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Institute of Marine Sciences
University of North Carolina
Chapel Hill and Morehead City, N. C.

Annual Report For 1970-1971

To

National Oceanographic and Atmospheric Agency,

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"Optimum Ecological Designs for Estuarine Ecosystems in North Carolina"

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ABSTRACT

This is the third annual report from an investigation of the ecological systems which develop when estuarine waters are enriched with sewage wastes. Faculty and students from the University of North Carolina have studied various phases of community structure and metabolism of six experimental brackish-water ponds, three of which receive treated sewage wastes, and of a small tidal creek and its salt marshes. In this report are chapters on productivity, carbon metabolism, the phosphorus budget, nitrogen, and bacterial heterotrophy; on the standing crops of phytoplankton, decapod crustaceans, fishes, meiofauna, foraminifera, insects, molluscs, and birds; on calcium analysis; and on growth and reproduction of algae. The waste ponds have developed into productive, well-integrated, but slightly unstable systems. They perform some of the functions of tertiary treatment and hold promise for production of harvestable seafood protein.

INTRODUCTION

Estuaries and Human Wastes

Estuaries have developed and evolved over geological time. Their form, their hydrographic characteristics, their sediment distributions, their communities of organisms have all been "self-designed." As far as the communities of organisms are concerned, they have developed to high levels of productivity and diversity in the face of relatively large excursions of the environment--changes in salinity, changes in inundation by tides, wide temperature excursions, strong current flows, and associated scouring.

Man is a newcomer, geologically speaking, to estuaries. He has affected current flows, turbidity, sediment distribution, and contributed large amounts of numerous pollutants. His effects of estuaries are many; this project focuses on only one of his effects, domestic pollution. Given a reasonable chance, that is to say, if shocks are not too severe, estuaries should adapt to new conditions imposed by man. This project will indicate what loads of domestic sewage effluent can be assimilated and what type of ecological system will "self-design."

Organic wastes, both municipal and industrial, are released into most of the estuaries and bays of the Atlantic seaboard of the United States. The resulting problems of eutrophication or pollution have been frequently documented, but space permits listing of only a few examples related to sewage or similar wastes. A number of workers (Ryther 1954; Ryther, Yentsch, Hulburt, and Vaccaro 1958; Lackey 1963; Barlow, Lorenzen, and Myren 1963) have investigated the ecological results of pollution of Great South Bay, Long Island, largely by duck farm wastes, namely the increased nutrients, the increased phytoplankton and benthic plant populations, the wide oxygen excursions with anoxic conditions sometimes occurring on the bottom, and the failure of the oyster industry. Studies of Raritan Bay, New Jersey (Jeffries 1962, 1964; Patten 1962, Dean and Haskin 1964), demonstrated the reduction in diversity of phytoplankton, zooplankton, and benthic fauna caused by pollution; pollution abatement in this bay resulted in increased biotic diversity. The Chesapeake Bay and its tributaries have been studied recently (Brehmer 1964; Shapiro and Ribeiro, 1965; Carpenter, Pritchard, and Whaley 1969); very high levels of P and N enter the bay and eutrophication, with resultant oxygen stresses near the bottom, is severe. Finally, studies of the subtropical waters of Biscayne Bay, Florida, (Lynn and Yang 1960; McNulty 1961) have shown that warm waters are also easily loaded beyond their capacity to function. A general conclusion from these studies is that

enrichment with sewage wastes greatly enhances primary productivity, but this increased energy is not channeled effectively into seafoods of direct interest to man. Furthermore, bacterial and viral contamination of the waters and of shellfish, water turbidity, noxious odors, and windrows of seaweeds on the beaches combine to reduce significantly the recreational value of polluted estuaries.

Self Design

If whole estuaries have self-designed, then smaller systems should also have this capacity. Six tenth-acre ponds have been built, 3 of which are controls while 3 receive sewage wastes from the town of Morehead City. Polluted and control ponds have sea water and freshwater added in such proportions that the salinities are the same and relatively constant (~15‰). A standpipe in the center of each pond carries away the overflow. The ponds are in effect tertiary treatment, or oxidation, ponds. The specifications of the ponds and the sources and nature of the water were given in the annual report for 1969-70.

The ponds have been seeded with microscopic plants and animals that pass through the pumps and by adding large numbers of freshly caught fish, zooplankton, and macroinvertebrates such as crabs and shrimp. Spartina grass has been planted and some sessile invertebrates such as oysters have been set out.

Measurements have been made for more than 2 years--temperature, salinity, insolation, and plant nutrients; standing crops of phytoplankton, zooplankton, fish, crabs, snails, oysters, bacteria, and insects; and metabolic rates have been assessed through measurement of phytoplankton primary productivity, metabolism of organic substrates by microbes, nitrogen balances and phosphorus kinetics, bottom algae productivity, and growth rates of selected species of crabs, fish and shrimp (see annual report 1969-70 and this report).

An objective of these studies is a description of productivity, nutrient cycling, and community development in sufficient detail to understand and manage such small ponds for water quality improvement (tertiary treatment) and for production of seafoods (aquaculture). Preliminary diagrams of the standing crops of organisms, the reservoirs of organic and inorganic substances in the water, and the fluxes of matter and energy between various compartments have been made.

Tertiary Treatment of Sewage

The flow of sewage, either raw or following primary or secondary treatment, is increasing in quantity both as a function of increases in populations that are being provided with sewer services and increases in water uses of which some major portion becomes part

of the wastewater flow of a community. Raw sewages are now seldom discharged directly to rivers and streams. Public and administrative requirements no longer allow for such casual use of receiving waters. When sewage wastes, whether receiving primary or secondary treatment are discharged to an estuarine environment, the response in terms of nutrient enrichment may be different than that of a stream because the estuarine ecosystem is far more complex in terms of available organisms, both plant and animal, that might respond to the enhanced nutrient flow. Thus, the use of the sewage oxidation ponds to carry mineralization of sewage wastes to the ultimate levels provides control mechanisms for the utilization of these nutrients in recycling them into the environment and possibly deriving from them plant or animal harvest which may be of substantial economic value. The ponds at Morehead City are unique in that they represent one of the very few examples of oxidation ponds operating in an estuarine or brackish water ecosystem. Preliminary data on the effects of these ponds on the carbon (J. Day), phosphorus (H. McKellar) and nitrogen (S. Masarachia) of treated sewage are found in this report and in Kuenzler (1971).

Aquaculture (Mariculture)

The world's rich estuarine systems hold the promise of greater food production for an increasing human population. Increased flow of effluents from sewage treatment plants in many coastal areas is adding plant nutrients to surrounding waters, often, however, with deleterious effects to the naturally occurring ecosystems. Utilization of such nutrients through conversion to usable food appears to hold great potential. Studies conducted during the present project show that sewage treatment effluents can support high levels of primary productivity. The next step lies in development of aquacultural techniques for converting such productivity to a dependable harvest of mollusks, crustaceans, or fishes.

Interest and activity in aquaculture has reached an unprecedented level in many nations. Many countries have succeeded in growing species of shrimp, mollusks and fishes, in most cases in natural waters. Pond culture in the United States has been successful in rearing catfish and trout, but little attention has been directed to estuarine animals.

Information on aquaculture in marine waters (mariculture) is scarce in the United States. Literature on the subject in journals devoted to publication of results of biological research is practically nonexistent although collateral information on topics of vital concern to mariculturists is widespread in the primary literature. Iversen (1968) took a broad look at aquacultural practices the world around, and Ryther and Matthiessen (1969)

provided a stimulating synopsis of potentials that may be realized from mariculture. The majority of technical literature concerning aquaculture is published in Japanese (Won Tack Yang, person. commun.).

Culture of marketable crustaceans for the home consumer is still experimental in this country. Pilot studies of pond and tank culture of penaeid shrimp have been in progress for over a decade in the southern United States. In some experiments larvae or postlarvae borne on the tide have been trapped in impoundments and held for observation on growth rates; in others ripe females have been captured, held until spawning occurred, and the eggs hatched, the larval stages passed, and juveniles allowed to grow to maturity. Allowing tides to sweep shrimp and fish larvae into impoundments for entrapment until growth to maturity and easy capture is a practice widespread in tropical parts of the Orient (reviewed by M. D. Beeston in this report). A more advanced technique of rearing penaeid shrimp from ripe females captured in the wild was pioneered by the Japanese, specifically Dr. Motosaku Fujinaga, who has developed the culture to a profitable business. Mass culture of some brachyuran crabs has also been successful for experimental purposes in tanks and small ponds.

Profit in mariculture of shrimp depends on low cost labor and a market commanding high prices, as shrimp used in the Japanese tempura and suki dishes. At present such a market is nonexistent in the United States.

Examples of current studies in the United States are provided by the Biological Laboratory of the National Marine Fisheries Service at Galveston (Lindner and Stevenson, 1970) where a multifaceted shrimp research program is pursued. Here under favorable captive conditions shrimp will spawn and larvae develop to a stage suitable for transport to experimental sites for planting. Work on physiological requirements of larvae is coupled with work on mass culture techniques. Experiments with artificial foods are conducted in hope of producing sexual maturation in F_1 generation shrimp. Only males have showed apparent (external) signs of maturity. On artificial foods the white shrimp has grown more rapidly than the brown shrimp.

A commercial venture on the Gulf Coast of Florida proposes to apply Japanese techniques to the growing of shrimp (U.S.T. 1970). A large area of shallow water is enclosed by a plastic net fence, fish are removed, and young shrimp are introduced. A yield of 1,000 lb/acre·year ($\sim$100g/m^2·year) is anticipated by feeding trash fish to the growing shrimp; lower yields may be expected if shrimp must depend on in situ productivity. However, if the operation proves successful, we may expect further development of this industry.

Our experimental ponds have thus far proved satisfactory for the growth of blue crabs and Palaemonetes shrimp (Beeston, this report). An economic analysis has not yet been made, but the annual production of blue crabs in these ponds is undoubtedly at least an order of magnitude too low for them to be of commercial interest. At present Palaemonetes are not valued for human consumption, but it is possible that they might be desirable for flavoring dishes or, if very abundant, as a livestock feed supplement. The valuable Peneid shrimps for some reason did not survive and grow in the polluted ponds; studies are continuing in an effort to determine what conditions might favor their growth.

Many problems remain in proving the worth of mariculture of crustaceans as a profitable venture in the U. S. A combination of abundant source of larvae, favorable water supply in impoundments protected from storm flooding, control of disease and predators, availability of secondary sewage effluent to stimulate primary productivity may provide appropriate conditions.

Oysters and mussels of several species are of considerable food value around the world. Natural populations have generally been overharvested, but with protection and by utilization of a variety of "farming" practices, very large yields can be obtained from suitable areas (Walford 1958). The minimum labor necessary in many places in the U. S. is collection of oyster spat on old shell or other cultch material at the proper place and season and the spreading of these young oysters on the bottom at suitable places in the estuary where growth is rapid but predators and diseases are not severe. The development of a hatchery system to provide a source of seed oysters has shown promise in the New England and mid-Atlantic states. More elaborate procedures may also be used, even to the extent of hanging oysters in the water below rafts. Raft-culture of oysters presents many problems in proper selection of location, competitive activities from fouling organisms, and further need for technical proficiency.

A number of species of edible fish grew in our control ponds, but not in the waste ponds (Beeston, this report; Hyle, this report). A detailed review of fish culture in ponds enriched with sewage has just appeared (Allen 1970) showing abundant evidence that fishes can be reared in such ponds. Commercial success has apparently not yet been achieved in the United States, but there is enough promise to encourage continued studies.

The economic aspects of tertiary sewage treatment and mariculture have not yet been investigated so it is not known whether single-purpose or multiple-purpose ponds would prove most desirable. Our ponds have developed into relatively complex ecosystems with many species of plants and animals. Whether simplification through human control in order to emphasize one or another goal would be more profitable than permitting development of diverse and stable assemblages that provide alternate values and yields is not yet known. There are many interesting studies yet to be made.

Scientific Reports Resulting from This Project

Papers Presented and Published

1. Day, J. W., Jr., C. M. Weiss, and H. T. Odum. 1970. The carbon budget and total productivity of estuarine oxidation ponds receiving secondary sewage effluent. Second International Symposium of Waste Treatment Lagoons. Kansas City, Missouri. August 22-25. To be published in Proceedings; in press.

2. Kuenzler, E. J. 1970. Aspects of phosphorus cycling in brackish waters. Symposium: "Algae and Pollution, Experimental Studies of Eutrophication." A.I.B.S., University of Indiana, Bloomington. August 27.

3. Kuenzler, E. J. 1970. Aspects of phosphorus cycling in brackish waters. Symposium Honoring Dr. E. P. Odum: "Toward Relevant Ecology." University of Georgia, Athens, Georgia. October 2-3. To be published in Proceedings; in press.

Theses and Dissertations

1. Beeston, M. D. 1971. Decapod crustacean and fish populations in experimental marine ponds receiving treated sewage wastes. Master of Science Thesis, Curriculum in Marine Sciences, U.N.C., under the direction of Dr. A. B. Williams.

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McNulty, J. K., E. S. Reynolds, and S. M. Miller. 1960. Ecological effects of sewage pollution in Biscayne Bay, Florida; Distribution of coliform bacteria, chemical nutrients, and volumes of zooplankton. In: Biological problems in water pollution. Robert A. Taft San. Eng. Center. Tech. Rep. No. W60-3. pp. 189-202.

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Log of Activities and General Notes

W. Laughinghouse, M. Smith, and E. J. Kuenzler

As in previous years, we have maintained a log book in which investigators enter all their activities that have a general influence on the ponds (seeding, harvesting, and significant disturbances of the sediments, for example) as well as general notes concerning the ponds (visible changes in color, failure of pumps, freezes, oxygen crashes, and fish mortalities, for example). A brief abstract from this log book is given in Table 1. The temperatures in all ponds were generally similar on any given day. The annual temperature range was approximately 30°C; the superimposed diel temperature changes were generally 4-6°C (Fig. 1, 2). Seasonal temperature increases usually occurred gradually, but fall cooling often occurred quickly (September 26-29; October 14-16; November 3-5 and 23-25).

Salinities were measured with an Industrial Instruments Inc. electrodeless induction salinometer. Highest salinities existed in Bogue Sound, especially during dry periods in summer. Generally lower and more variable salinities were found in Calico Creek, but the pattern follows that of Bogue Sound (Fig. 3). Because of the irregular sampling intervals, the values for Calico Creek should only be considered as representative; during low water in times of significant rainfall salinities much less than 20‰ often occur.

Salinity of the pond water was maintained more constant during 1970 than in previous years in spite of frequent pump failures and occasional heavy rains or backflooding during high water of spring tides (Fig.3-7). For the most part the pond water was between 15 and 20‰.

The flow rates of water into the ponds were obtained by measuring the volumes delivered per unit time by the seawater pumps and by sewage or tap water pipes. The times of pump operation were automatically recorded so that the monthly volumes could be calculated. Turnover rates for the ponds (Fig. 8) were calculated by dividing the total volume of water entering each pond per month by the volume of that pond (annual report, Odum and Chestnut 1970). Turnover time for each pond was more constant throughout 1970 than during 1969, but they were not the same for each pond; C-1 and P-1 had the fastest turnover rates whereas C-3 and P-3 had the slowest turnover rates.

Table 1. Record of events in ponds, April 1970-January 1971

Date	Activity
4/10/70	Last entry in 1969-1970 annual report.
4/11/70	Plexiglass plates placed in all ponds by Dowds.
4/16/70	All further Winkler data will be from Mrs. Smith's data.
5/ 5/70	Seined in P2 and C2 for Palaemonetes, found more in P2 than in C2.
5/ 8/70	Color change in P2 and P3, brownish green, visibility increasing.
5/11/70	P2 and P3 very clear, 2 dead mullet in P3, O_2 crash.
5/12/70	P1 about 15 small fish found dead, and 3 dead crabs.
5/18/70	C1 - 4 snapping shrimp found.
5/21/70	P1 and P2 dead algae floating up from bottom.
6/16/70	Man from sewer plant said that secondary primary tank is cleaned once a week. This makes the output appear dirty for about 20 minutes.
7/ 3/70	Sound pump out. Will have to be replaced.
7/17/70	Sound pump out. Replaced, 1730. Salinity about 15.5ppt.
8/ 3/70	P1 - Winklers averaged 108 mg/l, 19 egrets and bitterns feeding in P1 this A.M.
8/ 5/70	Juncus from Dill Creek planted in bank of every pond. D. Marshall.
9/15/70	Thermal stratification in C ponds noticed with warmer water on bottom - 34°C.
9/17/70	C3 - removed about 1 dozen dead spot and flounder. C2 - removed 1 dead flounder.
9/18/70	C3 saw 32 dead spot (?). C pond salinities dropping.
9/19/70	C3 - another dead flounder. C1 - 1 dead spot.
9/24/70	Primer line to sound pump on, the cause of low salinities(12ppt).
9/27/70	P3 - 2 dead mullet.
9/29/70	C pond seeded by pump. Gastrosaccus dissimilis <10 Pseudodiaptomus coronatus m Hexapanopeus angustifrons megalops <10 Uca megalops <10 Alpheus zoeae <10 Porcellanid zoeae <10 Mysidopsis bigelowi <10
10/ 1/70	P ponds seeded by pump. Pseudodiaptomus coronatus m Hexapanopeus angustifrons megalops X Uca megalops X Brachyuran (grapsoid?) zoea 1 Mysidopsis bigelowi <10

10/14/70 C ponds again seeded by pump.
C3 Pseudodiaptomas coronatus
Cumacean
Corophium
Sammarids

10/14/70 C2 Pseudodiaptomis coronatus
Hippolyte pleuracanthe juv.
C1 same as C3 and Uca megalops

10/15/70 Ponds seeded by seine hauls from ocean.

	Penaeus setiferus		Panaeusaztecus	
P1	4	avg. 5.77gr.	16	avg. 2.13gr.
P2	4		16	
P3	4		16	
C1	3		16	
C2	3		16	
C3	3		16	

Penacus duorarum avg. 1.71gr. - 29 each pond.
Callinectes sapidus - P ponds-3 each, C ponds-4 each.
Alpheus heterochelis - 1 adult into P2.
P ponds seeded by pump.
P1 Pseudodiaptomus coronatus
P2 Pseudodiaptomus coronatus
Corophium
Annelid fragments
P3 Pseudodiaptomus coronatus
Mysidopsis bigelowi

10/17/70 Gobiosoma bosci found in P3.
10/28/70 P3 back flooded.
11/2/70 Suspect that P3 has backflooded each high tide since 10/28/70.
12/4/70 C1 removed 50 Palaemonetes.
12/11/70 Mixing tank for C ponds cleaned out.
12/30/70 Heavy snow last night, ponds iced over.
1/12/71 1430 - Sewer pump shut off due to low salinities. Still off.
1/20/71 All ponds iced over this morning.
1/26/71 Sound pump out.
1/29/71 Sound pump repaired and relocated at the end of the dock.
This year as last year the salinities in the P ponds dropped quite a bit during January. The rains during January do not evaporate but continue to run off into Calico Creek for days and keeps the creek salinity low. Even with the sewer pump off the creek water cannot keep the salinities.

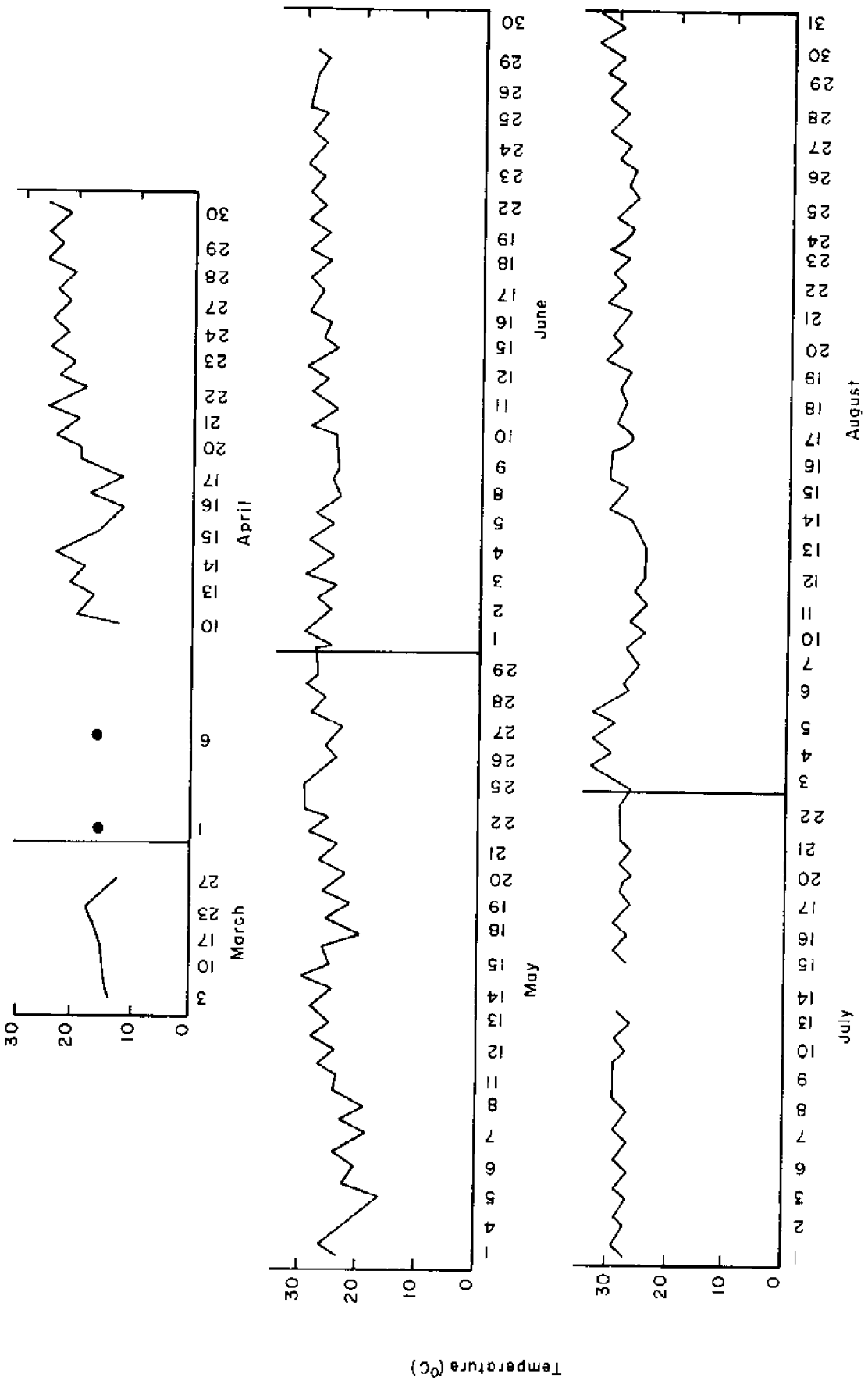

Figure 1. Mean temperatures for all control and waste ponds during March–August 1970.

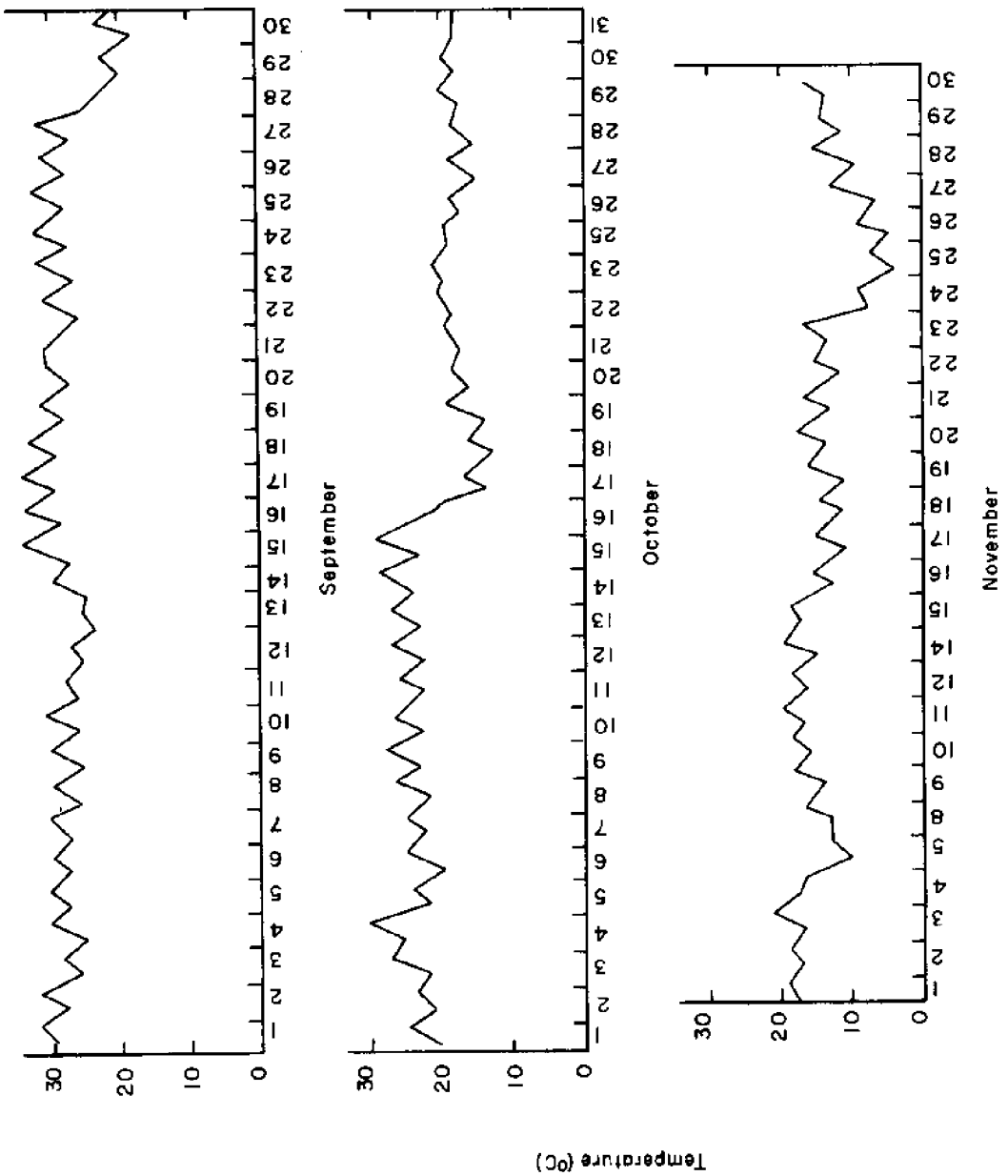

Figure 2. Mean temperatures for all control and waste ponds during September-December 1970.

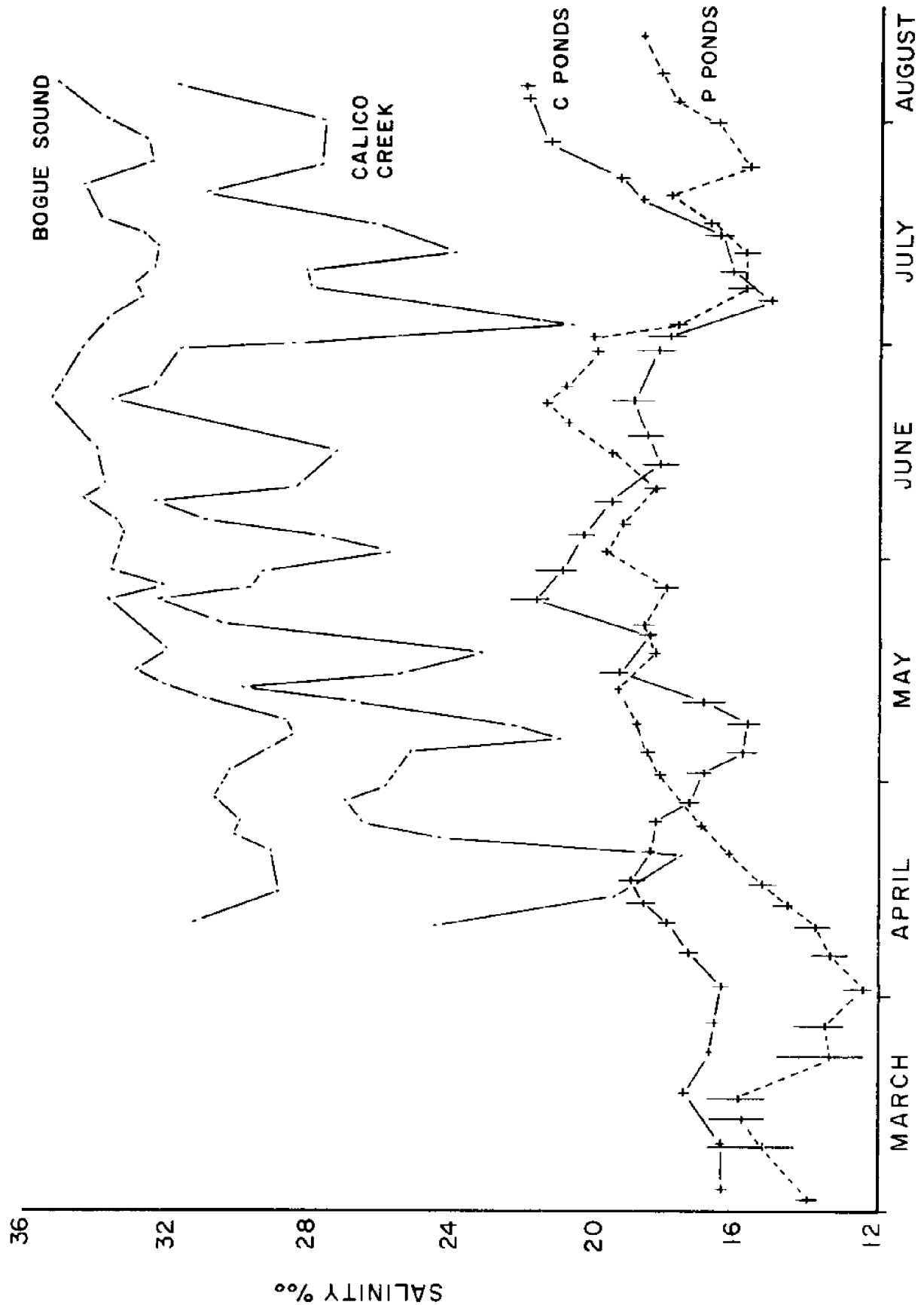

Figure 3. Salinities in Bogue Sound and Calico Creek during April-August 1970. Salinities (means and ranges) in the C-ponds and P-ponds during March-August 1970.

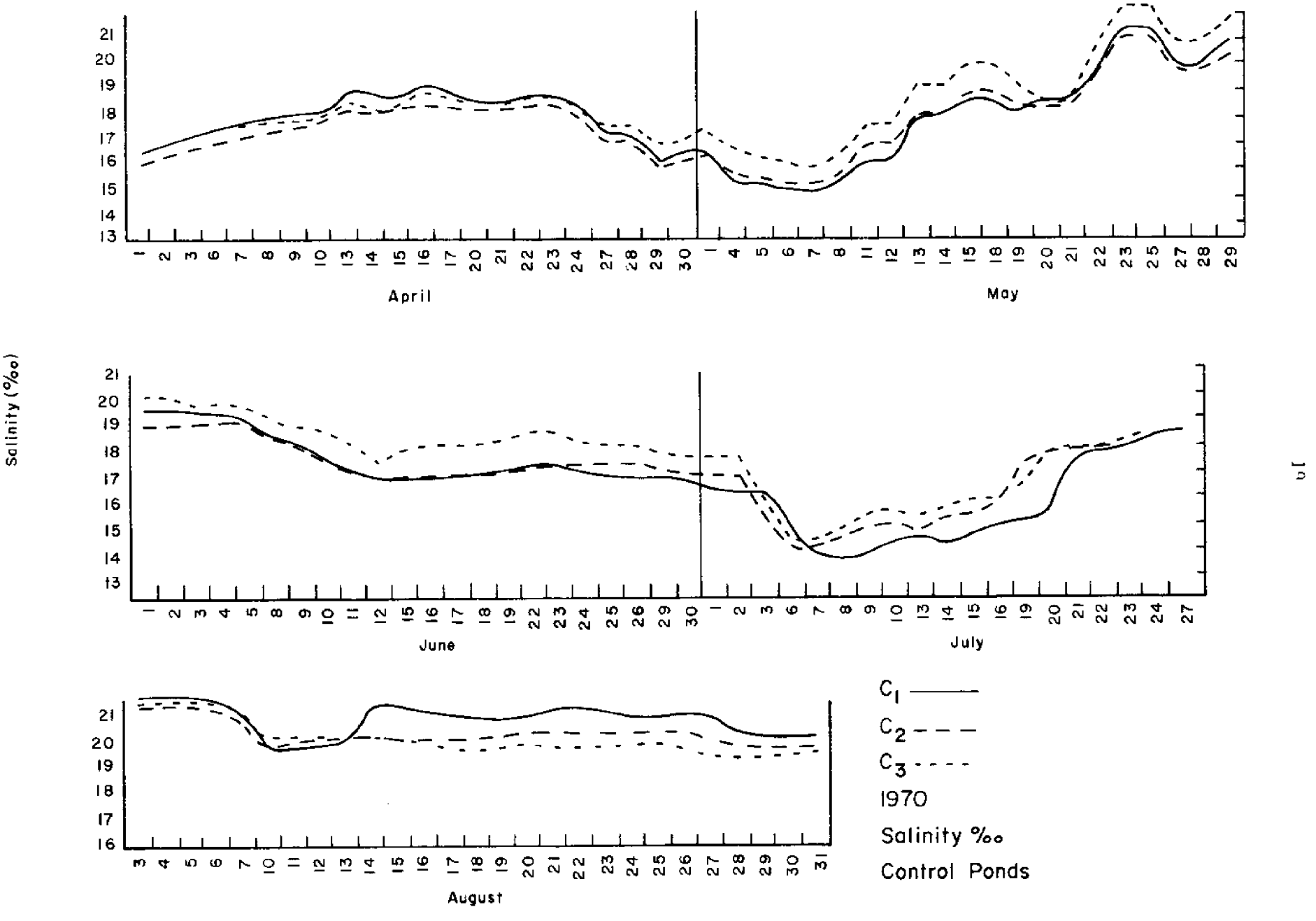

Figure 4. Saninities in the control ponds April-August 1970.

