THE EFFECTS OF DOMESTIC SEWAGE EFFLUENT AND NUTRIENTS ON PRODUCTIVITY IN MICROECOSYSTEMS

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PREFACE

The objectives of this experiment were to measure variation in changes in species diversity, net production, respiration, chlorophyll a, chlorophyll b, chlorophyll c, astacin pigments, dry weight, ash-free weight, Margalef's ratio, among laboratory microecosystems receiving different levels of domestic sewage.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

CHAPTER I

INTRODUCTION

The ecosystem is a natural unit with both abiotic and biotic components interacting to produce an exchange of materials (Odum, 1959). The ecosystem shows a high degree of interrelationships between its parts, it displays homeostasis by maintaining a constant flow of materials, and it reestablishes itself after a disturbance unless the noise is too great (Bray, 1958). It has both structure and function. Biotic and abiotic components make up the structure. The function of the ecosystem is measured by rate processes such as gross production and respiration. Domestic sewage contains excessive amounts of nutrients such as phosphates, nitrates, and associated nitrogen compounds which change the structure and function of the ecosystem.

Both organic and inorganic nitrogen is discharged by domestic sewage treatment plants. Nitrogen compounds are produced primarily by the decomposition of organic wastes. Talling (1965) reported algal blooms in Lake Victoria with nitrate nitrogen rarely above 10 $\mu g\ \ell^{-1}$. Sawyer (1947) concluded that a 0.30 mg ℓ^{-1} concentration of inorganic nitrogen could cause algal blooms. If the concentration of nitrate nitrogen is kept below about 0.3 mg ℓ^{-1} and the concentration of total nitrogen does not exceed 0.6 mg ℓ^{-1}, algal blooms can be avoided in polluted areas (Mueller, 1953). However, the total nitrogen in conventional effluent treatment plants exceeds 0.6 mg ℓ^{-1}. Nitrate is the form of nitrogen

usually measured. Strickland et al. (1969) found that ammonia was assimilated before nitrate in cultures of diatoms and dinoflagellates. This indicates that all forms of inorganic nitrogen should be measured.

Phosphates in domestic sewage effluents are mostly from biodegradable detergents. The optimum range of phosphorus concentration was from 0.009 to 17.8 mg ℓ^{-1} for many algal cultures (Chu, 1943). Machenthun (1969) suggested that algal blooms can be prevented if total phosphorus does not exceed 100 μg ℓ^{-1} at any point within streams and 50 μg ℓ^{-1} where waters enter a lake, reservoir, or another body of water. The concentration of domestic sewage effluent exceeds these limits. Phosphates are released from detergents by bacteria (Tilton, 1970). Bacteria control the aquatic cycle of phosphates to a great extent. Rigler (1956) stated that up to 95% of phosphorus added may be removed within a few hours by bacteria.

Allen (1955) found that 1 to 2 g ℓ^{-1} of dry weight of algae can be grown in domestic sewage cultures. Outdoor oxidation ponds were found to yield 0.5 g ℓ^{-1} maximum (Allen, 1955).

Plant pigments may be used to determine biochemical diversity. After the addition of sewage effluent, chlorophyll a is expected to rise and the carotenoid pigments are expected to be low. As succession proceeds, the carotenoid pigments increase in concentration. The ratio of chlorophyll a (green plant pigments) and the carotenoids (yellow plant pigments) is called Margalef's ratio or the yellow/green ratio (Odum, 1959). Chlorophyll a and the carotenoid pigments can be measured at absorption spectra of 665 nm and 430 nm, respectively. The 430/665 ratio is expected to be low initially and then increase as succession occurs.

Nitrogen concentration beyond 0.6 mg ℓ^{-1} inhibits chlorophyll formation in green algae (Machenthun, 1969). Chlorophyll values up to 2.5 g m^{-2} are found in polluted situations (Odum et al., 1958).

Many species make up a stable ecosystem. Addition of sewage reduces the number of species. No list of species has ever been established as pollution indicators for a universal situation because of differences in the physicochemical environments of the world (Gaufin and Tarzwell, 1952). The following mathematical index has frequently been used to measure species diversity ($\bar{d}$) and indicate the quality of the environment (Shannon and Weaver, 1963):

$$\bar{d} = - \sum_{1}^{S} (n_i/n) \log_2 (n_i/n),$$

where n_i is the individuals of i, s is the total number species in a community, and n is the total number of individuals. Wilhm (1970) states that the $\bar{d}$ values varies between three and four in clean-water areas and is usually less than one in polluted areas.

The objectives of this experiment were to measure variation in changes in species diversity, net production, respiration, chlorophyll a, chlorophyll b, chlorophyll c, astacin pigments, dry weight, ash-free weight, Margalef's ratio, among laboratory microecosystems receiving different levels of domestic sewage.

CHAPTER II

METHODS

A split-split plot analysis was used to study the effects of domestic sewage on laboratory systems growing in different nutrient levels. Nutrients were the main plot, sewage the subplot, and time the sub-subplot.

The basic experimental unit was 1.24 ℓ of pond water in a glass container painted black on the outside. The pond water was taken from a farm pond located 10 miles north of Stillwater, Oklahoma, in Section 7, Township 20N, 2E, Payne County. The pond water was analyzed by the Oklahoma State University Cooperative Extension Service for nitrate-nitrogen (NO_3-N) and phosphate phosphorous (PO_4-P). The domestic sewage effluent was final activated sludge effluent from the Oklahoma City Northside Plant for Water Pollution. Analysis of the domestic sewage effluent was conducted by A. Sneider, Oklahoma City Northside Plant for Water Pollution.

Microcosms were housed in a light-proof controlled environment chamber under 40-watt Gro-Lux fluorescent lights. The Gro-Lux lights emit wavelengths of light in the blue and red bands of maximum light absorption by algae (Bernier, 1962). The lights were allowed to stabilize for 8 weeks before the pilot and main studies began. Light intensity was measured with a G. M. Manufacturing photometer. The chamber was evenly lighted and contained aluminum foil reflectors on each side. Each fluorescent light was regulated by a model J Ohmite rheostat and activiated by a 120 volt Bendix transformer. The length of the

photoperiod was 12 hr, from 0600 to 1800 hours, and regulated by an automatic timing switch.

Pilot Study

A pilot study was conducted to determine concentrations of nutrients and sewage for further testing. Twenty-four experimental units were used to evaluate responses at three concentrations of nutrients, four concentrations of domestic sewage, and two time periods. The pond water had a TDIS of 160 mg ℓ^{-1}, 0.1 mg ℓ^{-1} NO_3 -N, and no measurable phosphate. The three nutrient concentrations had the following N/P (nitrogen/phosphorus) ratios: 0/0 in N1 (nutrient concentration 1), 12/2 in N2, and 120/20 in N3. The lowest concentration produced slight algal growth and the upper level is considered an enriched state. The two higher nutrient concentrations contained a 6:1 N/P ratio. The N/P in nature varies from 5.5:1 to 10:0 in phytoplankton and submerged plants (Mackenthun, 1969). The carrier compounds for N and P were $Ca(NO_3)_2 \cdot 4H_2O$ and $Na_2HOP_4 \cdot 7H_2O$.

Prior to adding the domestic sewage effluent, the microcosms were allowed to stabilize in the chamber until the net production (Pn) was approximately equal to nighttime respiration (Rni). Beyers (1963b) found a mean Pn/Rni ratio of 1.05 in his stabilized microcosms. Wilhm and Long (1969) found that this ratio was reached 35 days after nutrients were added. A Pn/Rni ratio of approximately one was found after 28 days in the pilot study. After stabilization, four levels of sewage were applied to two replicate microcosms at each nutrient level. The analysis of the sewage is given in Table I. Sewage level 1 (S1) contained 400 ml of deionized water and no sewage, S2 contained 100 ml of sewage

and 300 ml of deionized water, S3 contained 200 ml of sewage and 200 ml of deionized water, and S4 was 400 ml of sewage.

TABLE I

ANALYSIS OF SEWAGE

Component	Concentration (mg ℓ^{-1})	
	Pilot Study	Main Study
Biological oxygen demand	38.0	37.0
Suspended solids	68.0	40.0
Dissolved oxygen	0	0
Total solids	2400.0	1516.0
Total volatile solids	1148.0	292.0
Alkalinity	180.0	180.0
Total nitrogen	28.0	28.4
pH	7.4*	7.6*
PO_4 - P	33.0	38.0

*Negative logarithm of the hydrogen ion concentration.

The pilot study was conducted under a mean light intensity of 220 ft-c (0.03 ly min^{-1}) and a temperature range of 25.0 to 30.8 C with a mean temperature of 27.5 C. Diels were run on day 14 and 21 after adding sewage. Measurement of pH was made at 3-hr intervals during a 24-hr

period. The diel pH measurements were plotted and 25 hourly values were computed (Beyers et al., 1963) to determine respiration and productivity.

Biomass and pigment samples were taken 21 and 33 days after adding the domestic sewage. A Waring blender was used to break up masses of algae and diluted to appropriate volumes. Mechanical stirring was used while 250 ml samples were withdrawn and filtered by Millipore membranes of .22 and 5 μ pore size to determine the dry weight. With increasing concentrations of biomass, greater dilutions were used. Dried and tared filters were used in the filtration and were washed with deionized water to remove suspended solids. The filters and their residues were dried in an oven at 100 C for at least 48 hr. Samples of 250 ml were used to determine chlorophylls a, b, c, and astacin and carotenoid pigments. Samples from each experiment were filtered through a 0.45 and a 5 μ Millipore filter to provide a comparison of chlorophylls a, b, c, and astacin and carotenoid pigments. The methods of Strickland and Parsons (1965) were followed for pigment extraction. A Beckman DBG spectrophotometer was used to determine light extinctions using 1 or 4 cm light paths. The pigments were analyzed using the equations of Parsons and Strickland (1965).

The pilot study suggested the two intermediate sewage loads produced moderate stress on the systems, while S4 produced an oxygen deficit due to the high BOD in the effluent. It was decided that S1, S2, and S3 would be used in the main study.

Main Study

Water in the main study was collected on November 25, 1967, from the same pond used as a source in the pilot study. The pond water had

a TDIS of 100 mg ℓ^{-1}, no measurable phosphate, and 0.1 nitrate (Table II). Pond water was collected in three plastic barrels, each corresponding to one replicate. The water in each barrel was further divided into three portions, each corresponding to a main group or nutrient level. Each portion was treated with one of the three nutrient levels used to fill 21 microcosms. Thus, each of the three nutrient levels was composed of 63 microcosms.

TABLE II

CONCENTRATION OF TOTAL DISSOLVED INORGANIC SOLIDS (TDIS), NITRATES, AND PHOSPHATES FOR THE MAIN STUDY

	Concentration (mg ℓ^{-1})		
Treatment	TDIS	Total Nitrogen	PO_4-P
N1S1	100	0.1	0
N2S1	100	12.1	2.0
N3S1	100	120.1	20.0
N1S2	247	2.9	3.7
N2S2	247	15.0	5.7
N3S2	247	123.0	23.7
N1S3	394	5.7	7.3
N2S3	394	17.8	9.3
N3S3	394	125.8	27.3

A Pn/Rni ratio of approximately one was measured 35 days after establishing the microcosms. Then, each main group of 21 jars was subdivided randomly into three subgroups of seven microcosms each. One subgroup was treated with S1, the second with S2, and the third with S3. Each subgroup was then randomly designated for seven time periods or sub-subgroups.

During the main study, diurnal variation in temperature was usually less than 6 C. Mean daily temperature was 25.2 C. The mean light intensity was 220.5 ft-c.

Observations were made at 2, 4, 8, 16, 32, 64, and 128 days after the sewage was added. Three experimental units at each nutrient concentration versus each sewage level were analyzed to determine biomass, pigments, and diel pH changes. Methods used in analyzing parameters were the same as in the pilot study. In addition, plankton samples were withdrawn from microcosms after their respective diels. The 250 ml samples were added to an equal portion of Transeau's solution (Prescott, 1964). The plankton sample and an equal volume of saturated mercuric chloride solution were allowed to stand overnight, to precipitate the phytoplankton. The supernatant was poured off and the remainder was then poured into a graduated cylinder and washed with deionized water. The remainder of the supernatant was decanted and an equal volume of preservative was added to the precipitate. The drop method (Prescott, 1942) and standard techniques (A. P. H. A., 1960) were used to count the organisms.

The objective of the main study was to study responses over time. It was assumed that seven time periods would be required to observe trends in responses. Available space for housing experiment units

necessitated eliminating either a nutrient or sewage level in the main study. Because of similarity of response between S3 and S4, S4 was eliminated in the main study.

CHAPTER III

RESULTS AND DISCUSSION

Pilot Study

Net production (Pn) in the pilot study was usually highest in microcosms supplied with low concentrations of nutrients (N2) and lowest in systems receiving no nutrients (N1) (Table IV). Nutrients were probably limiting production in N1, while the N3 systems were over-fertilized. The addition of low amounts of sewage (S2) stimulated production in N1 and N3, but depressed production in systems receiving low nutrient levels. Maximum production was attained in N2 and N3 systems receiving intermediate concentrations of sewage and high amounts of sewage in N1 systems. In comparing production of systems receiving intermediate and high concentrations of sewage, the response was increased by the high concentrations in the systems receiving no nutrients, depressed in N2 systems, and unchanged in N3 systems.

Respiration (Rni) was similar to net production (Pn) in all microcosms except the high nutrient systems receiving low and intermediate levels of sewage and the N1 systems receiving high levels of sewage (Table III).

Biomass accumulation methods were limited in systems receiving no nutrients, whereas high levels of nutrients generally depressed dry weight accumulation (Table III). The addition of sewage stimulated production of biomass, but high levels of sewage were generally limiting.

TABLE III

MEAN RESPONSES TO SEWAGE AND NUTRIENT LEVELS OF THE TWO TIME PERIODS IN THE MICROCOSMS DURING THE PILOT STUDY

Source	Volume (ℓ)	Temp (oC)	Light (ft-c)	TDIS (mg ℓ^{-1})	NO_3* (mg ℓ^{-1})	PO_4-P (mg ℓ^{-1})	Pn (g CO_2 m^{-2} per 12 hr)	Rni (g CO_2 m^{-2} per 12 hr)	Pn/Rni	Dry Weight (g m^{-2})**	Pn/Dry Weight	Chlorophyll a (g m^{-2})	Chlorophyll b (g m^{-2})	Pigment Diversity (D430/D665)
N1S1	1.24	27.5	220	160	0.1	0	0.10	0.09	1.07	7.4	0.014	0.007	0	3.37
N1S2	1.24	27.5	220	353	2.3	2.7	0.80	0.78	1.01	30.4	0.026	0.079	0.009	2.68
N1S3	1.24	27.5	220	547	4.6	5.3	0.70	0.67	1.08	10.3	0.068	0.146	0.011	2.42
N1S4	1.24	27.5	220	934	9.2	10.6	1.41	1.48	0.77	15.9	0.089	0.080	0.008	2.93
$\bar{x}$							0.75	0.76	0.99	16.0	0.049	0.078	0.007	2.85
N2S1	1.24	27.5	220	160	12.1	2.0	1.87	1.80	1.03	63.6	0.029	0.124	0.087	2.45
N2S2	1.24	27.5	220	353	14.5	4.7	0.76	0.76	1.00	82.7	0.009	0.187	0.009	2.50
N2S3	1.24	27.5	220	547	16.7	8.3	2.78	2.76	1.01	44.1	0.063	0.200	0	2.07
N2S4	1.24	27.5	220	934	21.3	12.6	2.07	2.13	0.96	69.7	0.030	0.125	0.006	2.43
$\bar{x}$							1.87	1.85	1.00	65.1	0.033	0.182	0.026	2.33
N3S1	1.24	27.5	220	160	120.1	20.0	0.80	0.70	1.17	42.8	0.002	0.287	0.039	2.46
N3S2	1.24	27.5	220	353	122.5	22.7	1.39	1.62	0.82	58.3	0.023	0.291	0.040	2.60
N3S3	1.24	27.5	220	547	124.7	25.3	1.56	1.81	0.79	127.0	0.012	0.354	0.038	2.71
N3S4	1.24	27.5	220	934	129.3	30.6	1.52	1.57	0.95	60.2	0.026	0.386	0.003	3.04
$\bar{x}$							1.37	1.42	0.94	72.2	0.016	0.328	0.031	2.71
NiS1							0.93	0.87	1.08	38.0	0.015	0.128	0.036	2.76
NiS2							1.06	1.05	0.99	57.1	0.019	0.186	0.020	2.57
NiS3							1.68	1.75	0.96	60.7	0.046	0.233	0.017	2.40
NiS4							1.67	1.73	0.89	48.9	0.044	0.226	0.006	2.80

*The concentrations of the pilot study are total nitrogen.

**These means are from days 21 and 33, the other means are from days 14 and 21.

Maximum dry weight was attained by low levels of sewage in N1 and N2 systems and by intermediate levels of sewage in high nutrient systems.

The ratio between net production and dry weight was generally highest in systems receiving no nutrients and lowest in N3 systems (Table III). Maximum ratios were obtained by the addition of intermediate levels of sewage in N2 systems, and high levels of sewage into N1 and N3 systems.

The concentration of chlorophyll a at any particular sewage level increased with nutrient level (Table III). Chlorophyll a increased in concentration with increasing sewage in high nutrient systems, whereas maximum concentration was attained with intermediate levels of sewage in N1 and N2 systems.

Concentrations of chlorophyll b were usually highest in microcosms receiving high levels of nutrients (Table III). The concentration of chlorophyll b generally decreased with sewage level. Maximal concentrations of chlorophyll b were attained by adding intermediate sewage in N1 systems, no sewage in N2, and low levels of sewage in N3.

Pigment diversity or the ratio between absorbance of 430 and 665 was lowest in the N2 systems (Table III). Minimum values of diversity were observed in N1 and N2 systems after the addition of intermediate amounts of sewage. Maximal pigment diversity was attained in N1 systems receiving no sewage, low levels of sewage in N2 systems, and high levels of sewage in N3 systems.

Main Study

Community Composition

Seventy-four species of various groups of organisms were identified in the microcosms: Cyanophyta (17 species), Chrysophyta (8), Euglenophyta (3), Chlorophyta (35), Thallophyta (1), Cladocera (1), Copepoda (1), Gastrotricha (1), Ostracoda (1), Protozoa (4), and Rotifera (3), (Table IV). Species of Cyanophyta and Chlorophyta were the most numerous in several other studies involving microcosms. Cooke (1967) found 25 species of algae in enriched microcosms and 64% belonged to one of these two groups. Three species each of Cyanophyta and Chlorophyta were observed by Kehde (1970) in artificial streams and three and seven species, respectively, were found by Beyers (1963a) in microcosms established with water from the San Marcos River. In laboratory systems receiving different nutrient levels, Wilhm and Long (1970) identified 12 species of Cyanophyta and 11 species of Chlorophyta.

Species of Cyanophyta and Chlorophyta were the most abundant in several studies of natural environments. Species of Chlorophyta were more numerous than any other group of phytoplankton in clear ponds (Leake, 1935; Knudson, 1970), in organic enriched ponds (Ewing and Dorris, 1970), in organic polluted streams (Hofstetter and Mangold, 1970), and in sewage oxidation ponds (Parker, Samsel, and Obeng-Asamoa, 1971). Species of Cyanophyta were dominant in various lakes and ponds, especially during the late summer and fall (Smith, 1924; Wesenburg-Lund, 1905; W. West and G. S. West, 1909).

