 13: 45-49.
- VERDIUN, J. 1964. Changes in western Lake Erie during the period 1948-1962. — Verhandlungen Internationale Vereinigung Limnologie, 15: 639-644.
- VRHAN, O. 1932. Bietrage zur Kenntis der Stickstoffossimilation von Chlorella und Scenedesmus. — Jahrbuch für wissenschaftliche Botanica, 75: 1-44.
- WALLACE, N. M. 1955. The effect of temperature on the growth of some freshwater diatoms. — Notulae Naturae, Academy of Natural Sciences of Philadelphia, No. 280: 11 p.
- WARINNER, J. E. AND M. L. BREHMER. 1966. The effects of thermal effluents on marine organisms. — Air and Water Pollution International Journal, 10: 277-289.
- WARREN, K. S. 1962. Ammonia toxicity and pH. Nature, 195(4836): 47-49.
- AND S. SCHENKER. 1960. Hypoxia and ammonia toxicity. — American Journal of Physiology, 199(6): 1105-1108.
- WILLIAMS, O. B., C. R. GRONINGER, AND N. F. ALBRITTON. 1952. The algicidal effect of certain quaternary ammonium compounds. — *Producers Monthly*, 16(8): 14.
- WILLIAMS, L. G. AND D. I. MOUNT. 1965. Influence of zinc on periphytic communities. — American Journal of Botany, 52(1): 26-34.
- WILLMER, E. N. 1956. Factors which influence the acquisition of flagella by the amoeba, Naegleria gruberi. — Journal of Experimental Biology, 33: 583-603.
- WRIGHT, R. T. AND J. E. HOBBIE. 1966. Use of glucose and acetate by bacteria and algae in aquatic ecosystems. — Ecology, 47(3): 447-464.
- WURSTER, C. F. 1968. DDT reduces photosynthesis by marine phytoplankton. — Science, 159: 1474-1475.

- WURTZ-ARLET, J. 1961. Effect of certain surface active synthetic compounds on the growth of a protococcales. — Journal of Water Pollution Control Federation, 33: 687.
- YONGUE, W. H., JR. AND J. CAIRNS, JR. 1971. Micro-habitat pH differences from those of the surrounding water. — Hydrobiologia, 38(3-4): 453-461.

AND J. CAIRNS, JR. 1971. Colonization and succession of freshwater protozoans in artificial substrates suspended in a small pond in North Carolina. — Notulae Naturae, Academy of Natural Sciences of Philadelphia, No. 443: 13 p.

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