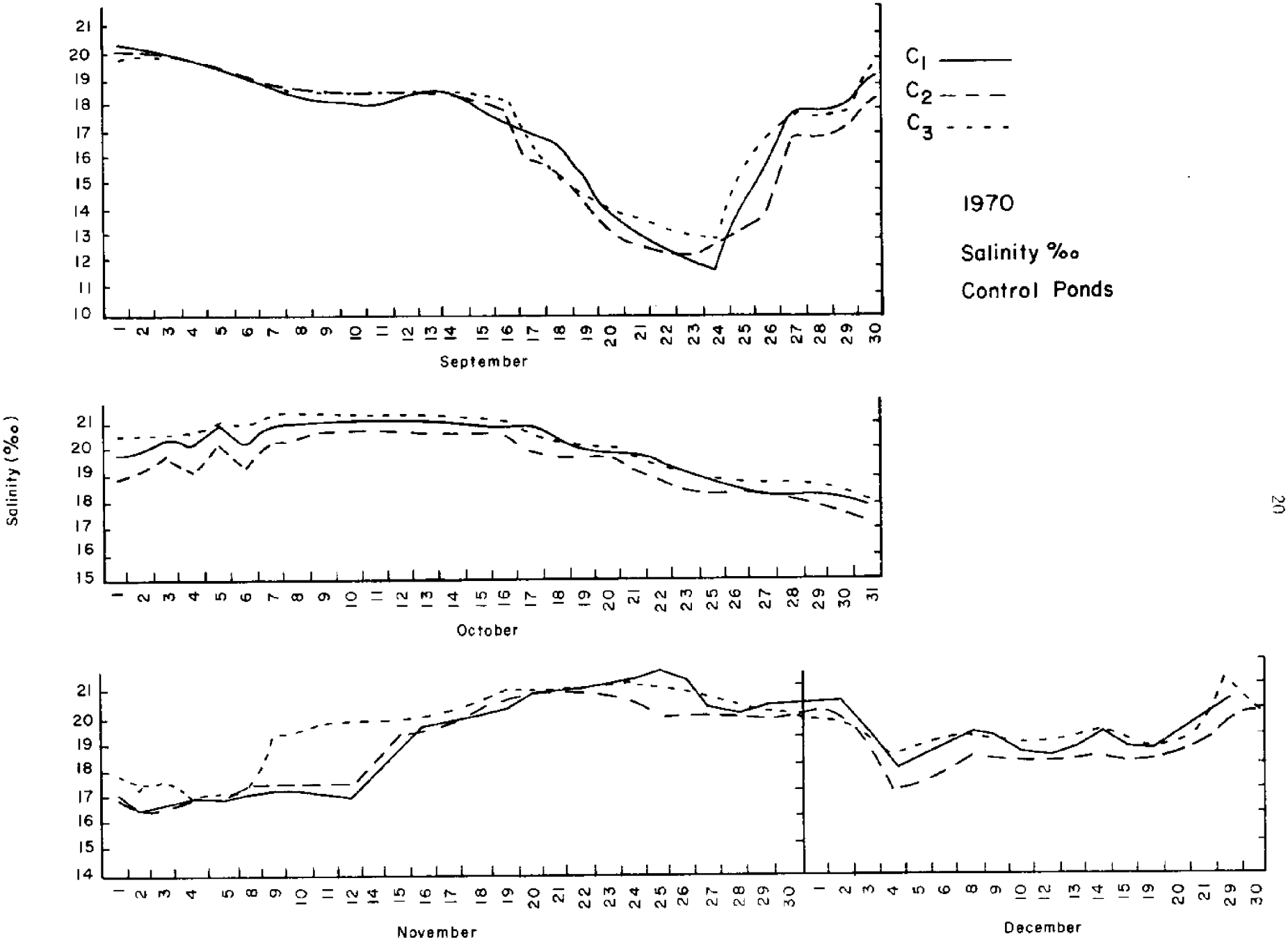

Figure 5. Salinities in the control ponds September-December 1970.

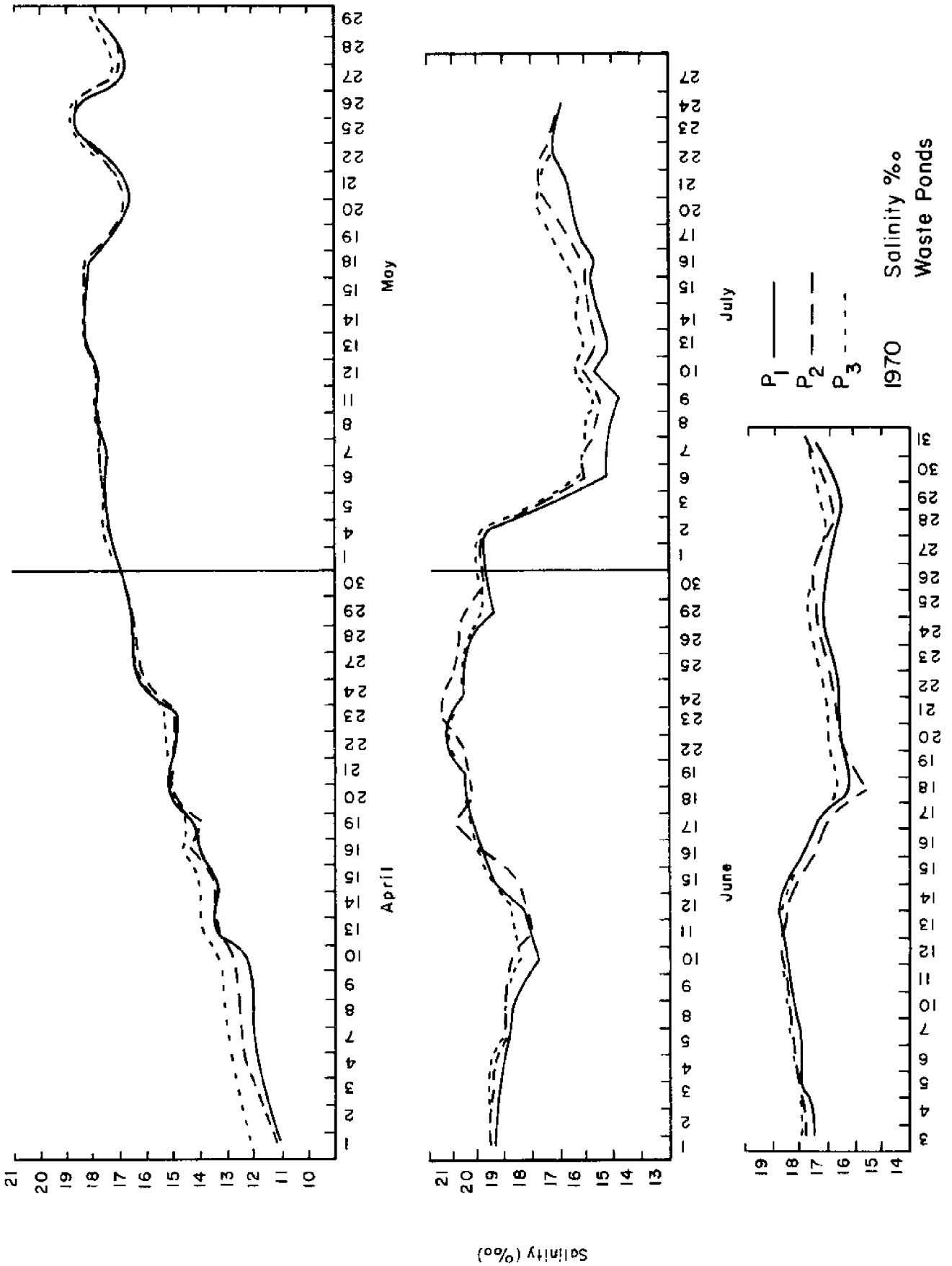

Figure 6. Salinities in the waste ponds April-August 1970.

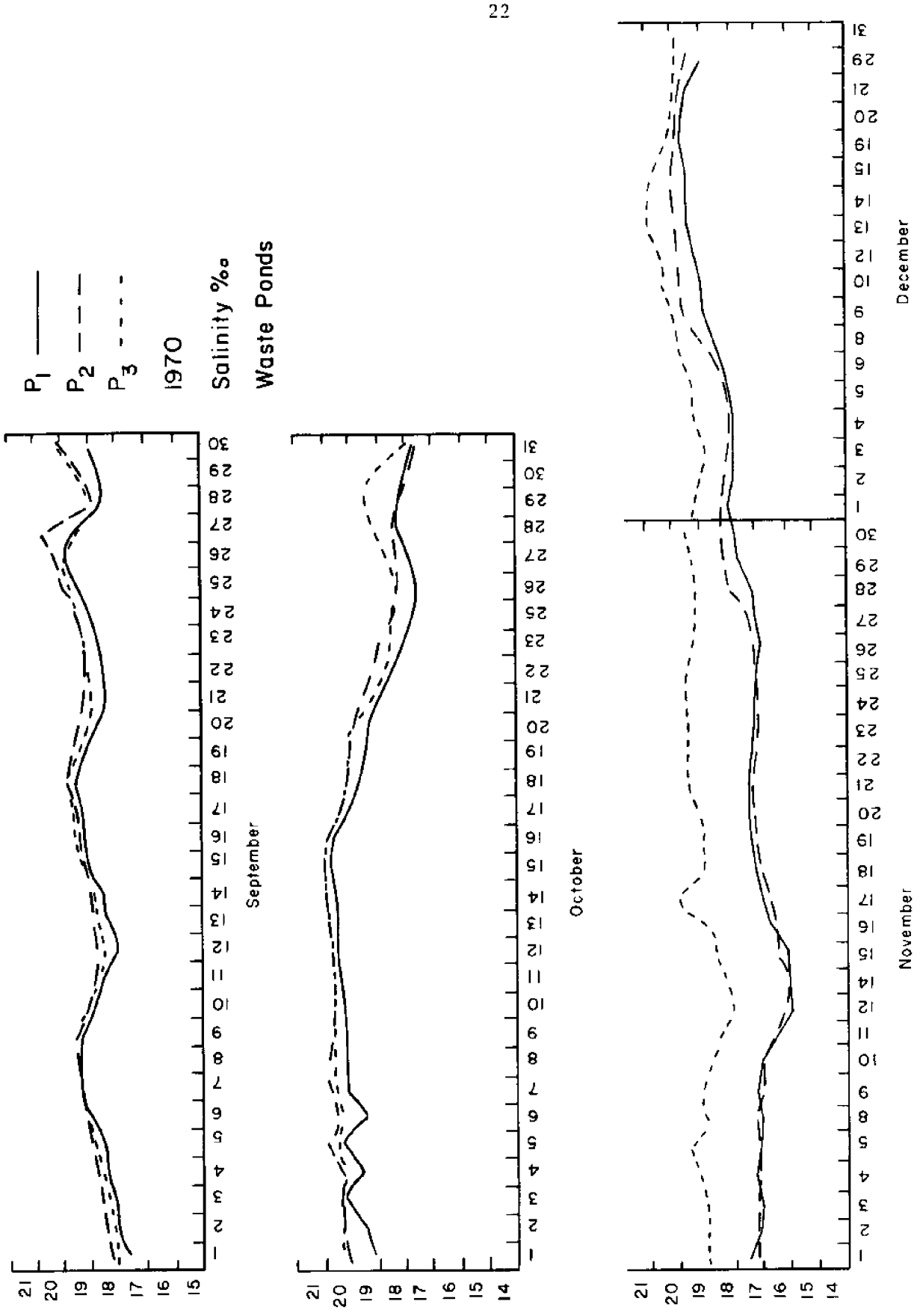

Figure 7. Salinities in the waste ponds September-December 1970.

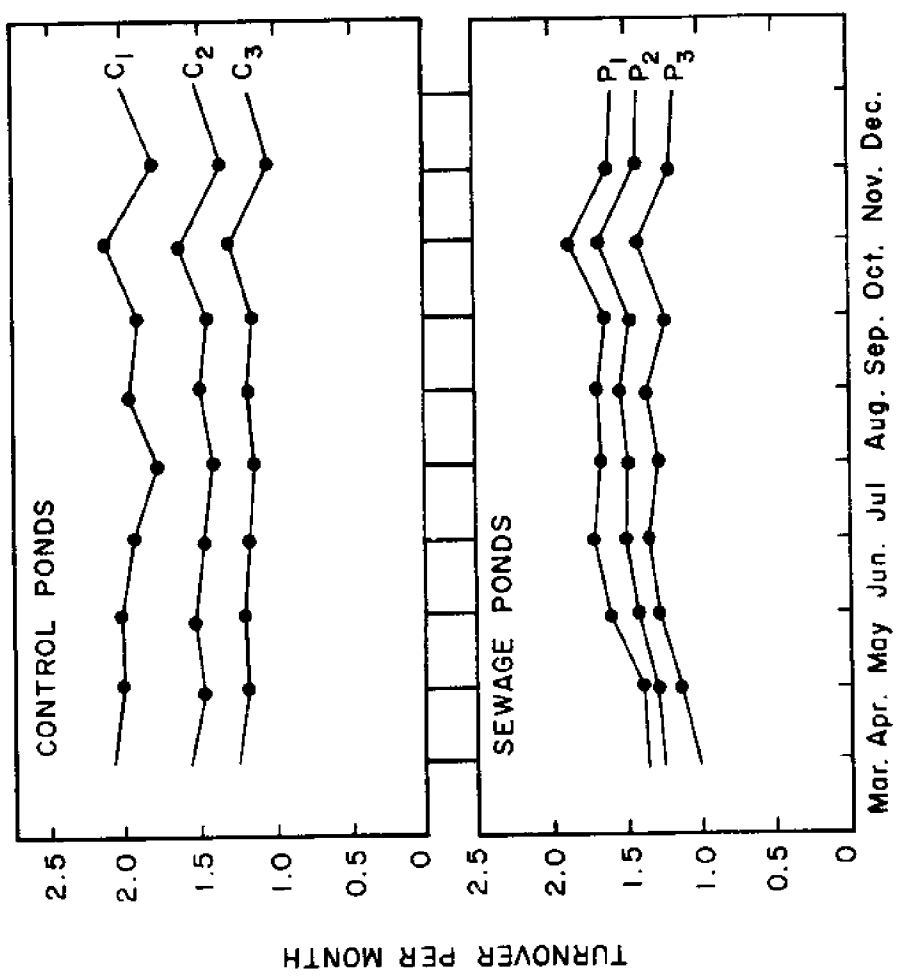

Figure 8. Turnover rates for control ponds and waste ponds March-December 1970.

Productivity of Marine Ponds Receiving Treated Sewage
from a thesis in progress by Martha Smith
Advisory Professor: Dr. H.T. Odum

Introduction

Through studies of productivity a means of providing solutions to two immediate problems of our society is being attempted. First, with the problem of overpopulation maximum use of more resources for food may become important to prevent food shortage. Second, increased sewage pollution is confronting us. We need a means for cheap processing and to provide beneficial use from the sewage.

To understand these potentials and problems ecosystems receiving treated sewage are being developed and studied.

This particular study is an effort to establish the amount of productivity occuring in six experimental ponds, three receiving treated sewage at Calico Creek in Moreheas City, North Carolina and three controlled ponds at Bogue Sound near the Institute of Marine Sciences in Morehead City, North Carolina. In this study the three waste ponds receiving effluents are designated P_1, P_2, and P_3. The three controlled ponds are referred to as C_1, C_2, and C_3.

This research includes the measurement of dissolved oxygen through duirnal curve records with emphasis on influencing parameters such as temperature, light, salinity and pH.

A continuous study was begun on 1 April 1970 and will exist through 1 April 1971, in an effort to show a comparison between the productivity of the waste ponds with the control ponds and to show a seasonal comparison of productivity within these systems.

According to previous studies before 1 April 1970 there is very little vertical turbulence in small shallow ponds such as these. Also, the diffusion constant of these ponds was previously studied by John Day who showed that the diffusion rates are low enough to be negligible in the computation of production.

Methods

To measure the overall metabolism of these six ponds dissolved oxygen values representative of each pond are taken and the results plotted.

Three water samples (60 ml. each) are taken just below the surface in BOD bottles from each of the six ponds at 0600 AM and at 1630 PM the minimum and maximum times of oxygen concentration established by hourly sampling over a twenty-four hour period at least once each month. (Fig. 1) Care was taken not to allow any bubbles to enter the samples. To assure the water in the ponds is mixing and has similar concentrations of dissolved oxygen, the samples are taken at three different stations in each pond.

The procedure used in the measurement of dissolved oxygen in the water expressed in milligrams per liter is a modification of the Winkler method by Barnes (1959).

Samples of 50 milliliters each are used in the titration procedure. The mean results in milligrams of oxygen per liter for each pond is recorded and plotted daily for the minimum and maximum concentration. These diurnal results are used to determine the net photosynthetic rate and night respiration. During the day only the excess of gross photosynthesis over daytime respiration can be measured. This excess is termed net photosynthesis. Respiration in this study is the night time respiration.

Net photosynthesis is the difference between the mean reading in the morning samples and the mean reading in the afternoon samples. This difference of dissolved oxygen expressed in milligrams per liter is converted to grams per square meter per day by multiplying the difference by the mean depth of each pond.

Night respiration is determined by the difference between the mean dissolved oxygen reading in the afternoon samples and the mean dissolved oxygen reading of the samples the next morning multiplied by the mean depth of the respective pond. Assume the mean readings for day 1 are: AM sample = 2.5 mg/l
PM sample =17.5 mg/l
and for day 2:AM sample = 4.5 mg/l
also, the mean depth of this assumed pond is 0.40 m. Then, the net photosynthesis P_n = (17.5-2.5) · 0.40m = 6.0 $g O_2/m^2/day$

and night respiration R = (17.5 - 4.5) · 0.40m = 5.2. $g^0 2/m^2/day$. Therefore photosynthesis exceeds respiration and the P_n/R is 1.1.

The mean depths for the ponds in consideration have been established as P_1 = 0.37m; P_2 = 0.41m; P_3 = 0.48m; C_1 = 0.49m; C_2 = 0.48m; and C_3 = 0.39m. (Odum and Chestnut, 1969-1970)

Parameters

At each time samples are taken the temperature, pH, salinity and amount of light are measured.

The temperature variation diurnally and seasonally is a major factor in shallow waters such as these six ponds. The temparature is measured by calibrated thermometers.

The pH has a large range in productive ponds. Photosynthesis by dense algal vegetation sometimes raises the oxygen content and the pH of the water. The pH is taken as a check on oxygen patterns and to indicate unusual conditions of acidity or alkalinity. A portable Beckman pH meter is used.

The salinity is measured by a Salinometer, model RS 5-3 of Beckman Inc. The salt water is mixed with fresh water in the controlled ponds or with effluents from the treated sewage plant in the waste ponds which flow into mixing tanks. A salinity comparable to estuaries, between 10 and 25 ppt, is being maintained.

For correlating productivity with light, light is measured by an integrating photometer. The mark IV Solameter, Talley Co., is the insolation meter being used. The insulation received is the difference between the ampere-hour readings from one evening to the next evening multiplied by the factor calibration: Ampere hours X 177 = calories per square centimeter per day.

Acknowledgments

I would like to acknowledge my appreciation to Bruno Marino, Jane Joyner and Bill Laughinghouse for their assistance in collecting data.

Results

Diurnal measurments of dissolved oxygen show a much greater range in the waste ponds than in the control ponds. This comparison is best shown in Fig. 2. The respective ponds seldom had the exact amount of production on the same day but the graph shows in a matter of days or weeks the waste ponds have followed a similar pattern and those of the control ponds have reached similar high or low production curves.

Since these ponds follow a similar metabolic pattern a representative sample of each group was taken to show a seasonal variation in daily range, net photosynthesis and respiration, and their influencing parameters.

The range of P_n/R seems to indicate both of these systems are in balance.

Summary

1. The waste ponds rates of metabolism exceed that of the control ponds.
2. Temperature seems to have a definite influence on productivity.
3. Light intensity shows a variation in production patterns.
4. Diffusion constants have already been calculated for these ponds and were found to be low enough that they were negligible.
5. The mean depths of these ponds were previously determined.
6. The oxygen range and influencing parameters are shown in Table 1.
7. The mean values of net production and night respiration and other corresponding data is shown in Table 2.
8. Presence of algal blooms shown in Table 3 correlate with productivity for respective months.
9. The P_n/R for the control ponds is 1:1.
10. The P_n/R for the waste ponds is 1:1.

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Table 1. COMPARISON OF SEASONAL RANGE OF DATA FOR WASTE PONDS (P) AND CONTROL PONDS (C)

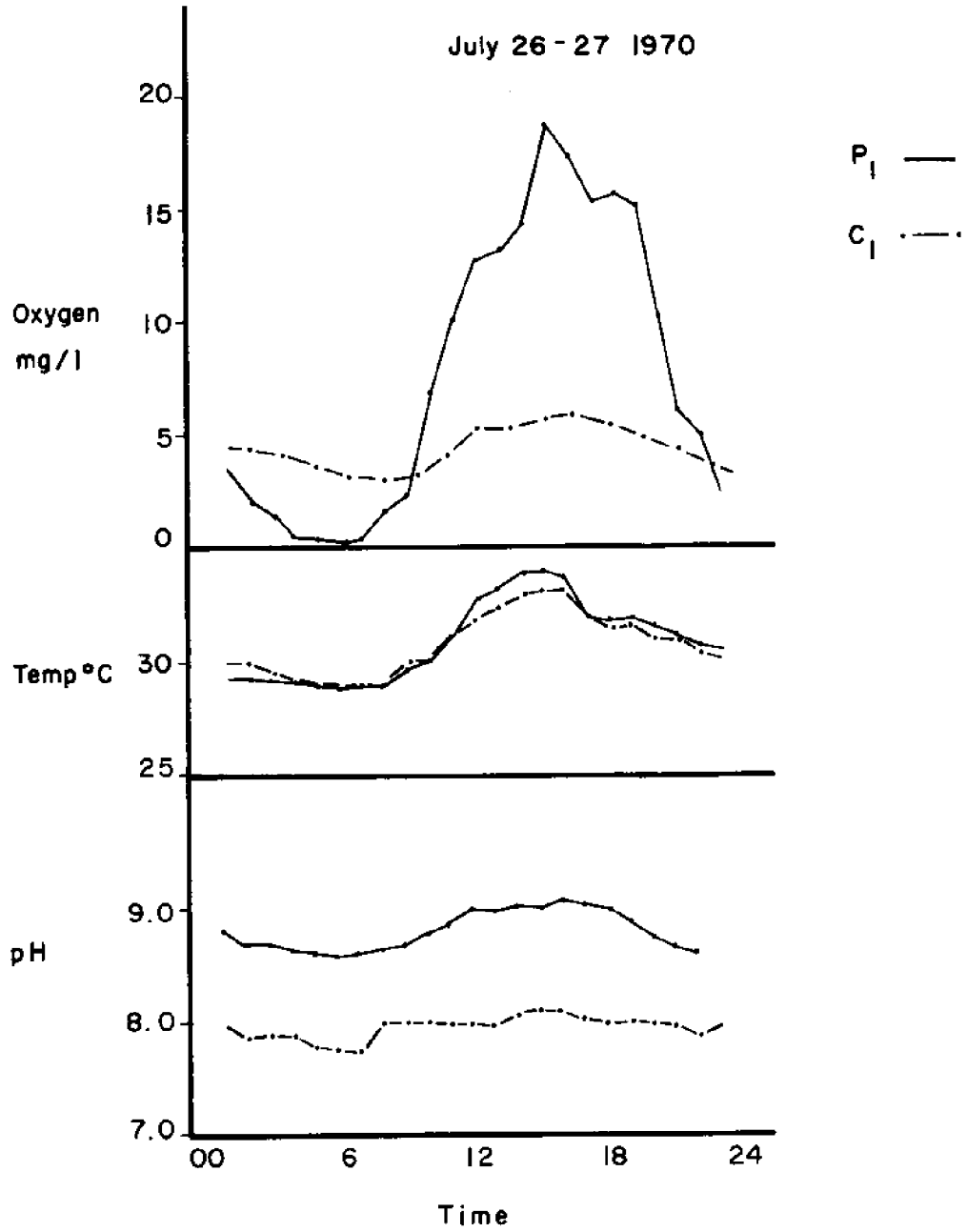
Mean		Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.
Oxygen mg/l	C	5.3-10.0	3.7-9.9	1.3-10.4	.32-9.7	2.1-11.5	3.9-14.9	4.34-12.8	6.7-13.8
	P	2.5-18.9	.29-24.1	.58-20.7	.39-16.4	.08-26.1	.30-30.3	..32-29.3	1.9-26.0
Net Photosynthesis $g/m^2/day$	C	.00-1.4	.52-2.6	.06-2.8	(-.63)-3.1	.47-3.7	.23-2.9	(-.53)-5.6	.00-2.4
	P	.00-7.2	.06-8.9	.42-7.1	.41-5.5	1.5-12.3	.73-11.5	(-.21)-9.9	(-06)-6.11
Respiration $g/m^2/day$	C	.00-1.4	.58-2.3	(-.83)-2.6	.03-3.4	.84-2.9	.01-3.3	(-.33)-5.7	.00-5.7
	P	.00-6.3	(-.05)-6.6	.61-7.7	.55-5.5	1.7-10.6	.44-10.3	.21-9.9	(-.08-8.2)
Light $cal/cm^2/day$	C&P	132.7-580.7	79.6-759.3	152.2-630.1	212.4-838.9	152.2-1083	35.4-601.8	17.7-497	40.7-315
Salinity o/oo	C	16-19.3	15.1-22.3	17.6-21.0	14.8-19.5	15.6-22.1	11.7-20.3	17.1-21.5	16.7-21.4
	P	12.2-17.6	17.6-19.8	18.0-21.7	15.0-20.2	17.5-21.8	17.5-21.1	17.5-21	15.9-19.6
Temperature ^oC	C	13.8-26.8	17.2-30.2	23.6-31.4	27.0-30.2	25.0-35.0	19,3-35.0	14-29.3	4-22.0
	P	12.0-26.8	16.4-30.2	23.8-31.2	27.0-30.2	24.6-35.0	18.0-34.0	13-29.8	3-21.5

Table 2. COMPARISON OF SEASONAL MEAN DATA FOR WASTE PONDS (P) AND CONTROL PONDS (C)

Mean		Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.
Oxygen mg/l (am)	C	6.86	5.28	4.12	2.15	4.80	6.13	6.55	8.66
	P	6,53	4.59	2.39	1.20	1.42	1.97	5.77	10.23
Oxygen mg/l (pm)	C	8.63	8.12	7.33	4.90	8.93	9.63	9.08	10.12
	P	14.83	12.52	10.70	7.22	15.08	16.68	15.40	17.56
Net Photosynthesis $g/m^2/day$	C	0.77	1.28	1.48	1.14	1.88	1.37	1.22	0.74
	P	3.44	3.33	3.46	2.46	5.65	6.15	4.18	3.30
Respiration (Night) $g/m^2/day$	C	0.84	1.32	1.53	1.21	1.84	1.33	1.29	0.73
	P	3.52	3.36	3.44	2.46	5.58	6.21	4.18	3.00
Light cal/cm^2	C&P	400.66	518.40	501.56	467.71	343.92	411.76	266.36	222.31
Salinity %	C	17.9	18.3	19.0	17.0	19.7	17.2	19.9	19.2
	P	15.6	18.7	19.9	17.1	18.5	19.0	19.7	17.8
Temperature °C (am)	C	18.1°	22.7°	25.8°	27.0°	27.4°	27.3°	20.5°	13.8°
	P	18.7°	22.9°	25.9°	27.1°	27.3°	26.7°	20.1°	12.8°
Temperature °C (pm)	C	23.2°	26.8°	29.1°	29.2°	31.1°	30.5°	23.2°	16.5°
	P	23.1°	26.9°	28.8°	29.2°	30.5°	30.3°	23.3°	16.4°

TABLE 3. DESCRIPTIVE COMPARISON OF WASTE (P) AND CONTROL (C) PONDS

		April	May	June	July
Algal blooms	C	Very Dense	Negligible	Latter half of month	Present in all C ponds
	P	Sparse	Sparse	Latter quarter of month	Present in all P_1
Color	C	Fairly clear Green to brown	Fairly clear Green to brown	Brown/green first half C_2 Green (June 10)	All C ponds green
	P	Pea Green Not Clear	Brown Clear	Brown and clear first Half turn green June 24	P_1 Green P_2, P_3 Brown/Green
Light Penetration (Secchi disc)	C	Range 47-100 cm Mean 65.94	Range 32-80 cm Mean 50.58	Range 48-84 Mean 61.5	Range 43-100 Mean 65.3
	P	Range 15-19 Mean 16.39	Range 15-100 Mean 48.14	Range 41-81 Mean 59.9	Range 28-72 Mean 43.5
Grass	C	Ruppia (C_3)	Ruppia (C_2 & C_3)	Ruppia C_2 & C_3	Ruppia small amount (C_2) Very abundant (C_3)
	P	Spartina growing profusily	Spartina growing profusily	Spartina Distichlis	Spartina



Salinity = 21.4 ‰

Light = 361.08 cal/cm^2/day

Figure 1: Diurnal oxygen curve of P_1 and C_1 for July 26-27, 1970.

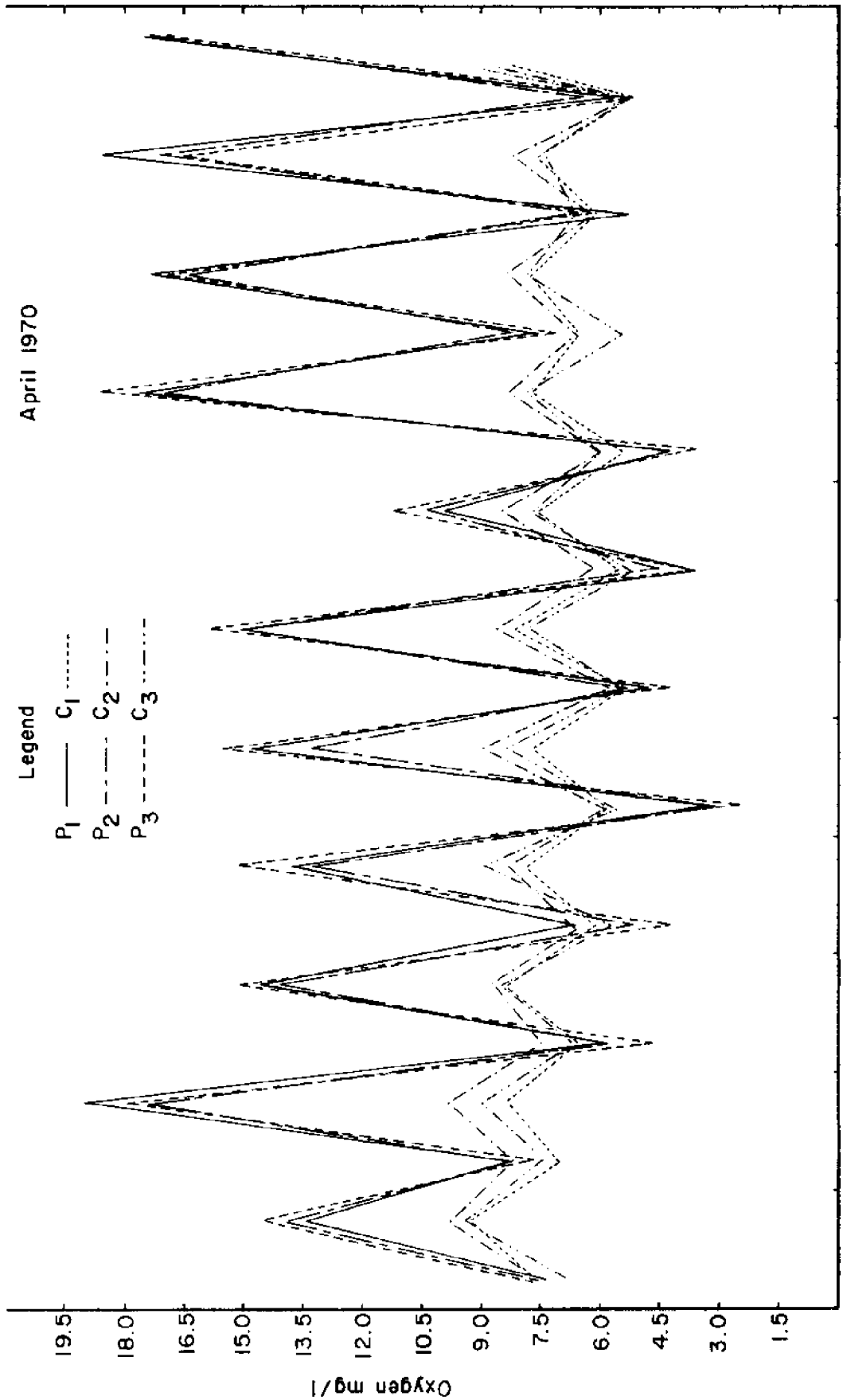


Figure 2: Comparison of the daily range of oxygen of the three waste and three control ponds. Measurements were made at 0600 and 1800 between 16 and 30 April 1970.