Species of Cyanophyta were the most abundant in enriched microcosms and Chlorophyta was the dominant group in control microcosms (Mitchell

TABLE IV

PHYTOPLANKTON, BACTERIA, AND ZOOPLANKTON SPECIES OBSERVED IN MICROCOSMS

Cyanophyta
- Anabaena variabilis Kuetzing
- Aphanothece sp.
- Chroococcus rufescens (Brebisson) Naegeli
- Gleocapsa rupestris Kuetzing
- Gleocystis vesiculosa Naegeli
- Gloeothece linearis Naegeli
- Lyngbya aerugineo - caerulea (Kuetz) Gomont
- Merismopedia tenuissimum Lemmerman
- Microcystis aeruginosa Kuetz
- Nostoc paludosum C. A. Agardh
- Oscillatoria limosa (Roth) C. A. Agardh
- Oscillatoria curviceps C. A. Agardh
- Phormidium retzii (C. A. Agardh) Gomont
- Rapidiopsis curvata Fritsch
- Rhabdoderma sigmoidea fa. minor Moore and Carter
- Rivularia naematites (D. C.) C. A. Agardh
- Spirulina Norstede Gomont

Chrysophyta
- Cymbella cistula (Hemprich) Grunow
- Fragillaria capucina Desmazieres
- Fragillaria crotonesis Kitton
- Navicula confervacea Hustedt
- Navicula exigua Hustedt
- Navicula hungarica Hustedt
- Navicula radiosa Kuetzing
- Rhopalodia gibba (Ehr.) O. Mueller

Euglenophyta
- Euglena sp.
- Trachelomonas hispida (Perty) Stein

Chlorophyta
- Ankistrodesmus falcatus (Corda) Ralfs
- Ankistrodesmus convolutus Corda
- Arthodesmus sp.
- Characium Pringsheimii A. Braun
- Chlamydomonas sp.
- Chlorella sp.
- Chlorococcum humicola (Naegeli) Rabenhorst
- Closterium venus Nitzsch
- Cosmarium angulosum Brebisson
- Cosmarium granatum Brebisson
- Crucigenia apiculata (Lemmermann) Schmidle
- Crucigenia tetrapedia (Kirchner) Wm. and G. S. West
- Euastrum sp.

TABLE IV (Continued)

Eudorina sp.
Gonium sociale (Duj) Warming
Kirchneriella obesa (W. West) Schmidle
Microspora sp.
Mougeotia capucina (Borg) C. A. Agardh
Mougeotia scalaris Hass
Oedogonium Pringsheimii Cramer
Pandorina morum (Mueller) Bory
Pediastrum biradiatum Meyen
Pediastrum duplex Meyen
Pediastrum tetras (Ehrenb.) Ralfs
Protococcus viridis G. A. Agardh
Scenedesmus bijuga (Turp.) Lagerheim
Scenedesmus dimorphus (Turp.) Kuetzing
Scenedesmus incrassatulus Bohlin
Scenedesmus longus var. Naegelii (de Breb) G. M. Smith
Sorastrum spinulosus Naegeli
Spirogyra sp.
Straurastrum paradoxum Meyen
Tetrahedron sp.
Tetraspora lubrica (Roth) C. A. Agardh
Volvox sp.

Thallophyta
Chlorobium sp.

Cladocera
Bosmina longirostris (O. F. M.)

Copepoda
Cyclops sp.

Gastrotricha
Chaetonotus sp.

Ostracoda
Cypricerus reticulatus (Zadd.)

Protozoa
Enchelydium fusidens Kahl
Holophyra simplex Schewiakoft
Paraholosticha herbicola Kudo
Paramecium sp.

Rotifera
Keratella cocclearis (Gosse)
Philodina sp.
Rotifers sp.

and Buzzell, 1971). This was generally true in the present study during the early time periods, but Thallophyta was generally the dominant group after day 8 (Table V). Although considerable variation existed in the dominant group between systems receiving no nutrients and those receiving low and high levels of nutrients during the early part of the present study, little variation existed among sewage levels of a particular nutrient level. In microcosms receiving no nutrients, Chlorophyta was the dominant group on day 2 and 4 at all sewage levels. Chlorophyta decreased abruptly after day 4 and Thallophyta became the abundant group. Cyanophyta was the dominant group in the systems receiving no nutrients on only one occasion. In systems receiving low and high levels of nutrients, Cyanophyta was generally the dominant group from day 2 through day 16 at all sewage levels. The percent composition of Chlorophyta dropped abruptly after day 4 in systems receiving no sewage and after day 2 in systems with sewage added. The presence of detergents in the sewage may have inhibited the growth of Chlorophyta in N2 and N3 systems of the present study.

Chrysophyta were lowest in abundance throughout the present study. Most diatom species acclimate at temperatures between 20-30 C (Chu, 1943). Patrick (1969) reported that Chrysophyta were the dominant species between 20-30 C in some selected unpolluted environments. Temperature variations in the present study in the upper limits of this range probably eliminated many species of diatoms. Pearsall (1932) stated that diatoms are more abundant in high concentrations of nitrates and phosphates. Diatoms were in low concentrations in all treatment levels in the present study. Chu (1943) reported a marked inhibition of Chrysophyta growth above 26.2 ppm nitrate-nitrogen.

TABLE V

PERCENTAGE COMPOSITION OF TOTAL BACTERIA AND PHYTOPLANKTON NUMBERS AT VARIOUS NUTRIENT X SEWAGE LEVELS IN EACH TIME PERIOD

Day	Division	Nutrient x Sewage Levels								
		N1S1	N1S2	N1S3	N2S1	N2S2	N2S3	N3S1	N3S2	N3S3
2	Cyanophyta	23	22	1	55	57	73	55	47	47
	Chrysophyta	7	14	1	3	2	15	3	8	5
	Chlorophyta	69	46	67	41	28	6	41	25	33
	Thallophyta	1	18	31	1	13	6	1	20	15
4	Cyanophyta	20	7	7	30	27	47	30	47	57
	Chrysophyta	3	3	8	2	4	6	2	5	11
	Chlorophyta	45	74	56	51	7	8	51	9	3
	Thallophyta	32	16	28	9	62	39	17	37	29
8	Cyanophyta	39	30	10	54	57	29	54	70	53
	Chrysophyta	6	13	13	4	5	7	4	2	3
	Chlorophyta	7	7	17	1	1	3	1	1	1
	Thallophyta	48	50	60	42	38	61	41	27	43
16	Cyanophyta	43	50	43	54	59	28	54	52	57
	Chrysophyta	7	5	9	5	3	7	5	3	1
	Chlorophyta	2	4	8	2	3	18	2	5	3
	Thallophyta	48	49	40	36	38	47	39	40	39
32	Cyanophyta	21	21	30	59	22	42	9	28	40
	Chrysophyta	9	6	3	1	1	3	1	2	5
	Chlorophyta	8	15	7	8	14	4	13	24	3
	Thallophyta	62	58	60	32	63	51	77	46	52
64	Cyanophyta	15	22	34	27	22	30	27	22	27
	Chrysophyta	3	1	3	1	8	4	1	1	1
	Chlorophyta	37	3	24	6	32	10	6	39	14
	Thallophyta	45	74	42	38	38	56	66	38	58
128	Cyanophyta	11	38	19	17	34	29	17	30	24
	Chrysophyta	1	1	1	1	1	1	1	1	1
	Chlorophyta	49	12	11	33	11	16	33	9	9
	Thallophyta	39	49	69	46	47	54	49	60	66

Margalef (1958) reported three to four complete cycles of phytoplankton during a year in a bay with flowing waters. Each cycle passed through the following stages: (1) small-celled diatoms and Chlorophyta - 0.5 to 2.2, (2) large-celled diatoms - 0.2 to 0.5, (3) larger free-swimming species - less than 0.2. These cycles were not observed in the present study except the Chlorophyta in step 1. Small unicellular Chlorophyta (e.g., Crucigenia sp.) and Cyanophyta (e.g., Gleocystis vesiculosa) were abundant initially. Large numbers of diatoms were not observed during the present study. The filamentous Chlorophyta and Cyanophyta were more abundant at the end of the experiment. The photosynthetic bacterium (Chlorobium sp.), a free swimming organism, was the predominant form from day 32 to day 128 (verification by D. Dunn, Oklahoma Christian College). Chlorobium sp. was reported in other studies (Czeczuga, 1965; Juday and Manning, 1941) and sewage oxidation ponds (Godniew and Winberg, 1951).

Species of algae have been classified according to their pollutional status by Whipple et al. (1948). Spirulina and Aphanotheca were listed as polysaprobic by Whipple and the former group was collected only in high nutrient conditions in the present study. Apanotheca was taken from systems with all levels of nutrients and sewage except S2. Species collected in the present study that were listed as mesosaprobic by Whipple were: Oscillatoria limosa, Navicula radiosa, Euglena sp., Trachelomonas hispidia, Protococcus sp., Chlorella, and Gonium sociale. The first two species were found only in high nutrient conditions, while the rest were observed in microcosms receiving all nutrient levels. Groups collected in the present study that were classified as oligosaprobic were: Cymbella cistula, Fragillaria crotonesis, Navicula hungaria,

Rhopalodia gibba, Pandorina morum, Pediastrum duplex, Pediastrum tetras, and Volvox sp. Volvox sp. was taken only from high nutrient systems, while the other species were taken from all treatments.

Palmer (1962) listed genera of algae that are tolerant of organic pollution and clean water environments. Examples included Chlamydomonas, Euglena, Navicula, Oscillatoria, Phormidium, and Synedra. In the present study, Chlamydomonas was taken from N1 and N2 systems, while Euglena and Oscillatoria limosa were taken from all treatment levels.

Hutchinson (1969) gives a classification of lakes by phytoplankton types classified according to dominant species. Fragillaria crotonesis was listed as a eutrophic diatom plankter and was observed in microcosms of all treatment levels in the present study. Pediastrum and Scenedesmus were listed as eutrophic plankton indicators and Cosmarium as a eutrophic desmid indicator. Pediastrum was found in systems of all treatment levels except N3S1 in the present study. Scenedesmus and Cosmarium were in microcosms of all treatment levels.

The rotifers, especially Keratella cochlearis, were the most abundant zooplankters observed in the present study. Keratella cochlearis is perhaps the most common planktonic perennial rotifer of lakes in the temperate region (Hutchinson, 1967). This species was considered generally to be eutrophic by Pejler (1957). Keratella sp. and Polyarthra sp. were the predominant zooplankton species during the late spring in enriched ponds (Ewing and Dorris, 1970). Edmondson (1964) recorded higher reproductive rates for Keratella cochlearis about 15 C than for many other species. Hutchinson (1967) reported maximum density of Keratella cochlearis in the summer and rare winter occurrence in Lake Glan, Sweden. The mean daily temperature (25.2 C) of the present study

probably favored this species. A trend in zooplankton could not be observed in the present study since a homogenizer was used to separate algal clumps and many zooplankton were destroyed or mutilated beyond recognition.

Species Diversity

The main effects of sewage, nutrients, and time on species diversity, $\bar{d}$, were not significant ($P = 0.05$) (Table VI). Since main effects were averaged over a wide variety of conditions, this finding was expected. Although the interaction between sewage and nutrients was not significant, the other two, two-factor interactions were.

A significant interaction existed between sewage and time (Figure 1). Diversity in systems receiving no sewage averaged over all nutrient levels increased through day 64, while diversity in S2 and S3 systems decreased through day 8. The greatest variation over time was measured in S3 systems. Significant differences were found between sewage levels on days 2 and 16. Species diversity on day 2 for the S1 level was significantly lower than the systems receiving low and high sewage levels. On day 16, diversity in S3 systems was significantly greater than for the S1 level, while S2 was not different from the other levels.

The interaction between nutrients and time on species diversity was significant (Figure 2). Little change existed after day 16 in responses of N3 systems. Species diversity of systems receiving no nutrients was significantly lower on day 2 than the other levels. The mean $\bar{d}$ of N1 systems on day 8 was significantly higher than in N3 microcosms, but not the N2 systems. Other levels of nutrients and time were not significant.

TABLE VI

MAIN STUDY TABULATED (P = 0.05 AND 0.01) AND CALCULATED VALUES OF F

Variable	F	R Replicates	A Nutrients	B Sewage	C Time	AB Nutrients x Sewage	AC Nutrients x Time	BC Sewage x Time	ABC Nutrients x Sewage x Time
	Tab (0.05)	6.94	6.94	3.89	2.19	3.26	1.85	1.85	1.63
	Tab (0.01)	18.00	18.00	6.93	2.99	5.41	2.37	2.37	1.96
Species Diversity ($\bar{d}$)	Calc.	0.55	0.26	3.30	0.84	0.53	1.85*	1.90*	1.03
Pn	Calc.	1.03	5.16	5.21*	6.96**	8.22**	1.19	1.00	1.00
Rni	Calc.	1.00	9.92*	5.38*	11.77**	3.51*	0.97	1.48	1.41
Pn/Rni	Calc.	2.57	6.44	0.17	4.72**	0.34	1.21	0.58	0.88
Dry Weight	Calc.	2.80	24.19**	4.88*	18.15**	0.26	0.88	1.67	0.39
Ash-free Weight	Calc.	0.78	33.67**	4.92*	21.90**	1.92	1.09	2.20*	0.43
Pn/Dry Weight	Calc.	2.11	0.92	0.72	1.06	0.40	1.07	1.05	1.04
Assimilation Number	Calc.	1.90	23.15**	11.42*	21.73**	1.29	2.69**	1.16	0.69
Chlorophyll a	Calc.	0.30	437.93**	25.07**	17.34**	1.87	2.21*	1.85*	1.11
Chlorophyll b	Calc.	1.00	2.33	3.89*	15.14**	1.71	1.37	1.51	1.41
Chlorophyll c	Calc.	0.33	4.61	7.04**	14.29**	0.61	1.01	1.58	0.59
Non-Astacin Pigments	Calc.	0.12	25.08**	1.59	11.69**	2.89	2.34*	0.88	1.36
Astacin Pigments	Calc.	2.93	6.36	2.21	29.61**	0.16	1.05	1.81	0.47
Pigment Diversity	Calc.	0.25	4.85	11.72**	7.79**	3.86*	1.78	1.11	0.76

*These F values are significantly different at the 95 percent level.

**These F values are significantly different at the 99 percent level.

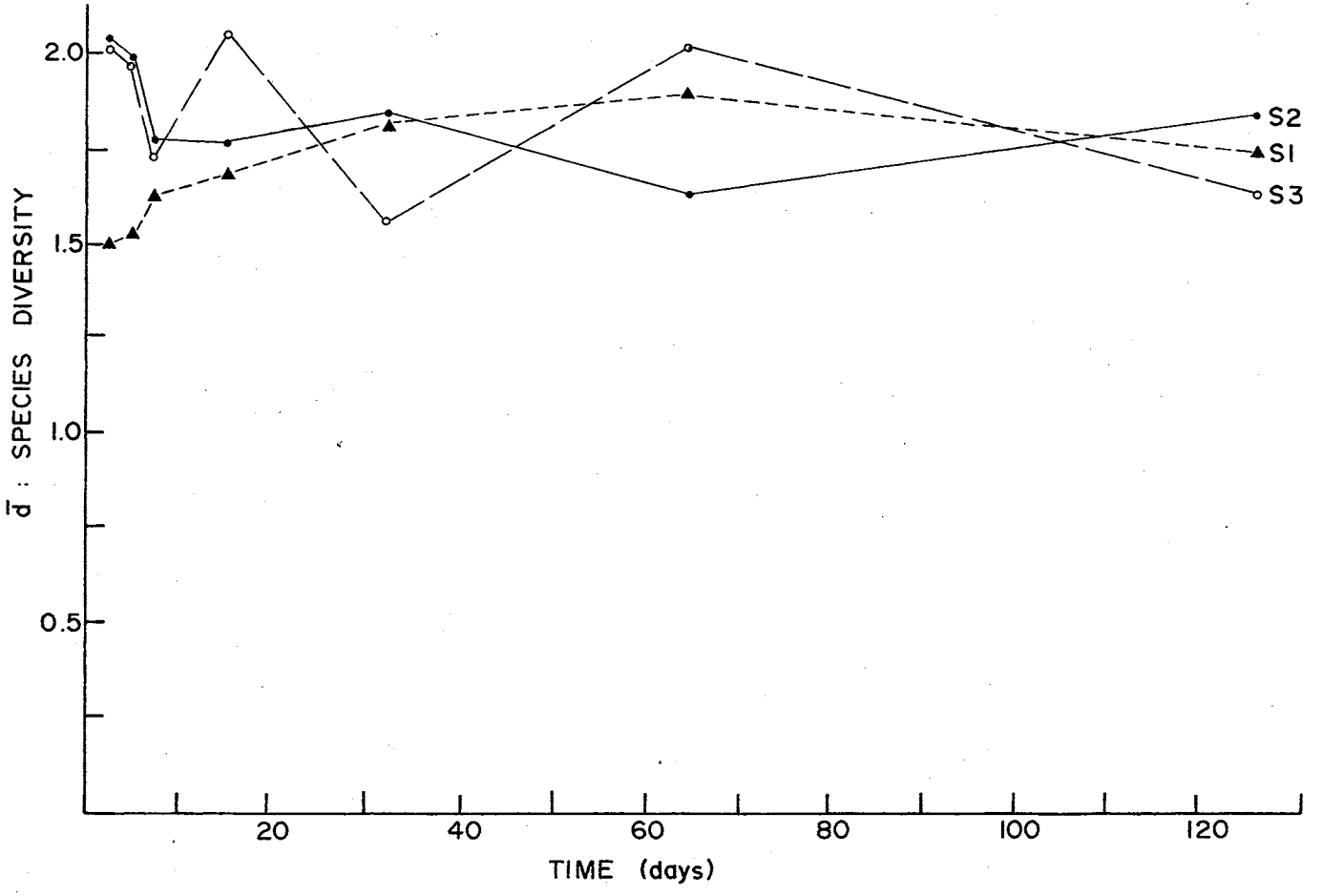

Figure 1. Temporal Variation of Species Diversity ($\bar{d}$) Values of the Three Sewage Levels Averaged Over All Nutrient Levels

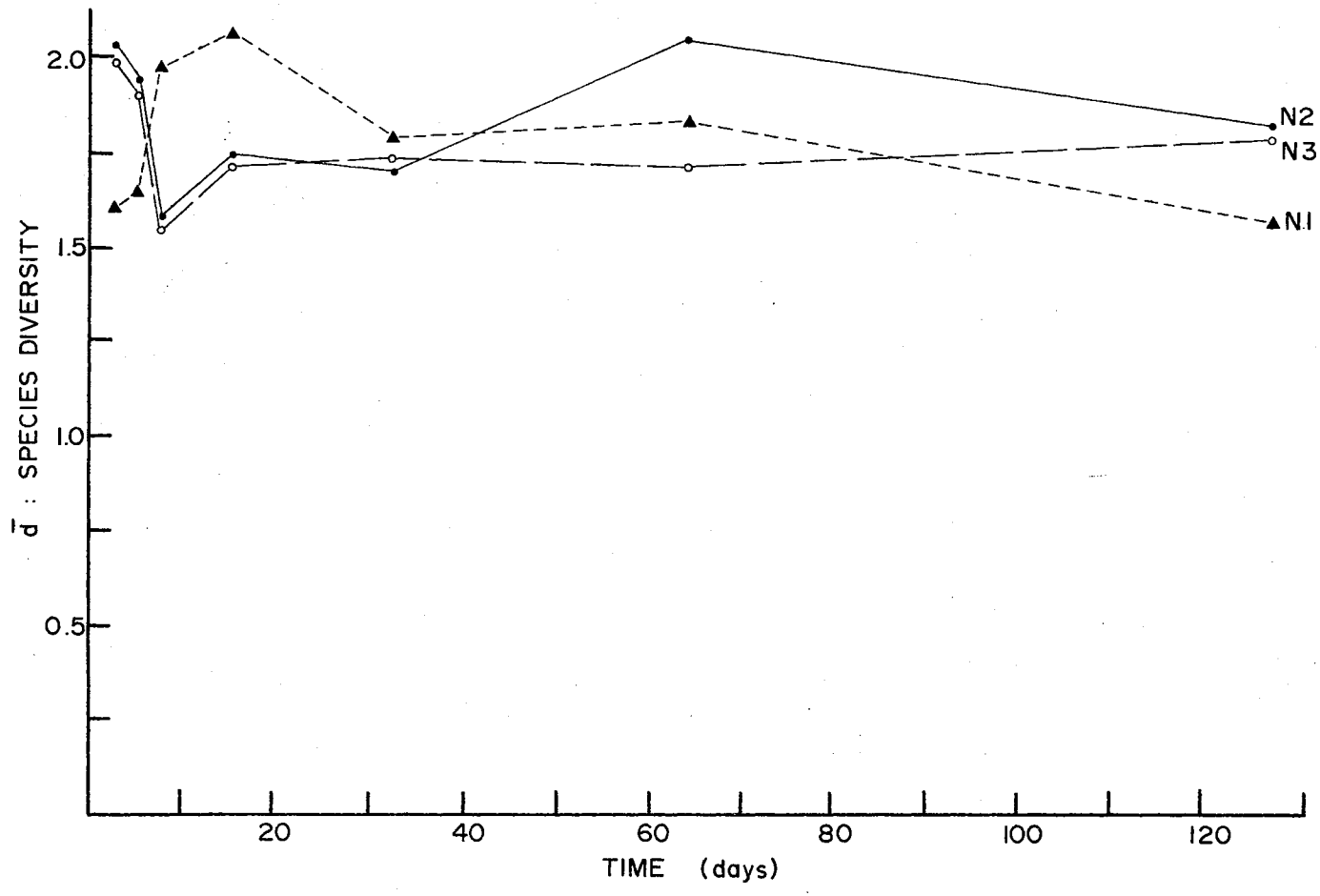

Figure 2. Temporal Variation of Species Diversity ($\bar{d}$) Values of the Three Nutrient Levels Averaged Over All Sewage Levels

Oligotrophic lakes probably have algal diversity indices ($\bar{d}$) from 0.7 to 1.0 and eutrophic lakes 0.3 or less (Mitchell, 1971). Mitchell added 0.6 liters of activated sludge effluents to 5.4 liters of water and 0.5 liters of mud from an oligotrophic impoundment of microcosms. Species diversity approached eutrophic conditions by day 42. The effluents in the present study were more concentrated and the diversity index decreased by 0.25 in the systems receiving low and high sewage levels by day 8 (Figure 1). The trends reported by Mitchell (1971) were not observed in the present study.

Staub et al. (1970) used species diversity ($\bar{d}$) of phytoplankton as an index of industrial and domestic pollution in streams near Memphis, Tennessee. Heavily polluted areas had values less than 1, moderately polluted areas values of 1-2, lightly polluted areas of 2-3, and clear water areas exceeded 3. The diversity values in the present study were between 1 and 2, suggesting moderate pollution according to Staub's classification (Table VII). The low values of the present study were probably affected by dominance. An annual phytoplankton species diversity mean of 0.94 was reported for a heavily polluted upstream steam station while the downstream clean-water stations had an annual mean of 1.23 (Cooper, 1972). Gale and Low (1971) reported $\bar{d}$ values of phytoplankton less than 1 in the winter and values greater than 3 in summer in the Mississippi River near Ft. Madison, Iowa.

Productivity was related to species individual relationship of phytoplankton in Silver Springs, Florida (Yount, 1956). In the present study, species diversity generally showed no relationship to productivity, with a -0.02 correlation.

TABLE VII

COMPARISON OF MEAN STRUCTURAL AND FUNCTIONAL PARAMETERS MEASURED IN MICROCOSM STUDIES

Source	Volume (ℓ)	Temp. (°C)	Light (ft-c)	TDIS (mg ℓ$^{-1}$)	NO3 f (mg ℓ$^{-1}$)	PO4-P (mg ℓ$^{-1}$)	Species-Diversity ($\bar{d}$)	Pn (g CO2 m^{-2} per 12 hr)	Rni	Pn/Rni	Dry Weight (g m^{-2})
Main Study a											
N1S1	1.24	25.2	220.5	100	0.1	0	1.65	0.23	0.21	1.10	21.4
N1S2	1.24	25.2	220.5	247	2.9	3.7	1.96	0.87	0.81	1.07	55.4
N1S3	1.24	25.2	220.5	394	5.7	7.3	1.82	0.81	0.78	1.04	68.0
$\bar{x}$							1.80	0.64	0.60	1.07	39.1
N2S1	1.24	25.2	220.5	100	12.1	2.0	1.75	1.01	1.05	0.96	41.6
N2S2	1.24	25.2	220.5	247	15.0	5.7	1.85	1.06	1.14	0.93	67.5
N2S3	1.24	25.2	220.5	394	17.8	9.3	1.97	0.97	0.94	1.03	85.1
$\bar{x}$							1.85	1.01	1.04	0.97	68.0
N3S1	1.24	25.2	220.5	100	120.1	20.0	1.69	1.27	1.11	1.14	58.0
N3S2	1.24	25.2	220.5	247	123.0	23.7	1.78	1.21	1.25	0.97	83.2
N3S3	1.24	25.2	220.5	394	125.8	27.3	1.87	1.07	1.05	1.02	85.7
$\bar{x}$							1.78	1.18	1.13	1.04	79.4
NiS1							1.70	0.84	0.79	1.07	46.6
NiS2							1.85	1.05	1.07	0.99	64.3
NiS3							1.88	0.95	0.92	1.03	74.3
Beyers (1963)	3	23	1,000	*	*	*	*	1.38	1.26	1.09	*
Butler (1964) b	10	23	400	75	11.0	5.0	*	1.40	1.46	0.96	26.3
	10	23	400	150	22.0	9.0	*	1.05	0.80	1.31	33.2
	10	23	400	300	44.0	18.0	*	1.22	1.25	0.98	57.6
	10	23	400	600	88.0	35.0	*	0.61	0.56	1.09	105.0
Wilhm and Long (1969) c	1	23	215	400	1.2	0.2	*	0.17	0.17	1.04	11.5
	1	23	215	400	12.0	2.0	*	1.12	1.13	0.98	27.5
	1	23	215	400	120.0	20.0	*	1.09	1.11	1.00	56.5
Kehde (1970) d	174	30-34	250	*	120.0	20.0	1.41	*	*	*	44.0
Spangler (1970) e	*	5-25	2671-7580	7893-29020	2.2-8.0	0.9-2.2	*	*	*	*	*

a Means of responses measured between 0 and 128 days.

b Means of responses measured between 21 and 49 days.

c Means of responses measured between 39 and 109 days.

d Means of responses measured between 0 and 92 days.

e Means of responses measured between 0 and 365 days.

f The concentrations of the main study are total nitrogen.

* Data not available.

TABLE VII (Continued)

Ash-free Weight (g m^{-2})	Pn/Dry Weight	Assimilation Number (Pg/Chlorophyll a)	Chlorophyll a (g m^{-2})	Chlorophyll b (g m^{-2})	Chlorophyll c (g m^{-2})	Non-astacin Pigments (SPU m^{-2})	Astacin Pigments (SPU m^{-2})	Pigment Diversity (D430/D665)
16.4	0.011	3.15	0.007	0.001	0.003	0.003	0.002	4.69
34.0	0.016	2.35	0.058	0.008	0.018	0.020	0.009	3.29
46.6	0.012	1.55	0.099	0.010	0.026	0.043	0.010	3.21
31.5	0.013	2.35	0.054	0.006	0.015	0.022	0.007	3.73
51.7	0.024	2.90	0.033	0.002	0.007	0.013	0.007	3.41
61.7	0.016	1.39	0.096	0.010	0.022	0.031	0.009	3.03
76.8	0.012	1.21	0.097	0.006	0.020	0.037	0.010	2.91
63.0	0.015	1.83	0.075	0.006	0.016	0.027	0.009	3.11
47.9	0.022	1.52	0.114	0.011	0.030	0.052	0.007	2.96
68.0	0.015	1.45	0.158	0.009	0.037	0.064	0.013	2.85
66.8	0.012	0.80	0.163	0.015	0.039	0.060	0.013	2.76
60.5	0.016	1.25	0.148	0.012	0.036	0.059	0.011	2.86
37.8	0.018	2.53	0.054	0.004	0.013	0.023	0.005	3.68
54.2	0.016	1.73	0.104	0.009	0.026	0.039	0.010	3.06
63.0	0.013	1.19	0.120	0.010	0.028	0.046	0.011	2.95
*	*	*	*	*	*	*	*	*
*	0.054	1.48	0.22	*	*	*	*	*
*	0.033	1.55	0.097	*	*	*	*	*
*	0.022	0.51	0.46	*	*	*	*	*
*	0.005	0.34	0.24	*	*	*	*	*
*	0.014	1.42	0.015	0.002	0.010	0.011	0.055	2.48
*	0.042	3.10	0.087	0.002	0.025	0.043	0.021	2.48
*	0.021	0.68	0.33	0.041	0.089	0.134	0.041	2.55
29.4	*	*	0.07	*	*	*	*	2.90
22.4-41.1	*	*	0.093-0.281	0.003-0.016	0.021-0.068	0.049-1.54	0.005-0.020	2.53-2.98

Temperature is one of the major factors required for optimum growth in species and species development. Algae from temperate waters generally require an optimum temperature between 20 and 25 C (Fogg, 1965). Kullberg (1968) reported an inverse relationship between temperature and species diversity in thermophilic algae between 20 and 60 C. Yount (1956) reported proximity to the optimum had the greatest influence on species diversity. In the present study, the mean temperature of water varied from 20.4 C to 27.6 C over time. High temperatures in the present study may have decreased values of species diversity.

Detergents in the activated sludge effluent may have decreased values of species diversity. Carthy and Arthur (1968) found 4-6 mg ℓ^{-1} active detergents in most effluents. Filamentous green phytoplankton were killed by the addition of 6.25 mg ℓ^{-1} detergents to algal cultures (Boney, 1968). One-half to two-thirds of total phosphorus per liter in domestic waste water is from detergent phosphates (Mitchell, 1971). Few filamentous algae were present in the initial stages of microcosms receiving the addition of low and high levels of sewage.

Net Production

The AOV for net production (Pn) revealed that significant interaction existed between nutrients and sewage (Table VI).

A general increase in net productivity was observed with an increase in nutrients with sewage held constant (Table VII). Mean net production in the main study varied from 0.23 to 1.27 g $CO_2 m^{-2}$ per 12 hr^{-1} and increased with nutrient level. The addition of low levels of sewage stimulated production in N1 systems and had little effect in N2 and N3 systems. Maximum production was attained when S1 was added to

the high nutrient systems. The values of productivity for S1 and S3 are significantly lower ($P = 0.05$) than S2 (Table VII) at a constant nutrient level. The lower value for the high sewage level was probably due to over-fertilization while the S1 value was due to nutrient deficiency.

Temporal changes in net production of the different nutrient levels are shown in Figure 3. Net production increased rapidly in all systems initially and then decreased abruptly in N2 and N3 systems, while subsequent change in N1 systems was slight. The rapid increase followed by a decrease was reported in other studies in natural systems (Ryther et al., 1958; McAlister, 1961). The rapid increase of net production initially is probably due to availability of nutrients. The increase in Pn in all levels of nutrients between days 64 and 128 was probably influenced by the increase of nutrient regeneration by grazers (Figure 4). Phytoplankton is directly correlated to the rate of nutrient regeneration (Ketchum et al., 1958; Pomeroy, 1969).

The interaction of nutrients x sewage was expected since nutrients essential for Pn are present in nutrients and sewage. The N1S1 value for Pn was significantly lower ($P = 0.05$) than the other treatment levels (Figure 5). This finding was expected since no nutrients were added to the N1S1 systems.

Values obtained in the present study were similar to values reported in other microcosm studies. Wilhm and Long (1969) used levels of nutrients and light comparable to the levels used in the present study (Table VII) and reported values similar to those in the present study. Beyers (1963) reported considerably higher values of production in microcosms receiving high light intensity.

Lower production values in the present study were probably due to

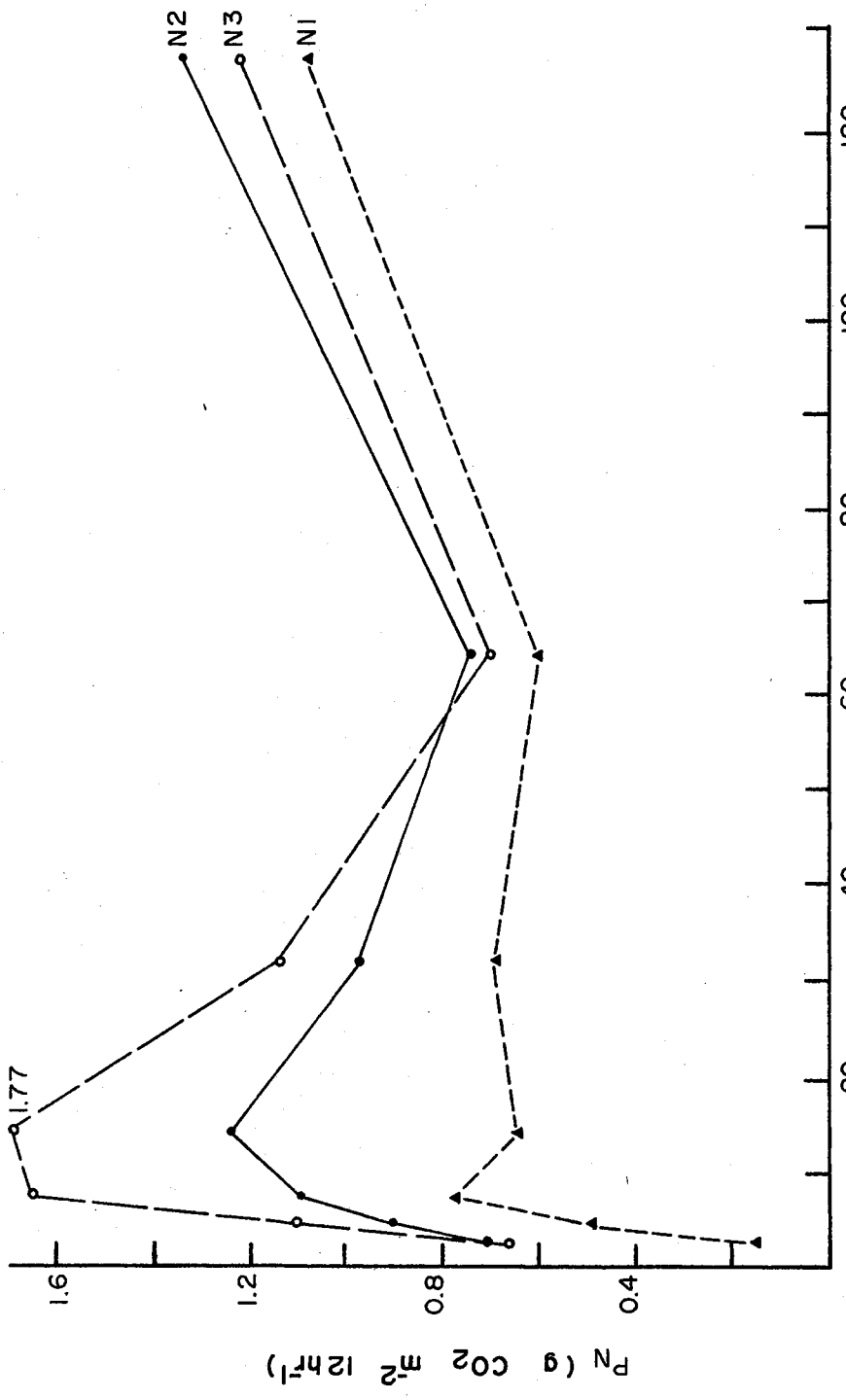

Figure 3. The Mean Fn Values of the Three Nutrient Levels Showing Temporal Variation