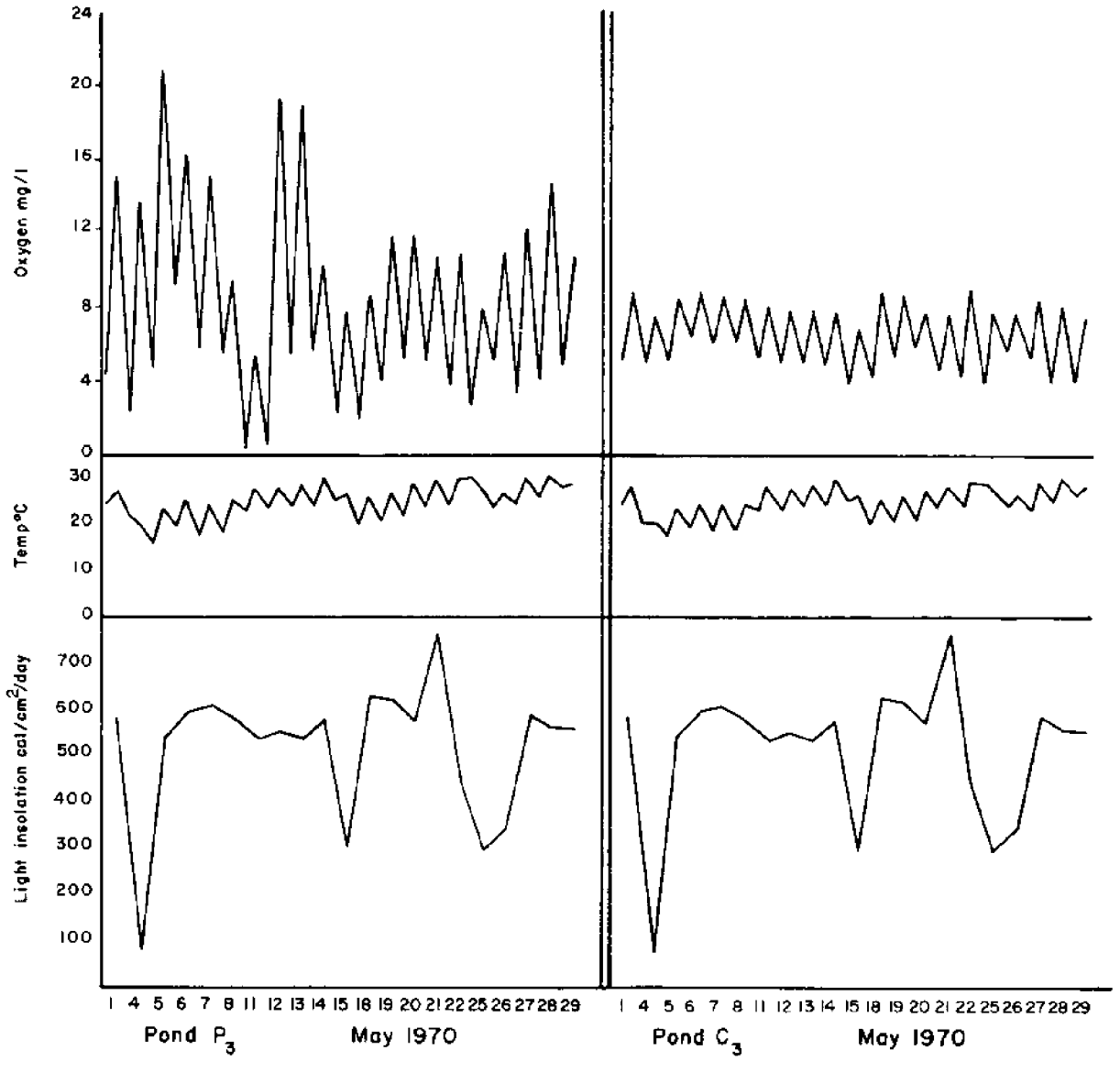


Figure 3: Representative spring comparison of a waste pond and a control pond and influencing parameters.

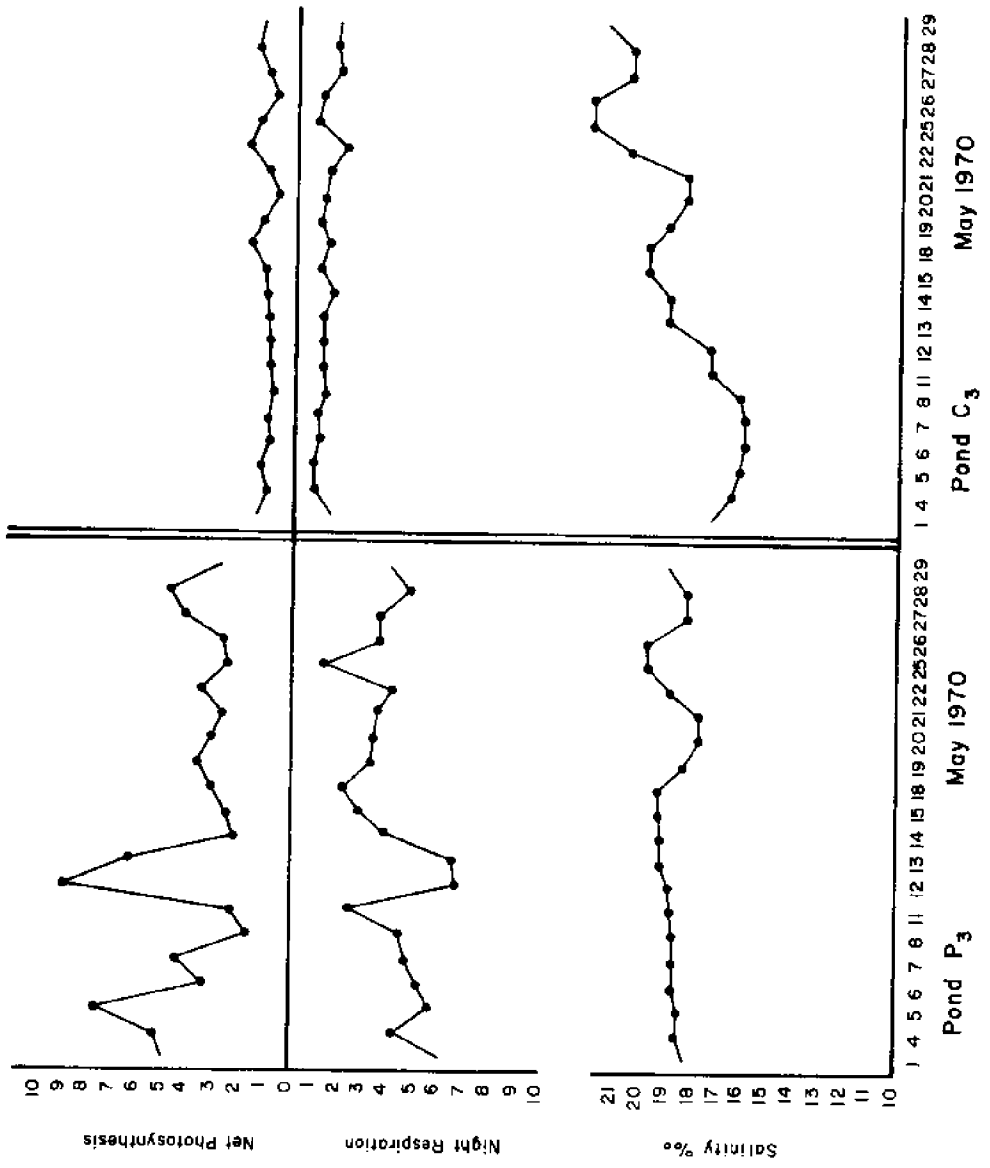


Figure 3. (continued)

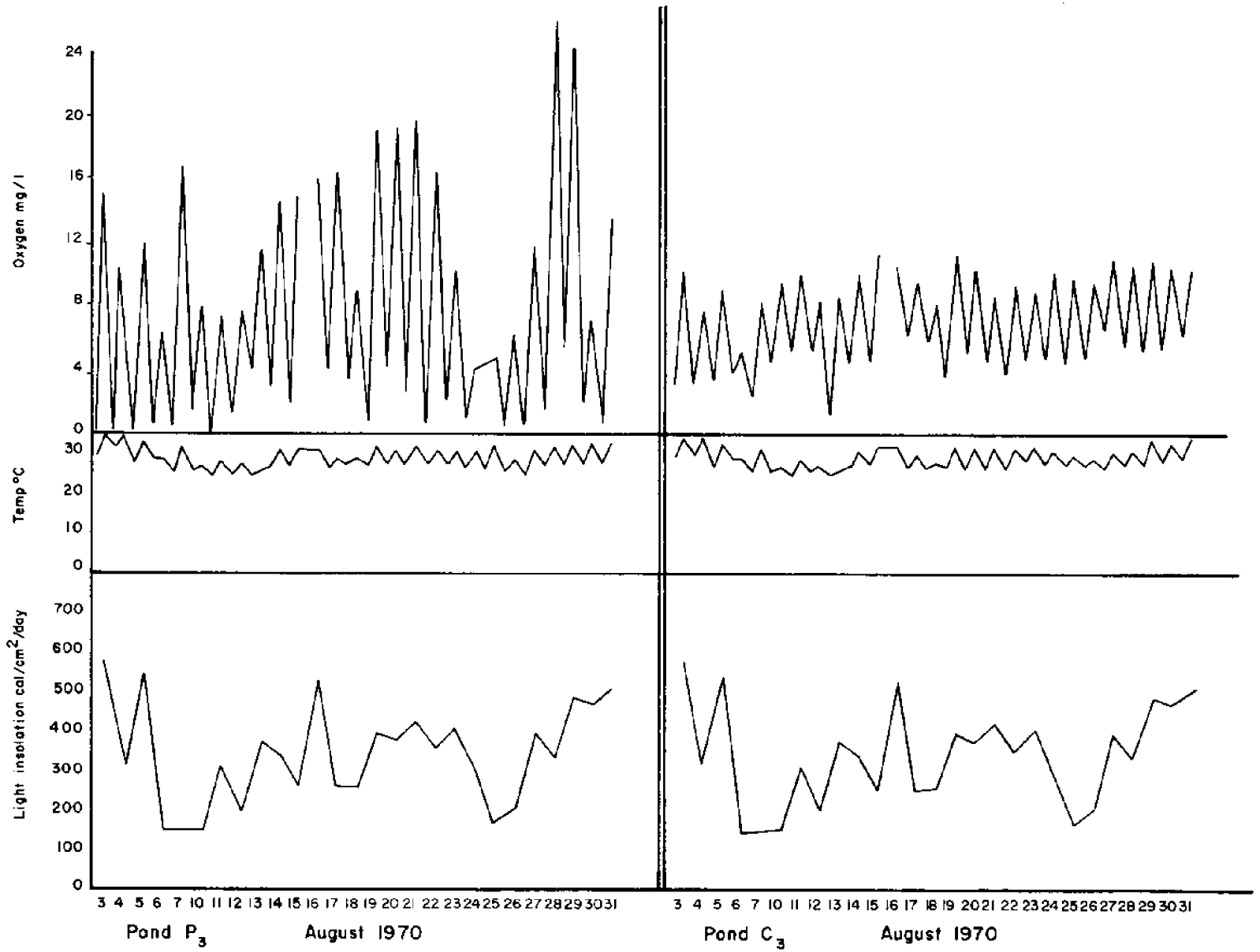


Figure 4: Representative summer comparison of a waste pond and a control pond, and influencing parameters.

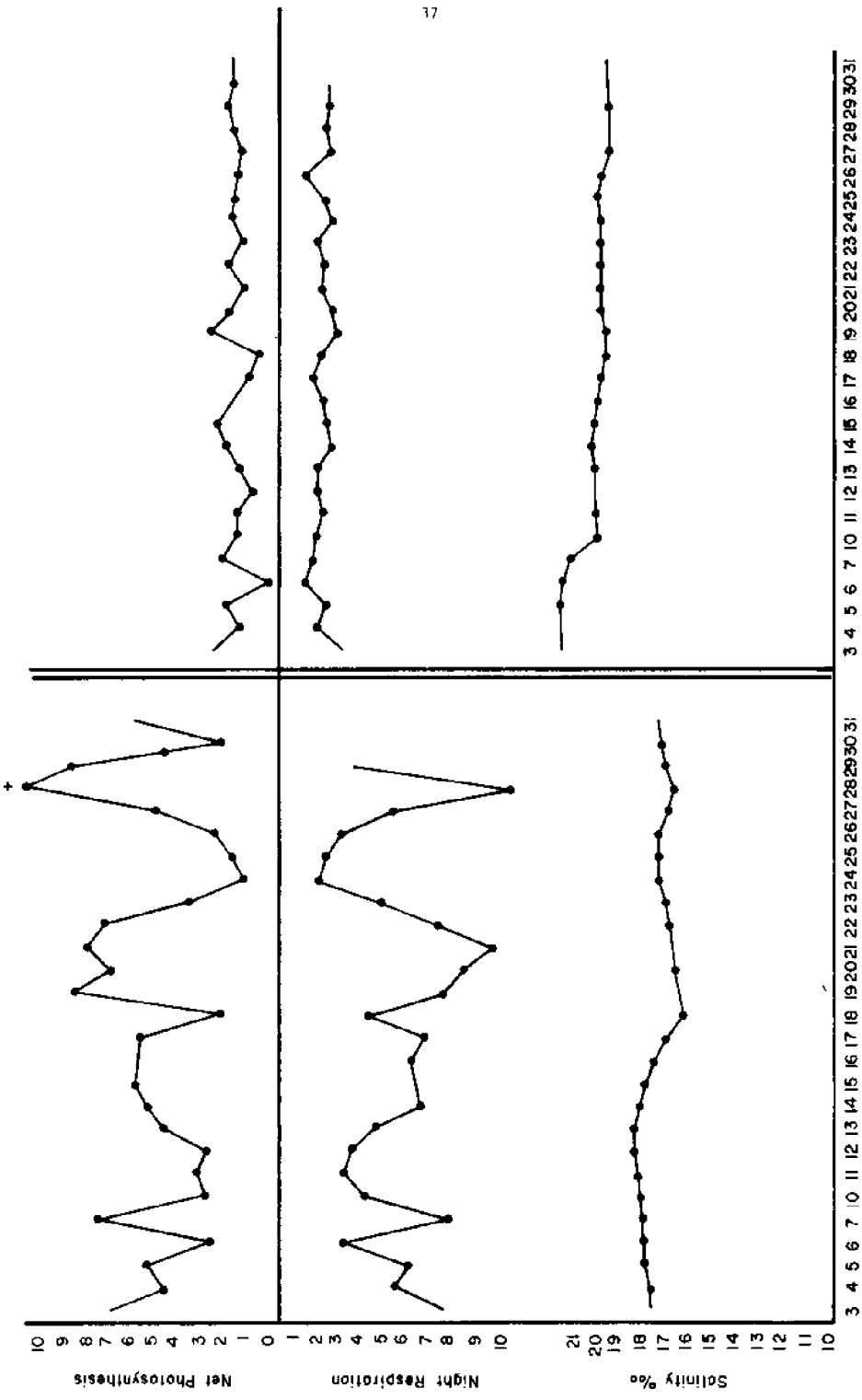


Figure 4. (continued)

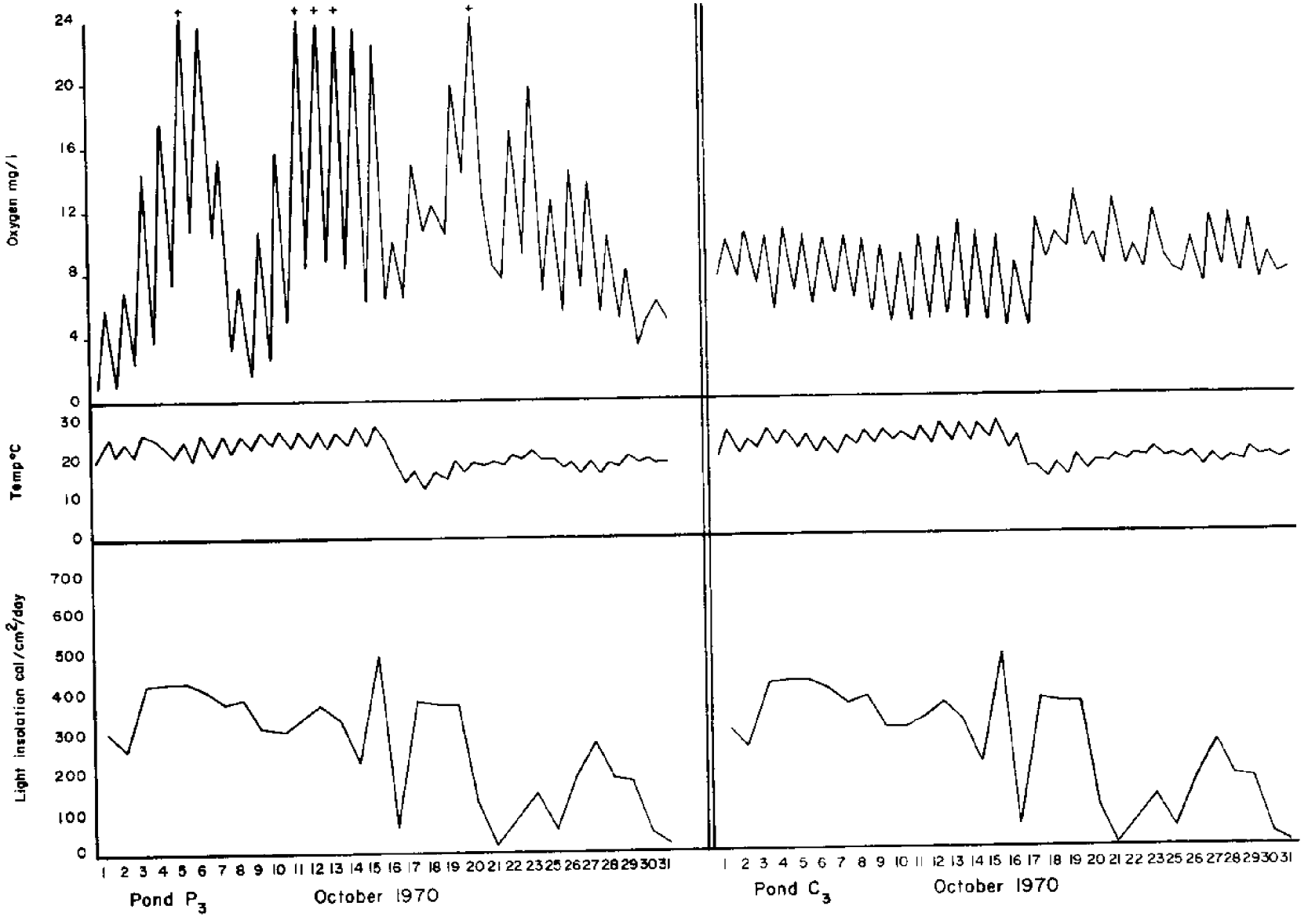


Figure 51. Representative fall comparison of a waste pond and a control pond and influencing parameters.

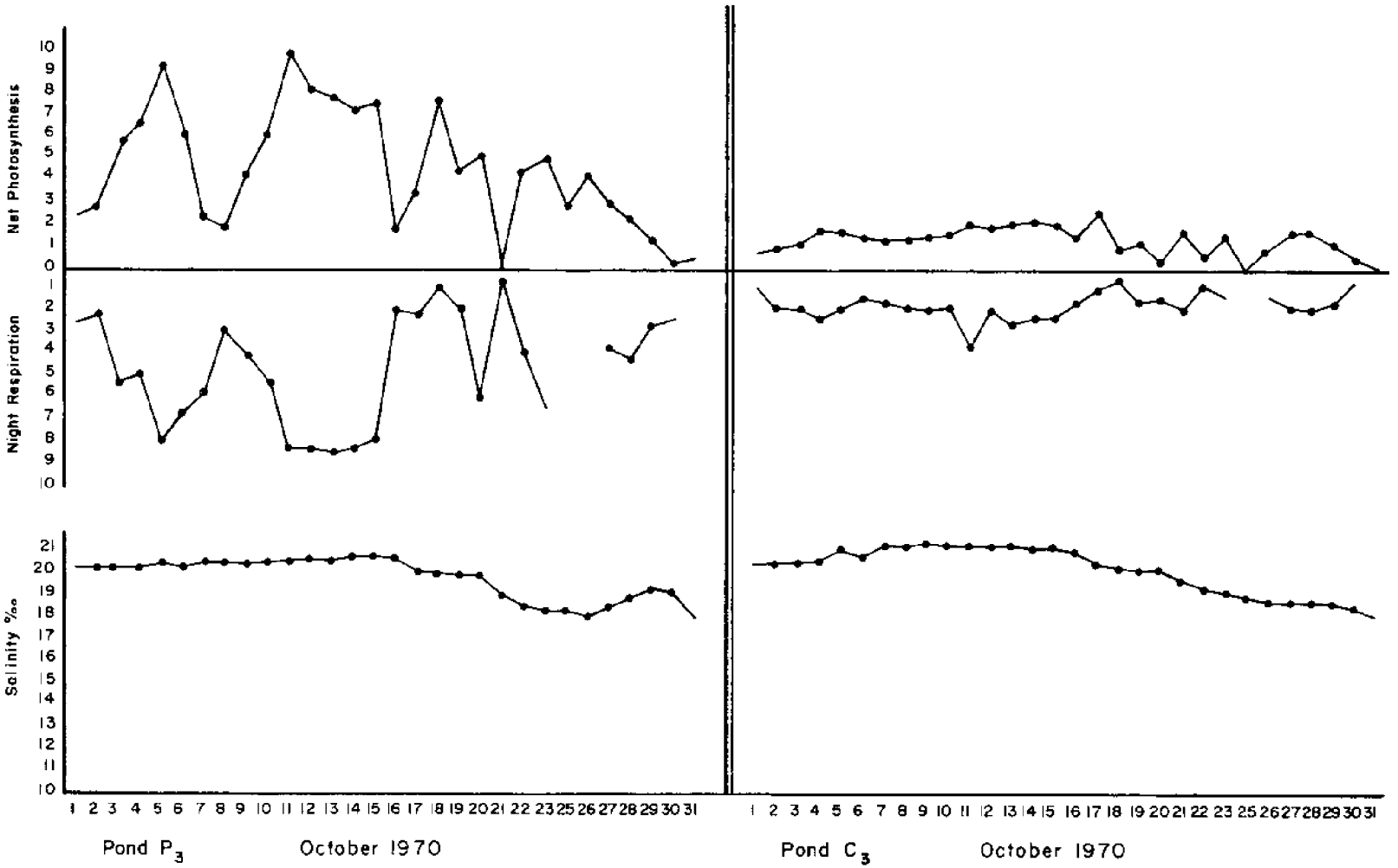


Figure 5. (continued)

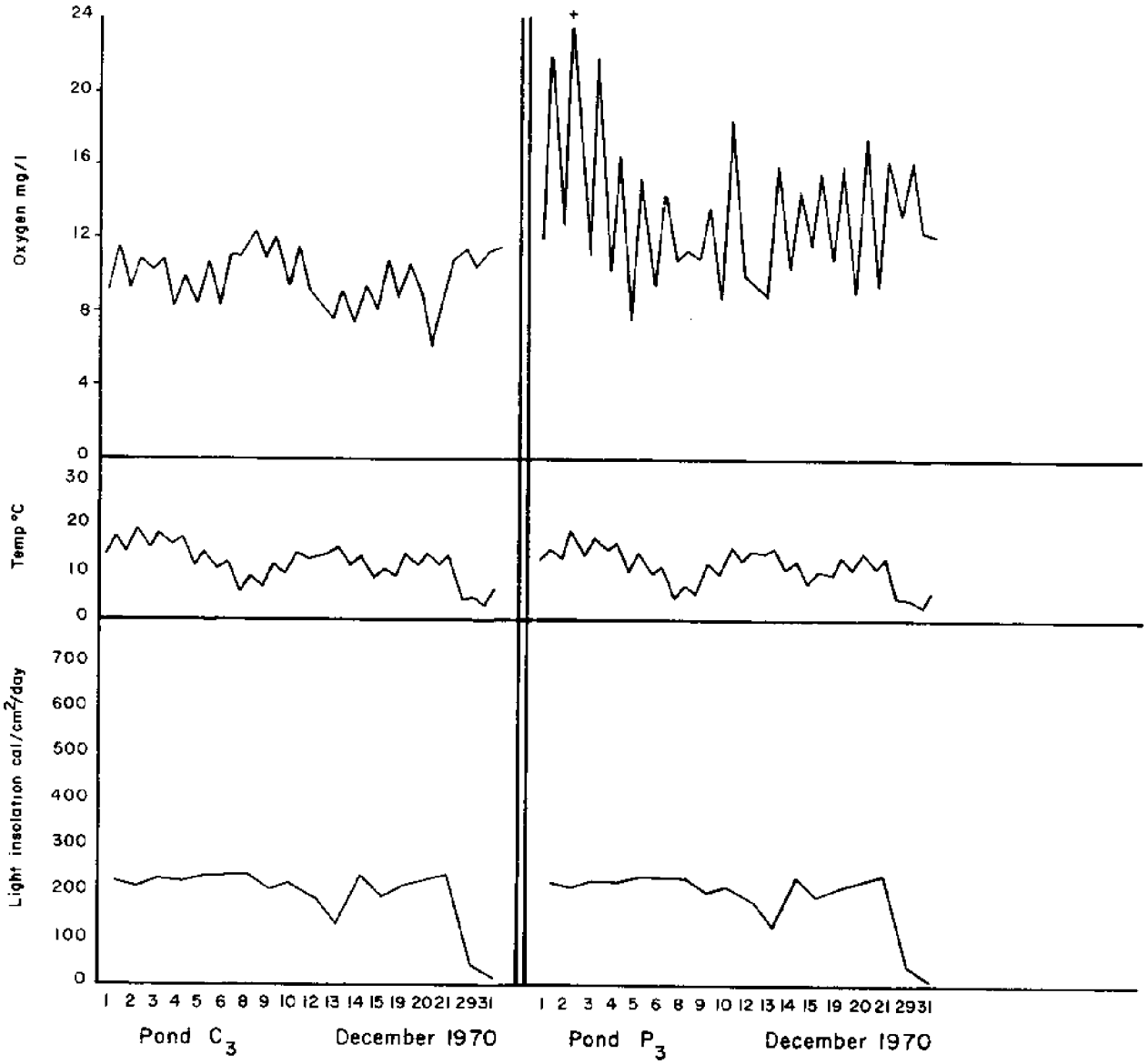


Figure 6: Representative winter comparison of a waste pond and a control pond and influencing parameters.

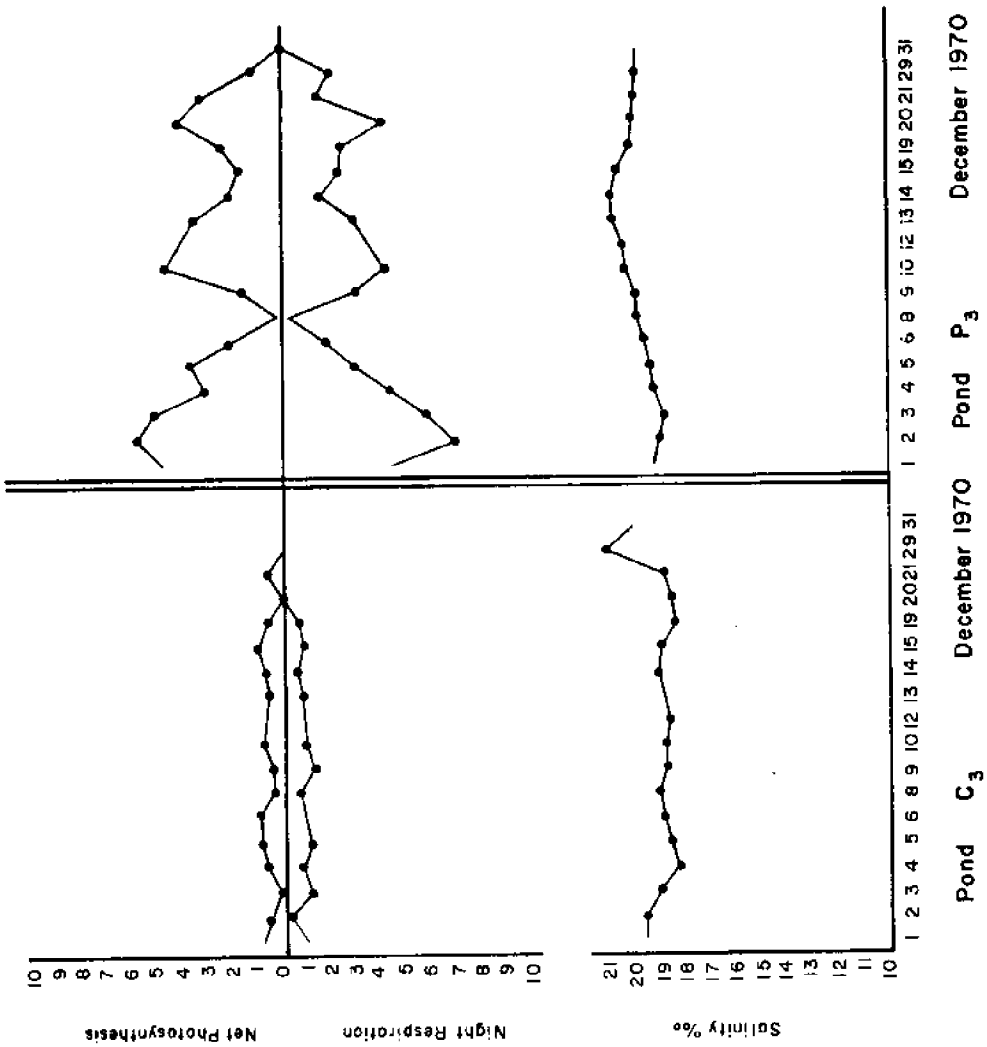


Figure 6. (continued)

CARBON METABOLISM OF THE SEA GRANT PONDS

by John Day *
Curriculum of Marine Sciences

INTRODUCTION

This is a report of studies of the carbon stores and flows in the sea grant ponds. Measurements have been made of the carbon levels in the water and in the sediments. The amount of carbon entering and leaving the ponds; from the mixing tank and diffusion from the atmosphere, and losses down the drain.

Knowledge of these flows and storages will give a better understanding of the structure and functioning of the biological community and can aid in determining to what extent the goals of the sea project have been realized.

MATERIAL AND METHODS

The methods used in measuring the carbon levels in the water and carbon dioxide diffusion are discussed in detail in the last annual report (Day, 1970). The methods used in determining the carbon metabolism and the budget of the ponds follows.