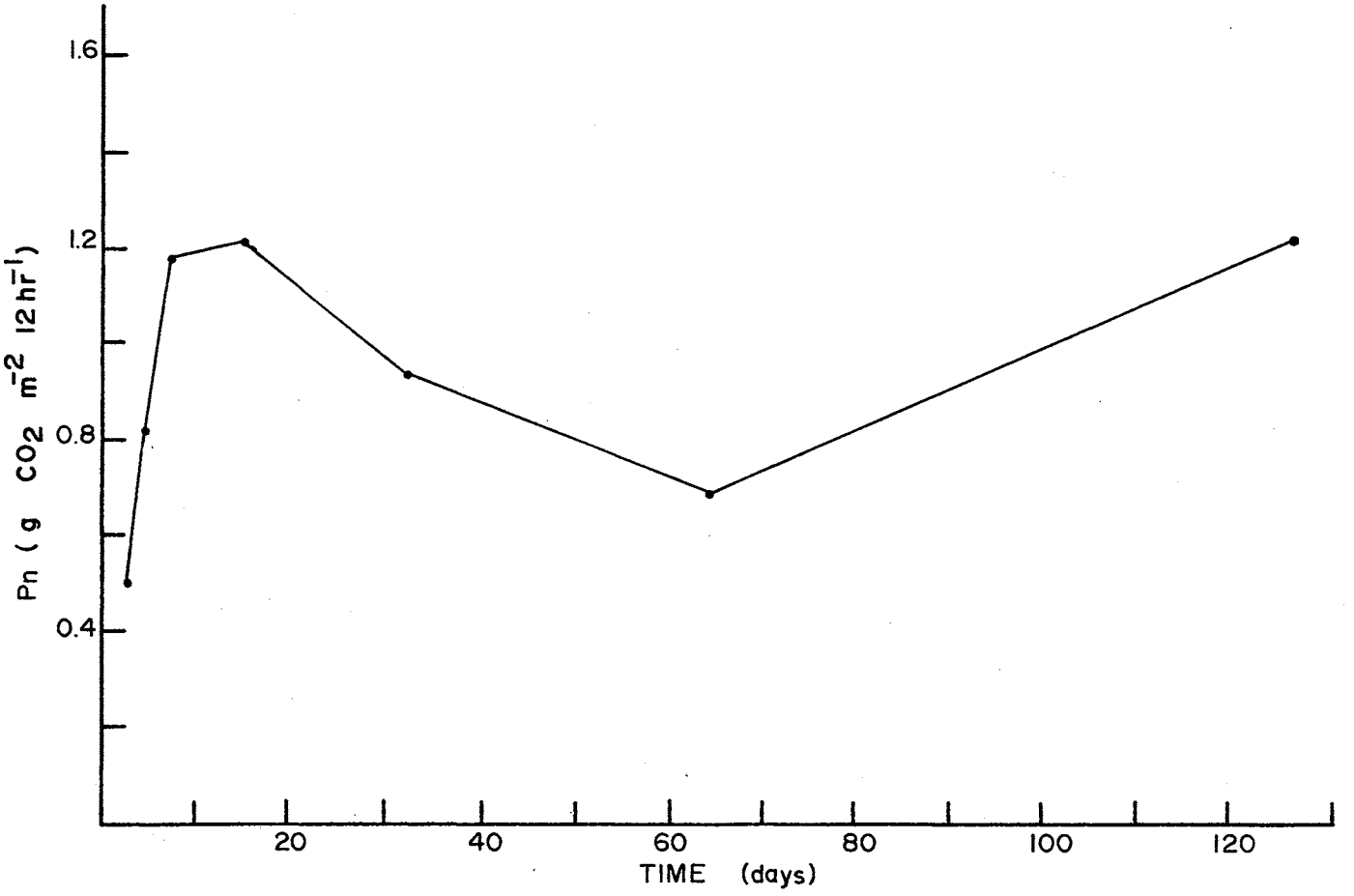

Figure 4. The Temporal Variation of Pn

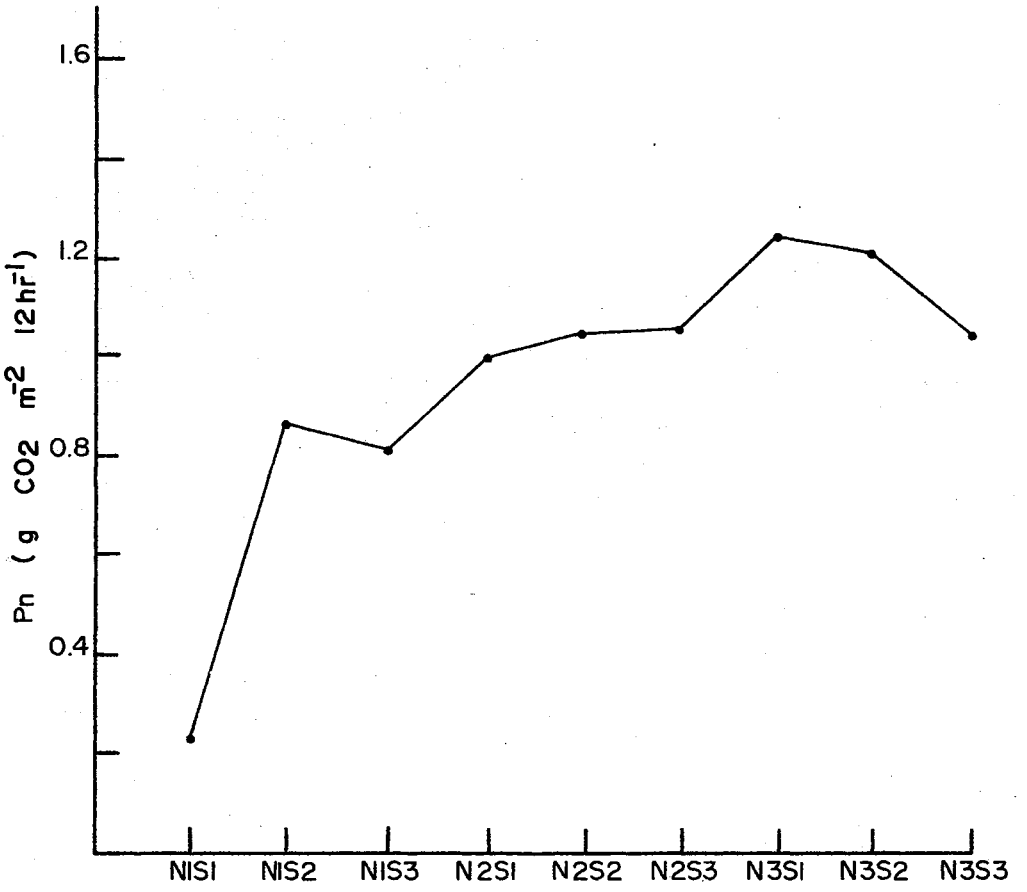

Figure 5. The Effect of Various Treatment Levels on Pn

high temperatures (Table VIII). Warinner and Brehmer (1972) reported a great reduction in the primary production of phytoplankton in river water with an ambient water temperature of 25 C and a diel temperature rise above 3.5 C. In the present study, the experimental lighting system increased the diel temperature a maximum of 6 C and temperatures near 30 C were sometimes observed. The present study probably adds additional evidence to Warinner and Brehmer's (1972) observations that high temperatures and increases in diel water temperature depress net production. The responses of Pn in a marine grass pond and Canyon Ferry Reservoir were comparable to values in the present study (Table VIII).

In selected polluted communities, the net production varied from -12.90 to 27.09 g 12 hr^{-1} (Table VIII). Communities receiving sewage supported higher productivity than those polluted with oil refinery effluents, possibly because sewage decomposition yields higher levels of nutrients (Copeland and Dorris, 1964). The values of the present study were closer to the values found in lakes than to sewage and oil oxidation ponds. However, systems in the present study were not subject to continuous stress and were allowed to recover.

Respiration

Table VI shows the AOV for nighttime respiration (Rni) with significant main effects for sewage, nutrients, and time.

The Rni data were similar to the net production data with an increase in nutrients when the sewage was held constant (Table VII). The highest values for Rni were observed in microcosms at the S2 level when the nutrient level is held constant (Table VII). Mean Rni values in the systems were similar to responses of net production except the high

TABLE VIII

A COMPARISON OF COMMUNITY METABOLISM WITH DIFFERENT LEVELS OF NITRATES AND PHOSPHATES

Source	NO_3 (mg ℓ^{-1})	PO_4 (mg ℓ^{-1})	Pn (g CO_2 m^{-2}/12 hr)	Rni	Pn/Rni
Unpolluted					
Goose Lake (Wetzel, 1966)	0.15	0.69	2.67	*	*
Eniwetok Atoll (Odum and Odum, 1955)	0.02	0.04	19.25	13.75	1.40
Silver Springs (Odum, 1957)					
Winter	0.46	0.05	7.15	1.93	4.13
Spring	0.46	0.05	41.25	3.44	11.99
Marine Grass Pond (Odum, Silers, Beyers, and Armstrong, 1963)	*	0.08	1.38	2.61	0.53
Canyon Ferry Reservoir (Wright, 1959)					
Euphotic Zone	*	0.04	0.33	2.80	0.12
Artificial Stream (McIntire and Phinney, 1965)					
Shade Adapted	0.20	0.08	1.53	3.47	0.44
Light Adapted	0.20	0.08	2.59	2.21	1.17
Polluted					
Sollerod Sø Denmark (Wetzel, 1966)	enriched-sewage	enriched-sewage	5.24	*	*
Farm Pond Below Swine Barn (Copeland and Whitworth, 1963)	enriched	enriched	3.99	6.09	0.66
Six Sewage Oxidation Ponds (Bartsch and Allum, 1957)	enriched-sewage	enriched-sewage	*	15.18 to 24.74	*
Oil Refinery Holding Ponds-Arkansas City, Kansas (Dorris, Patterson, and Copeland, 1962)					
Oil Pond 3	20.9**	6.55**	- 9.49	29.7	-0.32
Oxidation Pond 2			-12.90	35.42	-0.36
Oxidation Pond 3			- 8.21	25.51	-0.32
Oxidation Pond 4			- 6.52	17.79	-0.37
Sewage Oxidation Ponds (Ostwald et al., 1957)	enriched-sewage	enriched-sewage	17.05 to 27.09	*	*
Borax Lake (Wetzel, 1964)					
Phytoplankton	enriched	enriched	0.91	*	*
Periphyton	enriched	enriched	2.68	*	*
Eutrophic Lake Suwa (Hogetsu and Ischimura, 1954)	enriched	enriched	2.39	0.96	2.49

*This data is not available.

**The nitrogen and phosphorus data are the chemical analysis of the effluent before it was passed through the ponds. Nitrogen was measured as ammonia only. When the effluent passed through the final pond (oxidation pond 4), the concentration of ammonia was reduced by 84% in the summer and 57% in the winter.

nutrient microcosms receiving S1. The Rni value for N3S1 was lower than the Pn value.

The temporal trend for nighttime respiration was similar to the Pn data (Figure 6). The nighttime respiration (Rni) value for day 2 was significantly lower ($P = 0.05$) than day 16 and day 128 (Figure 6). This result was expected since biomass was lower in the initial stages of the present study. None of the other values were significantly different.

An interaction in sewage x nutrients existed (Table VI). This interaction was expected since the increased availability of nutrients through sewage and nutrients caused increased biomass in the systems. With increased biomass in the systems over time, respiration increased. The Rni sewage x nutrients interaction was similar to that observed for Pn (Figure 7).

Other investigators using the microcosm approach reported similar values for respiration. Values (0.21 to 1.25 g CO_2 m^{-2} 12 hr^{-1}) of microcosms in the present study compare favorably with those using similar levels of nutrients (Wilhm and Long, 1969). A mean Rni of .372 g CO_2 m^{-1} 12 hr^{-1}, reported by Cooke (1967), for enriched microcosms was within the Rni range of the present study.

Values of Rni in various communities (Table VIII) receiving various amounts of nitrates and phosphates ranged from 1.93 to 35.42 g $CO_2 m^{-2}$ 12 hr^{-1}. The highest rates of respiration were reported in sewage oxidation (Bartsch and Allum, 1957) and industrial treatment ponds (Dorris, Patterson, and Copeland, 1962). These high rates of respiration were probably due to the high oxidation rate of organic substances.

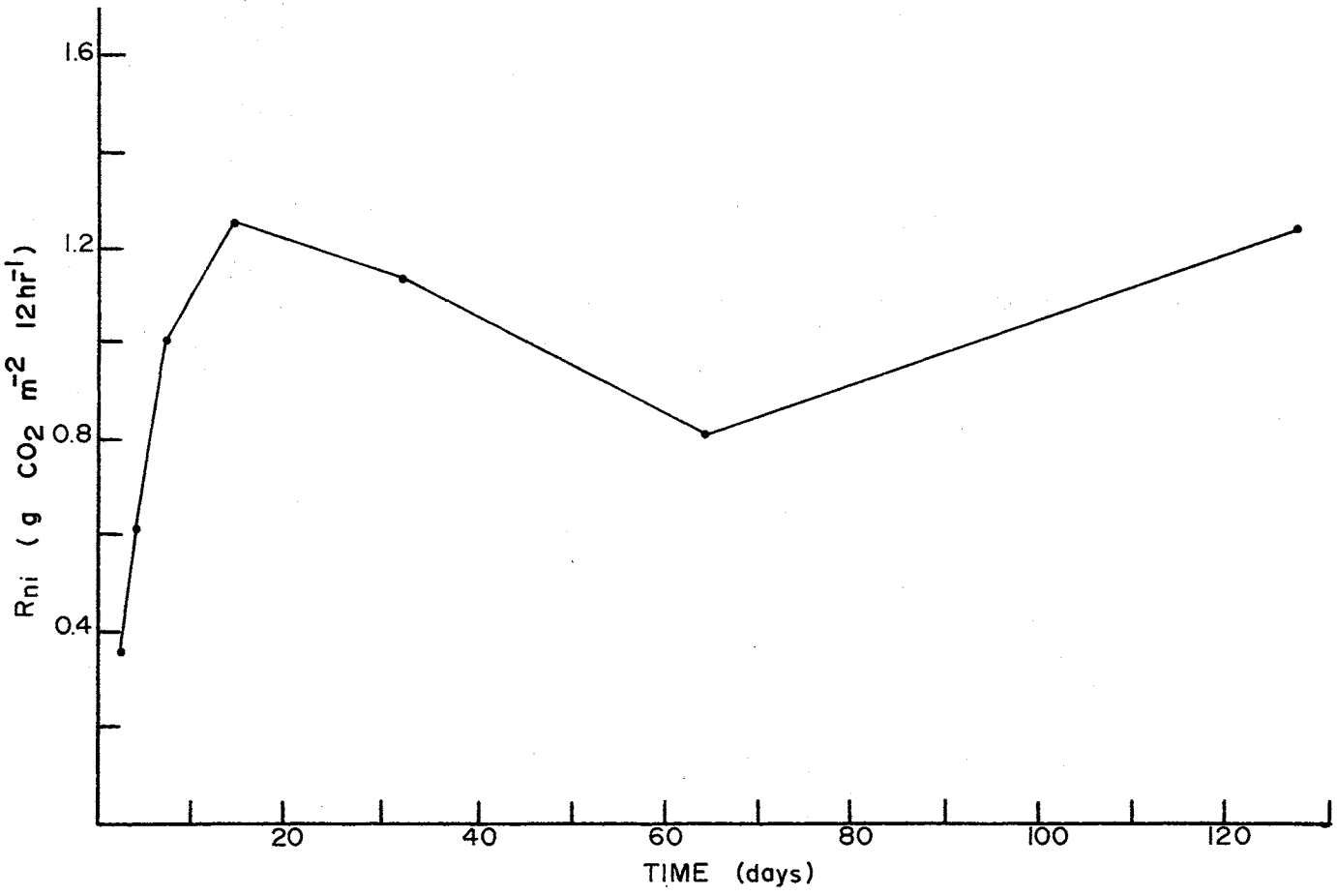

Figure 6. The Change in Nighttime Respiration Over Time

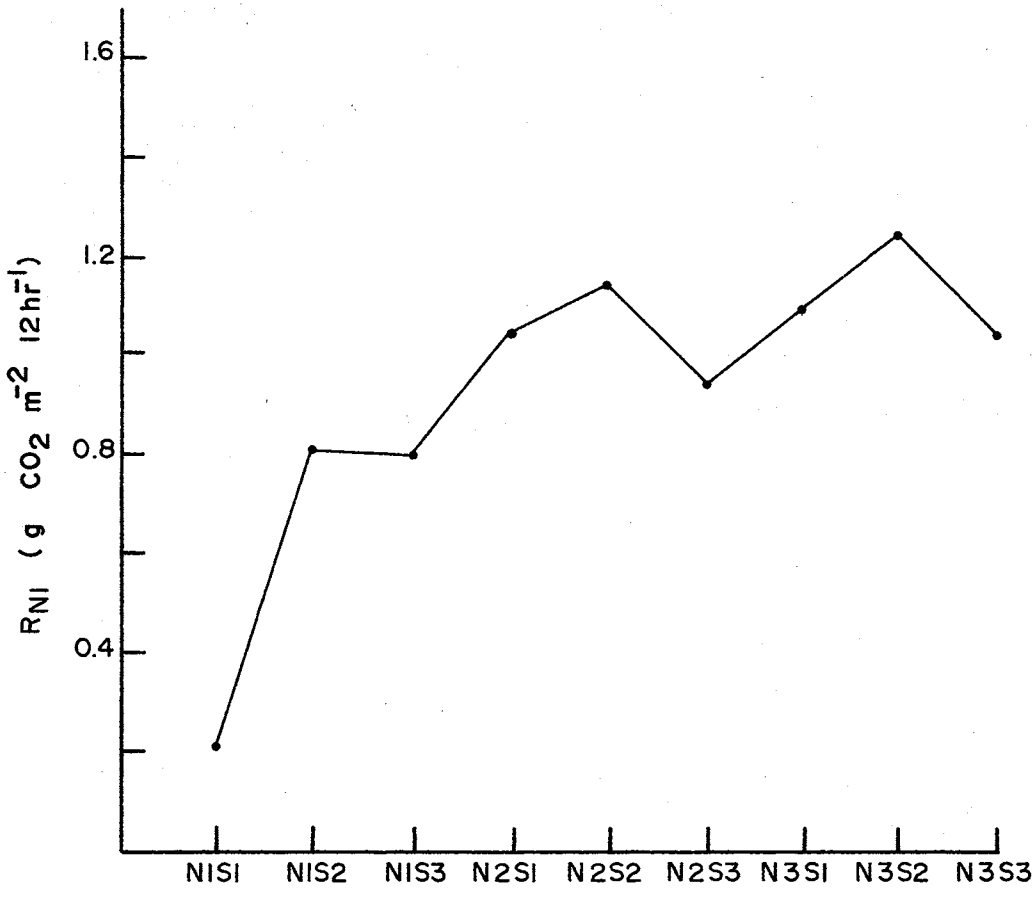

Figure 7. The Change in Nighttime Respiration With Varying Nutrient and Sewage Levels

Production to Respiration Ratio

No interactions (Table VI) in the net production to nighttime respiration ratio (Pn/Rni) were found and time was the only significant main effect.

The highest value for Pn/Rni was observed on day 4 (Figure 8) with a general decrease until day 32 and little change after that date, values on day 4 were significantly ($P = 0.05$) higher than the other values for Pn/Rni (Figure 8). The net production value (Figure 4) was higher than the Rni value (Figure 6) on day 4 because the initial increase of Pn was more rapid than Rni.

The mean Pn/Rni in the present study varied from 0.93 to 1.14 (Table VII). The net production to respiration ratios observed in the present study were similar to those observed in other microcosm studies (Beyers, 1963; Butler, 1964; Wilhm and Long, 1969 - Table VII) and an artificial light adapted stream (Table VIII). Cooke (1967) reported a Pn/Rni value of 1.22 in enriched microcosms.

In organically polluted communities, the Pn/Rni ratio is usually less than 1 (Odum, 1969). The negative net production to respiration ratios in the oil refinery holding ponds was probably caused by high concentrations of organic matter in the ecosystem (Table VIII). The Pn/Rni ratios in the present study probably would be heterotrophic if there had been a continuous addition of sewage.

Dry Weight

The AOV for dry weight showed main effects for nutrients, sewage, and time, but no interactions were significant (Table VI). The mean dry

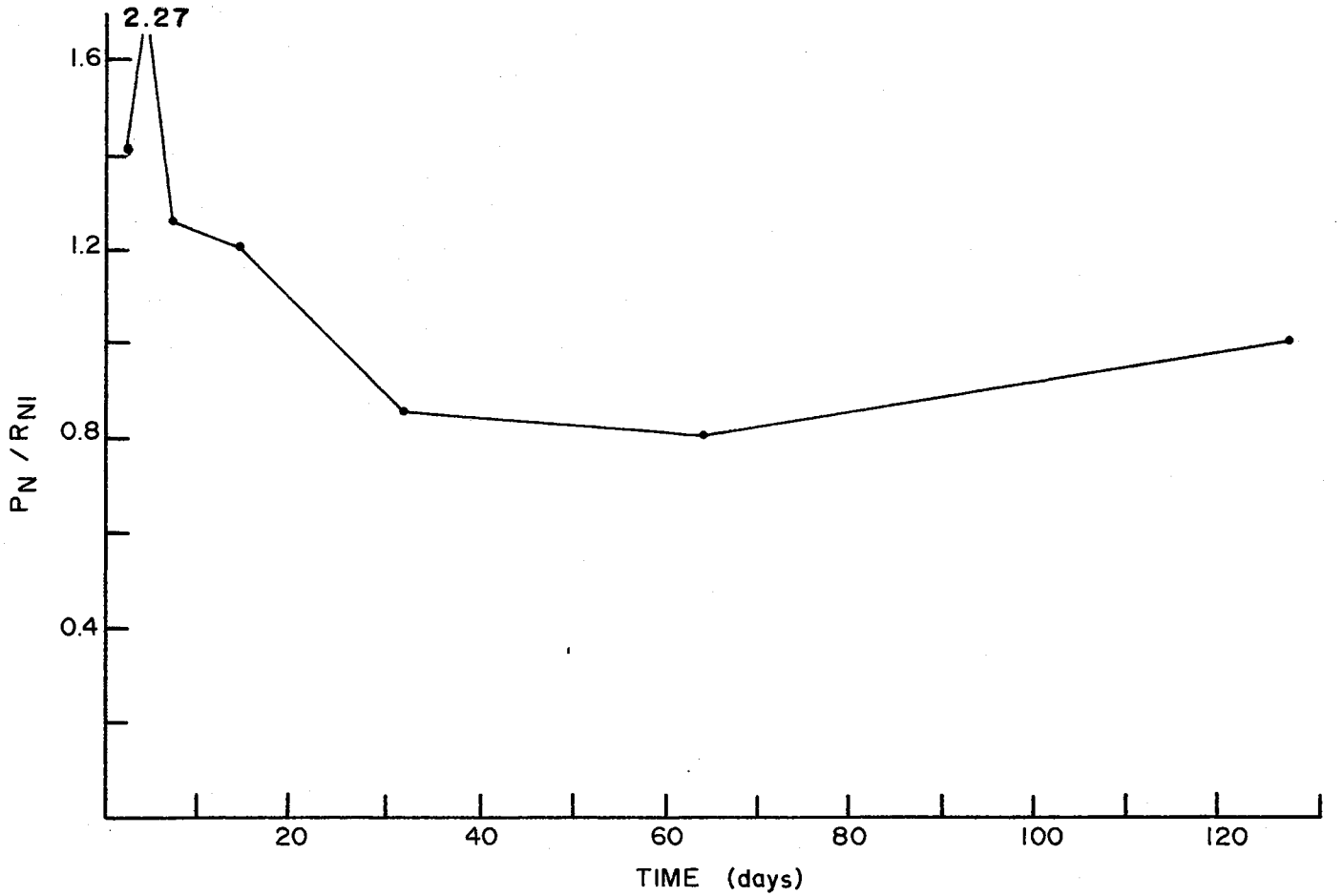

Figure 8. The Effect of Time on Net Production/Nighttime Respiration (Pn/Rni)

weight values for nutrients and sewage were all significantly different ($P = 0.05$).

A general increase in dry weight with an increase in nutrients was observed when sewage was held constant (Table VII). Wilhm and Long (1969) reported high significant differences ($P = 0.01$) in dry weight among systems receiving different levels of nutrients. Increased availability of nutrients within optimal ranges allows increased growth in phytoplankton.

When the nutrient level was held constant in the present study, a sewage increase resulted in an increase in dry weight at the N1 and N2 levels. At the N3 level, S2 and S3 are similar and greater than S1 (Table VII). The higher sewage levels cause higher biomass due to increased availability of nutrients in the N1 and N2 levels of microcosms, but may be limiting at the high nutrient levels.

Dry weight increased abruptly through day 16, exhibited a slight decrease between days 16 and 32, and then increased slightly throughout the remainder of the study (Figure 9). The abrupt increase of dry weight through day 16 was probably due to the initial uptake of nutrients after the sewage was added. The decrease from day 16 to 32 could be due to nutrient depletion. Grazing organisms were first observed on day 32 and increased through day 128. The appearance of grazers suggest that these organisms were regenerating nutrients and resulting in increased levels of growth after day 32. Mitchell (personal communication) observed the same phenomena in sewage treated microcosms.

Greater biomass was produced in microcosms receiving high levels of nutrients, sewage, and TDIS (Table VII) than in systems receiving other levels of nutrients, sewage, and TDIS. Higher dry weights at higher

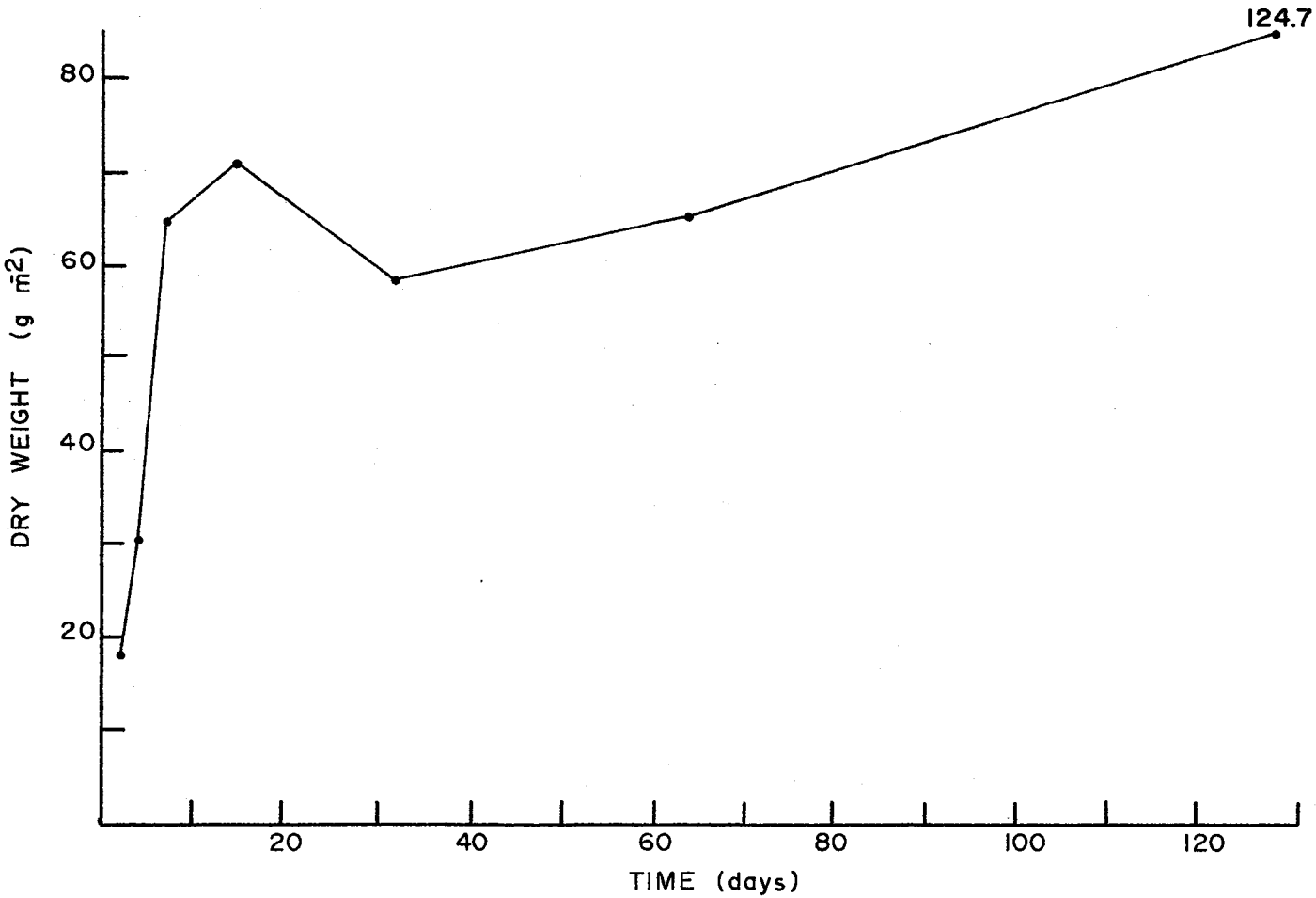

Figure 9. The Mean of the Variation of Dry Weight Over Time

levels of nutrients and TDIS were reported by Butler (1964) while, Wilhm and Long (1969) found higher dry weight with increasing nutrients in microcosms. Using similar nutrient levels, a mean dry weight value of 58.0 g m^{-2} was found in N3S1 microcosms of the present study compared to 44.0 g m^{-2} in an enriched artificial stream (Kehde, 1970). The mean dry weight yields of S2 and S3 (1.04 and 1.34 g ℓ^{-1}) on day 128 in the present study compared favorably with dry weights of 1-2 g ℓ^{-1} from sewage cultures (Allen, 1955).

Dry weights from the present study compared more favorably with euthrophic Lake Suwa than other community in Table IX. The dry weights of phytoplankton in Sylvan and Goose Lake were probably higher due to greater light intensity, high phosphate levels, and high TDIS. The dry weight of macrophytes in the Test and Chest Rivers was greater due to high inorganic concentrations.

Ash-Free Weight

Main effects in sewage, nutrients, and time were observed in the present study for ash-free weight (Table VI).

An increase in nutrients with sewage held constant caused N2 to have a greater ash-free weight than N1 (Table VII). The N1 mean was significantly different ($P = 0.05$) from the low and high nutrient levels. Lower ash-free weight in N3 than in N2 indicates over-fertilization. The ash-free weight data from the different sewage levels with nutrients constant were similar to that observed for dry weight in the present study (Table VII).

Interaction of ash-free weight was present in sewage x time (Table VI). The trend for temporal variation of ash-free weight was similar to

TABLE IX

BIOMASS VALUES FROM SELECTED POLLUTED AND UNPOLLUTED COMMUNITIES

Source	NO_3 (mg ℓ^{-1})	PO_4 (mg ℓ^{-1})	Dry Weight (g m^{-2})	Ash-Free (g m^{-2})
Polluted				
Eutrophic Lake Suwa (Hogetsu and Ishimura, 1954)	enriched	enriched	28.8	18.2
Fertilized Ponds				
Pond 1	0.5	0.039	*	13.5
Pond 2	1.2	0.039	*	23.5
Pond 3	1.6	0.115	*	101.5
Pond 4 (McIntire and Bond, 1959)	3.7	0.354	*	39.5
Unpolluted				
Sylvan Lake (Wetzel, 1966)	0.2	2.40	590.0	*
Goose Lake (Wetzel, 1966)	0.2	0.69	561.4	*
Test River (Owens and Edwards, 1962)	3.6	0.04	233.1	88.3
Chess River (Owens and Edwards, 1962)	3.8	0.04	323.1	130.9

*Data not available.

the trend for dry weight. Ash-free weight increased in S1 and S2 except from day 16 to 32 (Figure 10). The high sewage produced a similar trend except the decrease occurred from day 32 to day 64. Interaction appears to have occurred from day 8 throughout the remainder of the experiment. Significant differences ($P = 0.05$) between sewage levels were observed on days 16, 32, and 64. On day 32, all three levels of sewage were significantly different ($P = 0.05$) in ash-free weight.

The mean ash-free percentage of dry weight for N1, N2, and N3 are 80%, 93%, and 76%, respectively, and the mean of all values was 82.7%. The low nutrient level produced the most ash-free weight. Strickland (1960) reports the ash-free percentage of dry weight from field values to be 85% $\pm$ 5% for green algae. Kehde (1970) reports ash-free percentages of 62.6% and 58.5% in an artificial stream with a mixed Chlorophyta and Cyanophyta composition.

A mean of 12.88 g m^{-2} was found in an enriched microcosm study (Cooke, 1967). A value of 19.2 g m^{-2} (40% of the ash-free weight) was found in enriched N3S1 of the present study. Kehde (1970) reported a mean value of 29.4 g m^{-2} ash-free weight in an artificial stream compared to 47.9 g m^{-2} in N3S1. The higher value in the present study was probably due to the increased length of the present experiment, 128 days compared to 92.

Ponds 1, 2, and Lake Suwa (Table IX) compared favorably with the lower nutrient levels of the present study. The other ponds and the two river communities were higher than the values of the present study. Other factors such as light and temperature probably caused differences in the ash-free weights of the river communities and ponds 3 and 4.

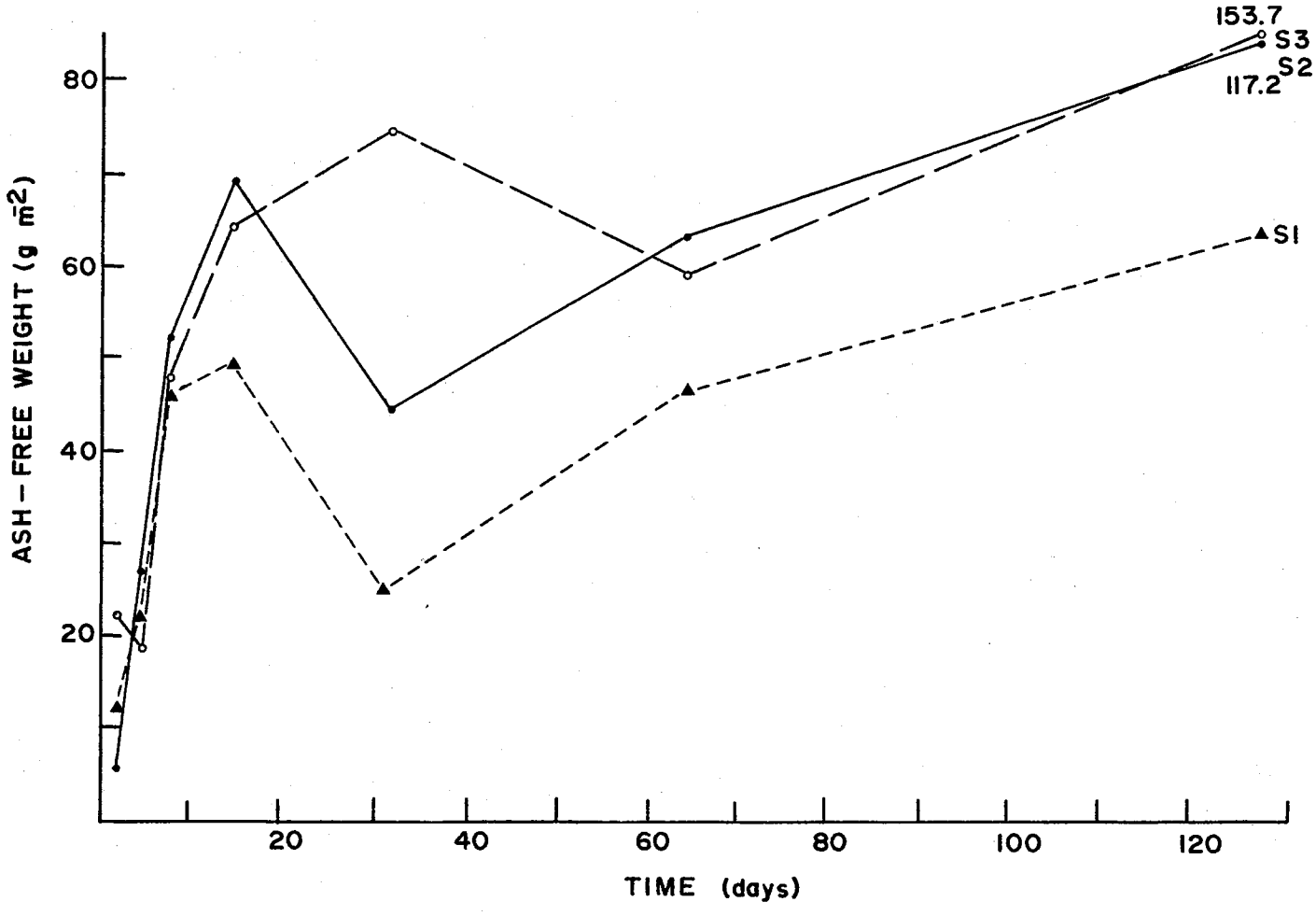

Figure 10. The Temporal Variation of Ash-Free Weight at the Three Sewage Levels

Pn/Dry Weight

No main effects or interactions were found in the Pn/dry weight (Pn/B) ratios of the present study (Table VI).

Pn/dry weight values are similar from the nutrient levels in the present study (Table VII). The net production/dry weight values from the low and high nutrient levels with S1 was higher than S2 and S3 at these nutrient levels. This may indicate a greater efficiency of biomass at the S1 level.

When the nutrient level is held constant, an increase in sewage causes a decrease in Pn/dry weight in N2 and N3. In N1 systems, S1 and S3 are similar and less than S2 (Table VII).

Temporal variation in Pn/dry weight was slight and the different nutrient levels were not significantly different ($P = 0.05$). Margalef (1958) reported three to four complete cycles of phytoplankton during the year in a bay with flowing waters. Each cycle had the following stages and Pn/B ratio: (1) small-celled diatoms and Chlorophyta - 0.5 to 2.0, (2) large celled diatoms - 0.2 to 0.5, (3) larger free-swimming species - less than 0.2. This succession was not observed in the present study except the Chlorophyta in step 1. Other investigations (Butler, 1964; Wilhm and Long, 1969) involving microcosms studies did not observe the trends described by Margalef. Cooper (1972) reported no spatial or temporal trend in the Pn/B ratio in a polluted stream.

Assimilation Numbers

The effect of nutrients, sewage, time, and nutrients x time of assimilation numbers were highly significant ($P = 0.01$) (Table VI).

The mean assimilation number values for sewage were all significantly different ($P = 0.05$).

Assimilation numbers generally decreased with an increase in nutrients when sewage was held constant (Table VIII). A decrease in assimilation numbers was observed with an increase in sewage when nutrients were held constant (Table VII). The addition of higher levels of sewage caused lower assimilation numbers due to increased chlorophyll (Odum, 1958).

The interaction of nutrients x time was significant (Figure 11). The nutrient levels were not significantly different on days 2, 16, 64, and 128. On days 4 and 8, the assimilation number of N1 was different from the low and high levels of nutrients. N1 and N2 were different from the high level of nutrients on day 32. These results suggest that the lack of nutrients in the N1 levels inhibited chlorophyll formation at the beginning of the experiment. The significant differences of assimilation numbers in N1 and N2 from the high level of nutrients appeared to be caused by the rapid formation of chlorophyll in the N3 systems as the result of the availability of nutrients.

The assimilation numbers reached their maxima on day 4 in N1 and N3 systems and day 16 in the N2 systems with a general decrease afterwards (Figure 11). This finding indicated a decrease in the efficiency of chlorophyll per unit biomass. The same phenomenon was noted by Wilhm and Long (1969). The observation that N1 generally has a higher assimilation number on each date suggests a greater efficiency in production. Cooke (1966) reported the assimilation numbers increased and then leveled off. The values in the present study were similar to those reported by Butler (1964) and Wilhm and Long (1969).