Carbon Metabolism of the Ponds

The carbon metabolism of the ponds was determined using the diurnal rate of change concept of Odum (1956) and Odum and Hoskin (1958). Diurnal curves of dissolved inorganic carbon concentrations and rate of change curves were plotted. Carbon metabolism was calculated from the inorganic carbon diurnal curves. Values for total inorganic carbon were used rather than free CO_2 because changes in free CO_2 do not reflect the total changes in inorganic carbon because much of the CO_2 produced is shifted into bicarbonate or carbonate (Beyers, 1963a). Daily values for carbon metabolism were calculated by subtracting the lowest reading on the curve from the highest reading, the difference being in mg/l or g/m^3. The area based metabolism was then calculated.

$$g\ C/m^3 \times \text{avg. depth of pond (m)} = g\ C/M^2 12\ hrs. \qquad (1)$$

The increase in carbon over the night was nighttime respiration and the decrease in inorganic carbon during the day was the net photosynthesis. These figures give the total changes in inorganic carbon and must be corrected for CO_2 diffusion to obtain the actual respiration and photosynthesis. The amount of carbon gained or lost by diffusion was calculated by integrating the area under the diffusion rate curve for the day being considered. Values above the zero line were carbon lost and values below the line were carbon gained. The area

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* Under the supervision of Dr. C. M. Weiss.

under a curve was obtained by xeroxing the graph of the curve under consideration and cutting the portion under the curve and weighing it and comparing it with the weight of a known value. The accuracy of this method was determined by cutting identical squares from the xeroxed sheet and comparing the variation among them. The differences between the squares was about 2%. Values for carbon lost from the pond were added to the respiration values and subtracted from the photosynthesis values. P/R ratios were calculated and the molar equivalent of O_2 was determined by multiplying by the molar ratio of 32 g O_2/12 g C.

Annual Carbon Budget

In order to determine overall flows of carbon through the ponds a budget was constructed for the year September 1969 through August 1970. The budget presented here is an averaged budget for each set of ponds. The concentrations of carbon in the ponds and the flows through the ponds are calculated by averaging measured values for flows and concentrations in the individual ponds. The concentrations of carbon in the ponds, the flows from the mixing tank, down the drain, and across the air-water interface are presented on a monthly basis for total carbon, inorganic and organic carbon.

The values for the concentrations of carbon in mg/l (= g/m^3) in the ponds for each month was determined by integrating the area under the averaged curve for each species of carbon for each month in the two sets of ponds (figures 1 and 2). The concentrations, presented as g/pond and g/m^2, were calculated as follows.

$$(g/m^3) \times (\text{avg. volume in } m^3) = \text{grams carbon/pond} \qquad (2)$$

$$(g/m^3) \times (\text{avg. depth in m}) = \text{grams carbon}/m^2 \qquad (3)$$

Values for average depth and average volume were obtained by averaging the mean depths and volumes for the three ponds in each set.

The input for the control ponds was obtained by multiplying the concentration of carbon in the input water by the rate of flow of the water into the ponds. The concentration in the input water was calculated by integrating the area under the annual curve for the C-mixing tank for each month. The amount of water flowing into each pond, in gal/month, has been measured by Perry Parks and Bill Laughinghouse. An average input for each month was obtained by averaging the three separate inputs. The input flows, presented as g/month and g/m^2/day, were calculated as follows.

$$(g/m^3) \times (\text{gal/month} \times 3.785 \text{ l/gal} \times m^3\text{l}/1000 \text{ l}) = \text{g Carbon/month} \qquad (4)$$

$$(\text{grams/month}) \times (1 \text{ month/y days}) \times (m^{-2}) = \text{g Carbon/m2/day} \qquad (5)$$

For y the number of days in the month under consideration was used and for m^{-2}, the average surface area for each set of ponds was used.

The calculation of the input to the P-ponds was not as straight forward because of the mixed pumping schedule. The volumes of creek water, sewage effluent, and mixed waters pumped each month into the P-ponds are available as above. Values for carbon concentrations in each of these water types were calculated by averaging all individual measurements made on each one. The inputs of each type to the three ponds were averaged. The total input of carbon each month was calculated by first determining the amount of carbon input for each of the water types using (4) and (5) and then adding the three together.

The amount of carbon lost or gained through CO_2 diffusion was determined as follows. For the control ponds there was constant outward diffusion. The average loss per day was determined by finding the area under the average diffusion curve for the control ponds (Curve C in fig. 5a) which is in g C/m^2/day. This value can be multiplied by the appropriate number of days and the average surface area of the C-ponds to obtain g carbon/month.

In the waste ponds diffusion was constantly inward during the Monodus bloom and assumed a more normal diurnal pattern at other times of the year. For November through April, the average curve of diffusion in the P-ponds during the bloom(Curve PB fig. 5a) was used and the flow rates were calculated as in the control ponds. For May through September, the average curve for the P-ponds during non-bloom periods was used (curve P-NB, fig. 5a). For the month of October when the Monodus was just beginning the curve for P-2 on October 4 was used (fig. 5a). It is assumed that this curve is a good approximation of the intermediate conditions which existed at that time.

In calculating the amount of carbon lost down the drain, there were no measurements of water flow down the drain and several assumptions had to be made. For the waste ponds it was assumed that the amount of water going down the drain was equal to the amount of water entering the ponds. This assumption is based on the fact that the P-ponds frequently were backflooded from Calico Creek. Examination of the sea grant log book shows that back flooding occurred usually during the period of spring tides each month. It is probable that this is not a complete record since the backflooding would be recorded only when it was observed. The log book also shows that on several occasions water entered the pond by running over the banks as well as through the standpipe. Since the water in the creek is of a higher salinity, most of it would sink under the pond water and displace it. Evaporative losses are negligible when compared to the amount of water entering from backflooding.

For the control ponds, the amount of water going down the drain was calculated from evaporation and precipitation data. Daily precipitation records are kept by the staff of the Institute of Marine Sciences. A curve for evaporation was constructed from measurements made during the year. Daily values, in mm/day, were plotted and a curve was drawn through the points. An average figure for evaporation for each month was read from the curve. The assumption was made that no evaporation occurred on days when precipitation was greater than

the average evaporation for a particular month, and that evaporation was the average on all other days of the month. The number of days when evaporation exceeded precipitation was multiplied by the average evaporation for that month to obtain the total evaporation for that month in mm. Since one mm evaporation equals one liter/m^2, the amount of water loss from the ponds per month in evaporation was calculated as follows.

$$\text{mm evaporation} = \text{liters}/m^2 \times (\text{surface area in } m^2) \times \frac{(\text{gal})}{3.785\ \text{l}} = \frac{\text{gal}}{\text{month}} \qquad (6)$$

This value was subtracted from the amount of water entering the ponds from the mixing tank to obtain the amount of water going down the drain. Having the amount of water going down the drain and the concentration of carbon in the water the rates of carbon flow can be calculated using (4) and (5).

RESULTS

The averaged annual variations of carbon in the waste and control ponds are shown in figures 1 and 2. During the winter there was an intense bloom of small green alga, Monodus. The characteristics of the waste ponds were so different during the bloom that the year can be conveniently divided into two parts; the period of the Monodus bloom and the rest of the year when the bloom was absent.

The effect of the bloom on carbon levels in the waste ponds can be seen in figure 1. During September and October both inorganic and organic levels fluctuated around 25 mg/l. In the early part of November the organic levels began increasing while the inorganic carbon decreased. For much of the bloom period the inorganic carbon was less than 5 mg/l while the organic level increased steadily, with occasional fluctuations. By the last week in April the average organic concentration reached 73 mg/l. Then the levels fell rapidly, approaching 20 mg/l by May 15. Concurrently the inorganic levels rose to 20 mg/l by May 15 and 40 mg/l by June 15. The total carbon in the water dropped from 75 mg/l to 40 mg/l in the two week period. These changes in carbon during the first two weeks in May are due to the fact that the Monodus bloom died in the first week in May. The drop in total carbon may simply represent the flushing of the algal cells, since the turnover time of the ponds is about two weeks. Another possible explanation is that the dead cells settled to the bottom and were consumed by benthic heterotrophs. The increase in inorganic carbon in the month and a half following the death of the bloom may be due to increased respiration from the metabolism of the plant material. With the onset of the bloom the average pH rose from 8.5 to above 10 and at the time of the crash the pH dropped to less than 8.5 in three weeks (figure 4). Likewise, the carbonate alkalinity shows the effect of the Monodus bloom (figure 3). During the bloom the alkalinity was less than one milliequivalent/liter

(meg/l) while at other times of the year it was about 3.5 meq/l.

The annual variations of carbon in the control ponds are presented in figure 2. Organic levels are low year round being slightly lower January through March. There is a slight decrease in the total carbon during the colder months of the year. In May and June there were two peaks of inorganic carbon and a small rise in the organic concentration in May. Two large peaks were measured in the input water which correspond to the two peaks measured in the ponds. The steep decline in the second peak is explained by the fact that the fresh water pump was broken at that time and only sea water was being pumped. The carbonate alkalinity evidenced a similar pattern because the fresh water had a higher alkalinity than the sea water (fig. 3). The reason for the high inorganic values in the input waters is not clear. The dir ctor of the Morehead City water plant reported that a new water line was opened in the last two weeks in April and resulted in higher water pressures and a little more chlorine in the water. It is interesting to note that the crash of the Monodus bloom began almost immediately after the large peak in inorganic carbon. The carbonate alkalinity, like the total carbon shows a slight decrease during the colder months (fig. 3). The pH remains almost constant over the year (fig. 4).

Diffusion

The results of the diffusion studies on the sea grant ponds are presented in figures 5, 6 and 7. The graphs for the control ponds (labeled C in the three figures) were obtained by averaging all of the individual studies. The data for the P-ponds was divided into two groups; those studies made during the Monodus bloom (labeled P-B) and the studies conducted at other times of the year (labeled P-NB). In addition, the diurnal record of P-2 on October 4 1969 is included in figures 5 and 6. This study was conducted when the Monodus bloom was just beginning and is included because it shows a transition between the two sets of P-pond data.

The diurnal variations in the rate of CO_2 diffusion are presented in Fig. 5a. Positive values indicate outward diffusion and negative values indicate inward diffusion. In balanced systems, CO_2 normally diffuses out at night due to respiratory production and in during the day because of photosynthetic uptake. The averaged graph of the P-ponds during the non-bloom period comes closest to this condition. Although there is a net diffusion out, diffusion is into the ponds in the late afternoon. Carbon dioxide diffused out of the control ponds continuously because of the high dissolved CO_2 levels which always exceeded saturation. There was a small diurnal variation with the diffusion being greatest during the pre-dawn hours. During the Monodus bloom there was a continual diffusion of CO_2 into the P-ponds. The diurnal curve is well developed; the inward diffusion being greatest during the latter part of the day. The net productive character of these ponds during the bloom means that there is a net loss of CO_2 from the aqueous phase which must be replaced by diffusion from the atmosphere. The record for P-2, October 4, is intermediate between the two averaged graphs of the P-ponds.

Averaged diurnal pH curves are presented in fig. 5b. All of the curves have the typical diurnal shape. The average for the P-ponds during the bloom is about 10 with a range of 0.4 pH units. This range would be higher if there were no CO_2 diffusion. The range for the P-ponds during the non-bloom period is about 0.5 pH units with a mean of about 8.3. The diurnal range is lowest for the control ponds (less than 0.3 pH units). The higher ranges in the P-ponds is indicative of their higher productivity.

The average diurnal variations in the concentration of dissolved CO_2 are presented in Fig. 6. The concentrations are plotted on a log scale to encompass the large variations encountered in the ponds. The highest concentrations are found in the control ponds. This is due to the tap water entering the ponds which is oversaturated with inorganic carbon. This explains the constant outward diffusion in the control ponds. The dashed line is the saturation concentration of CO_2 in equilibrium with the atmosphere. The concentration of CO_2 in the P-ponds during non-bloom periods varies above and below saturation. During the Monodus bloom the CO_2 concentration was between 10^{-7} and 10^{-8} moles/liter. Notice that there is a sharp drop in the morning and very little variation during the day.

The averaged diurnal variations in the diffusion constant for carbon dioxide, k, are presented in fig. 7. Recall that the units of k are for approximately a 0.03% atmosphere of CO_2 and not 100% as is often reported in the literature. The stripped and hashed areas are values of k reported by Sugiura, et al. (1963) for moderately stirred and static systems, respectively. The values of k for the P-ponds during the bloom were mostly greater than k for a moderately stirred system. The values were higher toward the end of the 24 hour period. There is a distinct diurnal pattern for the P-ponds during the non-bloom period. Values for k are low during the pre-dawn and light hours and towards the end of the 24 hour period. The averaged curve for the control ponds is low, being just above the values for a static system, and without any significant diurnal variation.

Carbon Metabolism of the Ponds

Examples of diurnal variations and rate of change of total inorganic carbon are presented in figures 8 and 9. The record of P-1, Aug. 2-3, is shown in fig. 8. There was more inorganic carbon at the end of the diurnal indicating that respiration exceeded photosynthesis in the preceeding 24 hours. The rate of photosynthesis was about constant until 1300 hours and decreased after that. The rate of respiration was highest before midnight and declined in the early morning hours. Photosynthesis exceeded respiration during the study of P-3 on Aug. 29-30 (fig. 9). The rate of photosynthesis increased until about 1400 hours and then decreased. The rate of respiration was highest from sundown to midnight and did not increase after 0400 hours.

The results of calculations of carbon metabolism are presented in Table 1. Non-corrected and corrected values for nighttime respiration and net photosynthesis, diffusion corrections, and P/R ratios are included. Both respiration and photosynthesis are generally higher in the P-ponds. Values for nighttime respiration range from 0.23 to

2.25 g C/m^2/12 hrs in the P-ponds and from 0.67 to 1.37 g C/m^2/12 hrs in the control ponds. Values for net photosynthesis range from 0.47 to 2.68 in the P-ponds and from 0.42 to 1.08 in the control ponds. P/R ratios were all less than one for the control ponds, and ranged from 0.46 to 4.79 in the waste ponds. Corrections for diffusion were small for the waste ponds during the non-bloom period, but significant in the control ponds and the P-ponds during the Mondus bloom because of the unusual diffusion patterns.

Annual Carbon Budget

Data on the carbon budget of the two sets of ponds is presented in Tables 4 and 5 and in figures 10 and 11.

The inputs of carbon to the ponds should be equal to the losses. For the waste ponds the total input is greater than the total export. The input includes carbon from the mixing tank and a net flow of carbon from the atmosphere. The export is carbon lost down the drain. The excess input is 25115 g/year or 12.78%. On a daily basis this excess flow amounts to 0.142 g C/m^2/day. The opposite situation occurred in the control ponds. The calculated total export of carbon from the ponds is greater than the total input. The excess export is 19435 g carbon/year or 9.56%. On a daily basis this flow is equal to 0.101 g C/m^2/day. The flows and storages of carbon in the waste ponds are shown in figure 10. The concentration in the ponds and the loss from the ponds show the effect of the bloom. During the winter months most of the carbon in the ponds was organic carbon and hence most of the export was organic carbon. The high inorganic carbon in the input reflects the concentration in the input wastes as well as the mixing tank.

The budget data for the control ponds (figure 11) shows none of the large fluctuations that occurred in the waste ponds. Organic levels in the pond, and the amounts input or loss does not vary greatly.

DISCUSSION

These shallow basins, receiving large inputs of organics and nutrients with relatively high metabolic rates and periodic blooms are similar to many other systems which receive sewage wastes. Many oxidation ponds and tertiary treatment lagoons are similar to the sea grant ponds. Weiss (1965) and Hartley and Weiss (1970) have studied oxidation ponds in Durham, N. C. The species diversity of phytoplankton was low and there were often persistent blooms. Dissolved oxygen underwent large daily excursions and often approached low values at night. The ponds were shallow with an average depth of 2.4 feet and O_2 and temperature isopleths indicated that the ponds destratified daily. Bartsch and Allum (1957) studied treatment lagoons in 99 communities in the Northern Plains states. The average depth of all the ponds was 1.2 m. The ponds, which were net productive, had shallow eutphotic zones and were often green in color. There were large diurnal fluctuations in oxygen. There was sometimes less production in the afternoon and they suggested that low CO_2 was responsible. Curves of O_2 with depth indicated that the ponds mixed each day.

Weiss and Wilkes (1969) reviewed the literature on estuarine ecosystems receiving sewage wastes. Heavy loads of both organic matter and nutrients were common. There were often heavy photoplankton blooms which consisted of one or few species which replaced the normally more diverse flora. Species diversity was low in the polluted waters and increased with decreasing pollution. Copeland and Wholschlag (1968) added artificial sewage to estuarine microcosms constructed with mud, water, and organisms from Redfish Bay, Texas. The artificial sewage was added at 0.01, 0.1, 1 and 10% of the total volume of the microcosms. One set of microcosms was stirred to simulate the wind conditions of the Texas coast. A 1% sewage addition both stirred and non-stirred microcosms had a photosynthetic rate which was about 50% higher than the control. The community respiration increased by a similar amount. The authors commented that "such increased production is seldom advantageous to man. Many times the system reacts by exclusion of top carnivores because of instability or slight toxicity." Pratt (1950) added commercial fertilizers to salt water ponds. Standing crops and diurnal pulses of phosphate were increased. Several studies have been made on the sewage pollution in Biscayne Bay, Fla. McNulty, et al. (1960) found that phosphate and nitrate concentrations were higher close to the mouth of the Miami River where most of the sewage wastes enter the Bay. Lynn and Yang (1960) found that organic levels in the sediments were higher on the mainland side of the Bay where most of the sewage outfalls are located. Patten (1962) found that species diversity of phytoplankton in Raritan Bay, New York and New Jersy, decreased up bay toward increased pollution.

One well studied example of pollution of an estuarine system which has many parallels to the present study is the case of Moriches Bay and Great South Bay, Long Island, New York. These bays resemble the

ponds in several respects. They are shallow, have a long flushing time on the average of once a month, and have very small tidal ranges. In the past several decades a large duck farming industry has grown up on the shore of Moriches Bay and several of the streams entering the bay. The wastes of these farms enter the bay. With the rise of the duck farms there was a concurrent decline in a prosperous oyster fishery. Subsequent to the introduction of the duck farms, intense annual blooms of two species of small green algae, Nannochloris and Stichococcus. These two forms have been identified from the control ponds. The annual blooms in Moriches Bay began in the spring and persisted until late fall. Rhyther (1954) studies the ecology of these blooms. Cell counts reached 10 million/ml and secchi disk transparency was reduced to less than one foot. The duck farm wastes had a high nitrogen to phosphorus ratio and the small forms did well because they could use organic nitrogen. Before the rise of the duck farms the phytoplankton consisted of mixed populations of diatoms, green flagellates, and dinoflagellates. The persistence of the small forms is in marked contrast to seasonal patterns of succession found in non-polluted estuaries. The only time there was any significant other phytoplankton was in the winter when the temperature was too low for the small forms. It was found that the oyster industry declined because the oyster could not exist on the small form alone. Diatoms which were normally a significant portion of their diet were shaded out by the intense blooms.

Barlow, et al. (1963) studied the eutrophication of the Forge River, a short, shallow tributary of Moriches Bay. The main fresh water input to the stream was wastes from the duck farms. They found high phosphate levels in the water and that the greater part of organic matter in the water was plant cells. The P/R ratio of 1.1 to 1.2 was high enough to maintain the plant populations.

There are several similarities between the waste ponds and the conditions in the bays. Although the Monodus occurred in the colder months of the year, there were similar concentrations of cells and reduction of light penetration. Most of the organic matter was in the plant cells and there are fairly high concentrations of phosphate. The P/R ratio was high enough to increase cell numbers even though the ponds were flushed on the average of every two weeks. Like those in Moriches Bay, the oysters in the waste grew poorly and had high mortalities.

Odum, et al. (1963a) conducted a series of experiments in man-made ponds at Port Aransas, Texas, which are similar to the sea grant studies. The different systems studied included artificial reefs, a blue green algal mat, and turtle grass. The water in the reef ponds was circulated with pumps and a plankton system developed over the sessil reef consumers. When the ponds were first filled the organic levels in the water were between 5 and 10 mg/l. In about two months the levels had risen to between 19 and 30 mg/l, with approximately 75% being soluble. The diurnal range of organic carbon was about 4.5 mg/l. The alkalinity in the ponds was higher than the sea water because a high alkalinity fresh water was used to mix with the Gulf of Mexico water. Park, et al. (1958) measured alkalinities as high as 4.0 meq/l in several Texas bays. As in the case

of the sea grant ponds, this high alkalinity was due to input of high alkalinity fresh water. In the reef ponds, a heavy diatom bloom which caused very high pH values also lowered the alkalinity. Normally the plankton system in the reef ponds was dominated by small green nanoplankton. The records of metabolism in the reef ponds show rapid recovery after salinity shocks which caused the death of the oysters and other dominant populations. This ability of a system to substitute new populations with little interruption of photosynthesis and respiration was also noted by Odum, et al. (1963b) for field measurements in Nueces Bay, Texas. This type of fast substitution seems to have occurred in the waste ponds after the crash of the Monodus in May when the O_2 levels dropped to low levels for a few days and then resumed the large daily excursions. In the blue green algal mat ponds blue green algae dominated during warm weather with diatom populations taking over during cold weather. There were large daily fluctuations in dissolved oxygen, often going anerobic at night. Diurnal inorganic carbon ranges were as high as 7.7 mg/l. This is higher than any diurnal range measured for this study and is indicative of the extremely high productivities of blue green mats.

In a series of papers on the bays of Texas, H. T. Odum and his co-workers described naturally and man-stressed marine systems (Odum, 1963, 1967; Odum and Hoskin, 1958; Odum and Wilson, 1962; Odum, et al., 1963b). These systems ranged from the very polluted Galveston Bay to the relatively clean, but high salinity Laguna Madre and included such types as brackish water plankton systems, reef systems, blue green algal mats, turtle grass systems and hypersaline bays. Total phosphorus ranged from 40 ppb in the open Gulf to 150 ppb in hypersaline bays. Bays receiving waste discharges were generally higher ranging up to 2000 ppb. The metabolism in waste receiving bays ranged from very slight to very large values. Very high rates of metabolism in Galveston Bay in some instances were equal to or greater than the high natural values in turtle grass communities (40 to 60 g $O_2/m^2/day$). Very low values in some ship basins probably indicated toxic effects. Zooplankton diversities were highest (10 to 25 species per 1000 individuals) in non-polluted areas and lowest (1 to 10) in disturbed environments of the waste receiving bays.

The ranges of organic matter in aquatic ecosystems has been reported by several workers. As stated earlier, the levels of organic matter in the control ponds was usually less than 10 mg/l while the range in the waste ponds was from 20 to 75 mg/l. Wilson (1963) measured organic concentrations in various aquatic ecosystems ranging from one to 125 mg/l. The highest concentrations were found in brine ponds in Puerto Rico. Values as high as 50 mg/l were measured in the Texas bays. Duursma (1960) reviewed the literature on organic carbon in the sea and found that the range in the open sea was 0.04 to 8 mg/l. Corcoran (1952)* reported that some waters off California contained 20 mg/l. Collier, et al. (953) measured high concentrations of organics in Galveston Bay, Texas. Odum, et al. (1963a) reported organic levels between 19 and 30 mg/l in experimental reef ponds. Robertson and Powers (1967) reported 22-99 mg/l of dissolved organic material in the great lakes.

*This reference should read Fox, Issacs, and Corcoran (1952)

Odum, et al. (1963a) stated that "when high organic levels exist, it may be postulated that with restricted diversities among consumer networks, the organic levels must rise, thus stimulating micro-organisms metabolism to balance photosynthesis. It may be suggested that there is a general inverse relation between dissolved organic matter and diversity of consumer circuits among higher organisms." However, Kelly (1971) found that metabolism of organics by microorganisms was retarded by high pH. Therefore, the diversity of consumer networks in the waste ponds during the Monodus bloom may be even more restricted than Odum, et al., suggests. The results of the measurements of bottom metabolism suggests that the rate of metabolism was much less during the bloom.

Carbon Dixoide Studies

The theoretical and practical problems of gaseous diffusion across an air-water interface have received considerable attention (Bolin, 1960; Bolin and Eriksson, 1959; Eriksson, 1961; Kanwisher, 1960, 1963a, 1963b; Keeling, et al., 1965; Revelle and Suess, 1957; Sugiura, 1960; Sugiura, et al., 1963; Takahasi, 1961; and Hood and Ibert, 1960). In using the dome method for measuring diffusion it is assumed that the rate determining step is not transfer in the gas phase. Kanwisher (1963a) reported that diffusion is limited by the surface film of the water. Within this film, molecular diffusion is the only effective means of transport. This film may vary from 10 to 100 microns thick depending on the amount of turbulence in the water. Bolin (1960) also concluded that it is the aqueous boundry layer which is rate determining. He states that this is not unexpected since the gaseous diffusion constant is much greater than the constant in the liquid phase (10^{-2} cm^2/sec as compared to 3×10^{-5} cm^2/sec). Another way of stating this is that the mean free path of the gas molecules in the gas phase is much higher than in the liquid phase. Sugiura, et al. (1963) assumed that an inner tube floating on the sea surface with which they measured diffusion rates, would not significantly alter the turbulence of the water either at or below the sea surface. Turbulence of the water arises primarily from wind stress. Since this effect is realized over large areas, they concluded that the turbulence would not be minimized by the floating tube. From these considerations, it seems likely that use of the dome would not significantly effect the replacement of the surface water film.

Kanwisher (1960) found that changes in the partial pressure of CO_2 in the atmosphere do not have a large effect on the total inorganic carbon in seawater. He circulated seawater and air in a closed counter-current system and measured CO_2 in the air and total inorganic carbon in the water. He found that an increase of 10% of CO_2 in the air increased the inorganic carbon in the water by only 0.6%.

Diffusion Rate and Constant

Three general patterns were measured in the ponds (see figure 5). In the control ponds there was a constant outward diffusion of CO_2 due to the high concentrations of dissolved CO_2 which were consistently above saturation. In the waste ponds during the Monodus bloom

there was constant inward diffusion because the ponds remained undersaturated due to the high rate of uptake of inorganic carbon by the phytoplankton. The pattern for the waste ponds during the non-bloom period was intermediate between the two. On the average, CO_2 diffused out when the ponds became oversaturated at night and into the water in the afternoon when photosynthetic activity reduced the levels below saturation. Individual diurnals show that diffusion ranged from constant inward diffusion to constant outward diffusion, but the magnitude was always intermediate between the control ponds and the waste ponds during the Monodus bloom. There was a definite diurnal pattern of diffusion in all the ponds. In the waste ponds during the non-bloom period, CO_2 diffused out of the ponds at night and into the water during the day. For the control ponds the rate of diffusion out of the ponds was greater at night and for the waste ponds during the bloom the inward diffusion was greatest in the late afternoon. In general, the ponds were rarely in equilibrium with the atmosphere. Ibert and Hood (1958) reported that data collected from many areas of the ocean show that equilibrium seldom exists between carbon dixoide in the air and the water.

Values determined for the diffusion constant, k, ranged from slightly greater than values reported for static systems to greater than values reported for moderately stirred system (fig. 7). All measured values were well below those reported for the open ocean. All constants discussed here have the units of g carbon/m^2/hr/0.03% CO_2 atmosphere. Sugiura, et al. (1963) measured the diffusion constant for CO_2 in the laboratory and on the open sea and compared their results with values reported for static, moderately agitated systems and vigorously agitated systems. Values for static systems were 0.0017 and 0.001; values for moderately agitated systems were 0.014 and 0.017, and values for vigorously agitated systems were 0.355, 0.104, 0.074 and 0.049. The latter three values were measured for the sea. The data for static and moderately agitated systems are plotted in figure for comparison with values obtained in this study. Park, et al (1958) estimated a diffusion constant of 0.0055 for the shallow bays of Texas. Eriksson (1961) reported that the rate of exchange of CO_2 is lowest for fresh water lakes, higher for the open ocean and highest for rapidly flowing streams. In this respect, the ponds are most like the fresh water lakes. Hanya and Ishiwatari (1960) measured the absorption and escape of CO_2 in a system in which air flowed over a stream of moving sea water. For non-flowing conditions the diffusion constants were 0.0051-0.0064 for absorption and 0.0006-0.00097 for escape. For a flow of 3 cm/sec the constants were 0.0053-0.0072 for escape, and for a flow of 6 cm/sec the values were 0.0094-0.011 for absorption and 0.0084-.011 for escape. Thus k increased linearly with increasing flow but the constants for absorption were higher than the constants for escape. In computing k, they assumed that the exchange of CO_2 was a first order rate.

The values for k were consistently lower for the control ponds. At first consideration, it would seem that since the two sets of ponds have about the same average depth and are subjected to similar wind conditions, the diffusion constants should be about equal.

However, as mentioned in the previous paragraph, Takhisa and Ishiwatari found that the rate of escape was lower than the rate of absorption for the same conditions of turbulence. Thus the diffusion constants measured for the control ponds where there is constant escape of CO_2 might be expected to be lower. The next higher diffusion constants waste ponds during non-bloom periods, where there was both escape and absorption. The highest values were measured in the waste ponds during the Monodus bloom when there was constant absorption. Eriksson (1961) reported that the exchange of O_2 in the sea was greater in the water. It is possible that winter storms caused a higher degree of turbulence and thus a higher diffusion constant, which is a measure of turbulence. Odum and Wilson (1962) observed that the diffusion constant for O_2 increased with high winds.

There is a diurnal pattern of the diffusion constant in the waste ponds. The constant is low during the light hours and high during the first part of the night. The pattern is well developed for the ponds during the non-bloom period and less so during the bloom period. Several of the individual diurnals for the control ponds show the same pattern although the averaged curve does not. This pattern is apparently due to the fact that shallow ponds such as these tend to thermally stratify each day and mix at night. The mixing produces a higher diffusion constant.

Carbon Metabolism

In an aquatic system containing biological components, the CO_2 concentration will increase at night due to respiration and decrease during the day due to photosynthesis. The more productive the system, the greater this diurnal range of inorganic carbon. If the system is closed, the overall rate of photosynthesis must equal the overall rate of respiration. One may exceed the other for short periods of time, but in the long run they must be equal. In open systems, where there is a consistent imbalance of photosynthesis and respiration, there must be imports or exports to maintain the system. A body of water receiving untreated sewage wastes may have a P/R ratio less than one because the input is supplying additional organic material. In the waste ponds, the P/R ratio is consistently greater than one because there is net import of inorganic nutrients and carbon dioxide and a net export of organic carbon. Thus the carbon metabolism of aquatic systems can be determined from changes in inorganic carbon, but to fully understand these changes a knowledge of carbon dioxide diffusion is essential.

Beyers (1963a, 1963b) studied the carbon metabolism of small microcosms by following pH changes and correlating them to changes in total carbon dioxide. He plotted rate of change curves for 21 types of microcosms. In the simplest systems, with a 12 hour dark-12 hour light photo period, such as high temperature microcosms and brine microcosm, the carbon dioxide decreased very sharply just after the lights went off. The rate of change for the rest

of the day was close to zero. In more complex systems, the patterns of metabolism were not as well defined. In systems under natural illumination, maximum net photosynthesis usually occurred in the morning and there was sometimes an excess of respiration over photosynthesis in the late afternoon, resulting in a negative rate of net photosynthesis. In the simple systems the rate of photosynthesis was usually positive for the entire light period. In an oyster pond the greatest change in inorganic carbon occurred in the afternoon. All of the systems showed a decreasing nighttime respiratory rate. In these studies Beyers assumed that diffusion of CO_2 was not significant.

Park, et al. (1958) studied pH variations and carbon metabolism in several Texas bays. Graphs of diurnal changes in total carbon dioxide and rate of change curves are presented. Some of the diurnal curves for total carbon dioxide showed almost no change while others had large changes. The rate of change curves were variable. Some showed almost no change over the 24 hour period. Some showed the greatest changes in the post-dawn and dusk hours. Others showed the greatest decrease of inorganic carbon in the late afternoon. All of the curves showed a decreasing nighttime respiratory rate.

Similar metabolic patterns for carbon metabolism have been measured by Copeland and Dorris (1962) for oil refinery holding ponds, by Jackson and McFadden for Sanctuary Lake, Calif., by Odum, et al. (1963a) for man-made reef ponds, and by Odum, et al. (1963b) for several abnormal marine ecosystems of Texas.

In this study, as in the studies mentioned above, there was considerable variation of both diurnal curves for inorganic carbon and rate of change curves. The magnitude of the variations of inorganic carbon were generally smaller for the control ponds. Some of the rate of change curves showed the ideal pattern of Beyers (1963) with sharp changes in the post dawn and dusk hours and very little change the rest of the day. Most of the curves, however, show variable rates of change for photosynthesis and respiration. In general, the tendency was for the rate of photosynthesis to be highest in the morning and for the rate of respiration to be highest in the early part of the night and decreasing afterward. In several cases the greatest decrease in inorganic carbon was in the late afternoon.

In considering the photosynthetic parts of the curves it should be remembered that these are graphs of net photosynthesis (the difference between gross photosynthesis and daytime respiration). In some instances, the respiration was greater than photosynthesis giving a negative rate of photosynthesis for parts of the day. This could be due to increasing daytime respiration of decreasing photosynthesis, or both. Negative rates of photosynthesis were also measured by Beyers (1963), Park, et al. (1958) and Odum, et al. (1963a, 1963b). From the graphs presented here and the works discussed earlier it is obvious that nighttime respiration is not constant, so there is no reason to suspect that daytime respiration should be.