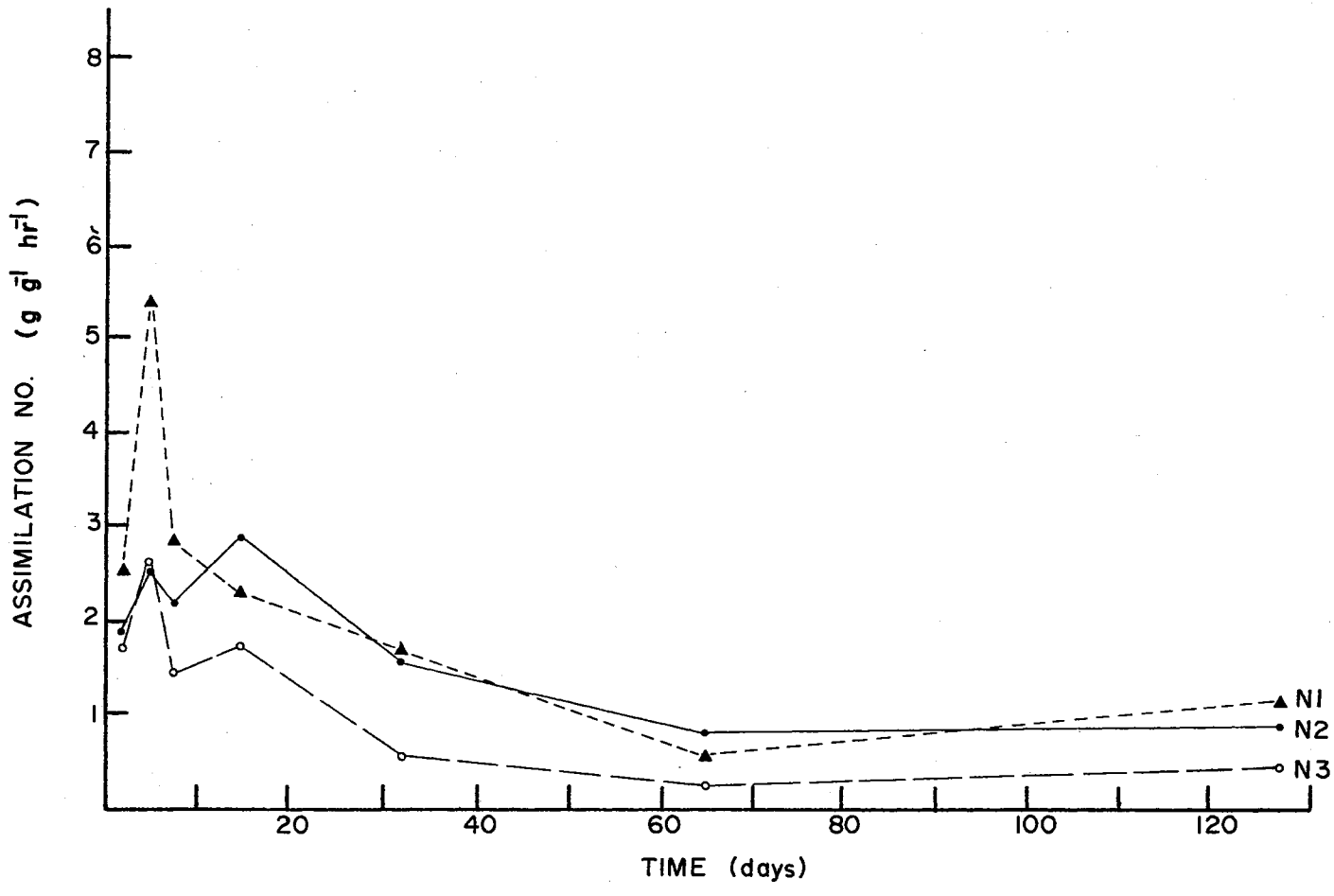

Figure 11. The Effect of Time on the Assimilation Numbers at the Three Nutrient Levels

Assimilation numbers in the present study were similar to those found in polluted and unpolluted communities with similar nutrient levels (Table X). Assimilation values of 5 and 7 were found in polluted Lake Washington (Edmondson et al., 1956) and in a fertilized tank (Edmondson and Edmondson, 1947).

Chlorophyll a

Chlorophyll a content was significantly affected ($P = 0.01$) by sewage, nutrients, and time (Table VI). Interactions were present in nutrients x time and sewage x time.

Chlorophyll a generally increased with an increase in nutrients when sewage was held constant (Table VII). The greater availability of nutrients resulted in higher levels of chlorophyll a (Figure 12). When the nutrient level is held constant, a sewage increase results in an increase in chlorophyll a at N1 but chlorophyll a is similar in S2 and S3 and exceed S1 in N2 and N3 systems (Table VII). Higher concentrations of nutrients in the upper sewage levels (S2 and S3) probably allowed the higher concentrations of chlorophyll a.

A general increase in chlorophyll a over time was observed in the present study except from day 8 to 16 (Figure 12). Higher concentrations of nutrients in the S2 and S3 sewage levels probably allowed a prolonged increase of chlorophyll a after day 32 and grazers had a greater chance of releasing the available nutrients of those levels (Figure 13).

Comparable values of chlorophyll a were observed in the present study and selected polluted and unpolluted environments (Table X). The values for chlorophyll a in the present study were slightly lower than

TABLE X

PIGMENT RELATED VALUES FROM POLLUTED AND NON-POLLUTED ENVIRONMENTS AND COMMUNITIES

Source	NO_3 (mg ℓ^{-1})	PO_4 (mg ℓ^{-1})	Assimilation No. (g g^{-1} hr^{-1})	Chlorophyll a (g m^{-2})
Polluted				
Lake Washington (Edmondson et al., 1956)	enriched	enriched	5	0.015-0.025
Sewage Pond (Kadoka, S. D., Bartsch and Allum, 1957)	enriched	enriched	2	1.5
Sewage Culture (Ludwig et al., 1951)	enriched	enriched	2	*
Borax Lake (Wetzel, 1964)	enriched	enriched	*	0.362
Unpolluted				
Long Island Sound (Riley, 1956)	*	*	1-2	0.1-0.6
Blue-gree algae mat in flowing Microcosm (Odum and Hoskin, 1957)	0.05	0.005	1.0-3.00	0.03-0.38
Artificial Stream (McIntire and Phinney, 1965)				
Shade Adapted	0.20	0.08	*	0.98
Light Adapted	0.20	0.08	*	0.70

*Data not available.

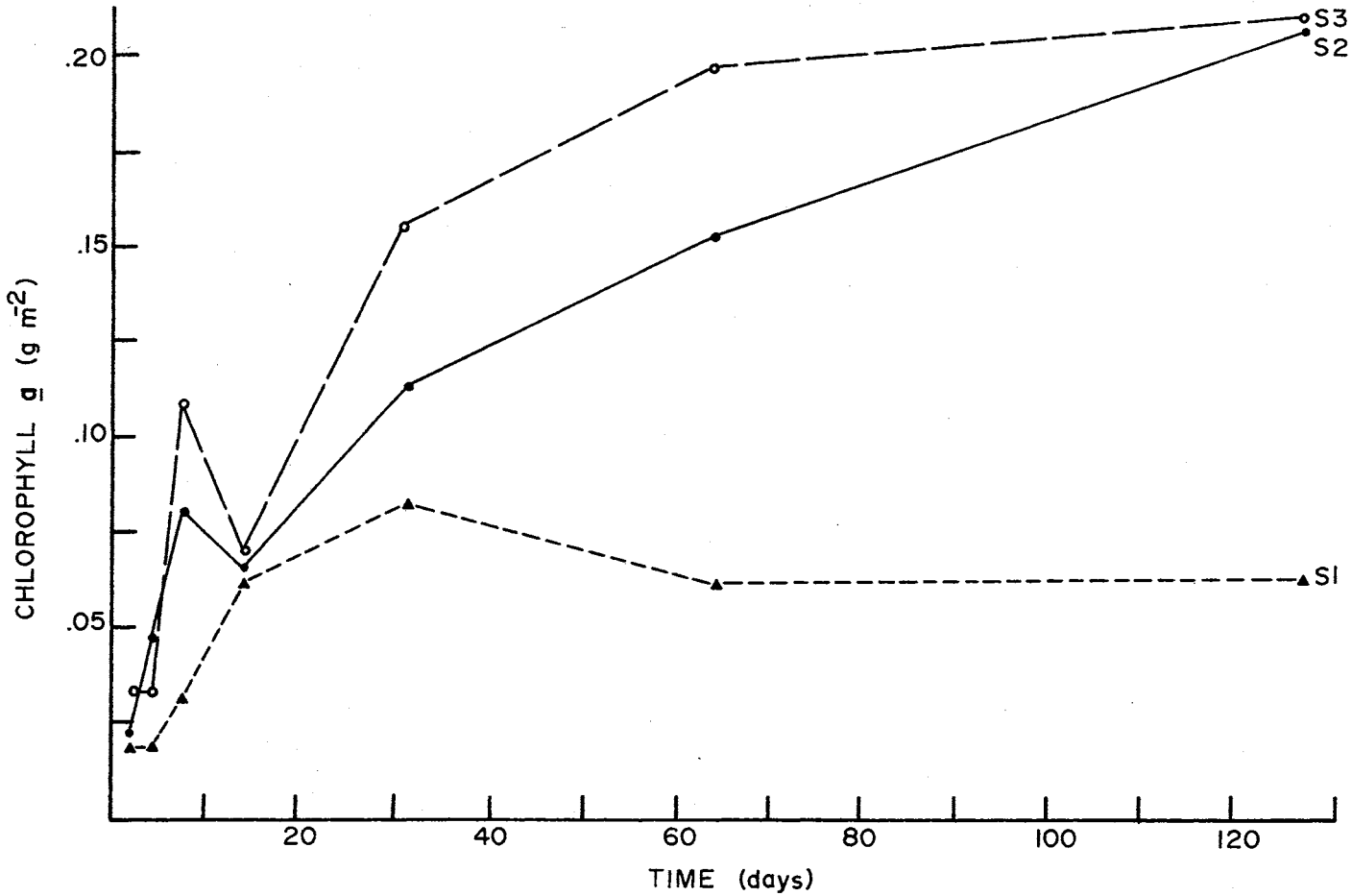

Figure 12. Temporal Variation of Chlorophyll a Values of the Three Sewage Levels Averaged Over All Nutrient Levels

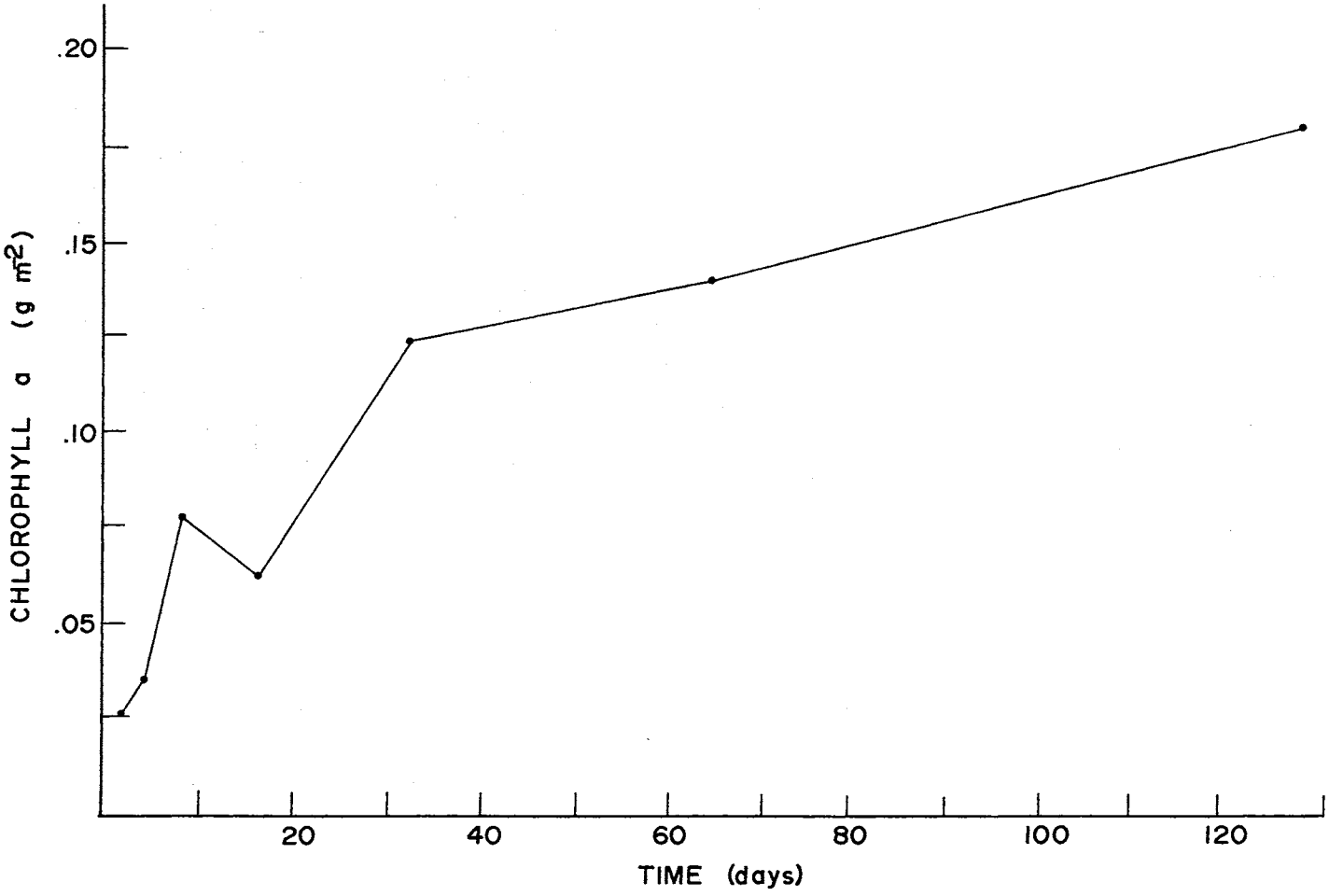

Figure 13. The Temporal Variation of Chlorophyll a

those observed by Wilhm and Long (1969) (Table X). The chlorophyll a value, determined in N3S1 (Table VII), was comparable to a value of 0.105 of an enriched nutrient system in microcosms of another experiment (Cooke, 1967). Butler's values (1964) for chlorophyll a were slightly higher than data in the present study (Table VII). However, Butler used a higher light intensity. The use of higher light intensities has resulted in higher concentrations of chlorophyll a (Odum, 1958).

Various correlations between chlorophyll a and other factors have been calculated. A significant correlation between chlorophyll a and Pn ($r = 0.46$) and between chlorophyll a and dry weight ($r = 0.64$) were calculated in the present study. Studies using a ^{14}C method by Odum (1958) in the North Atlantic revealed little correlation between Pn and chlorophyll a. Wilhm and Long (1969) found a higher correlation between Pn and chlorophyll a. Wilhm and Long (1969) found a higher correlation between chlorophyll a and dry weight than between chlorophyll a and Pn.

Chlorophyll b

The effects of sewage and time were significantly different ($P = 0.05$) for chlorophyll b (Table VI). Time had a greater effect on chlorophyll b than sewage.

The values of chlorophyll b among nutrient levels with the sewage level held constant are similar and not significantly different ($P = 0.05$) (Table VII). Concentrations of chlorophyll b exhibited little change through day 32 and increased after this time (Figure 14). The concentrations are not significantly different until day 16 and all values are different for each observation period after day 16.

Mean chlorophyll b values varied from 0.001 g m^{-2} to 0.048 g m^{-2} in

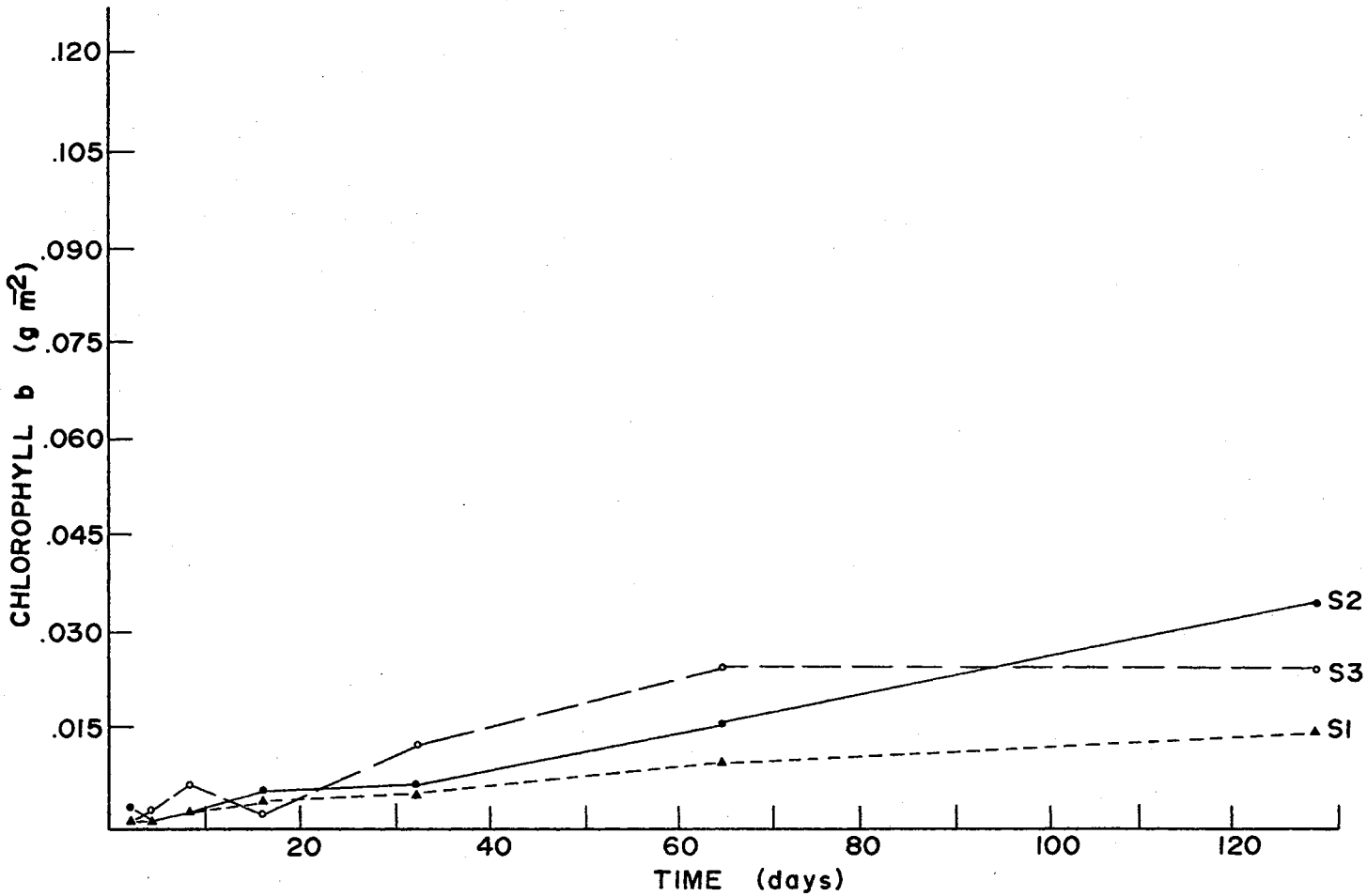

Figure 14. Temporal Variation of Chlorophyll b Values of the Three Sewage Levels Averaged Over All Nutrient Levels

the present study (Table VII). The mean chlorophyll b values of the present study were similar to a microcosm study by Wilhm and Long (1969) (Table VII). Comparable data of 0.003 to 0.016 g m^{-2} were found in a eutrophic lake (Spangler, 1970).

Chlorophyll c

The effects of sewage and time upon the concentration of chlorophyll c were significantly different at the ($P = 0.01$) level (Table VI).

The concentration of chlorophyll c generally increased with an increase in nutrients when the sewage level was held (Table VII). An increase in sewage generally results in an increase in chlorophyll c concentration, when the nutrient level is held constant (Table VII). However, at the N2 and N3 levels, the low and high sewage levels produced similar values of chlorophyll c. Chlorophyll c generally increased through time (Figure 15).

The range of the chlorophyll c values in the present study was from 0.001 to 0.091 g m^{-2} (Table VII). These values were within the ranges found in a microcosm study (Wilhm and Long, 1969) and in a eutrophic lake (Spangler, 1970).

Non-Astacin Pigments

The main effects of nutrients and time plus an interaction of nutrients x time were present in non-astacin pigments (Table VI).

When sewage is held constant, non-astacin pigments generally increased with an increase in nutrients (Table VII). An increase in non-astacin concentrations generally occurred with increases in sewage when nutrients are held constant (Table VII).