The values for carbon metabolism for the control ponds was generally less than the waste ponds. Net photosynthesis in the control ponds was 0.42-1.08 g $C/m^2/12$ hours and nighttime respiration was 0.68-1.37 g $C/m^2/12$ hours. The P/R ratios were 0.63-0.79. For the waste ponds the net photosynthesis was 0.47-2.68 g $C/m^2/12$ hours and the nighttime respiration was 0.23-2.25 g $C/m^2/12$ hours. The P/R ratios were 0.46-4.80. All P/R ratios for the waste ponds during the Monodus bloom were greater than one. Beyers (1963a, 1963b) reported values of nighttime respiration of 0.68-2.34 g $C/m^2/12$ hours and net photosynthesis of 0.62-2.42 g $C/m^2/12$ hours with P/R ratios of 0.8 to 1.47. Odum, et al. (1963a) reported values for gross photosynthesis and total respiration in man-made ponds of 1.2-12.3 g $C/m^2/day$ and 0.7-12.0 g $C/m^2/day$, respectively. Most of the values if halved for 12 hour values fall within the range of values obtained in this study. Similar levels of carbon metabolism were reported by Odum, et al. (1963b) for abnormal marine ecosystems of Texas. Park, et al. (1958) calculated P/R ratios of 0.3-1.0 for various Texas Bays.

The values of carbon metabolism reported in this study were corrected for carbon dioxide diffusion. The corrections were not significant for the waste ponds during the non-bloom period. But when abnormal diffusion patterns occurred as in the control ponds and the waste ponds during the Monodus bloom, the corrections were significant. The magnitude of these corrections is shown in Table 1. Several workers (Beyers, Odum, et al., and Park, et al.) have assumed that carbon dioxide diffusion was not significant when compared to the rates of photosynthesis and respiration, but the results of this study suggests that this assumption is not always warranted. Each situation must be studied separately.

Carbon Limitation

In discussions of nutrient limitation of phytoplankton, phosphorus and nitrogen have generally been considered the limiting nutrients (Sawyer, 1947; Weiss, 1970). Weiss stated that "it would appear. . . . that under heavy pollution conditions, all other factors being compatible for algal growth, the quantity of nitrogen rather than phosphorus determines the biomass of algae that might be expected to develop." Recently, several workers have presented evidence that CO_2 is limiting in heavy phytoplankton blooms (Lange, 1967; Kuentzel, 1969; Kerr, et al., 1970; and King, 1970). These workers contend that phytoplankton growth is limited by CO_2 production from bacterial metabolism. Edmonston (1970) studied phytoplankton and nutrients in Lake Washington after diversion of sewage. After six years, nitrogen had decreased to 80% and phosphorus to 23% of the original concentrations. CO_2 and alkalinity remained high. Plantonic chlorophyll in summer was related to winter concentrations of phosphates to eutrophication, stated that "although higher than normal macronutrient levels will generally produce an increase in levels of productivity or shift of trophic status, the specific interrelationships of each nutrient factor are exceedingly complex and remain unclear."

There was very low concentrations of inorganic carbon in the waste ponds during the Monodus bloom. The question arises as to whether these low levels were limiting. Since the amount of carbon from nighttime respiration and diffusion is known, the sum of these should be equal to the amount of carbon consumed in net photosynthesis if carbon is limiting (See Table 1). For P-1, 11-8-69, the nighttime respiration was 0.23 g $C/m^2/12$ hours, and the total carbon gained from diffusion was 0.75 g/m^2, thus the total inorganic carbon available was 0.98 g/m_2. The net photosynthesis was 1.10 g $C/m^2/12$ hours, which is 0.13 g C/m^2 more than was available. For P-2, 10-4-69, the total amount of inorganic carbon produced was 1.33 g C/m^2 and the total amount consumed in net photosynthesis was 1.40 g C/m^2; the difference being 0.07 g C/m^2. For P-3, 4-4-70, the total inorganic carbon produced was 1.37 g C/m^2 and the amount consumed in net photosynthesis was 1.56 g C/m^2, or 0.19 g C/m^2 more than was produced. In these three instances, consumption exceeded available inorganic carbon by 0.13, 0.19, and 0.07 g C/m^2. These differences are small enough to indicate that carbon may be limiting in the waste ponds during the Monodus bloom, but more determinations would have to be made before drawing any final conclusions.

Bottom Metabolism

Since there are such distinctive differences between the two sets of ponds, it would seem that such differences might be reflected in the sediments. However, the organic content of the sediments showed no consistent differences between the two sets of ponds or with seasonal changes (Table 2). In some cases, there is as much variation between duplicate cores taken on the same date as there is among the different sampling dates. Lynn and Yang (1960) have questioned the validity of organic determinations by the drying and firing method. They claim that water may remain in the sample even after drying for three days. This may account for some of the variation obtained in this study. Ryan and Hommersand (1970) have also made organic determinations of the sediments of the sea grant ponds. However, only the thin top layer of sediment was analyzed as opposed to one cm for this study and their values are generally lower than those obtained in this study.

The results of bottom nighttime respiration (Table 3) show that the rate of respiration was low in the waste ponds during the Monodus bloom and increased after the crash of the bloom in May. The rate for the control ponds was higher than the waste ponds in the winter, but less in May. Kelly (1971) found that high pH in algal microcosms retarded the metabolism of organic material. This seems to be the case in the waste ponds. The levels of bottom respiration were low during the bloom because of the pH and increased after the crash when pH levels were lowered. The large increase in organic carbon in the water of the waste ponds in May and June is probably the result of the high rates of metabolism.

Pond Budget

The data for the budgets for the two sets of ponds show that there is an excess loss of carbon from the control ponds and an excess input into the waste ponds. For the waste ponds (Table 4 and 6) it was assumed that the amount of water entering the ponds was equal to the amount of water going down the drain because of the frequent back-flooding from Calico Creek. There are seven instances of back-flooding recorded in the log book. However, flooding probably occurred much more often than this because flooding at night and at times when no one was at the ponds would not be recorded. For example, back-flooding was recorded on 13 and 15 October but not on 14 October. If the tides were high enough to cause flooding on 13 and 15 of October, it probably occurred on the 14th also. In addition, it is likely that flooding occurred on both high tides each day, but since one tide is usually at night, it would not normally be observed. Similarly, flooding was recorded on the 25 and 28 of November, but not on the two intervening days. On several occasions the water from Calico Creek was observed running over the banks into the ponds.

Since the water in Calico Creek has a higher salinity, much of it would probably sink under the lighter pond water which would be displaced when the tide went out. The water in Calico Creek also has a lower total carbon than the water in the ponds so that there would be a net loss of carbon at each back-flooding. Sample calculations follow which show the effect of back-flooding on the waste ponds during the Monodus bloom. It was assumed that the back-flooding increased the water level to 4 inches above the top of the stand pipe and that the concentration of total carbon in the ponds was 60 mg/l and the total carbon in the creek was 34 mg/l. The latter figure is the average of all determinations of total carbon in Calico Creek. 60 mg/l is an approximate average of the total carbon in the waste ponds during the Monodus bloom from Nov. through April. It was also assumed that the surface area of the pond was 486 m^2. A four inch back-flood would add 49086 liters of Calico Creek water to the pond containing 1669 grams of carbon. If this all sank under the pond water, 49086 liters of pond water containing 2945 grams of carbon would be lost. This results in a net loss of 1277 grams of carbon for one four inch episode of back-flooding. If 18 such floodings occurred during the six months of the Monodus bloom, there would be a net loss of 22986 grams of carbon, or enough to make the budget for the waste ponds balance.

In general, the budget for the waste ponds shows that there was a net input of inorganic carbon to the waste ponds and a net export of organic carbon. This reflects the net productive nature of the waste ponds.

The budget for the control ponds (Table 5 and 6) shows that there was an excess loss of 19435 g carbon/year. This is equivalent to a daily rate of 0.101 g C/m^2/day. The values for carbon metabolism in the control ponds show that on the average, the rate of photosynthesis was about 0.2 g C/m^2/day, less than the rate of respiration. Thus the P/R ratios are less than one. If the respiration consistently exceeds photosynthesis there must be an

additional input of organic carbon. Smith (1971) found that P/R ratios calculated from oxygen productivity values were close to one. The difference between the P/R ratios calculated from carbon and oxygen and the excess loss of carbon from the ponds can be explained if it is assumed that organic carbon from the sediments is being oxidized by anerobic bacteria. Lathrop (1970) studied bacterial populations in tidal mud flats bordering the inland waterway at Wrightsville Beach, N. C. The main types of organisms in the sediments were nitrate-reducing and dentrifying (those which produce N_2 as an end product) bacteria. Counts of both types of organisms were between 10^4 and 10^5 organisms/ml. Counts for sulfate reducers were less than 10^2/ml. The Wrightsville area he studied is much like Bogue Sound and it is likely that similar organisms are found there and perhaps in the control ponds. If these organisms were present in the sediments of the control ponds it could explain the different P/R ratios obtained from oxygen and carbon measurements. These organisms metabolize organic carbon by using nitrate as an oxygen source, and producing CO_2 (or an organic end product which could be used by aerobic bacteria to produce CO_2). Thus P/R ratios calculated from oxygen metabolism values would not be affected, but those calculated from CO_2 changed would. The endproduct of denitrifying bacteria is N_2 gas which would not be oxidized by oxygen in the water. Even if there were sulfate reducing or methane bacteria which do produce oxidizable endproducts (H_2S and CH_4 respectively) much of this would probably escape from the shallow ponds without being oxidized.

The high levels of organic matter in the sediments are the result of lining the ponds with marsh mud. If 37 g carbon/m^2 were obtained from the sediments each year, this would account for the discrepancy in the budget data. Although there was no disernable decrease in the organic carbon content of the sediments in the control ponds (see Table 2) only about 18 grams of carbon/m^2 would be needed for the six month period covered by the measurements and this is much less than the variation in Table 5. This extra input of carbon could also account for the low P/R ratios. The excess of respiration over photosynthesis is 0.2 g C/m^2/day and the excess loss calculated in the budget is 0.1 g C/m^2/day. Considering the assumptions made in calculating the budget, this is very close agreement. In summary, if anerobic bacteria were utilizing the organic matter in the sediments, the excess input of carbon to the control ponds and the low P/R ratios can be explained.

Generally, the budget for the control ponds shows little production of organic matter and a large export of inorganic carbon, both by diffusion and by export to the drain.

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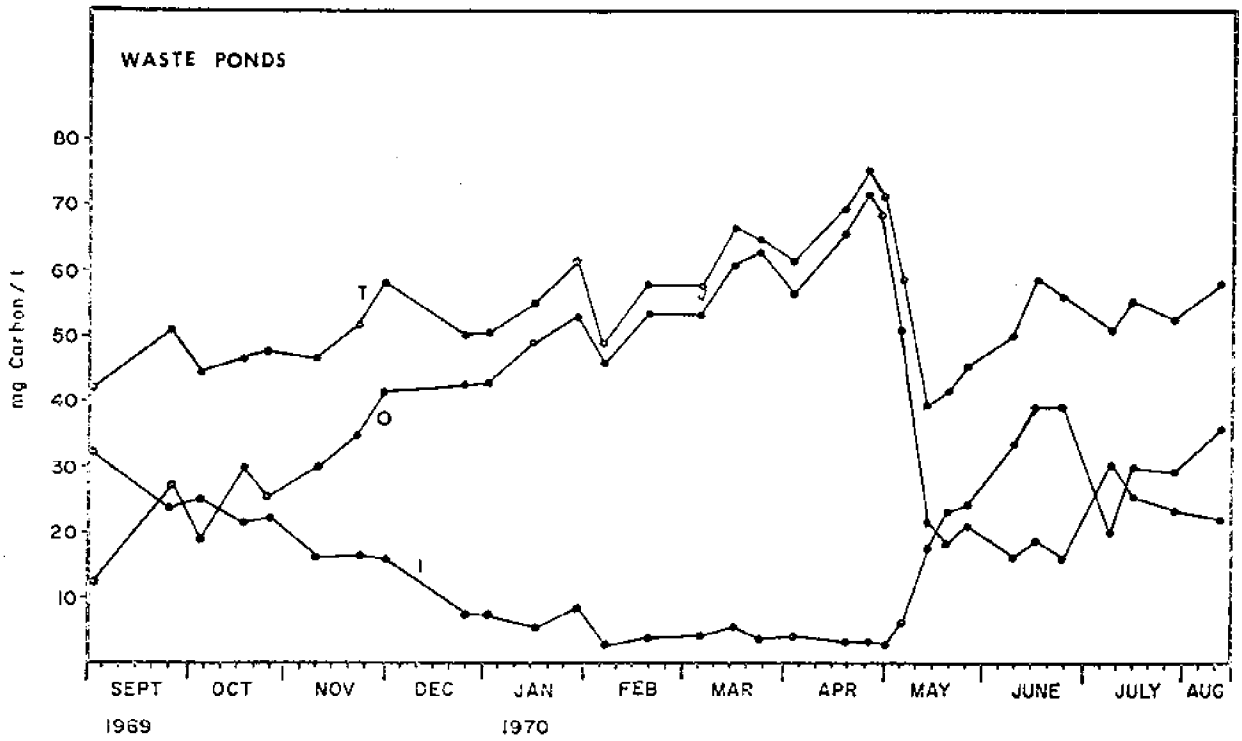

Figure 1. Averaged, annual variations of carbon in the waste ponds. T-total carbon, O-total organic carbon, I-total inorganic carbon.

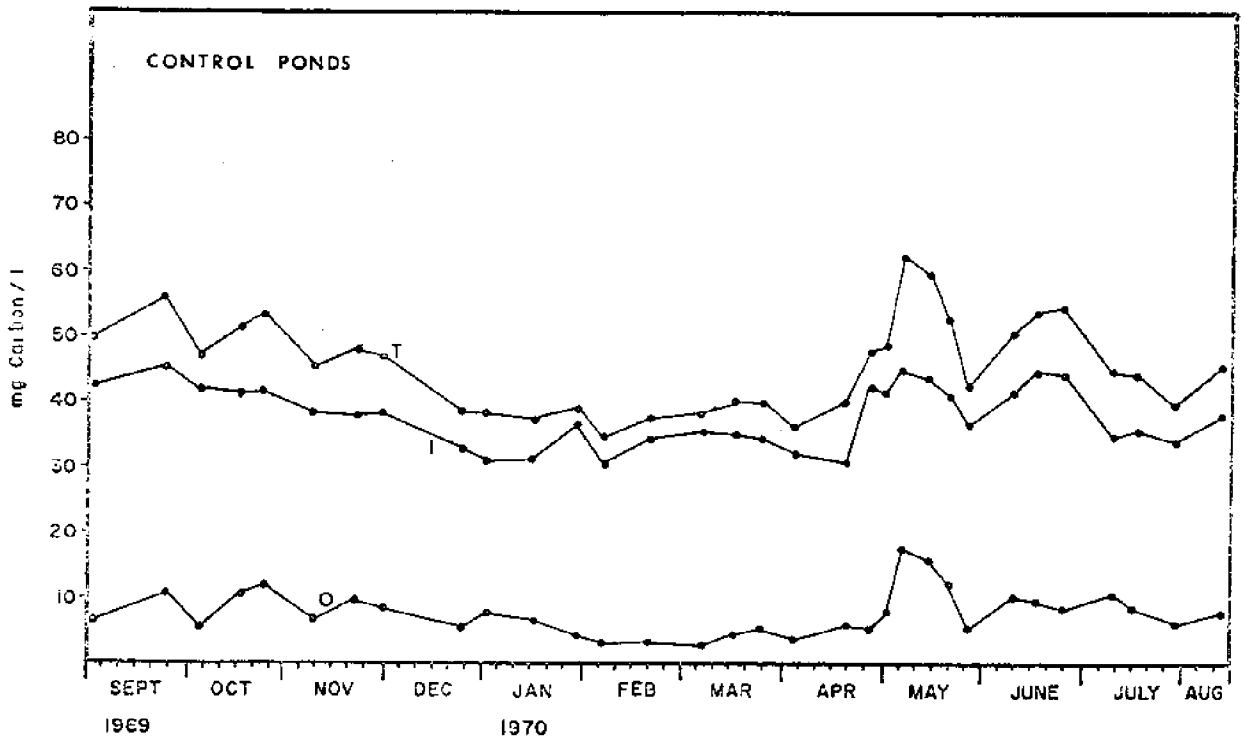

Figure 2. Averaged, annual variations of carbon in the control ponds. T-total carbon, O-total organic carbon, I-total inorganic carbon.

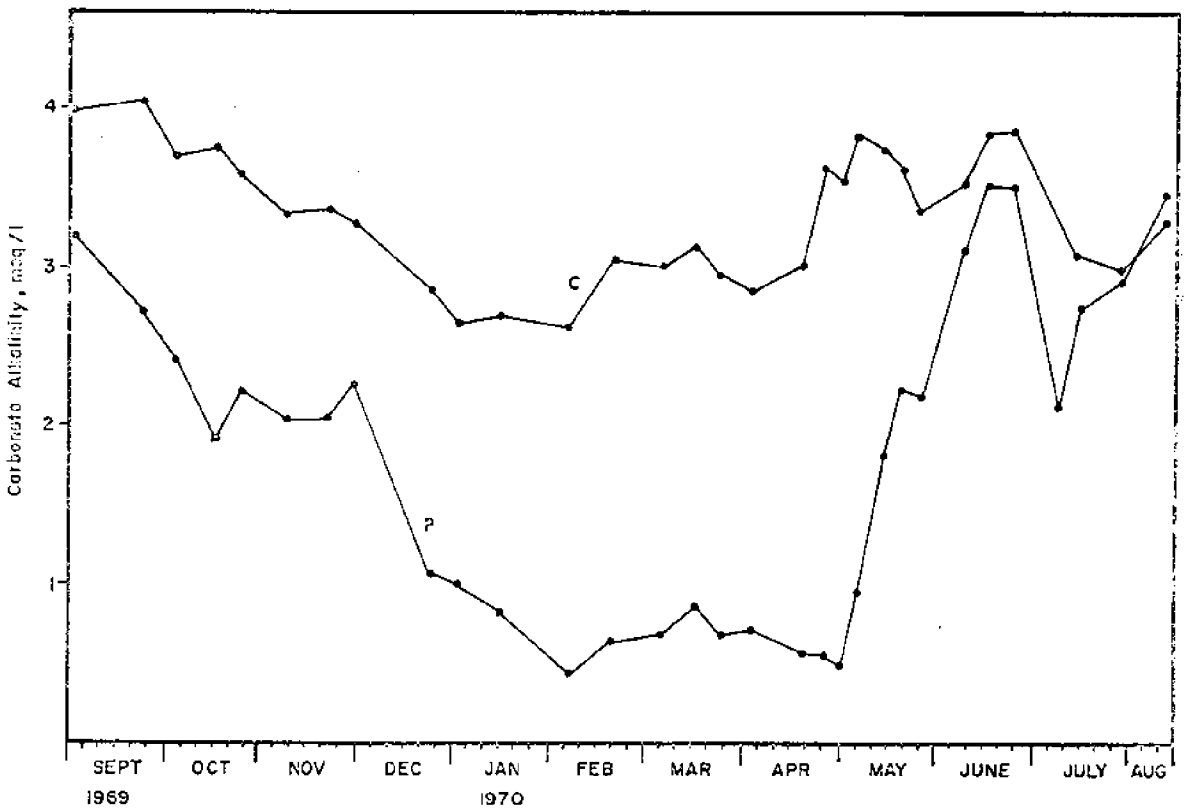

Figure 3. Averaged annual variations of carbonate alkalinity in the sea grant ponds. C-control, P-waste.

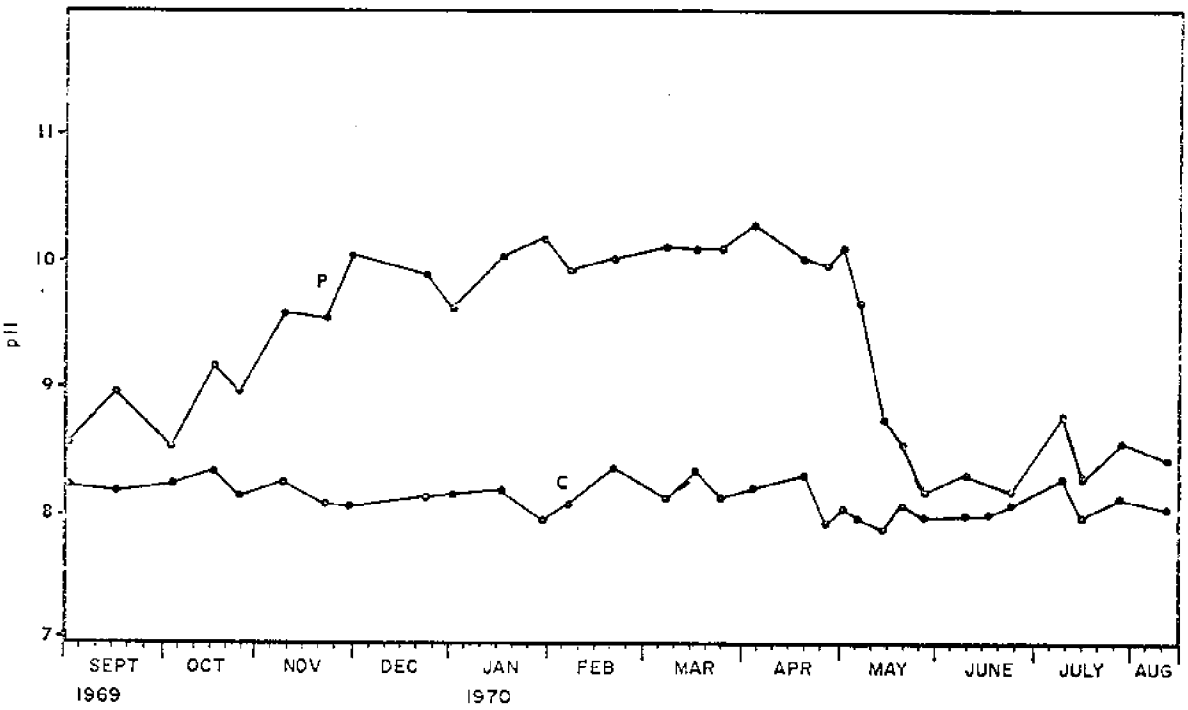

Figure 4. Averaged, annual variations of pH in the sea grant ponds. P-waste ponds, C-control ponds.

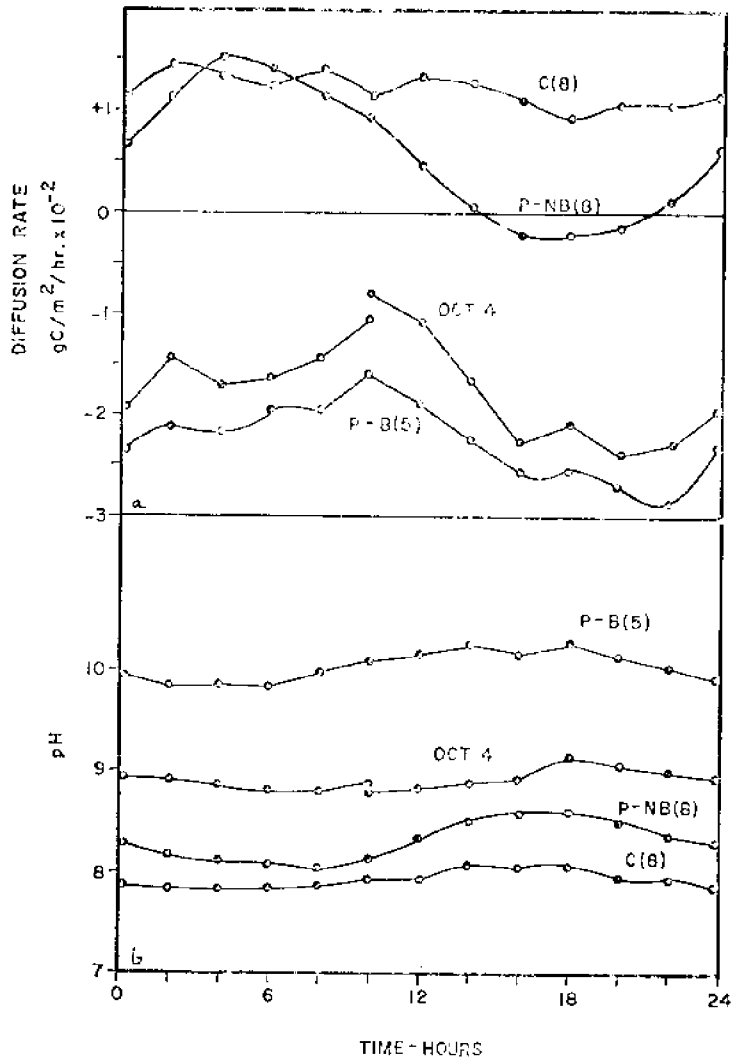

Figure 5. Averaged, diurnal variations of the diffusion rate of of CO_2 and pH in the sea grant ponds. C-control ponds, P-Nb-waste ponds when there was no Monodus bloom, P-B-waste ponds during the bloom. Numbers in parentheses refer to number of curves used to obtain the average.

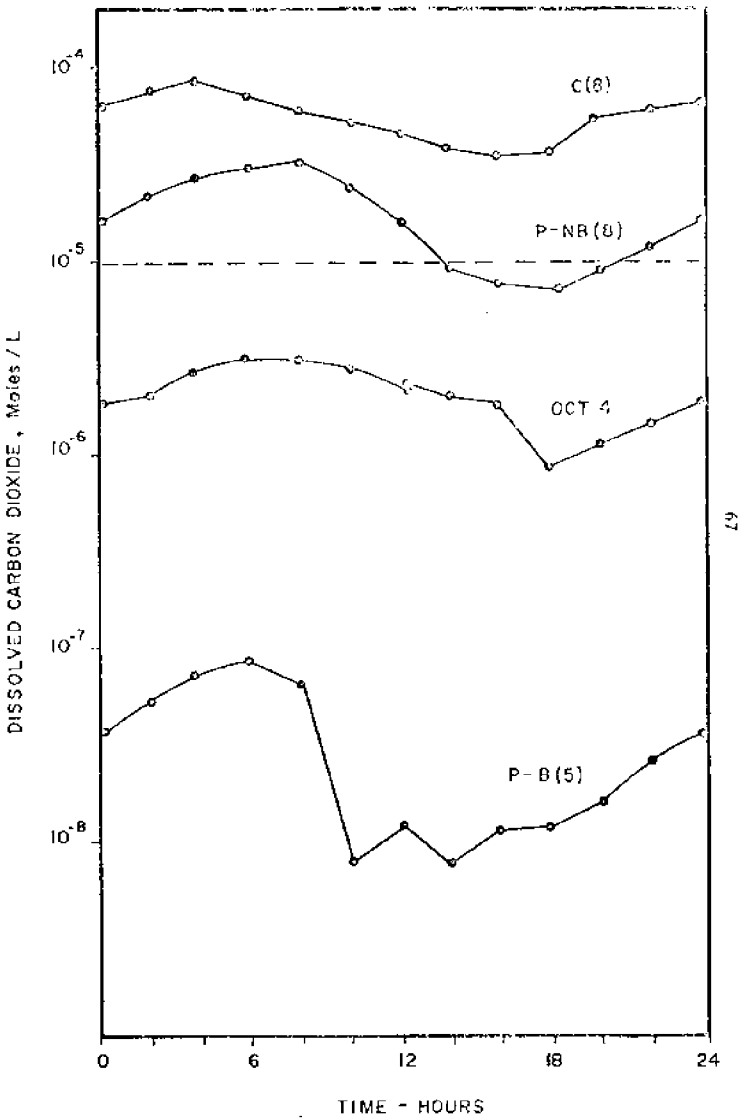

Figure 6. Averaged, diurnal variations in the concentration of dissolved carbon dioxide. Symbols have same meaning as figure 5.

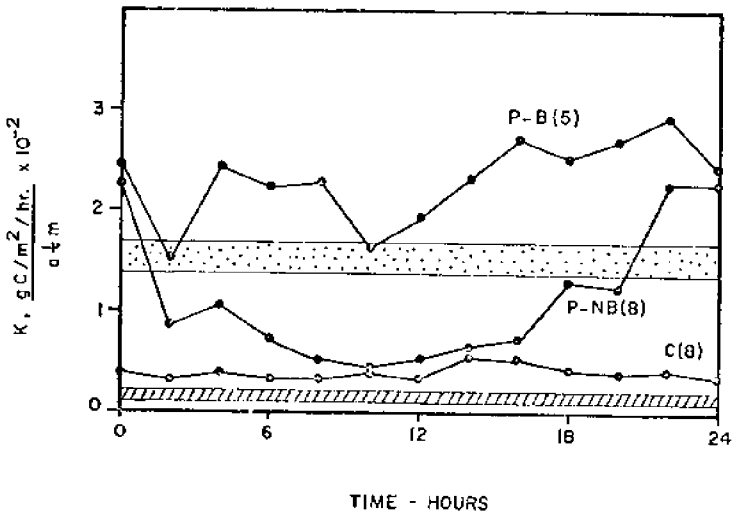

Figure 7. Averaged, diurnal variations of the diffusion constant for carbon dioxide. C-control ponds, P-NB-waste ponds when there was no Monodus bloom, P-B-waste ponds during the bloom, Oct 4-record of P-2 when the Monodus bloom was just beginning. Numbers in parentheses refer to the number of diurnal curves which were used to obtain the average curve. Stippled and cross-hatched areas represent values of k for moderately stirred and static systems, respectively, reported by Sugiura, et al. (1963).

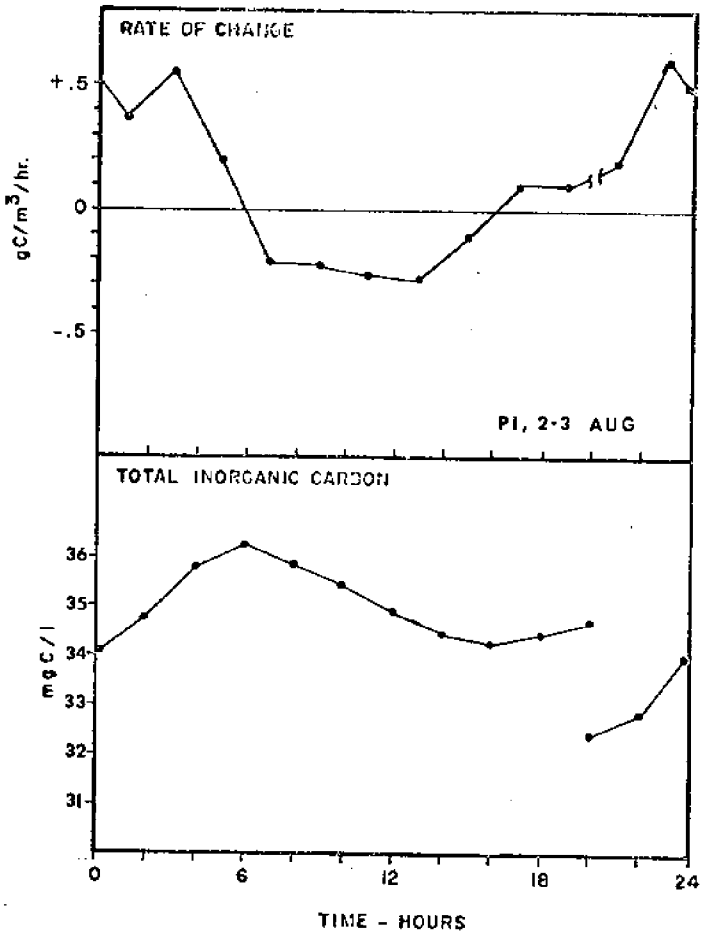

Figure 8. Diurnal variations and rate of change of total inorganic carbon in P-1, 2-3 Aug., 1970.

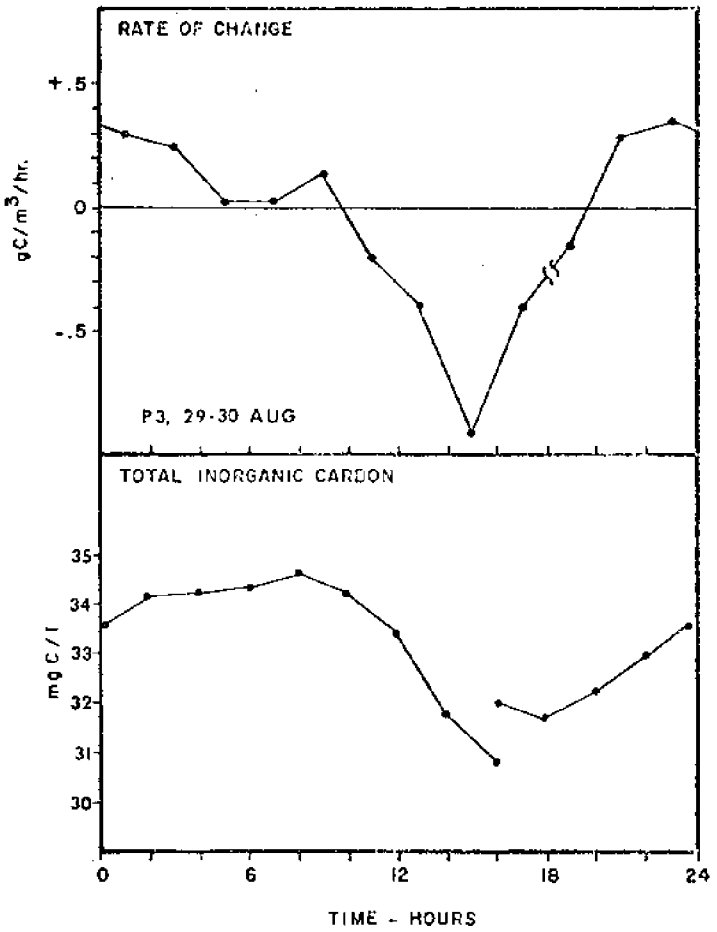

Figure 9. Diurnal variations and rate of change of total inorganic carbon in P-3, 29-30 Aug, 1969.

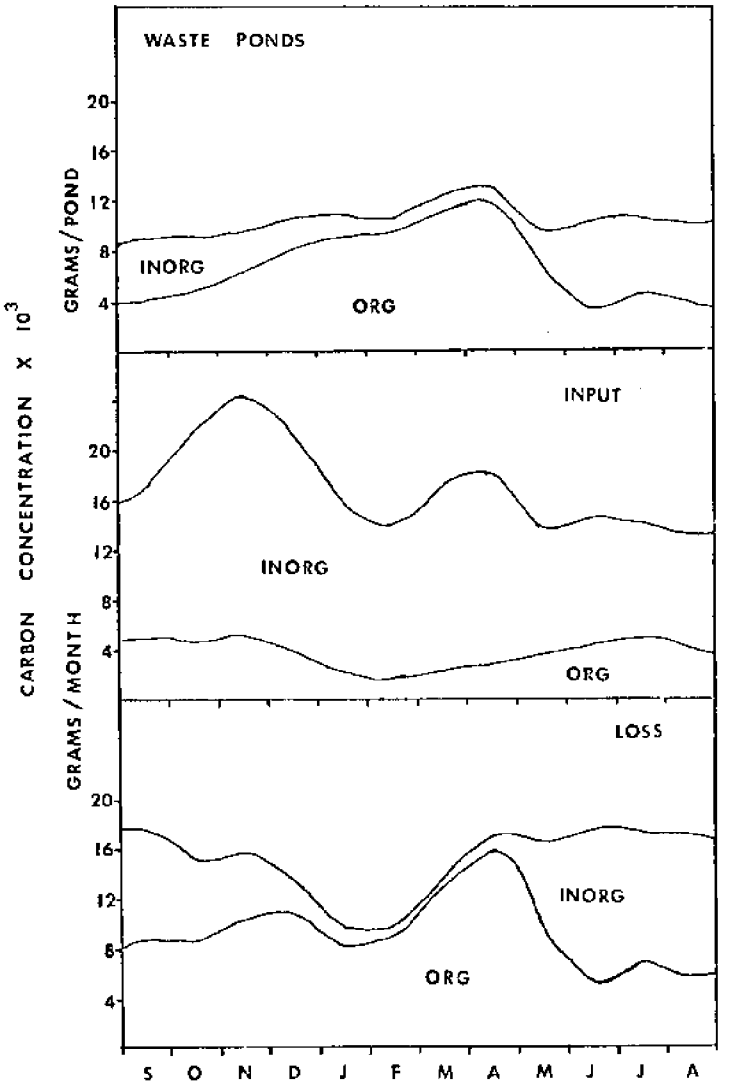

Figure 10. Annual carbon budget for the waste ponds showing average concentration in the ponds, and rates of input and loss of carbon. INORG-total inorganic carbon, ORG-total organic carbon.

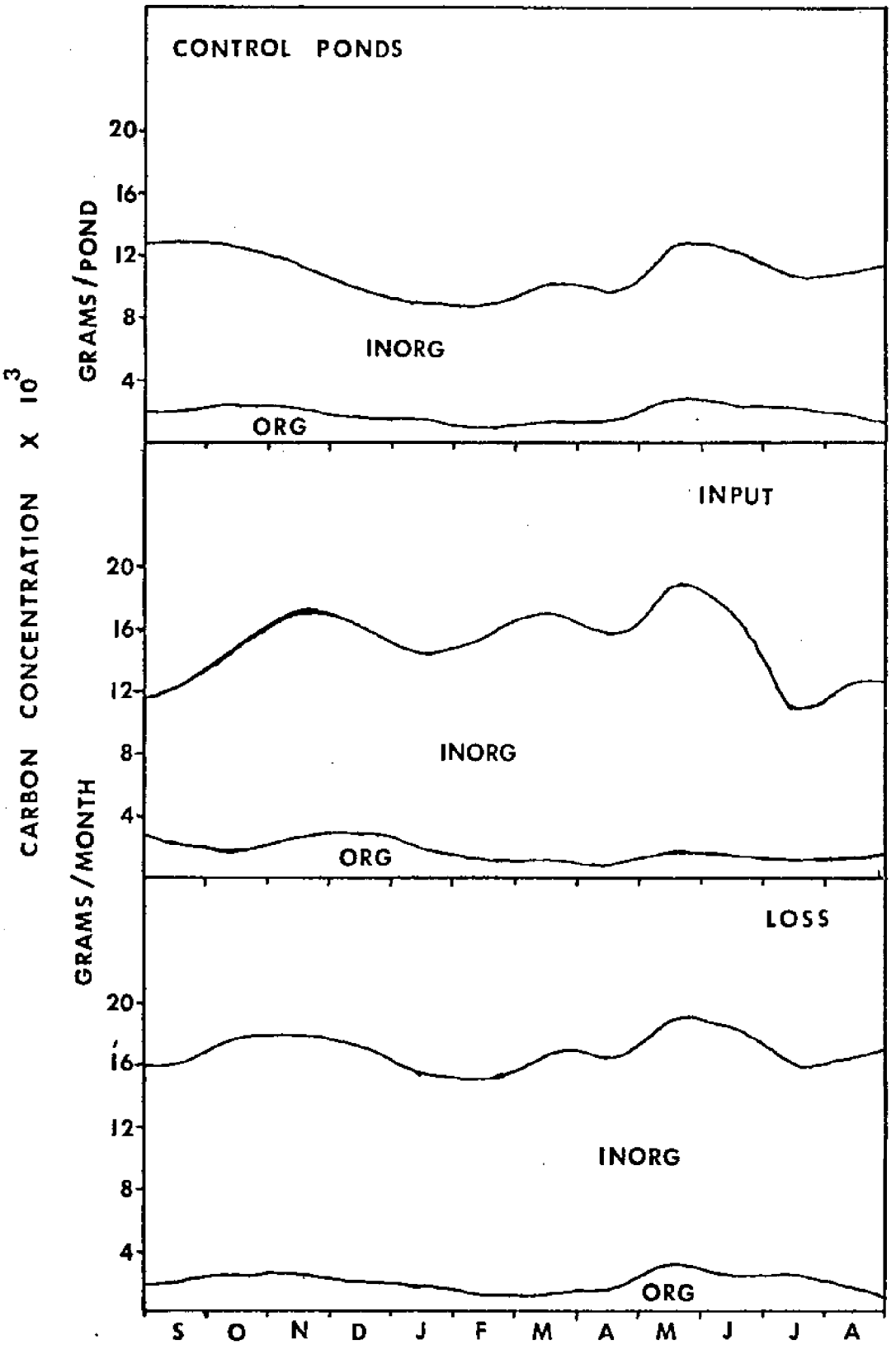

Figure 11. Annual carbon budget for the control ponds showing average concentration in the ponds, and rates of input and loss of carbon. INORG-total inorganic carbon, ORG-total organic carbon.

Table 1. Nighttime respiration, net photosynthesis, and P/R ratios for various sea grant ponds.

	Carbon metabolism								
	non-corrected		diffusion correction		corrected			molar O_2 equivalent	
Pond-date	R	P	R	P	R	P	P/R	R	P
C1 5-27	0.54	0.52	-0.13	-0.09	0.67	0.42	0.63	1.78	1.13
C2 8-5	0.34	0.58	-0.36	-0.14	0.70	0.43	0.62	1.87	1.15
C3 9-5	1.05	1.05	-0.12	-0.08	1.17	0.93	0.80	3.12	2.51
C3 5-25	1.17	1.17	-0.20	-0.10	1.37	1.08	0.79	3.65	2.88
P1 5-29	1.30	1.18	-0.02	-0.02	1.31	1.20	0.92	3.49	3.20
P1 8-2	1.41	0.74	-0.04	-0.08	1.45	0.66	0.46	3.87	1.76
P1 11-8	0.67	0.81	+0.44	+0.31	0.23	1.10	4.79	0.61	2.94
P2 10-4	1.11	1.19	+0.21	+0.21	0.91	1.40	1.54	2.42	3.73
P2 5-30	0.74	0.44	+0.05	+0.03	0.69	0.47	0.68	1.84	1.25
P3 8-30	2.02	2.74	-0.23	-0.06	2.25	2.68	1.19	5.99	7.14
P3 8-29	1.39	1.82	-0.33	-0.09	1.73	1.73	1.00	4.60	4.62
P3 4-4	1.15	1.34	+0.14	+0.22	1.01	1.56	1.54	2.69	4.16

All values for carbon metabolism are in g $C/m^2/12$ hours, values for oxygen are in g $O_2/m^2/12$ hours. For diffusion corrections + indicates carbon gained by inward diffusion, - indicates carbon lost through outward diffusion.

Table 2-Organic concentrations in sediments of the sea grant ponds in g/m^2

Pond	11/8/69	1/30/70	3/7/70	4/4/70	5/2/70	5/28/70
C-1	291.0	393.2	334.3	275.3	342.1	205.3
C-1	605.6	317.7	220.2	114.0	397.2	359.0
C-2	287.1	348.8	275.3	110.1	373.6	325.2
C-2	---	252.3	385.4	184.8	460.1	291.0
C-3	487.6	239.5	337.6	180.8	365.7	224.5
C-3	318.5	---	330.3	82.6	291.0	204.1
P-1	149.4	324.8	346.0	228.1	306.7	340.1
P-1	190.7	382.6	314.6	180.8	412.9	330.3
P-2	184.8	282.3	287.1	212.3	381.4	373.9
P-2	216.3	230.8	184.8	204.5	255.6	279.2
P-3	222.1	371.2	255.6	141.6	365.7	156.1
P-3	287.1	307.9	137.6	708.4	405.0	277.3

Table 3. Rates of nighttime respiration of the bottom.

Date	Pond	$gO_2/m^2/hr.$	$gC/m^2/hr.$
11/8/69	P-1	.0088	.0033
	C-1	.0149	.0056
1/30/70	P-2	.0024	.0009
	C-1	.0076	.0029
5/2/70	P-2	.0128	.0048
5/31/70	c-1	.0415	.0156
	C-2	.0287	.0108
	C-3	.0241	.0090
	P-1	.2092	.0785
	P-2	.1311	.0492
	P-3	.1612	.0605

Table 4 - Carbon budget for the period Sept, 1969 through Aug., 1970 for the waste ponds. + indicates carbon gained from diffusion, - indicates carbon lost through diffusion.

	total carbon		organic carbon		inorganic carbon	
month	g/m^2/day	g/month	g/m^2/day	g/month	g/m^2/day	g/month
INPUT						
Mixing tank						
S	1.15	16787	0.34	4923	0.82	11905
O	1.04	15638	0.39	4588	0.74	11101
N	1.15	16756	0.34	4895	0.82	11907
D	0.90	13473	0.26	3909	0.64	9600
J	0.51	7718	0.15	2194	0.37	5546
F	0.49	6619	0.12	1792	0.36	4845
M	0.62	9357	0.17	2563	0.45	6819
A	0.72	10469	0.20	2896	0.52	7610
M	0.90	13541	0.26	3871	0.64	10708
J	1.01	14629	0.29	4212	0.72	10453
J	0.94	14155	0.24	4581	0.67	10106
A	0.90	13650	0.26	3875	0.65	9912
grams/year		152792		44299		110512
Carbon Dioxide Diffusion						
S	-0.14	-1997	--	--	-0.14	-1997
O	+0.42	+6328	--	--	+0.42	+6328
N	+0.54	+7873	--	--	+0.54	+7873
D	+0.54	+8136	--	--	+0.54	+8136
J	+0.54	+8136	--	--	+0.54	+8136
F	+0.54	+7348	--	--	+0.54	+7348
M	+0.54	+8136	--	--	+0.54	+8136
A	+0.54	+7873	--	--	+0.54	+7873
M	-0.14	-2066	--	--	-0.14	-2066
J	-0.14	-1997	--	--	-0.14	-1997
J	-0.14	-2066	--	--	-0.14	-2066
A	-0.14	-1997	--	--	-0.14	-1997
grams/year		+43707				+43707
total input		196499		44299		154219
EXPORT						
Drain						
S	1.06	15399	0.47	8644	0.62	8983
O	1.03	15531	0.56	8511	0.51	7749
N	1.09	15902	0.73	10654	0.37	5374
D	0.90	13541	0.72	10874	0.18	2769
J	0.62	9310	0.54	8096	0.07	1113
F	0.68	9306	0.63	8548	0.05	724
M	0.95	14373	0.89	13373	0.07	1070
A	1.18	17186	1.11	16120	0.07	1066
M	0.94	14157	0.63	9523	0.30	4521
J	1.09	15876	0.37	5341	0.72	10564
J	1.03	15460	0.50	7512	0.54	8094
A	1.02	15342	0.40	5983	0.67	10100
total export		171384		113179		62074

Excess input = 25115 g/year = 0.142 g/m^2/day 12.78%error

Table 5 - Carbon budget for the period Sept, 1969 through Aug, 1970 for the control ponds. - indicates carbon lost through diffusion

	total carbon		organic carbon		inorganic carbon	
month	$g/m^2/day$	g/month	$g/m^2/day$	g/month	$g/m^2/day$	g/month
INPUT						
Mixing tank						
S	0.77	12238	0.13	2128	0.64	10209
O	0.92	15097	0.11	1731	0.87	14300
N	1.10	17590	0.16	2524	0.95	15097
D	0.98	16085	0.17	2784	0.81	13301
J	0.86	14139	0.07	1194	0.78	12788
F	1.08	15982	0.08	1170	0.99	14711
M	1.15	16976	0.08	1369	0.96	15685
A	0.97	15360	0.06	946	0.91	14376
M	1.20	19610	0.12	1930	1.09	17872
J	1.09	17227	0.11	1673	0.96	15270
J	0.68	11177	0.10	1632	0.58	9474
A	0.76	12459	0.10	1673	0.66	10860
total input		183940		20754		163943
EXPORT						
Drain						
S	0.79	11492	0.12	2068	0.69	10090
O	0.88	13191	0.16	2436	0.71	10755
N	0.91	13254	0.17	2418	0.74	10827
D	0.82	12373	0.14	2148	0.68	10256
J	0.69	10463	0.11	1730	0.59	8952
F	0.79	10694	0.08	1035	0.71	9688
M	0.80	12032	0.09	1280	0.71	10695
A	0.80	11712	0.10	1493	0.68	9926
M	0.98	14887	0.22	3268	0.76	11507
J	0.95	13797	0.17	2460	0.78	11311
J	0.74	11148	0.15	2316	0.60	9087
A	0.79	11880	0.12	1868	0.65	9831
grams/year		146923		24520		122925
Carbon Dioxide Diffusion						
S	-0.29	-4641	--	--	-0.29	-4641
O	-0.29	-4794	--	--	-0.29	-4794
N	-0.29	-4641	--	--	-0.29	-4641
D	-0.29	-4794	--	--	-0.29	-4794
J	-0.29	-4794	--	--	-0.29	-4794
F	-0.29	-4330	--	--	-0.29	-4330
M	-0.29	-4794	--	--	-0.29	-4794
A	-0.29	-4641	--	--	-0.29	-4641
M	-0.29	-4794	--	--	-0.29	-4641
J	-0.29	-4641	--	--	-0.29	-4641
J	-0.29	-4794	--	--	-0.29	-4794
A	-0.29	-4794	--	--	-0.29	-4794
grams/year		-56452				-56452
total export		203375		24520		179377

Excess export = 19435 g/year = 0.101 $g/m^2/day$ 9.56% error

Table 6 - Average monthly concentration of total, inorganic and inorganic carbon in the sea grant ponds

Waste Ponds

Date	Total Carbon		Org. Carbon		Inorg. Carbon	
	g/m^2	g/pond	g/m^2	g/pond	g/m^2	g/pond
S-69	19.66	9172	8.74	4076	11.47	5350
O	19.70	91924	10.79	5037	9.83	4586
N	21.38	9976	14.32	6683	7.22	3371
D	22.39	10446	17.98	8388	4.58	2136
J-70	23.18	10819	20.16	9408	2.77	1293
F	22.86	10584	20.83	9721	1.76	823
M	25.96	12112	24.15	11270	1.93	901
A	28.43	13269	26.67	12440	1.76	823
M	21.04	9819	14.15	6605	6.72	3136
J	22.47	10486	7.56	3528	14.95	6977
J	23.30	10407	10.84	5056	11.68	5448
A	21.76	10152	8.48	3954	14.32	6683
Control Ponds						
S-69	23.85	12720	4.05	2160	19.76	10536
O	22.91	12216	4.23	2256	18.68	9960
N	21.20	11304	3.87	2064	17.33	9240
D	18.41	9816	3.20	1704	75.26	8136
J-70	17.15	9144	2.84	1512	14.67	7824
F	16.74	8928	1.62	864	15.17	8088
M	19.04	10152	2.03	1080	16.92	9024
A	18.00	9600	2.30	1224	15.26	8136
M	23.99	12792	5.72	2808	18.54	9888
J	23.22	12384	4.14	2208	19.04	10152
J	19.71	10512	4.10	2184	16.07	8568
A	20.61	10992	3.24	1778	17.06	9096

THE PHOSPHORUS SYSTEM:
March-December, 1970

by Henry N. McKellar, Jr.*
Marine Science Curriculum, U.N.C.

INTRODUCTION

The increasing input of municipal wastes into coastal waters has altered the natural structure and functions of our estuaries. The evolution of new systems in response to this input has led to the decline of many valuable, brackish water species and has forced new considerations of our estuarine environment as a resource. In order to explore both the self-design of these new systems and also their possible capacity in processing and recycling mineral nutrients, three brackish water ponds, subjected to continual inflow from the Morehead City treatment plant, are being studied. Since phosphorus is both a major constituent of municipal waste and also an absolute requirement for life, the phosphorus system of these ponds has been an integral phase of this investigation.

In the past, much has been learned about phosphorus cycling in aquatic systems by tracing radioactively labeled phosphorus atoms through water compartments. Hutchinson and Bowen (1947), using tracer techniques, described the general movement of phosphorus in a small lake. This report led to more quantitative studies of phosphorus cycling in lakes (Hutchinson and Bowen, 1950; Hayes et al, 1952; Rigler, 1956, 1964) in which the concepts of transfer rates and turnover times have been developed. The phosphorus system is postulated as a dynamic equilibrium between dissolved and solid phosphorus fractions which include plankton, sediments, and littoral plants. Hayes et al comment on the general problem of eutrophication by noting cases in which continual additions of sewage to lakes kept the nutrients in the water above the level of equilibration with solids.

Phosphorus dynamics in a salt marsh system is presented in detail in a report by Pomeroy et al (1967) wherein they combine their own tracer studies with other data on salt marsh populations such as Uca, Fundulus, Penaeus, Palaemonetes, and Spartina as well as plankton and sediments. The equilibrium established between the plankton, bacteria, and the oxidized sediments was strongly dominated by the sediments and the role of deposit feeding populations in regenerating dissolved phosphorus was noted.

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*Principal worker on the phosphorus system under the direction of Dr. E. J. Kuenzler.

From work more closely related to our own, Abbott(1969) reports nutrient studies on hyperfertilized marine pond ecosystems. The ponds were constructed by capturing a small bayou channel between artificial levees and dividing the channel with cross levees. The ponds were then fertilized with reagent grade NaH_2PO_4 which was traced through the systems with ^{32}P. This study, again, points out the importance of the sediments as the "stock pile" of phosphorus in setting up equibrilibria. He describes the pond environment as a "steady-state ecological phosphorus processing machine" whereby incoming phosphorus is "assimilated and disseminated through various compartments of the machine". He regards an estuary as a 'nutrient buffer" which modulates wild fluctuations in the phosphorus economy.

Data presented in this report will show the effects of continual inflow of municipal waste on the general structure of phosphorus fractions in three waste ponds as compared to control ponds which receive low level phosphorus input. The seasonal variations of phosphorus uptake rates by the plankton are shown for the control and fertilized ponds and are related to seasonal plankton blooms with corresponding changes in phosphorus fractions and chlorophyll. Also, in a more direct effort to establish the phosphorus budget of the ponds, diurnal studies of phosphorus changes with respect to inflow, outflow, and internal phosphorus exchanges were conducted. Finally, a mathematical model of diurnal changes is hypothesized which can be used to simulate photostimulated exchanges between planktonic, consumer, and benthic storages. This data, hopefully, shows the basic structural and functional design in estuaries developing with treated sewage wastes and possibly indicates the role of brackish pond systems as a feedback mechanism from waste outputs into useful food production.

METHODS

Water samples for the seasonal study were taken on an approximate monthly basis and analyzed for plankton pigments, suspended particulate phosphorus(PP), dissolved inorganic phosphorus(DIP), and dissolved organic phosphorus(DOP). In conjunction with the phosphorus and chlorophyll conditions of the ponds, the instantaneous DIP uptake rate by plankton was also determined. Water for pigment and phosphorus analyses was taken from a 10 cm. depth in the center of each pond, carried to the lab in polyethylene bottles, stored in the refrigerator until they were filtered for analysis which was within one hour after collection.

Pigment Analysis. Pigment analyses were done by the method of Strickland and Parsons(1968)for chlorophyll a, b, c, and carotenoids; and by the method of Lorenzen(1967)for chlorophyll-a and pheo-pigments. Because of the high chlorophyll content of the three fertilized ponds (P1, P2, P3)only 25 ml. or less were needed for the analysis, whereas, 100 to 500 ml. from the three control ponds(C1, C2, and C3)was required. Analyses were done in duplicate except for C-pond samples during non-bloom conditions when 500 ml. were filtered for the analysis.

Planktonic DIP Uptake. Instantaneous uptake rates by plankton were measured by radiotracer techniques. 100 ml. of pond water were placed in a 125 ml. glass-stoppered bottle, labeled with 1-3 micro-Curies of carrier-free ^{32}P, and suspended to an approximate 10 cm. depth in the center of the pond to insure ambient temperature and light conditions. At specific time intervals the bottle was removed, 6 ml. were removed and filtered through a one-inch, .45 μ membrane filter, and the bottle was re-suspended until the next sample interval. Since the relative uptake in the C-ponds was usually very rapid, time intervals between samples were shorter for the C-ponds than for the P-ponds. However, the uptake experiments for both sets of ponds lasted for approximately one hour for each experiment. At some time during the experiment, a water sample was taken from the pond for phosphorus analysis.

The filters from each time interval were placed in planchets and the amount of radioactivity absorbed by the plankton during each interval was determined with a gas-flow proportional counter. Also, .3ml of the raw, labeled water was dried on planchets in quadruplicate to determine the total radioactivity of the experiment. By subtracting the activity absorbed by the plankton for each time interval from the total activity, the exponential decline of ^{32}P from solution due to plankton uptake was determined. The constant proportion of $DI^{32}P$ absorbed by the plankton per unit time is described as an exponential function characterized by its transfer coefficient, K.* Since previous work has shown that non-biological adsorption is negligible (Odum and Chestnut, 1969), the uptake rate, here, is the summed, active uptake by phytoplankton, zooplankton, and bacteria.

Phosphorus Analyses. Phosphorus analyses were performed by the methods of Menzel and Corwin (1965) and Murphy and Riley (1962) and used to determine suspended particulate phosphorus, dissolved inorganic phosphorus, and dissolved organic phosphorus as described in last year's report (Odum and Chestnut, 1969). Phosphorus concentrations in the fertilized ponds were high enough to necessitate dilution of samples with distilled water before analysis. The absorbance of all treated samples were read on a Hitachi Perkin-Elmer, UV-VIS spectrophotometer.

Diurnals. Diurnal experiments were run on P1 (28-29 July) and on C3 (11-12 Aug) to determine the daily phosphorus budget of the ponds. Phosphorus concentrations were determined both in pond water and inflow at sunrise, sunset, and times half-way between, along with DIP uptake experiments.

Estimates of inflow rate for each time interval were obtained by measuring the volume of inflow caught by a bucket held beneath the mouth of the inflow pipe for short periods of time. This was done 3 times for each sample and the inflow volume per hour was calculated from the average

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* $K = (\ln A_o - \ln A_t)/t$ where $A_o = DI^{32}P$ activity at time 0 and $A_t = DI^{32}P$ activity at time t

An evaporation estimate was obtained by partially filling a 100ml. beaker with pond water and floating it in the pond for the duration of the diurnal study. After 24 hrs. the beaker was retrieved and evaporation was measured. Given the area of the pond(Odum and Chestnut, 1969) the evaporation rate was estimated. Assuming no loss of pond water by seepage, the inflow rate minus the rate of evaporation was calculated as the outflow rate.

To estimate contributions of the plankton(phytoplankton, zooplankton, and bacteria), the bottom(sediments and algal mat), and large crustaceans and fish(consumers)to diurnal variations, two plastic tubes, enclosing a column of water approximately .5 m. deep, were inserted into the mud at sunrise. A rubber stopper was inserted into the bottom of one tube, thus enclosing only the water column and its suspended particulate matter. The other tube enclosed the water column plus an approximately 10cm. core of sediment with an approximate 30cm^2 surface area. At sunset, samples were taken from both tubes for phosphorus analysis. The tubes were then removed, emptied, and re-inserted to be sampled again the following sunrise. The changes observed in the tube inclosing only the water column were assumed to be net planktonic exchanges. The changes due to plankton flows were subtracted from the changes observed in the tube with exposed sediment to determine the net effects of bottom flows. Finally, the differences between changes in the tube with exposed sediment and the open water column were assumed to be phosphorus exchanges of fish and large crustaceans. This procedure was repeated for the interval from sunset to the next sunrise.

RESULTS AND DISCUSSION

Seasonal Variations in Chlorophyll-a

Previous reports on pond studies(Odum and Chestnut, 1969)indicate that both C and P ponds have matured to a certain degree since their construction in 1968 and undergo recurring seasonal cycles. Figure 1 shows the chlorophyll-a regimes from March to December, 1970 in relation to pond temperature.

In early March when pond temperatures were slightly below 10°C, the C ponds were in winter non-bloom conditions with chlorophyll-a concentrations below 2 mg/m^3, while chlorophyll in the P ponds was above 400mg/m^3, indicating the winter Mododus bloom(characteristic of the fertilized ponds). No chlorophyll data was available for April but by mid-May a reverse had occurred in that the bloom in the P ponds had declined to chlorophyll concentrations less than 25 mg/m^3 and a spring bloom had occurred in the C ponds with chlorophyll concentrations from 10 to 20 mg/m^3.

The C pond blooms persisted throughout the summer months with the peak occurring in C1 as late as 31 Aug. Concentration is C2 and C3 declined slowly after peaks in May and by December, chlorophyll-a in all control ponds showed winter, non-bloom conditions again. Note that C3 chlorophyll values were always lower than the other control ponds,

both in summer and winter. A prolific growth of Ruppia in C3 during the summer has converted the pond to a benthic productions system.

From studies on late afternoon and early morning dissolved oxygen values(Smith, this report), the decline of the winter P-pond bloom was actually a crash occurring approximately on 11 May when early morning oxygen values dropped below 1 mg/l. The P-ponds quickly recovered, however, and daily O_2 production returned to approximate winter conditions by 14 May. The recovery was shown by chlorophyll-a data although concentrations were still only 25 to 50% of winter concentrations. The crash represented a transition stage from the winter adapted system, with Monodus as dominant producer, to a summer team of producers with lower chlorophyll-a concentrations but comparable O_2 production.

These summer conditions in the P-ponds persisted through October with chlorophyll-a ranging from 50 to 150 mg/m^3. By 13 November, however, the winter system was taking over and by December, chlorophyll-a in P1 was higher than 300 mg/m^3.

Note **that the conversion back to the winter system was gradual and** involved no sudden crash of plankton producers as occurred in May with the conversion into summer conditions. Possibly, sudden environmental changes occurred in late April and early May(such as the temperature increase between 15 May and 17 June, shown in Fig. 1)which necessitated immediate elimination of Monodus before the summer production system had developed. This sudden winter to summer change required a transition period with low production and chlorophyll-a whereas the conversion back to the winter system was more gradual, allowing the **Monodus bloom** to develop **while** the summer producers declined.

Seasonal Phosphorus Conditions

Figures 2 and 3 show seasonal variations of relative DIP uptake rates by plankton with changes in phosphorus fractions in the C and P ponds. Points plotted on these graphs represent averaged, duplicate analyses of daytime phosphorus concentrations. Two points plotted for a phosphorus value on the same date represent the maximum and minimum values observed during a diurnal study, indicating the daily range as compared with seasonal variations. Diurnal patterns will **be discussed in** more detail below.

A rough correlation of PP in Figures 2 and 3 with chlorophyll in Fig. 1. **indicates** that PP was predominately phytoplankton although variations in zooplankton, bacteria, detritus, and even phosphorus within phytoplankton cells could cause the deviations from a constant chlorophyll/PP ratio.

C-Ponds. The bloom as indicated by chlorophyll-a, is concurrent with PP peaks in the C ponds of approximately 2 µg-at/l on 9 May. These peaks drop sharply and rise to intermediate values between 1 and 2 µg-at/l throughout the summer, although second PP peaks occurred in C2 on 7 July and in C1 on 2 September. PP concentrations in C3 were always lower than in C1 and C2, indicating that the phosphorus, as well

as chlorophyll-a, was dominated by the benthic system.

DIP concentrations in the C-ponds were always very low, usually below 0.01 µg-at/l when detectable and therefore, no seasonal pattern could be determined. Kuenzler and Ketchum(1962)showed that Pheodactylum tricornutum could remove DIP down to values as low as $7.2*10^{-10}$ which is well below the detection limits of analytical procedures. Uptake by benthic and planktonic producers is evidently so closely linked with metabolic release of DIP that the accumulation of a DIP pool is virtually impossible.

DOP values were below 1.0 µg-at/l from March to December and no seasonal pattern was detected. However, through ^{32}P experiments, DOP has been shown to be metabolically active in the C ponds.(Kuenzler, 1970) In the first hour of an experiment as much as 47% of ^{32}P assimilated by the plankton was excreted as $DO^{32}P$. Also plankton from C1 and C2 were shown to rapidly assimilate $DO^{32}P$ produced by culture algae. C3 did so more slowly. In earlier work on algae cultures, Kuenzler and Perras(1965)showed that phosphatase enzymes were produced by cultures when they became phosphorus deficient. The algae of the C-ponds are very likely phosphorus deficient and the production of phosphatases and the utilization of DOP could be important mechanisms in the phosphorus system.

The relative DIP uptake by plankton shows a rough correlation with PP. The relation is especially good for C1 where maximum rates coincide with PP peaks on 9 May and 2 September. This relationship holds less in C2 and C3 but nevertheless, the rates occurring during the summer bloom conditions are significantly higher that pre-and post-bloom rates. The highest rate observed(.67%/min.) occurred in C2 on 7 July and indicates an effective DIP turnover time(1/K) of approximately 1.5 minutes. This turnover is slightly faster than those demonstrated in laboratory studies of natural plankton samples by Nigler(1966) who showed DIP turnover times from 3.[illegible] to 5.8 minutes. The lowest rates observed in the C-ponds(appro... .001/min) occurred in C1 and C2 on 13 December and indicated a DIP turnover time of 700 minutes.

P Ponds. As in the case of the C-ponds, the PP concentrations in the fertilized ponds reflected seasonal conditions as indicatied by chlorophyll-a values, with a PP crash occurring in mid-May, followed by a slow increase over the summer to bloom conditions again in December. During winter Monodus bloom, the PP range(48.3-[illegible].2ug-at/l) was from 80 to 90% of the total phosphorus which compares to similar PP/total ratios in the C-ponds during the summer. However, the total phosphorus in the fertilized ponds was usually 50 times greater than in the C-ponds.