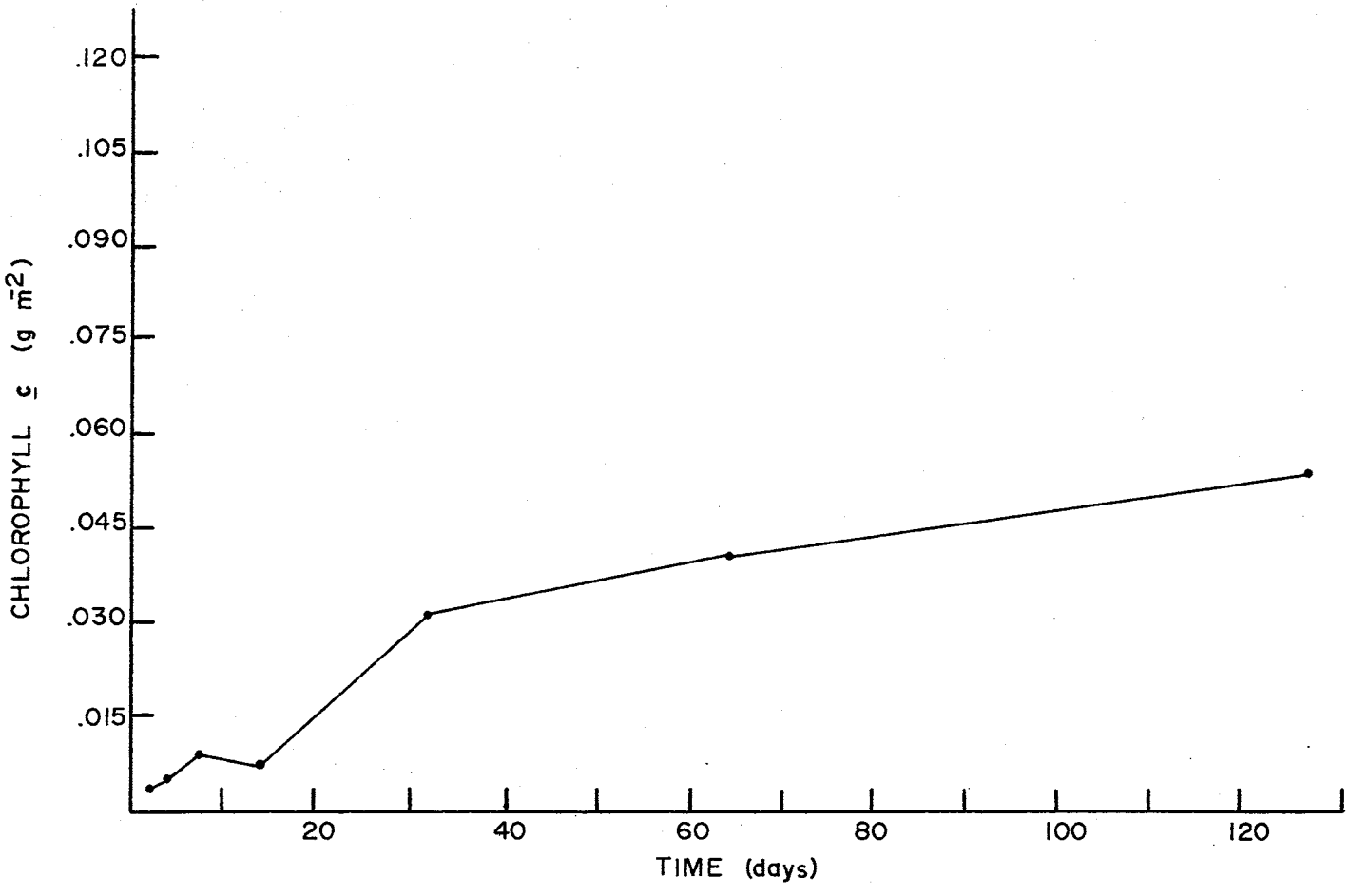

Figure 15. The Temporal Variation in Chlorophyll c Concentrations Averaged Over Nutrient and Sewage Levels

Non-astacin pigments generally increase through day 32 and appear to have reached an asymptote after this (Figure 16). No significant differences ($P = 0.05$) between the concentrations of non-astacin pigments among the nutrients levels were present on day 2. On the second observation date, N1 was different from N2, but not N3. All treatments were significant on day 8. The analysis of non-astacin pigments in N1 and N2 was significantly different from the high level of nutrients on days 16, 32, and 64. The last observation revealed N2 different from N3, but not N1.

Non-astacin pigment values in the present study were generally within the range of 0.011 to 0.134 found in microcosms by Wilhm and Long (1969). Spangler (1970) found a higher range of values from 0.049 to 1.54 in a eutrophic lake. These higher values may be due to individual variation and higher TDIS found in the lake or to the import of allochthonous materials from other sources (e.g., streams). Import was not possible in the present experiment because closed systems were used.

Astacin Pigments

Time was the only effect present ($P = 0.01$) for astacin pigment with no interactions (Table VI).

Little change occurred in the astacin pigment concentrations with an increase in the nutrient level with sewage held constant (Table VII). Astacin pigment values were similar with an increase in sewage when the nutrient levels were held constant (Table VII).

Little temporal variation in astacin pigment was observed until day 16 and a general increase occurred after this observation (Figure 17). No significant differences among mean astacin pigment temporal values

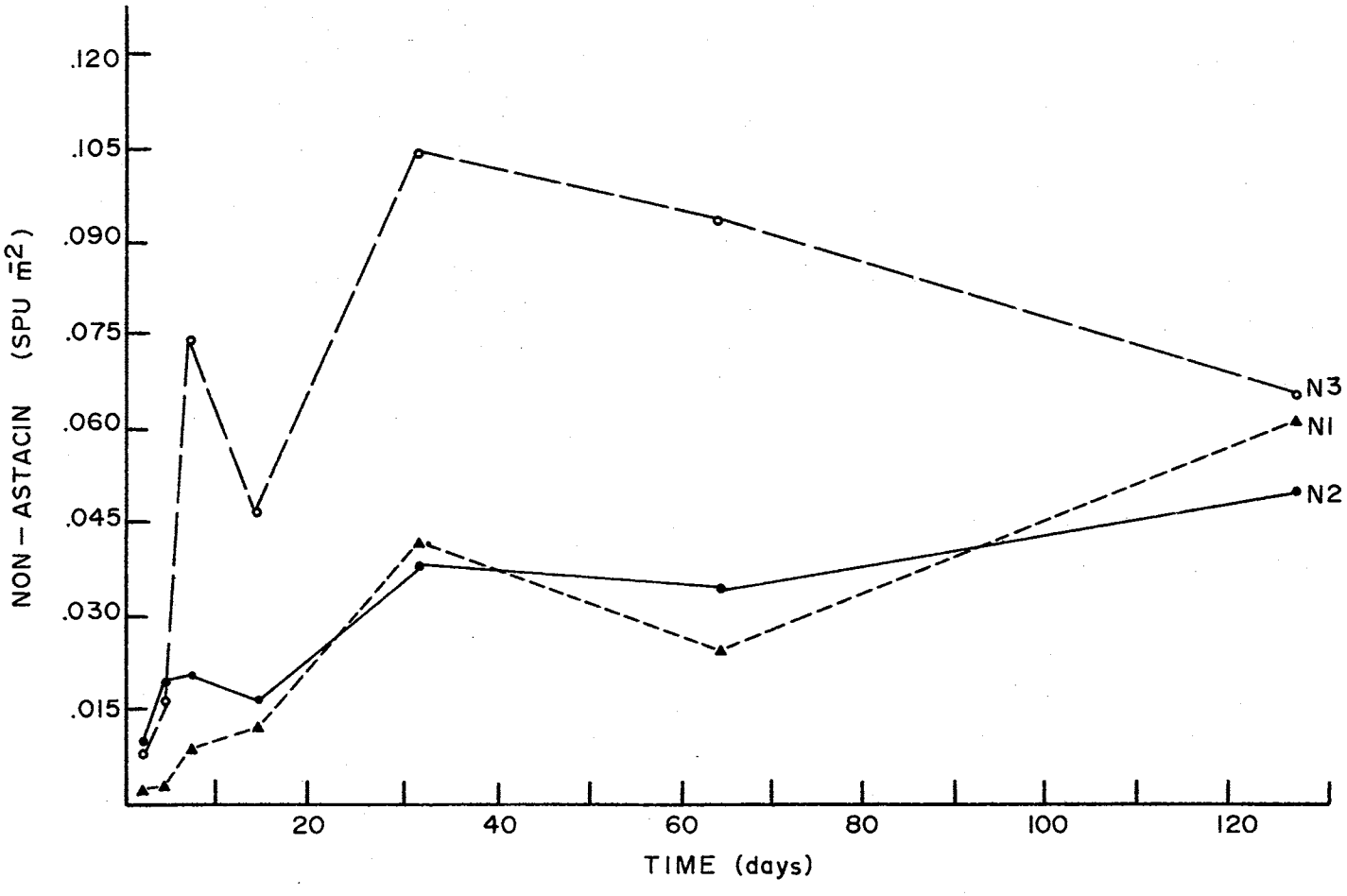

Figure 16. The Temporal Effect of the Three Nutrient Levels on Non-Astacin Pigments

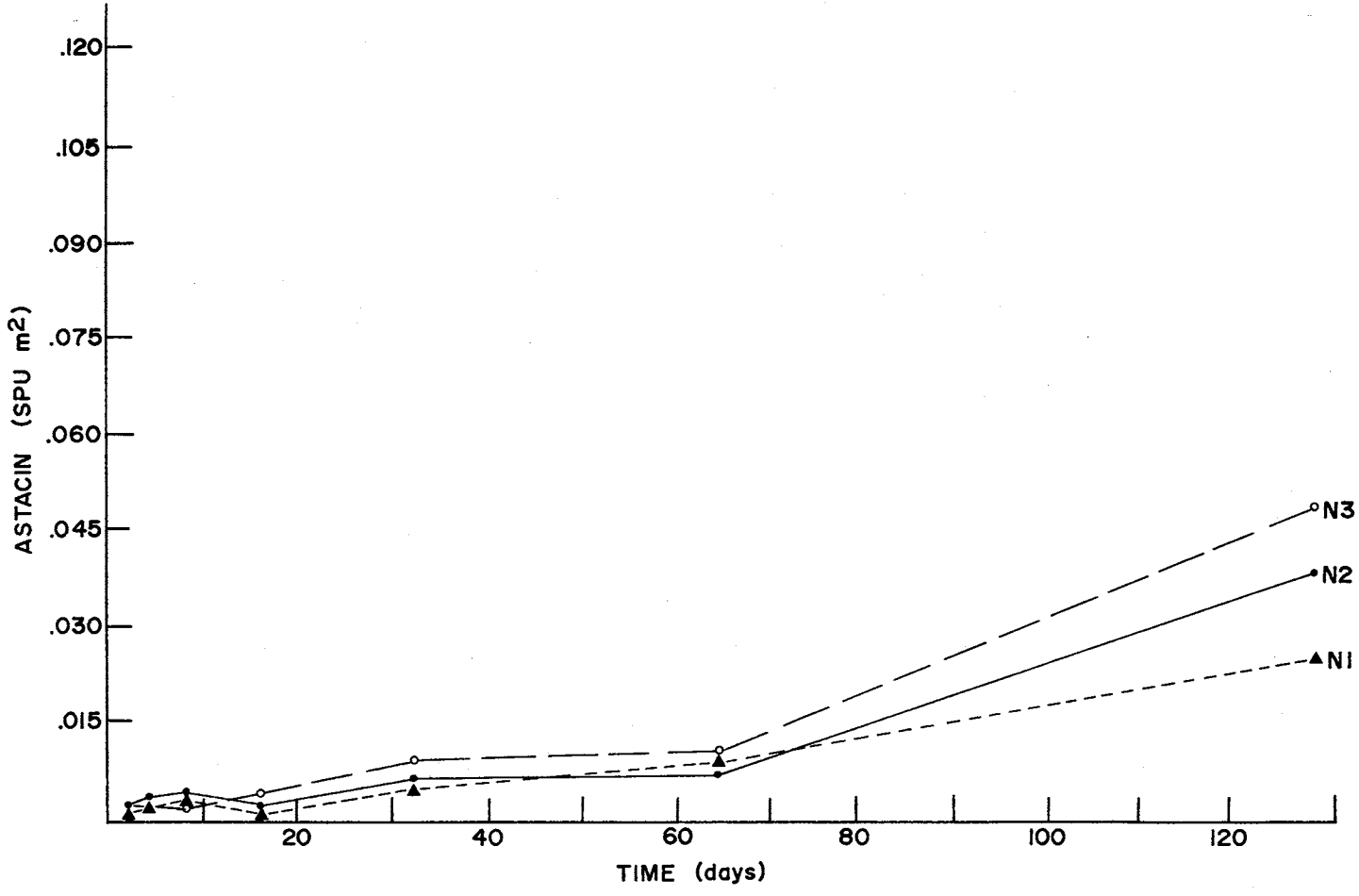

Figure 17. Temporal Variation of Astacin Pigment Values of the Three Nutrient Levels Averaged Over All Sewage Levels

occurred until day 32 (Figure 17). This study suggests that the addition of sewage had a deleterious effect on the primary consumers until day 32.

Astacin pigments in the present study are within the range of .005 to .020 SPU m^{-2} found in Lake Keystone and are generally lower than those in a previous microcosm study (Wilhm and Long, 1969). These findings may be due to the fact that Lake Keystone was constantly receiving domestic sewage which is deleterious to crustaceans, consequently reducing production of astacin pigments. Wetzel (1964) and Langford and Jermolajev (1966) state that grazing is indicated by an inverse relationship between astacin pigments and chlorophyll a. Grazing by crustaceans releases nutrients which may stimulate production in systems. Mitchell (personal communication) noted a direct relationship between zooplankton and chlorophyll a in microcosms receiving activated sludge effluent. In the present study the following positive relationships between chlorophyll a and astacin pigments were observed in microcosms of different levels of nutrients, sewage, and time: N1-0.952**, N2-0.948**, N3-0.471; S1-0.526; S2-0.855**, S3-0.774*, Time-0.774* (*P = 0.05, **P = 0.01).

Pigment Diversity

Main effects in sewage and time plus an interaction of sewage x nutrients were observed for pigment diversity (Table VI).

A decrease in pigment diversity was observed with an increase in nutrients with sewage level held constant (Table VII). When the nutrient level is held constant, a sewage increase results in a decrease in pigment diversity (Table VII).

Pigment diversity increased until day 4 in the three sewage levels and a general decrease was observed until day 16 (Figure 18). From day 16 to day 32, pigment diversity was lower in microcosms with higher sewage loads except on day 32. Margalef (1957) proposed the "yellow/green" ratio or pigment diversity at 430/665 nm. It has been proposed that successional and physiological changes in algal populations were reflected in changes of the "yellow/green" or pigment diversity (Margalef, 1963 and 1958). Pigment diversity was highest in older stable communities and lower in young, growing communities (Odum, 1963). The present study does not suggest the trend during succession reported by Margalef (1963 and 1958).

Mean diversity values in the present study range from 2.40 to 6.36 and compare with values of pigment diversity measured by other investigators. Values from 4.32 to 6.98 were reported for artificial lakes in Spain during the summer months (Margalef, 1964). Knudson (1970) reported values as high as nine in clear ponds in Central Oklahoma with lower values in turbid ponds. The values of the present study were within the range of data obtained from studies in shallow, turbid ponds (Knudson, 1970). The depth of the ponds probably affects pigment diversity. A mean diversity value of 2.9 was found in an enriched stream (Kehde, 1970). Values of 2.53 to 2.98 were found in eutrophic Lake Keystone (Spangler, 1970).

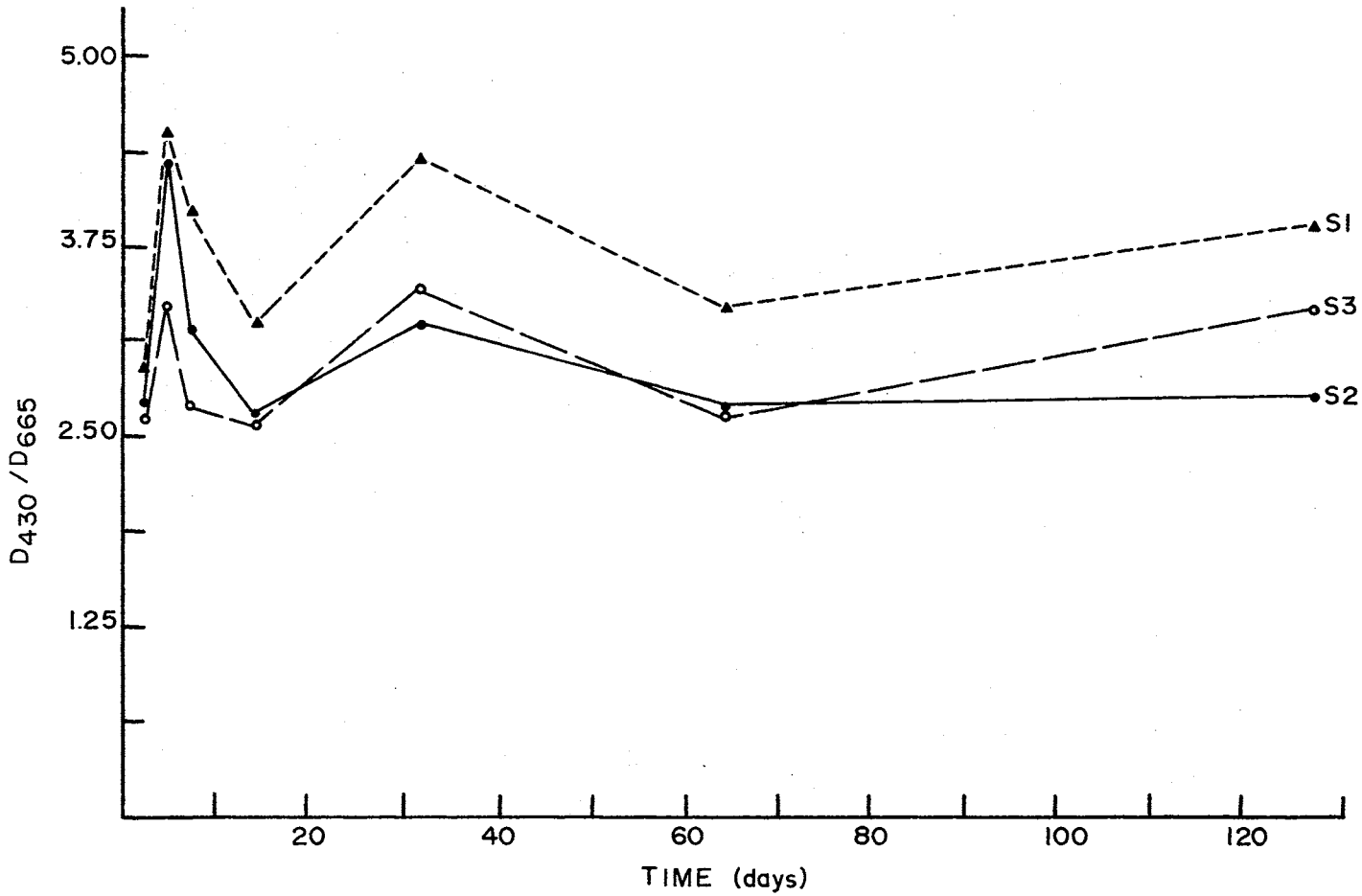

Figure 18. The Temporal Variation of Pigment Diversity at the Three Sewage Levels

CHAPTER IV

SUMMARY

1. The objectives of this experiment were to measure variation in changes in species diversity, net production, respiration, chlorophyll a, chlorophyll b, chlorophyll c, astacin pigments, dry weight, ash-free weight, net production/respiration, pigment diversity, among laboratory microecosystems receiving different levels of domestic sewage.

2. A split-split plot analysis was used in order to study the effects of domestic sewage on laboratory systems adapted to different nutrient levels. Nutrients were main plot, sewage the subplot, and time the sub-subplot.

3. The basic experimental unit was 12.4 ℓ of pond water in a glass container painted black on the outside. Microcosms were housed in a light-proof controlled environmental chamber under 40-watt Gro Lux fluorescent lights from 25 November 1967 to 26 April 1968.

4. Observations were made at 2, 4, 8, 16, 32, 64, and 128 days after the sewage was added. At each observational period, three experimental units at each nutrient level versus each sewage level were analyzed.

5. Seventy-four species of algae and zooplankton were identified in the microcosms. Species of Cyanophyta were the most abundant in enriched microcosms and Chlorophyta was the dominant group in control microcosms during the early time periods but Thallophyta was generally

the dominant group after day 8.

6. Similar values for species diversity were observed with an increase in nutrients when sewage was held constant and vice versa. The diversity values in the present study were between 1 and 2.

7. A general increase in net productivity was observed with an increase in nutrients when sewage was held constant. When the nutrient levels were held constant, the highest response of Pn occurred at the S2 level except for the high nutrient level in microcosms. Net production increased rapidly in all systems initially and then decreased abruptly in N2 and N3 systems, while the subsequent change in N1 systems was slight. The production values from selected field areas were higher than values in the present study due to high temperatures and diel temperature increases.

8. The trends for Rni were generally similar to the responses of net production.

9. Temporal variation was the only significant factor in the net production to nighttime respiration ratio.

10. The higher nutrient and sewage levels generally cause higher dry weight values due to the increased availability of nutrients. The temporal observations of dry weight generally increased except from day 16 to 32. The decrease from day 16 to 32 could be due to nutrient depletion. Grazers were first observed on day 32 and became more abundant thereafter. The trends for ash-free weight were similar to those observed for dry weight.

11. The ratio of net production to dry weight did not indicate any main effects or interactions.

12. Assimilation numbers generally decreased with an increase in

nutrients when sewage was held constant and vice versa. Higher nutrient and sewage levels probably caused lower assimilation numbers due to increased chlorophyll.

13. Chlorophyll a generally increased with an increase of sewage and nutrients. The concentrations of chlorophyll a are thought to be correlated with nitrogen concentration, which was more abundant in higher levels of nutrients and sewage.

14. The mean concentration of chlorophyll b for S1 was significantly different ($P = 0.05$) from S2 and S3. The concentrations of chlorophyll b exhibited little change through day 32 and increased after that time.

15. The same main effects were present in the chlorophyll c analysis that were observed for chlorophyll b.

16. An increase in non-astacin pigment concentrations generally occurred with increases in sewage when nutrients were held constant.

17. Little temporal variation in astacin pigments was observed until day 16 and a general increase occurred after this observation. This study suggests that the addition of sewage had a deleterious effect on the primary consumers until day 32.

18. The pigment diversity value for S1 was significantly different ($P = 0.05$) from the low and high sewage values. Pigment diversity increased until day 4 and then decreased until day 16. After day 16, the pigment diversity values appear to have stabilized. This study does not suggest the pigment diversity trend during succession reported by Margalef (1963 and 1958).

LITERATURE CITED

Allen, M. B. 1955. General features of algal growth in sewage oxidation ponds. Sacramento: State Water Pollution Control Board, 48 pp.

American Public Health Association. 1960. Standard methods for the examination of water and wastewater. 11th edition. American Public Health Association. New York.

Bartsch, A. F., and M. O. Allum. 1957. Biological factors in treatment of raw sewage in artificial ponds. Limnol. and Oceanogr. 3:77-84.

Bernier, C. J. 1962. Measurement techniques for radiant requirements of growing plants. Dallas: National Technical Conference of the Illuminating Engineer Society. 8 pp.

Beyers, R. J. 1962a. The metabolism of twelve aquatic laboratory microecosystems, Ph. D. Dissertation. Univ. Texas, 190 pp.

Beyers, R. J. 1962b. Relationship between temperature and the metabolism of experimental ecosystems. Science. 136:980-982.

Beyers, R. J. 1963a. The metabolism of twelve aquatic microecosystems. Ecol. Mono. 33:281-306.

Beyers, R. J. 1963b. A characteristic diurnal metabolic pattern in balanced microecosystems. Publ. Inst. Mar. Sci., Univ. Texas. 9:19-27.

Beyers, R. J., J. L. Larimer, H. T. Odum, R. B. Parker, and N. E. Armstrong. 1963. Directions for the determination of changes in carbon dioxide concentration from changes in pH. Publ. Inst. Mar. Sci., Univ. Texas. 9:454-489.

Beyers, R. J. 1965. The pattern of photosynthesis and respiration in laboratory microecosystems. IBP Symposium on Primary Productivity in Aquatic Ecosystems. 3:63-74.

Boney, A. D. 1968. Experiments with some detergents and certain intertidal algae, p. 45-54. In J. D. Carthy and D. B. Arthur, The Biological Effects of Oil Pollution on Littoral Communities. Field Studies Council 9, Devereux Court Strand, London.

Bray, J. R. 1958. Notes toward an ecological theory. Ecology. 39:770-776.

Butler, J. L. 1964. Interaction of effects by environmental factors on primary productivity in ponds and microecosystems. Ph. D. Thesis. Oklahoma State University. 189 pp.

Carthy, J. D., and D. B. Arthur. 1968. The Biological Effects of Oil Pollution on Littoral Communities. Field Studies Council 9, Devereux Court Strand, London. 188 pp.

Chu, S. P. 1943. The influence of the mineral composition of the medium on the growth of planktonic algae. J. Ecol. 31:109-148.

Cooke, G. D. 1967. The pattern of autotrophic succession in laboratory microcosms. Bioscience 17:717-721.

Cooper, J. M. 1972. Spatial and temporal variation in productivity, species diversity of periphyton in a stream receiving domestic and oil refinery effluents. Ph. D. Thesis. Oklahoma State University. 54 pp.

Czeczuga, B. 1965. Chlorobium limicola Nads. (Chlorobacteriaceae) and the distribution of chlorophyll in some lakes of the Mazur Lake District. Hydrobiologia. 25:412-423.

Dorris, T. C., D. Patterson, and B. J. Copeland. 1963. Oil refinery effluent treatment in ponds. J. Water Pollution Control Federation. 35:932-939.

Edmondson, W. T., and Y. H. Edmondson. 1956. Measurement of production in fertilized salt water. J. Mar. Res. 6:228-246.

Edmondson, W. T., G. C. Anderson, and D. R. Peterson. 1956. Artificial eutrophication of Lake Washington. Limnol. and Oceanogr. 1:47-53.

Edmondson, W. T. 1964. Reproduction rate of planktonic rotifers as related to food and temperature in nature. Ecol. Monogr. 35:61-71.

Ewing, M. S., and T. C. Dorris. 1970. Algal community structure in artificial ponds subjected to continuous organic enrichment. Amer. Midland Natur. 8:565-582.

Fogg, G. E. 1965. Algal Cultures and Phytoplankton. Ecology. Univ. Wisconsin Press, Madison. 126 pp.

Gale, W. F., and R. L. Lowe. 1971. Phytoplankton ingestion by the fingernail clam, Sphaerium transversum Say., in Pool 19, Mississippi River. Ecol. 52:507-513.

Gaufin, A. R., and C. M. Tarzwell. 1952. Aquatic invertebrates as indicators of stream pollution. Public Health Report. 67:57-67.

Gaufin, A. R., and C. M. Tarzwell. 1956. Aquatic macroinvertebrate communities as indicators of organic pollution in Lytle Creek. Sewage and Ind. Wastes. 28:906-924.

Godniew, T. N., and G. G. Winberg. 1951. O. pigmientach, zielonej bakteiu. Dok. A. M. Z. S., R. R. 76:909-912.

Goldman, S. 1953. Information Theory. Prentice-Hall, New York. 156 pp.

Hofstetter, A. M., and T. Mangold. 1970. A preliminary report on the aquatic algae from selected sites in Shelby County, Tennessee. Acad. of Sci. 45:8-11.

Hogetsu, K., and S. Ichimura. 1954. The ecological studies on the production of phytoplankton. Japanese J. of Bot. 14:280-300.

Hutchinson, G. E. 1967. A treatise on limnology. Vol. 2. Introduction to Lake Biology and the Limnoplankton. John Wiley and Sons, New York. 1115 pp.

Juday, R. E., and W. M. Manning. 1941. The chlorophyll content and productivity of some lakes in Wisconsin. Trans. Wis. Acad. Sci. 33:363-393.

Kehde, P. M. 1970. The effects of grazing by snails on community structure of periphyton in laboratory streams. Master's Thesis, Oklahoma State University. 34 pp.

Ketchum, B. H., J. H. Ryther, C. S. Yentsch, and N. Corwin. 1958. Productivity in relation to nutrients. Rapp T. Procus-Verbaux, Conseil. Perm. Intern. Exploration Mer. 144:132-140.

Kevern, N. R., J. L. Wilhm, and G. M. Van Dyne. 1966. Use of artificial substrata to estimate the productivity of periphyton. Limnol. and Oceanogr. 11:499-502.

Knudson, V. A. 1970. Community structure of plankton in clear and turbid ponds. Ph. D. Thesis, Oklahoma State University. 106 pp.

Kullberg, R. G. 1968. Algal diversity in several thermal spring effluents. Ecology. 49:751-755.

Langford, R. R., and E. G. Jermolajev. 1966. Direct effect of wind on plankton distribution. Verb. Internat. Verein. Limnol. 16:188-193.

Leake, D. V. 1935. The algae of Crystal Lake. Ph. D. Thesis, Oklahoma University. 72 pp.

Lewis, R. A. 1962. Physiology and Biochemistry of Algae. Academic Press, New York. 929 pp.

Ludwig, H. F., W. J. Ostwald, H. B. Gotaas, and V. Lynch. 1951. Algal symbiosis in oxidation ponds. I. Growth characteristics of Euglena gracilis cultured in sewage. Sewage and Industrial Wastes. 28:1337-1355.

McAllister, C. D., T. R. Parsons, K. Stephens, and J. D. H. Strickland. 1961. Measurements of primary production in coastal sea water using a large-volume plastic sphere. Limnol. and Oceanogr. 6:237-257.

McConnel, William J. 1962. Productivity relations in carbon microcosms. Limnol. and Oceanogr. 7:335-343.

McIntire, D. C., and C. E. Bond. 1959. Effects of artificial fertilization on plankton and benthos abundance in four experimental ponds. Ecol. Monogr. 28:303-312.

McIntire, D. C., and H. K. Phinney. 1965. Laboratory studies of periphyton production and community metabolism in lotic environments. Ecol. Monogr. 35:237-258.

Machenthun, K. M. 1969. The practice of water pollution. U. S. Government Printing Office, Washington. 193 pp.

Margalef, R. 1957. La teoria de la information en ecologia. Memo. Real. Acad. Cicncias Y Artes Barcelonia. 32:372-449.

Margalef, R. 1958. Temporal succession and spatial heterogeneity in phytoplankton. In Perspect in Mar. Biol. Univ. Calif. Press. pp. 323-349

Margalef, R. 1963. On certain unifying principles in ecology. Amer. Natur. 97:357-374.

Margalef, R. 1964. Correspondence between the classic types of lakes and the structural and dynamic properties of their populations. Verl. Internat. Verein. Limnol. 15:169-175.

Mitchell, D. 1971. Eutrophication of lake water microcosms: phosphate versus non-phosphate detergents. Sci. 174:827-829.

Mitchell, D., and J. C. Buzzell, Jr. 1971. Estimating eutrophic potential of pollutants. J. of the Sanitary Engineering Div. 4:453-465.

Mitchell, D. 1972. Eutrophication and phosphate detergents. Sci. 177:816-817.

Mueller, W. 1953. Nitrogen content and pollution of streams. Gesund. Heitsing. 74:256.

Odum, E. P. 1959. Fundamentals of Ecology. 2nd edition. W. B. Saunders, Philadelphia. 574 pp.

Odum, E. P. 1963. Ecology. Holt, Rinehart, and Winston, San Francisco. 152 pp.

Odum, H. T., and E. P. Odum. 1955. Trophic structure and productivity of a windward coral reef community on Eniwetok Atoll. Ecol. Monogr. 25:291-320.

Odum, H. T. 1956. Primary production in flowing waters. Limnol. and Oceanogr. 1:102-117.

Odum, H. T. 1957. Primary production measurements in eleven Florida Springs and a marine turtle grass community. Limnol. and Oceanogr. 2:85-97.

Odum, H. T., and C. M. Hoskin. 1957. Metabolism of a laboratory stream microcosm. Publ. Inst. Mar. Sci., Univ. Texas. 5:115-133.

Odum, H. T., and Walter Abbott. 1958. The chlorophyll "a" of communities. Publ. Inst. Mar. Sci., Univ. Texas. 5:65-96.

Odum, H. T. 1963. Primary production in flowing waters. 1956. Limnol. and Oceanogr. 1:102-117.

Odum, H. T., R. J. Beyers, N. Armstrong, and W. L. Siler. 1963. Experiments in engineering marine ecosystems. Publ. Inst. Mar. Sci., Univ. Texas. 9:373-404.

Owens, M., and R. W. Edwards. 1962. The effects of plants on river conditions: III Crop studies and estimates of net productivity of macrophytes in four streams in Southern England. J. Ecol. 50:157-162.

Palmer, C. M. 1962. Algae in Water Supplies. Public Health Service Publication #657. Superintendent of Documents, U. S. Government Printing Office, Washington 25, D. C. 88 pp.

Parker, B. C., G L. Samsel, and E. K. O. Asamoa. 1971. Effects of detergent protease enzymes on sewage oxidation pond phytoplankton. Bioscience. 21:1035-1047.

Parrish, F. K. 1968. Keys to water quality indicative organisms. Federal Water Pollution Control Administration, U. S. Department of the Interior. 195 pp.

Patrick, R. 1969. Some effects of temperature on fresh water algae, p. 161-198. In P. A. Krenkel and F. L. Parker, Biological Aspects of Thermal Pollution. Vanderbilt Univ. Press, Nashville, Tennessee.

Patten, B. C. 1962. Species diversity in net phytoplankton of Raritan Bay. J. Mar. Res. 20:57-75.

Pearsall, W. H. 1932. Phytoplankton in English Lakes II. The composition of the phytoplankton in relation to dissolved substances. J. Ecol. 20:241-262.

Pejler, B. 1957. Taxonomical and ecological studies on planktonic rotatoria from Central Sweden. K. svenskall Vetensk Akad. Handl., Fjarde Ser. Bd. 6, No. 5, 68 pp.

Pomeroy, L. R. 1969. Residence time of dissolved phosphate in natural waters. Sci. 131:1731-1732.

Prescott, G. W. 1942. Ecology of Panama Canal algae. Amer. Microscop. Soc., Trans. 70:1-24.

Prescott, G. W. 1964. How to Know the Fresh-water Algae. W. C. Brown, Dubuque. 272 pp.

Richards, F. R., and T. G. Thompson. 1952. The estimation and characterization of plankton populations by pigment analysis II. A spectrophotometric method for the estimation of plankton pigments. J. Mar. Res. 11:156-172.

Rigler, F. H. 1956. A tracer study of the phosphorus cycle in lake water. Ecology. 37:550-562.

Riley, G. A. 1956. Oceanography of Long Island Sound, 1953-54. IX Production and utilization of organic matter. Bull. Bing. Oceanogr. Coll. 15:324-343.

Ryther, J. H., C. S. Yentsch, E. M. Hulburt, and R. F. Vaccaro. 1958. The dynamics of a diatom bloom. Biol. Bull. 115:257-268.

Sawyer, C. N. 1947. Fertilization of lakes by agricultural and urban drainage. J. New England Water Works Ass. 61:109.

Shannon, C. D., and W. Weaver. 1963. The Mathematical Theory of Communication. Univ. of Illinois Press, Urbana.

Smith, G. M. 1924. Ecology of the plankton algae in the Palisades Interstate Park, Including the relation of control methods to fish culture. Roosevelt Wild Life Bull. 2:94-195.

Spangler, F. L. 1969. Chlorophyll and carotenoid distribution and phytoplankton ecology in Keystone Reservoir, Tulsa, Oklahoma. Ph. D. Thesis, Oklahoma State University. 61 pp.

Staub, R. J., W. Appling, A. M. Hofstetter, and I. J. Hass. 1970. The effects of industrial wastes of Memphis and Shelby County on primary planktonic producers. Bioscience. 20:905-915.

Steeman-Neilsen, E. 1955. The production of organic matter by phytoplankton in a danisk lake receiving extraordinary amounts of nutrient salts. Hydrobiologia. 7:68-74.

Strickland, J. D. 1960. Measuring the production of marine phytoplankton. Bull. Fish. Res. Board Can. 122:1-72.

Strickland, J. D. H., and T. R. Parsons. 1965. A manual of sea water analysis. Bull. Fish. Res. Board Can. Bulletin No. 125, 2nd ed.

Strickland, J. D. H., O. Holn-Hunsen, R. W. Eppley, and R. J. Linn. 1969. The use of a deep tank in plankton ecology: I. Studies of the growth and composition of phytoplankton crops at low nutrient levels. Limnol. and Oceanogr. 14:423.

Talling, J. F. 1965. The annual cycle of stratification and phytoplankton growth in Lake Victoria (East Africa). Int. Rev. Hydrobiol. 50:1-32.

Tilton, R. C. 1970. Microbial degradation of detergent phosphates. J. of Environ. Health. 32:523-527.

Warinner, J. E., and M. L. Brehmer. 1972. The effects of thermal effluents on marine organisms, pp. 381-393. In R. F. Ford and W. E. Hazen, Readings in Aquatic Biology. W. B. Saunders, Philadelphia.

Wesenberg-Lund, C. 1905. A comparative study of the lakes of Scotland and Denmark. Proc. Roy. Soc. Edinburg. 25:401-448.

West, W., and G. S. West. 1909. The British fresh-water phytoplankton, with special reference of the Desmid - plankton and the distribution of British Desmids. Proc. Roy. Soc. London B. 81:165-206.

Wetzel, R. G. 1964. Primary productivity of aquatic macrophytes. Verl. Int. Verein. Limnol. 15:426-436.

Wetzel, R. G. 1966. Productivity and nutrient relationships in marl lakes of Northern Indiana. Verl. Int. Verein. Limnol. 16:321-332.

Whipple, G. C., G. M. Fair, and M. C. Whipple. 1948. The Microscopy of Drinking Water. John Wiley and Sons, New York. 586 pp.

Wilhm, J. L., and Joseph Long. 1969. Succession in algal mat communities at three nutrient levels. Ecology. 50:645-652.

Wilhm, J. L. 1970. Range of diversity index in benthic macroinvertebrates populations. J. Federation Water Pollution Control. 42:221-224.

Wright, J. C. 1958. Limnology of Canyon Ferry Reservoir I. Phytoplankton-zooplankton relationships in the eutrophic zone during September and October, 1956. Limnol. and Oceanog. 3:150-159.

Yount, James L. 1956. Factors that control species numbers in Silver Springs, Florida. Limnol. and Oceanogr. 1:286-295.

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