An interesting seasonal relation between PP and DIP was observed corresponding to the conversion between summer and winter systems. After the summer regime had developed with relatively low PP values, DIP became the largest phosphorus pool, ranging from 56.3 to 85. µg-at/l which

represented from 70 to 80% of the total phosphorus. Because of lower rates of DIP uptake by the plankton and increased metabolic release from bacterial decomposition and consumer excretion, a DIP reserve accumulated in the water and remained in high concentrations throughout the summer. Only with recurrence of the Monodus bloom and higher uptake rates were DIP levels reduced below 25µg-at/l. This cycle was shown best in P1(see Fig. 3)since the winter blooms had not yet fully developed by December in P2 and P3.

Both the highest and lowest relative uptake rates were observed in P3. The highest rate(.0023/min.)was observed in mid-April, just before the crash in May, and corresponded to a turnover of DIP by plankton every 7.25 hrs. which is longer that the lowest turnover rates in the C-ponds(5 hrs.). The lowest uptake rate(approx. .0001/min.)was observed in December, before the winter production system had taken over. This very low rate indicated that the DIP pool was not used but oncein 6.1 days. The winter bloom had occurred in P1 by November and the DIP pool was being reduced as uptake rates climbed to over .002/min.

Algal reduction of dissolved nutrients from water in pond systems, as occurs during bloom conditions in this study, is the basis of engineering approaches to nutrient removal from sewage effluent(Rolich, 1969). Phosphorus removal during bloom conditions in some stabilization ponds coincide with high pH values which are characteristic of bloom conditions in our experimental ponds. However, Rolich notes that removal was probably the result of coagulation and adsorption as well as algal uptake. He notes that many pond systems are not dependable as nutrient removal mechanisms because of inadequate operating and control procedures.

Diurnal Studies

Figure 4 shows the overall diurnal phosphorus changes in P1 on 28-29 July with corresponding changes in relative DIP uptake rates(K). Also plotted with K in the upper graph, is the absolute, gross uptake rates(J)in µg-at/l·hr, calculated as J=K(DIP). Changes in DIP showed a distinct diurnal pattern in that the concentration dropped steadily during the day and rose again through the night. This pattern has been previously observed by Bruce and Hood(1959)in their studies of diurnal phosphate variations in Texas Bays. PP concentrations increased slightly through the 24 hour period but had no diurnal pattern. DOP underwent a net decrease from sunrise to sunset and a net increase overnight although these changes were not as distinct as those of DIP. Overall, the dominant diurnal phosphorus exchanges in P1 appeared to be the uptake and release of DIP.

The relative and absolute DIP uptake rates by the plankton followed DIP values in the water whereby declining DIP concentrations during the day corresponded to decreasing per-cent uptake and, consequently, less phosphorus being incorporated into algal tissue per unit time. This pattern of transfer indicates that on a short term basis with no population reproduction, the phosphorus system is controlled by passive storage kinetics whereby storage rates are proportional only to the

driving force(DIP concentration) and outflow from each compartment depends on amounts stored(Odum, 1969).

While no diurnal cycle was observed in C3(Fig. 5), the large phosphorus storages in P1 allowed both pronounced photostimulated fluctuations and identification of internal mechanisms of phosphorus exchange through the use of the plastic tubes.

Table 1 lists the net exchanges observed between the water and plankton, bottom, and consumer compartments. Note especially that net planktonic DIP uptake is positive and relatively constant during both day and night indicating that enough energy was stored in the plankton community during the day to allow incorporation of phosphorus through the night at rates similar to daytime rates.

Inflow and outflow estimates(Table 2) are small and indicate that the large DIP variations observed in P1 were caused by internal fluxes. The largest flux detected was the loss of DIP from the sediments at night which obviously caused the DIP increase in the water column at night. In fact, the net rate of DIP increase at night(.8µg-at/1.hr) almost doubles the net loss rate during the day(-.4µg-at/1.hr). These results indicate that ponds used for nutrient removal from sewage wastes would certainly not be effective if conditions were similar to those in P1 on 28-29 July.

Mathematical Model of Diurnal Phosphorus Exchanges

Based on data presented in Tables 1 and 2, a mathematical description of the phosphorus exchanges observed in P1 was formulated. Figure 6 serves as a basis in showing the storage compartments and paths of diurnal phosphorus exchange, using a combination of material and energy circuit languages(Odum, 1969). The circular modules at the left of the figure represent the source of phosphorus for the pond. The tank symbols represent passive storage compartments of the ponds, which change in concentration due to the fluxes shown between compartments. Some of the fluxes are photostimulated, shown in the diagram as the intersection of light energy source(I) and flows between certain compartments. These light stimulated exchanges will be discussed with the assumptions used in formulating the mathematical model. These assumptions are as follows:

(1) Planktonic DIP uptake was assumed to be constant both day and night. There was no diurnal photostimulation associated with this flow.

(2) Gross planktonic DOP uptake was assumed to be constant(.17)both day and night, with DOP release of .16µg-at/1.hr during the day, yielding a net daytime uptake of .01µg-at/1.hr shown in Table 1. DOP excretion by some marine plankton has been shown to be proportional to light intensity(Kuenzler,1970). Increasing photosynthesis during the day probably necessitates faster elimination of organic fluids from phytoplankton. This light stimulated flux is shown in Fig. 6 with the workgate module intersecting J-23(flux from compartment 2 to 3)with light energy.

(3) DIP release from the sediments is assumed to be constant (1.03) with a daytime uptake of 1.4µg-at/l.hr. yielding a net daytime uptake of 0.37µg-at/l.hr. as shown in Table 1. This uptake, presumably by the algal mat, is indicated by the light controlled work gate of J-14.

(4) DOP uptake by the bottom is also assumed to be light controlled with constant release of 0.10µg-at/l.hr. and a 0.06µg-at/l.hr. daytime uptake, yielding the observed net daytime release of 0.04µg-at/l.hr.

(5) DIP excretion by large consumers is assumed to be a photo-stimulated mechanism, indicated by the light-controlled switch module on J-51. Possibly higher dissolved oxygen concentrations during the day provide more fuel for the oxidation of ingested organic matter by consumers and the subsequent metabolic elimination of inorganic fluids.

(6) Data indicating DOP uptake by consumers led to the assumption of a constant DOP release of 0.17µg-at/l.hr. and a daytime uptake of 0.22 µg-at/l.hr. yielding the net daytime uptake of 0.05µg-at/l.hr. shown in Table 1. This light controlled mechanism is shown by the work gate on J-35.

(7) Consumer ingestion of PP was calculated as the sum of the phosphorus losses from the consumer compartment (.17µg-at/l.hr.) assuming a diurnal steady state.

Based on the diurnal exchanges shown in Fig. 5, differential equations were written describing the changes of phosphorus compartments with respect to time and are listed in Table 3. The multiplying effects of light energy on these changes is represented by (I) in the terms involving light-controlled fluxes. The transfer coefficients (K) in these equations bear the same number as the fluxes with which they are associated. For example, K12 is the transfer coefficient for plankton uptake of DIP (J-12).

An Electronics Associates TR-20 analog computer was programmed to simulate the exchanges described by these differential equations, whereby electrical circuits were designed to be analogous to the phosphorus system, in so far as it is described by the equations. To be installed properly into the electrical circuitry of the computer, these equations were scaled to fit the amplitude and time capacities of the machine. The scaled equations, listed in Table 4, assign computer values to the variables, which then can be incorporated into the machine for simulation. "B" in the scaled equations is the time scaling factor. Figure 7 shows the electrical circuitry of the entire analog program for the diurnal phosphorus simulation with the scaled variables noted.

Modules 1,2,3,4,and 5, corresponding to the compartments diagrammed in Fig. 6, are high-gain amplifiers, wired to integrate the inputs to the compartment. Outputs of some of the integrators were generated negative to conserve the use of other amplifiers used in different ways within the program.

Modules 9, 11, and 13 are multipliers which correspond to the work gate symbols in Fig 6, whereas module 19+20 is the switch mechanism corresponding to the switch symbol on J-51 in Fig. 5.

An integral part of a diurnal model is the generation of a function whose input variations into the program approximate diurnal changes

in light. The chopped sine serves this purpose and although the electrical machinery used to generate it are not shown in Fig. 7, its input into the program is indicated by + and - I. The circular modules are grounded potentiometers which correspond to the scaled transfer coefficients in Table 4.

Preliminary work with the analog computer simulation had indicated that this model can be a useful tool in understanding the phosphorus system of the ponds. By manipulating and altering transfer coefficients and initial conditions in the model, the time response of these changes can be explored. The model serves as a description of the pond phosphorus system as now understood and the computer can be used to understand the consequences of the model. Parker (1968) has found that such a model, based on weekly exchanges, was very informative in predicting the effects of increasing DIP inputs from fertilizer plant effluent on the phytoplankton, zooplankton, and salmon populations of Kootenay Lake.

Applications of the model presented in this report are being planned and, hopefully, a better understanding of the structure and function of the phosphorus in our experimental ponds will be gained through these simulation techniques.

I would like to express my appreciation to Larry Burns for his help and advice in programming and patching the computer for these preliminary simulation studies.

TABLE 1.

NET DIURNAL PHOSPHORUS FLOWS OBSERVED IN P-1
28-29 JULY, 1970
(ug-at/l/hr)

COMPARTMENT	FLOW	RATES DAY	NIGHT
PLANKTON	DIP UPTAKE	0.28	0.32
	DOP UPTAKE	0.01	0.17
BOTTOM	DIP UPTAKE	0.37	-1.03
	DOP RELEASE	0.04	0.10
CONSUMER	DIP EXCRETION	0.22	0.25
	DOP UPTAKE	0.05	-0.17

TABLE 2.

AVERAGED* P-1 INFLOW AND OUTFLOW
28-29 JULY, 1970
(ug-at/liter of pond water/hr)

PHOSPHORUS FRACTION	INFLOW	OUTFLOW
PP	0.06	0.05
DIP	0.08	0.10
DOP	0.01	0.01

*Diurnal inflows and outflows are assumed to be constant over 24 hrs. since the effects of daily input variations on the total phosphorus budget are dampened by mixing.

TABLE 3.

DIFFERENTIAL EQUATIONS DESCRIBING DIURNAL RATE-CHANGES OF PHOSPHORUS FRACTIONS

(1) $\frac{d\,Q1}{dt} = J2+(K51*Q5)+(K41*Q4)-(K12*Q1)-(K14*Q1*I)-(K16*Q1)$

(2) $\frac{d\,Q2}{dt} = J1+(K12*Q1)+(K32*Q3)-(K23*Q2*I)-(K25*Q2)-(K26*Q2)$

(3) $\frac{d\,Q3}{dt} = J3+(K23*Q2*I)+(K43*Q4)+(K53*Q5)-(K32*Q3)-(K35*Q3*I)-(K34*Q3*I)-(K36*Q3)$

(4) $\frac{d\,Q4}{dt} = (K14*Q1*I)+(K34*Q3*I)-(K43*Q4)-(K41*Q4)$

(5) $\frac{d\,Q5}{dt} = (K25*Q2)+(K35*Q3*I)-(K51*Q5)-(K53*Q5)$

TABLE 4.

SCALED DIFFERENTIAL EQUATIONS

(1) $\frac{d\,Q1}{dt\ 100} = \frac{J2}{100B} + \frac{(K51)}{10B}\frac{Q5}{10} + 2\frac{(K41)}{10B}\frac{Q4}{20} - \frac{(K12)}{B}\frac{Q1}{100} - 2\frac{(K14)}{2B}\frac{Q1}{100}(I) - \frac{(K16)}{B}\frac{Q1}{100}$

(2) $-\frac{d\,Q2}{dt\ 100} = -\frac{J1}{100B} - \frac{(K12)}{B}\frac{Q1}{100} - \frac{(K32)}{10B}\frac{Q3}{10} + \frac{(K23)}{B}\frac{Q2}{100}(I) + \frac{(K25)}{B}\frac{Q2}{100} + \frac{(K26)}{B}\frac{Q2}{100}$

(3) $\frac{d\,Q3}{dt\ 10} = \frac{J3}{10B} + 10\frac{(K23)}{B}\frac{Q2}{100}(I) + 2\frac{(K43)}{B}\frac{Q4}{20} + \frac{(K53)}{B}\frac{Q5}{10} - 10\frac{(K32)}{10B}\frac{Q3}{10} - \frac{(K35)}{B}\frac{Q3}{10}(I) - \frac{(K34)}{B}\frac{Q3}{10}(I) - \frac{(K36)}{B}\frac{Q3}{10}$

(4) $-\frac{d\,Q4}{dt\ 20} = -10\frac{(K14)}{2B}\frac{Q1}{100}(I) - \frac{(K34)}{2B}\frac{Q3}{10}(I) + \frac{(K43)}{B}\frac{Q4}{20} + 10\frac{(K41)}{10B}\frac{Q4}{20}$

(5) $-\frac{d\,Q5}{dt\ 10} = -10\frac{(K25)}{B}\frac{Q2}{100} - \frac{(K35)}{B}\frac{Q3}{10}(I) + \frac{(K51)}{B}\frac{Q5}{10} + \frac{(K53)}{B}\frac{Q5}{10}$

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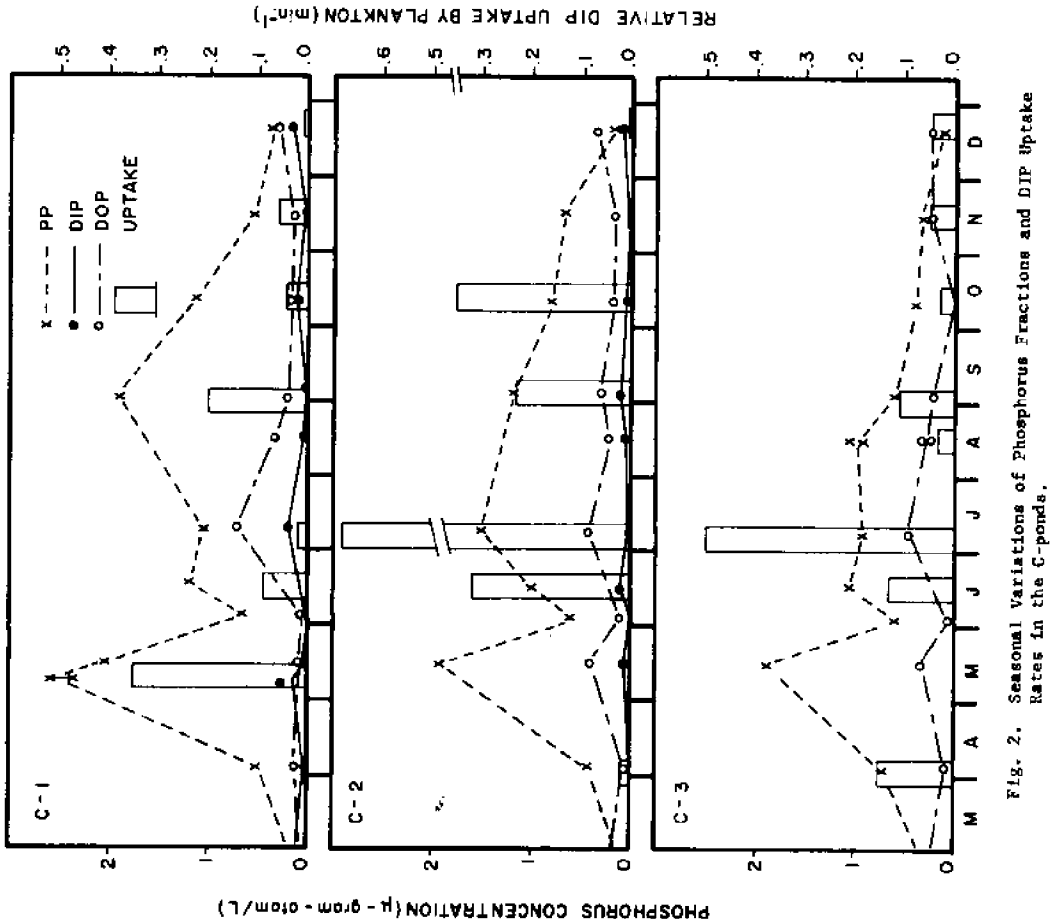

Fig. 2. Seasonal Variations of Phosphorus Fractions and DIP Uptake Rates in the C-ponds.

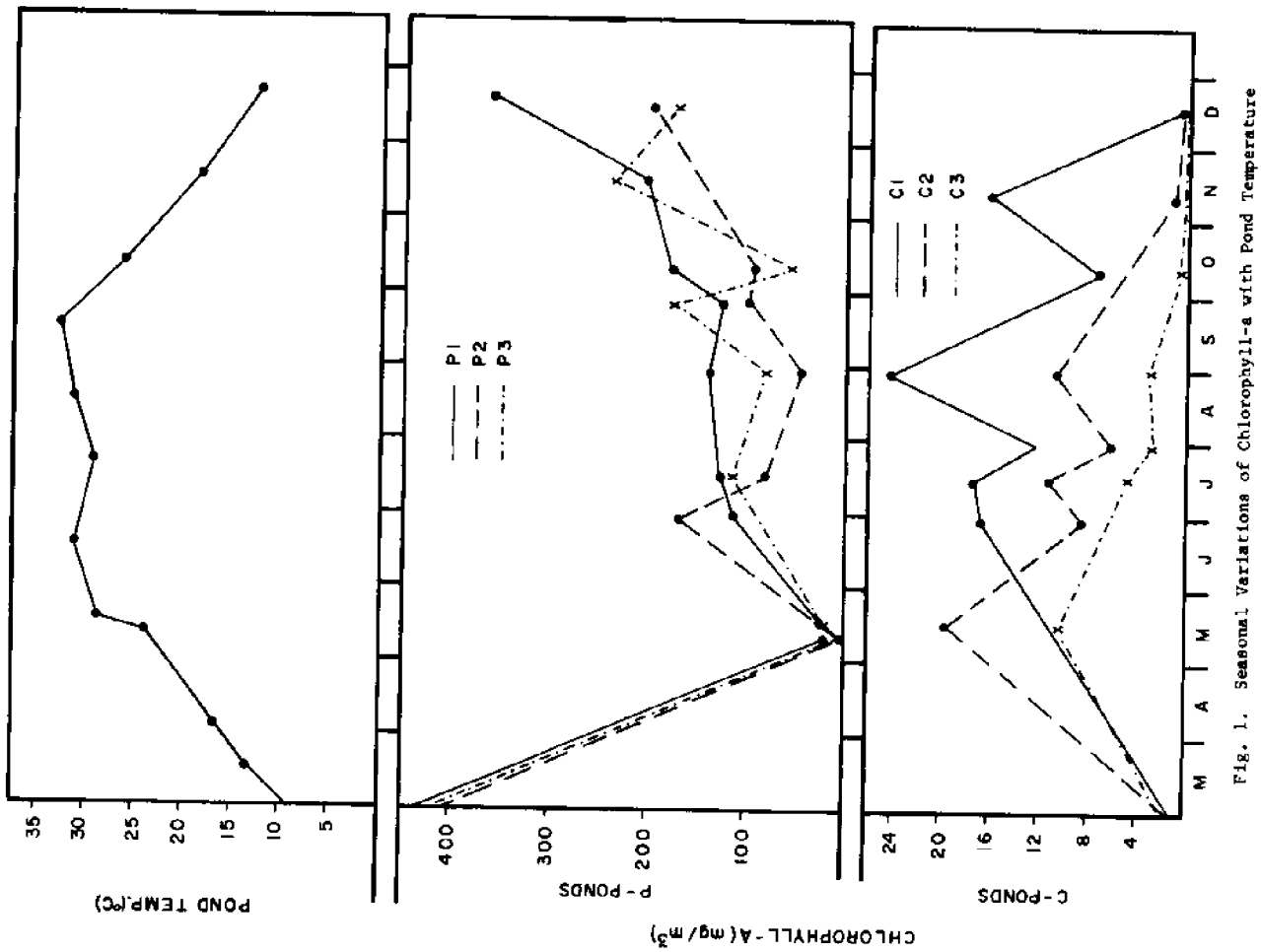

Fig. 1. Seasonal Variations of Chlorophyll-a with Pond Temperature

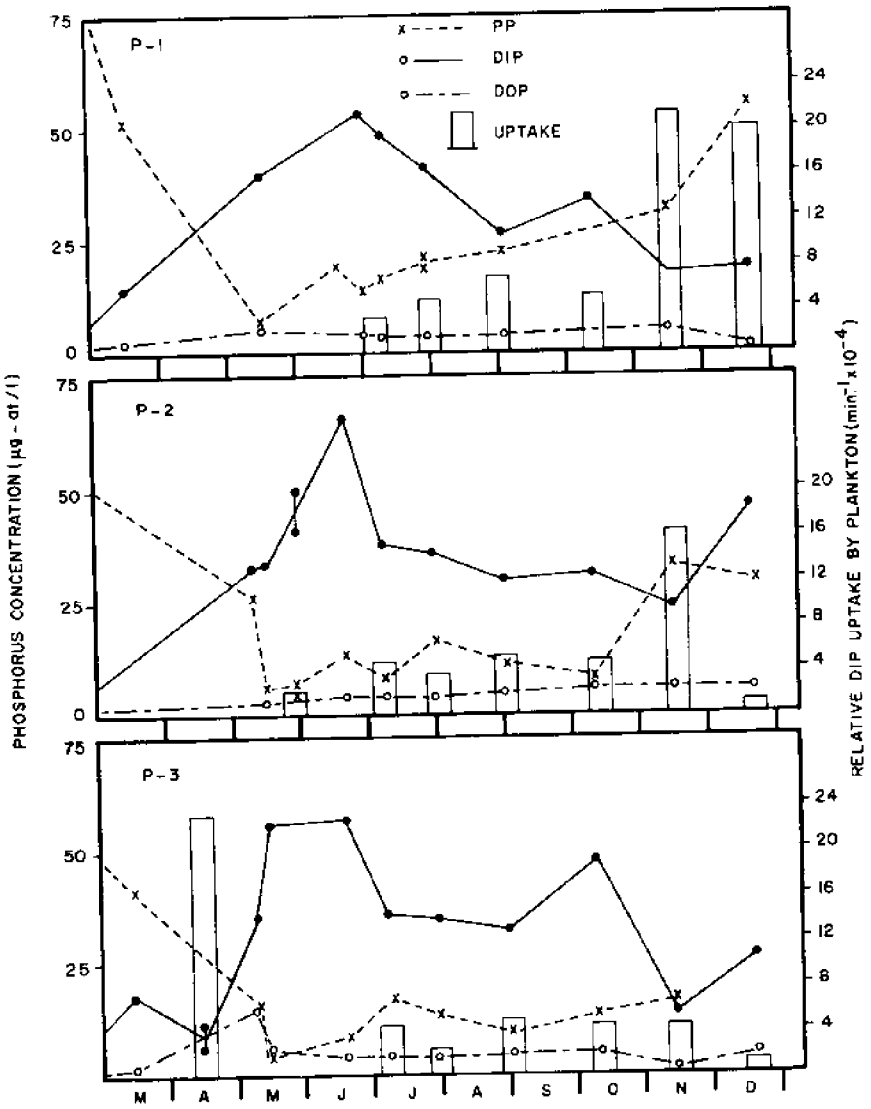

Fig. 3. Seasonal Variations of Phosphorus Fractions and DIP Uptake Rates in the P-ponds.

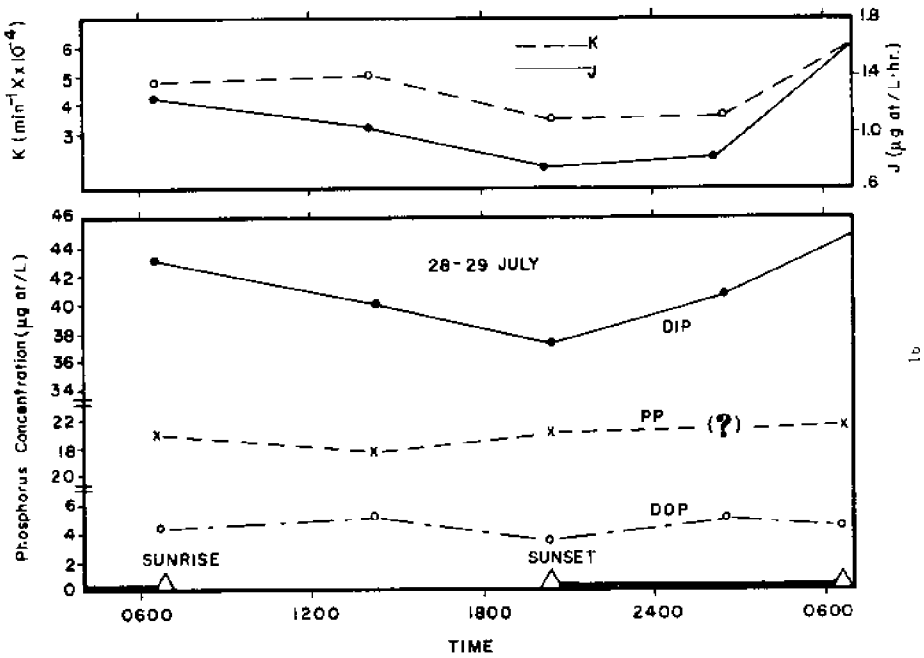

Fig. 4. Diurnal Phosphorus Variations in P-1.

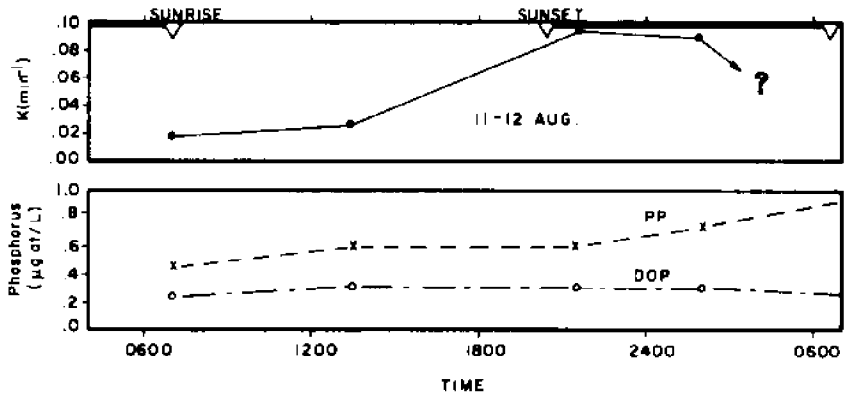

Fig. 5. Diurnal Phosphorus Variations in C-3.

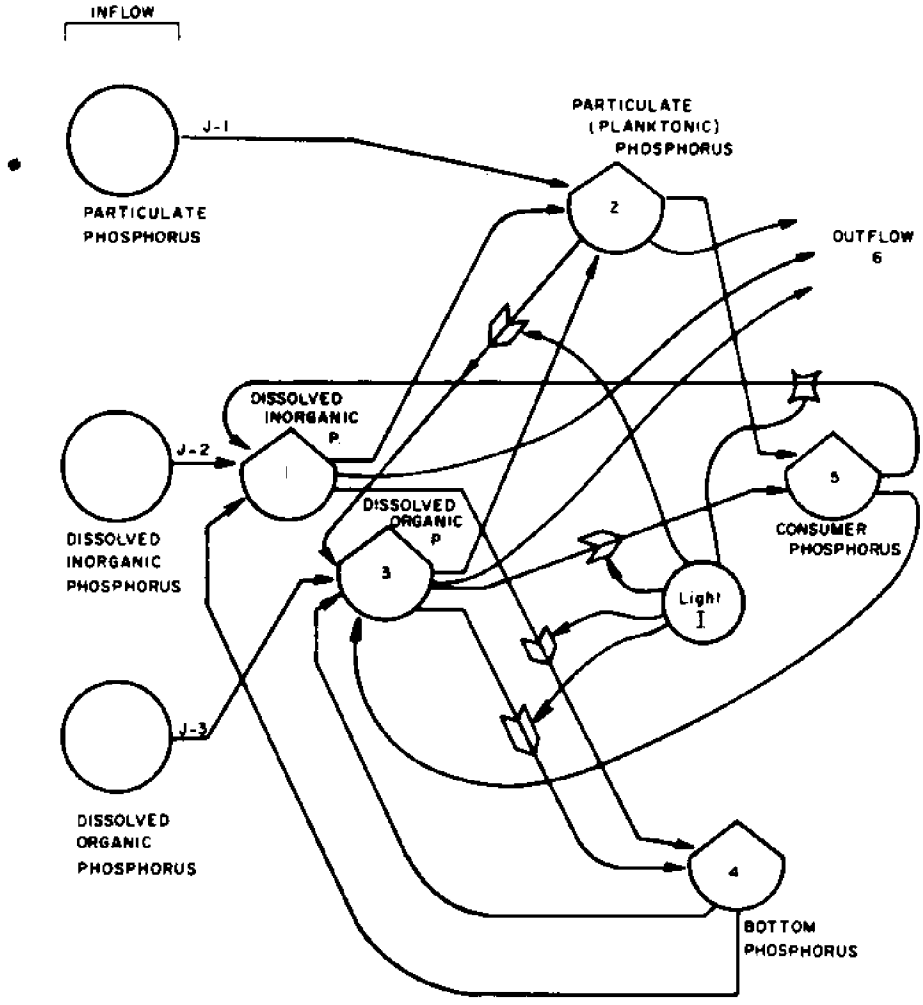

Fig. 6. Flow Chart of Diurnal Phosphorus Exchanges Detected on 28-29 July in P-1.

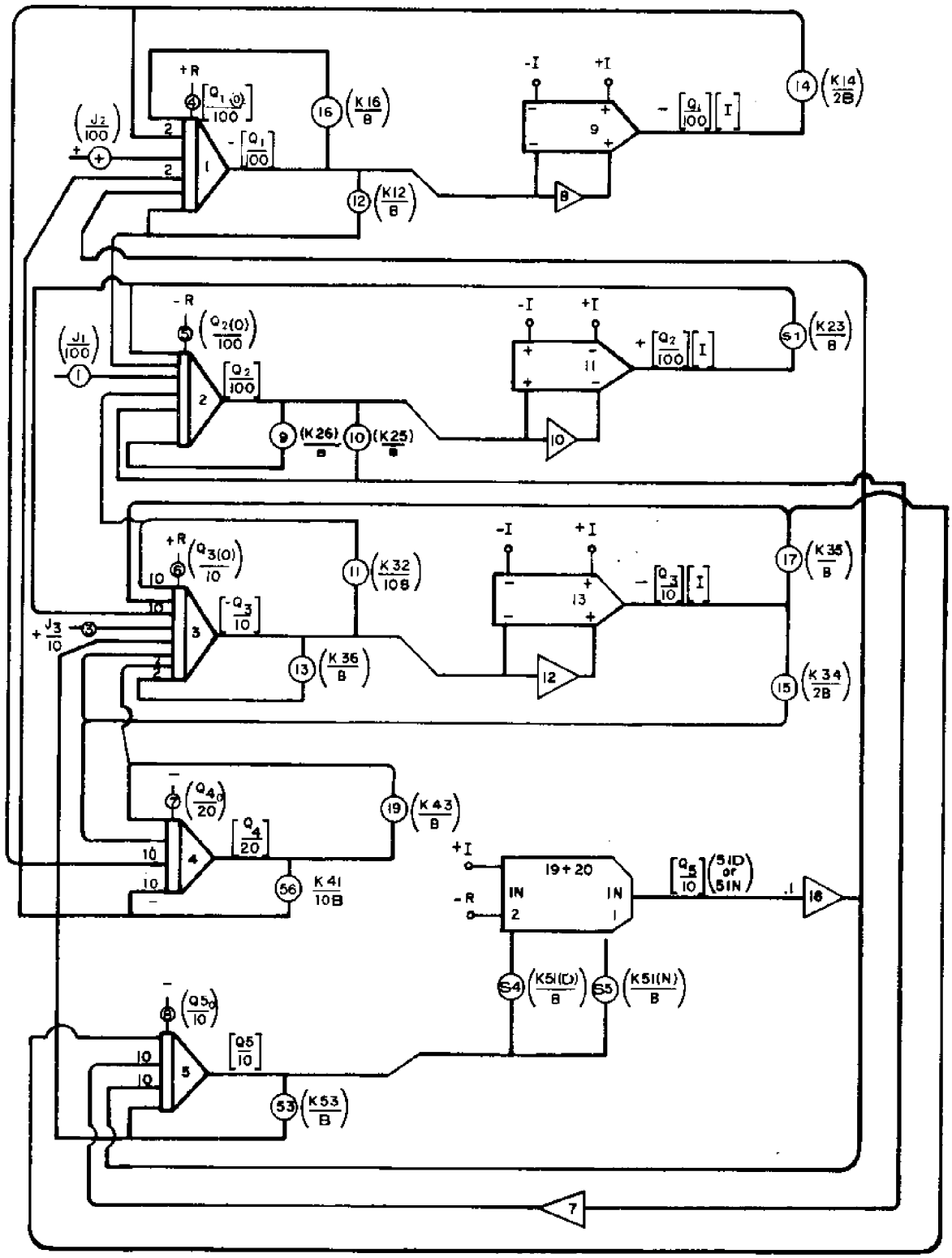

Fig. 7. Analog Computer Program for Diurnal Phosphorus Simulation.

A STUDY OF THE NITROGEN IN THE SEA GRANT PONDS FOR THE PERIOD OF 14 July TO 31 AUGUST, 1970

Sam C. Masarachia
under the direction of
Dr. C. M. Weiss

INTRODUCTION

Both organic and inorganic forms of nitrogen are found as waste products of metabolism. After passing through a primary sewage treatment plant, nitrogen is found to a great extent in the inorganic forms of ammonia (as NH_4^+), nitrate (NO_3^-), and nitrite (NO_2^-), as well as numerous unidentified organic forms. As a result of this, any study of the effects of treated sewage on natural waters should include work concerned with the various nitrogen forms. Although the Sea Grant ponds in Morehead City, N. C. have been studied for over two years, very little concentrated effort has been done on the nitrogen phase. Dr. W. J. Woods (1970) has been analyzing samples monthly since the onset of the study; P. Hebert (1970) made a very short study for Dr. C. M. Weiss in August, 1969.

This present study was done under the direction of Dr. C. M. Weiss in order to see how the nitrogen forms vary from day to day over a given period of time, to see what daily variations exist, and to set up a framework around which a total nitrogen "budget" might be described. All work described here was done in the period from 14 July to 31 August, 1970.

MATERIALS AND METHODS

Analyses for the various nitrogen forms were made on samples of water drawn from the Sea Grant ponds and from the mixing tanks. Water was taken in aliquots of approximately 100 ml in plastic screw-top bottles. The samples were fixed with $HgCl_2$, 4 mg to each sample, to stop biological activity. Unless they were to be analyzed immediately, all samples were frozen until needed. A record was kept of the times the samples were taken.

Preliminary or sample studies were analyzed at the Institute of Marine Sciences, Morehead City, with the technical help of Dr. W. J. Woods. Analyses were made for nitrate and nitrite as described by Mullins and Riley (1955), ammonia as described by Solorzano (1969), and micro-Kjeldahl digestion of organic nitrogen with selenium catalyst was followed by the ammonia method described. Except for the particulate organic nitrogen analyses, all samples were filtered through 0.45 μ Millipore filters.

The majority of the samples were analyzed for total organic nitrogen (filtered and unfiltered samples), nitrate, nitrite, and ammonia by Technicon AutoAnalyzers under the direction of Mr. Tony Owen and his staff at the Limnology Laboratory, School of Public Health, Chapel Hill. Samples were analyzed against standards made up in synthetic ocean water, diluted to the approximate saliniaty of

the ponds (less than 20°/oo), and against standards made from deionized water; difference between the two types of standards was found to be slight. The sensitivity of the nitrate/nitrite apparatus was not acceptable for the first set of samples, but this problem was corrected in later studies.

The first set of samples was taken during the week of 14-21 July, 1970. This study was to see how the various forms of nitrogen varied from day to day as a result of photosynthesis and respiration, as well as from the schedule of water inflow. Samples were drawn at dawn and dusk from the surface water of each of the ponds within 0.1 m of the outflow spout (as a measure of the water leaving the ponds) and from the surface water of each of the mixing tanks. Samples were analyzed by the autoanalyzers.

Another study was made on 30 July, 1970, to see if measureable horizontal concentration gradients exist between the inflow pipe and the opposite side of each pond. Samples were taken only from P2 and C2, and chemical analyses were done in Dr. Wood's laboratory.

A diel study was made on the surface waters of each of the ponds from 2000 on 4 August to 2000 on 5 August, 1970. Samples were taken at four hour intervals from the surface water of each pond and from both mixing tanks as described. The samples were analyzed on the autoanalyzers.

A second diel study was made from 0000 to 2200 on 18 August, 1970. With the help of Mrs. Martha Smith and Mr. Gene Walton, water samples were taken from ponds P3 and C3 near the bottom in the area of the shell reefs. Samples for the nitrogen study were taken at 0000, 0100, 0200, 0300, 0600, 1000, 1400, 1800, and 2200; the samples were analyzed by the autoanalyzers. The purpose of this study was to see what effect low nightly O_2 concentrations would have on the various nitrogen forms.

In order to study the extent of vertical stratification in the ponds, on 19 August, 1970, samples were taken from ponds P1 and C1 at the surface, at 40 cm depth, and 10 cm from the bottom at the deepest part of the ponds. Samples were taken by means of a corked plastic bottle secured to a meter stick. Samples were taken by suspending the bottle at the desired depth and then pulling the cork out with a string. For the first part of the study the samples were analyzed in the laboratory at Morehead City. After analyses had been completed and the results noted, a further study was undertaken from all of the ponds; samples were taken at the surface, at 30 and 60 cm depths, and 10 cm from the bottom at the deepest part. Surface samples were taken from the mixing tanks at the same time. These latter samples were taken on 21 August, 1970, and analyzed on the autoanalyzers.

RESULTS

The results of the various experiments are summarized in Figures 1-12 and in Table 1. Figures 1-5 show the main results from the dawn and dusk samples of 14-21 July, 1970. The diel studies are summarized in Figures 6-9 for the surface waters, and in Figures 10-12 for the bottom waters. Table 1 gives the results of the vertical

stratification study.

Some general results were noted from this short, concentrated study. Although the diel studies indicate that fluctuations of the concentrations of the various nitrogen species occur in a day, the overall levels in the ponds do not usually change significantly from day to day. The P-ponds tend to have higher concentrations of the various nitrogen forms. There is a change in concentration of nutrients with changes in depth, but the general pattern is not clear. In general the levels of measurable nitrite are of the same order as that of the nitrate; indeed there are samples which show more nitrite than nitrate. Horizontal concentration gradients exist while water is being pumped into the ponds, but the wind is sufficient to mix the waters shortly thereafter.

DISCUSSION

The main question asked when this study began was: "What is the nitrogen budget for the Sea Grant ponds?" Of course, for such a short study as this, no complete answer can be forthcoming without followup studies. However, an approximation figure may be determined by using the following steps:

1. Determine the volume of water flowing into the ponds for a given period of time, and the nitrogen content thereof.
2. Determine the volume of water flowing out from the ponds and the nitrogen content thereof.
3. Determine the loss of nitrogen compounds to the bottom in the form of organic sediments.
4. Determine the loss of nitrogen, chiefly as ammonia, to the atmosphere by diffusion.
5. Calculate the loss and gain of nitrogen compounds to the ponds due to such miscellaneous items as birds, domestic animals, rain, and human intervention (experimenters or trespassers).

Steps 1 and 2 were the main objects of this study. Step 3 is very difficult to assess, as several of the researchers do stir the ponds by their activity (i.e. Step 5). The diffusion of ammonia is presently being studied by Mr. M. Raps; personal communication from him indicates that this loss may not be significant. The miscellaneous gains and losses are likewise not determinable at this time; for the sake of simplicity I have assumed that the net change due to miscellaneous activity is zero.

From the information kept by Mr. W. Laughinghouse, inflow rates for the time period studied (14-21 July, 1970), water inflow rates may be summarized as follows:

C1	C2	C3	P1	P2	P3
16,300 1/day	11,600 1/d	10,400 1/d	2700 1/d	2600 1/d	2300 1/d

Note that for a few days of this study one of the water pumps for the C-ponds was broken, but that the concentrations of the various nitrogen species did not change drastically after the pump was fixed. For the week of 14-21 July, 1970, an "average" liter of water in each of the ponds would have contained the following:

(Note: all figures as mg of Nitrogen)

Pond	Particulate Organic N	Dissolved Organic N	NH_4+	NO_3-	NO_2-	Total N
P mixing tank	0.69	1.48	1.76	.005	.088	4.02
P1	1.35	0.71	0.29	.004	.005	2.36
P2	0.82	0.72	0.47	.005	.009	2.02
P3	1.09	0.77	0.64	.004	.016	2.52
C mixing tank	0.25	0.11	0.57	.003	.006	0.94
C1	0.22	0.37	0.34	.004	.008	0.94
C2	0.25	0.42	0.36	.004	.006	1.04
C3	0.20	0.48	0.29	.004	.005	0.98

Exact figures on the volumes of water flowing out from the ponds are lacking at this time. However, since the volume of water in the ponds is kept fairly constant by means of the outflow spouts, we can assume that the inflow volume equals the outflow volume minus evaporation; any leakage through the bottom mud is considered the same as outflow water. John Day (Odum and Chestnut, 1970, Introduction) calculated an average figure of 5 mm per day, or about 2×10^3 liters per day per pond, lost to the atmosphere as evaporation. For simplicity, as above, I assume that there is no nitrogen loss with the evaporated water; therefore all nitrogen not found in the overflow must be found in the sediments. The results of such calculations yield the following:

Pond	Inflow (I)	Outflow (O)	Loss to sediments (I-O)
P1	10.9 g N/day	1.7 g N/day	9.2 g N/day
P2	10.4	1.2	9.2
P3	9.2	0.7	8.5
C1	15.3	13.4	1.9
C2	10.9	10.0	0.9
C3	9.8	8.2	1.6

We must note that the above figure is at best a "ball park" estimate. A more accurate accounting of the inflow and outflow volumes would be in order to obtain more accurate figures. The above data are based solely on samples taken at dawn and dusk for the period of one week in the middle of the summer. Over longer periods of time more accurate numbers may be obtained. Work over the course of the seasons would give a better yearly accounting.

A second question that needs to be answered is whether nitrogen is a limiting factor in the ecology of the ponds. At first glance this may seem to be a foolish question with respect to the sewage ponds, since so much nitrogenous material is poured in every day. However, we must remember that sewage effluent contains high levels of organic carbon and of phosphorus compounds, and that the relative abundance of nutrients needs to be considered. We cannot answer the question of limiting factors at this time because of the lack of sufficient information, but there is some indication that the amounts

of nitrogen may be limiting under some conditions. Although N/P (nitrogen-phosphorus) ratios do not prove anything by themselves, Dr. W. J. Woods (1970) noted that the N/P ratios of the P-ponds were more like 2:1 than the 16:1 often quoted for natural waters.

One aspect of the inorganic nitrogen which is of note is that the nitrite levels are similar, if not higher, that the levels of nitrate. During the daylight hours the concentration of oxygen in the ponds is at the level of saturation or higher (Odum et al., 1970): one would expect that these levels of oxygen would favor the more oxidized forms such as nitrate. However, at night the oxygen values often drop to a level undetectable by the standard Winkler analysis. This low oxygen level during the night may be favoring the reduced forms of inorganic nitrogen in order to support respiration. This is one aspect that could use further study.

The diel studies show that the nitrogen concentration increases during the time of inflow of water from the mixing tanks, and then change during the course of the day; fluctuations in the sewage ponds are greater than those of the control ponds. There is some indication that the inorganic nitrogen forms are metabolized to organic compounds during the night, but the data are confused to some degree; the differences could be due to the stage of algal bloom in progress. The diel study of the bottom water tends to confirm that the low oxygen concentrations of the bottom are reflected by the excess of NO_2^- over NO_3^- as noted above; however, some bottom mud was included in several of the samples, so there may be some bias.

The mixing studies confirm that the ponds are well mixed horizontally by the wind; however, the vertical mixing tends to be more of a problem. As often shown for natural waters, vertical stratification exists in the Sea Grant ponds; the pattern of this stratification is confusing. Apparently the fact that the ponds are being stirred by various researchers at odd times is the greatest contributing factor to the problem.

RECOMMENDATIONS

I recommend that a more detailed study of the nitrogen forms be continued; at the very least samples should be taken at weekly intervals from each of the ponds as well as the mixing tanks. During the course of a bloom in each season semidaily (e.g. dawn/dusk) samples should be taken to follow the complete chemical cycle of an algal bloom. The triggering mechanisms of the start and demise of these blooms have not yet been described.

Another recommendation, which I have borrowed from Dr. E. J. Kuenzler and Dr. W.J. Woods, is that nitrogen enrichment studies should be undertaken on both sets of ponds. This method of study may prove valuable in the determination of whether nitrogen is a limiting factor in the ecology of the ponds.

For the more accurate determination of a budget of the nitrogen in the ponds, exact flow of water over a given time period should be measured; at intervals during this study samples should be taken, in replicate, for chemical analysis. A set of studies of a whole year would prove to be very valuable.

TABLE 1
VERTICAL STRATIFICATION STUDY

A. 19 August, 1970

Pond	Particulate Organic N	Dissolved Organic N	Ammonia	Nitrate	Nitrite
P1 0 cm	.305 mgN/l	.331 mgN/l	.056 mgN/l	.056 mgN/l	.003 mgN/l
33	.342	.262	.009	.031	.005
90	.347	.301	.037	.038	.004
C1 0cm	0	.119	.022	.027	.004
40	.004	.105	.022	.022	.004
80	0	.154	.036	.024	.006

B. 21 August, 1970

Pond	Ammonia	Nitrate	Nitrite
P1 0 cm	.070 mgN/l	0 mgN/l	.019 mgN/l
30	.090	.012	.012
60	.070	.019	.012
90	.400	.009	.013
P2 0 cm	.050	.021	.012
30	.060	.016	.011
60	.060	.005	.014
74	.320	.015	.014
P3 0 cm	.080	.046	.012
30	.020	.013	.008
60	.160	.008	.008
77	.040	.006	.007
C1 0 cm	.030	.056	.010
30	.350	.023	.013
60	.120	.013	.017
77	.130	.008	.013
C2 0 cm	.110	.024	.008
30	.140	.033	.013
60	.080	.004	.018
82	.050	.003	.014
C3 0 cm	.580	.016	.009
30	.130	.014	.010
60	*	.007	.011
81	.250	.008	.011

* sample lost

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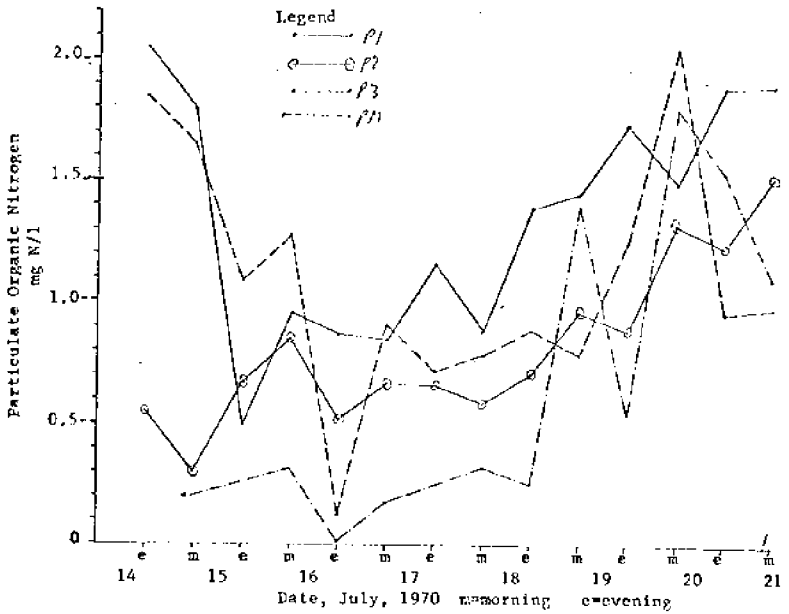

Figure 1. Particulate Organic Nitrogen P-ponds, 14-21 July 1970.

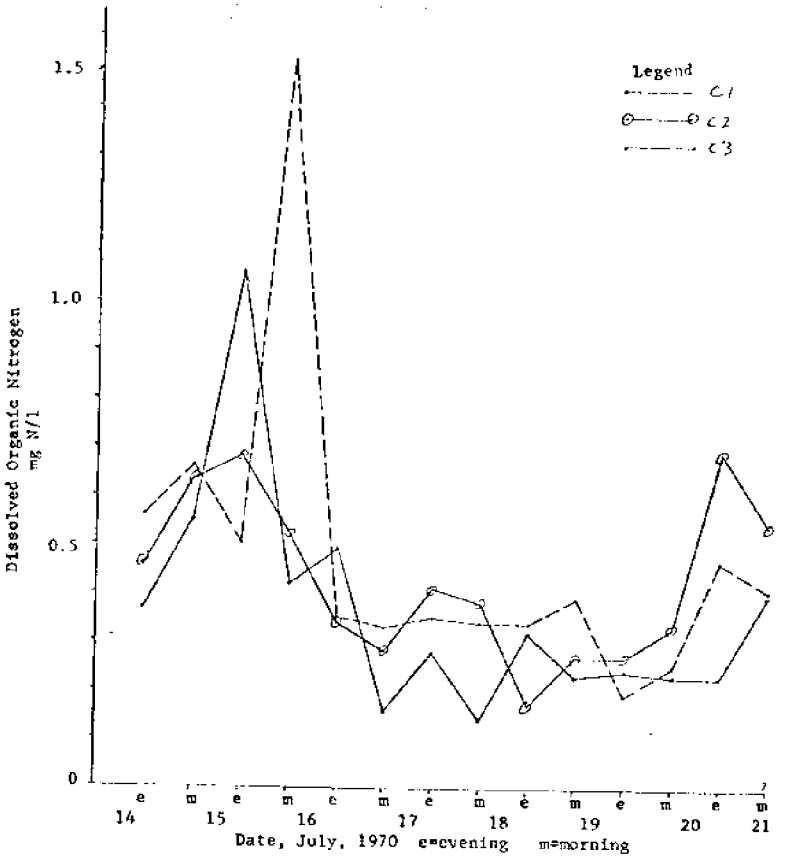

Figure 2. Dissolved organic nitrogen C-ponds, 14-21 July 1970.

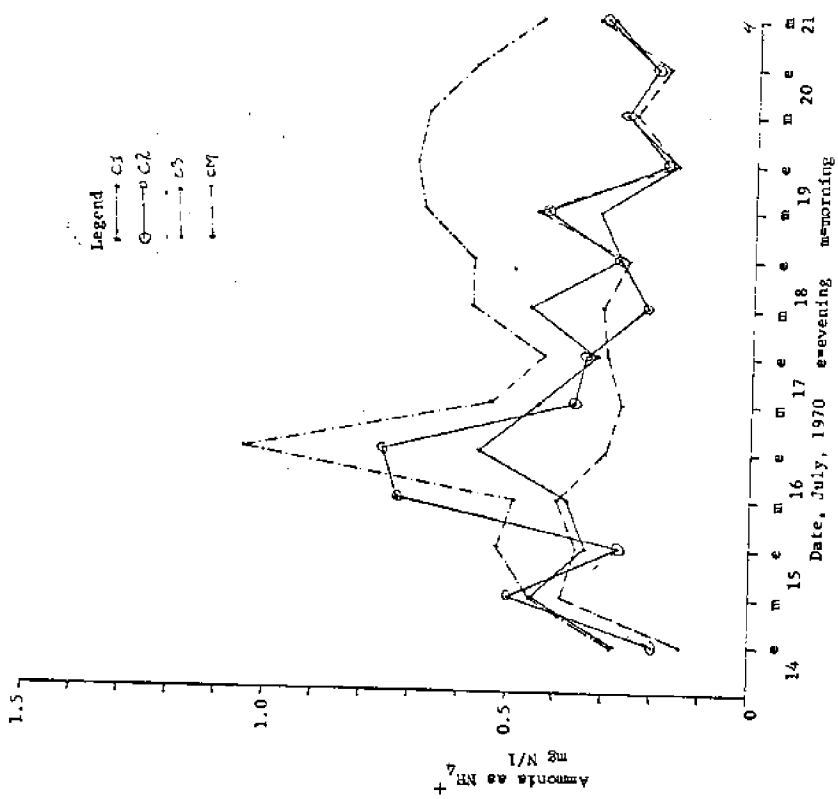

Figure 4. Ammonia Nitrogen C-ponds, 14-21 July 1970.

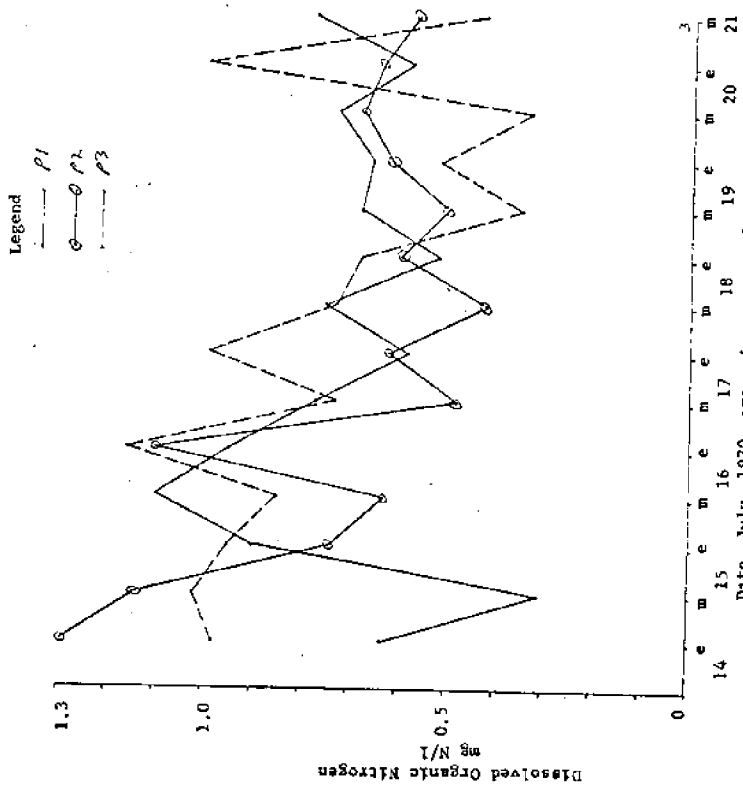

Figure 3. Dissolved organic nitrogen P-ponds 14-21, July 1970.

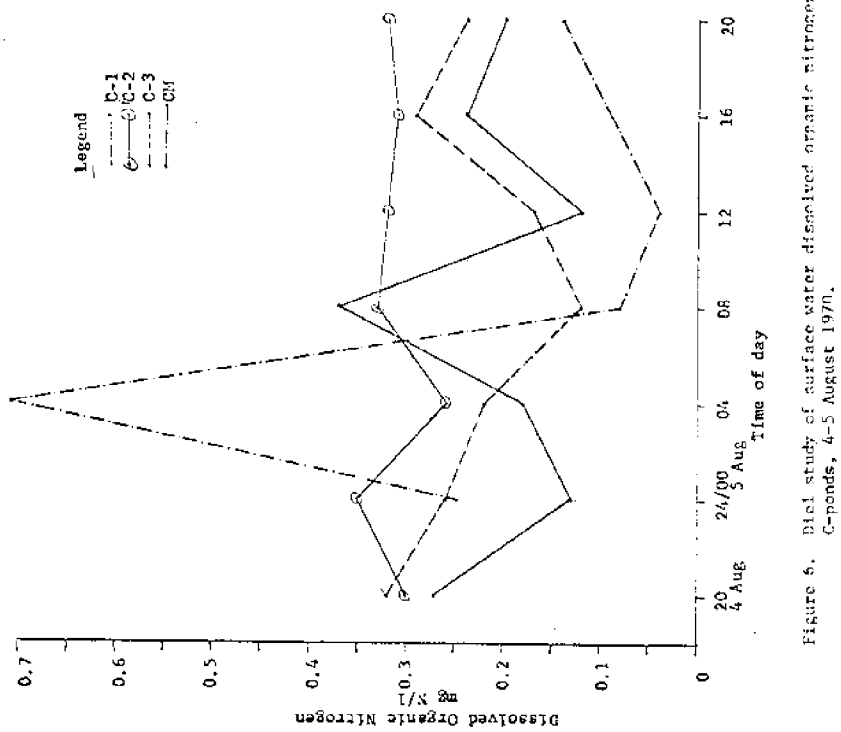

Figure 6. Diel study of surface water dissolved organic nitrogen C-ponds, 4-5 August 1970.

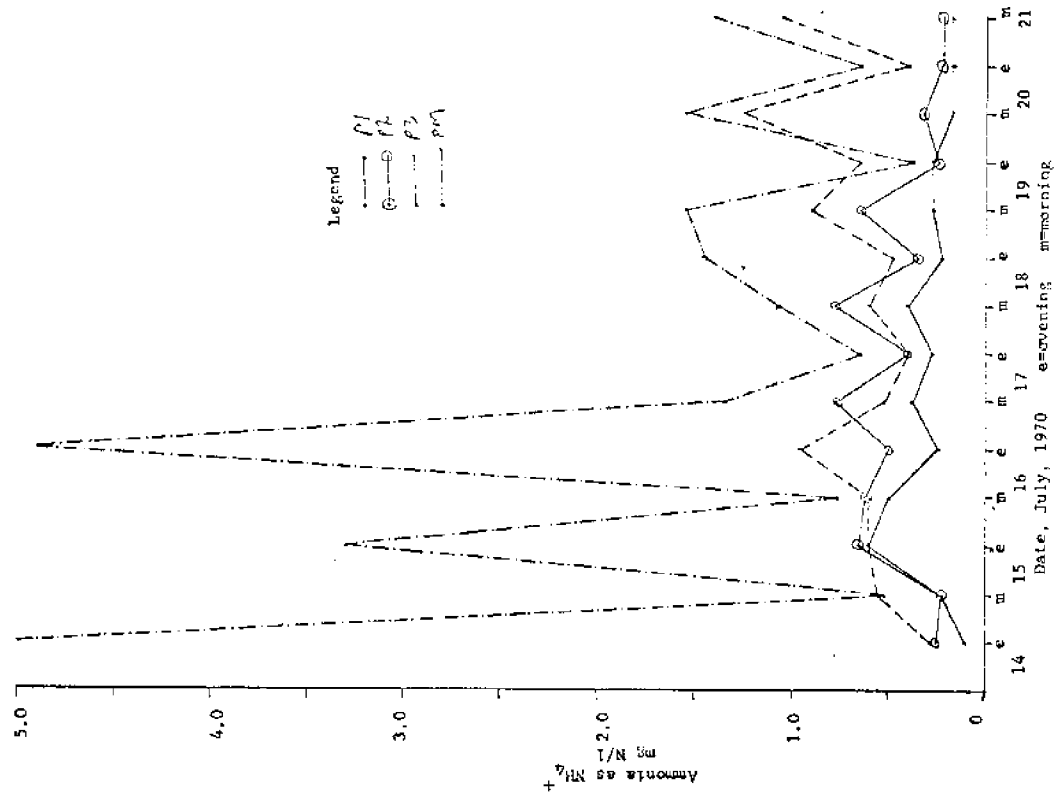

Figure 5. Ammonia nitrogen P-ponds, 14-21 July 1970.

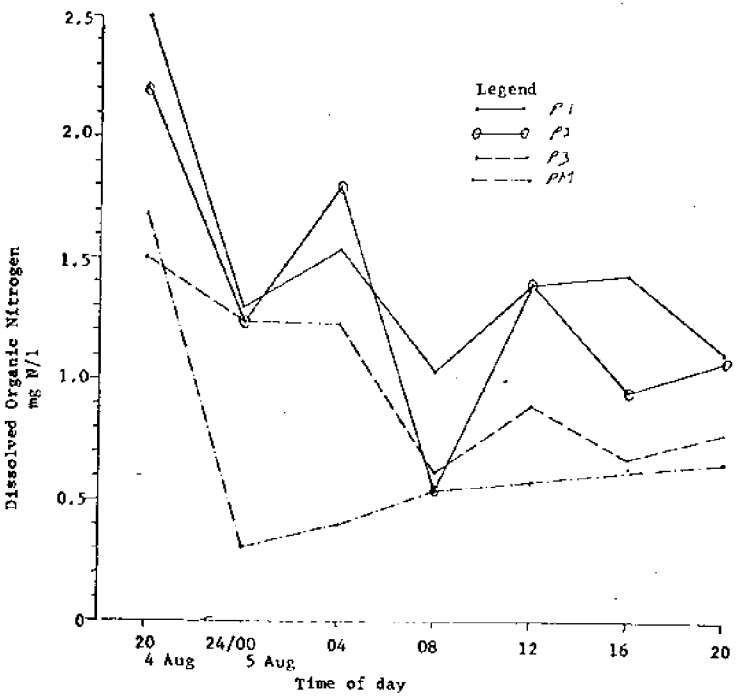

Figure 7. Diel study of surface water dissolved organic nitrogen P-ponds, 405 August 1970.

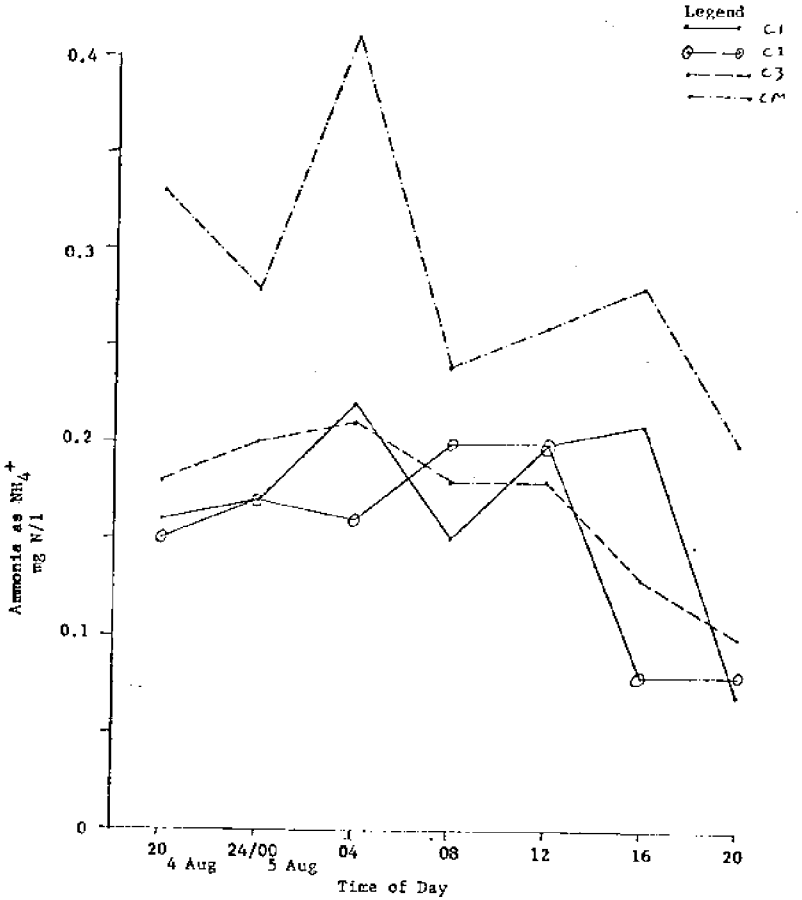

Figure 8. Diel study of surface water ammonia nitrogen C-ponds, 4-5 August 1970.

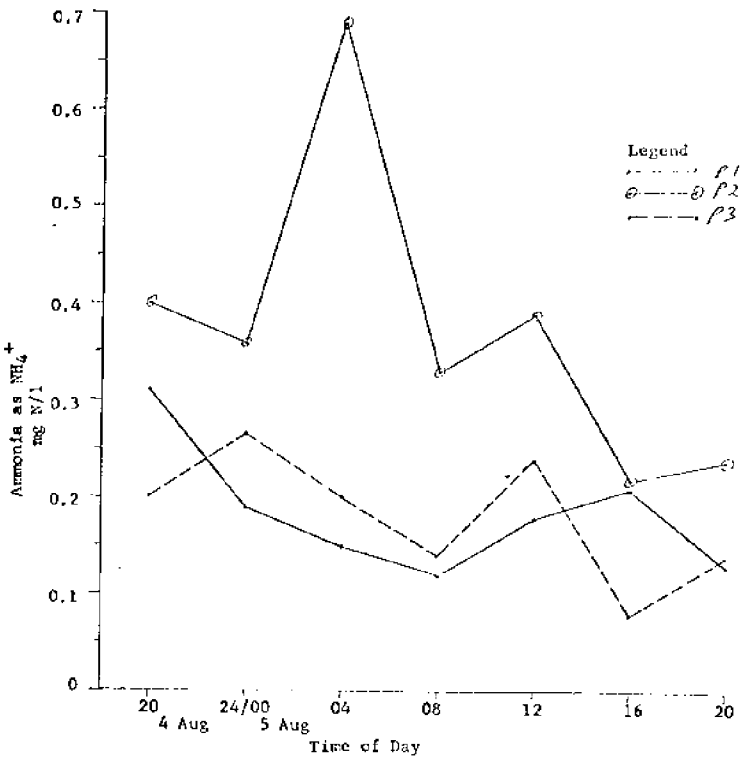

Figure 9. Diel study of surface water ammonia nitrogen P-ponds, 4-5 August 1970.

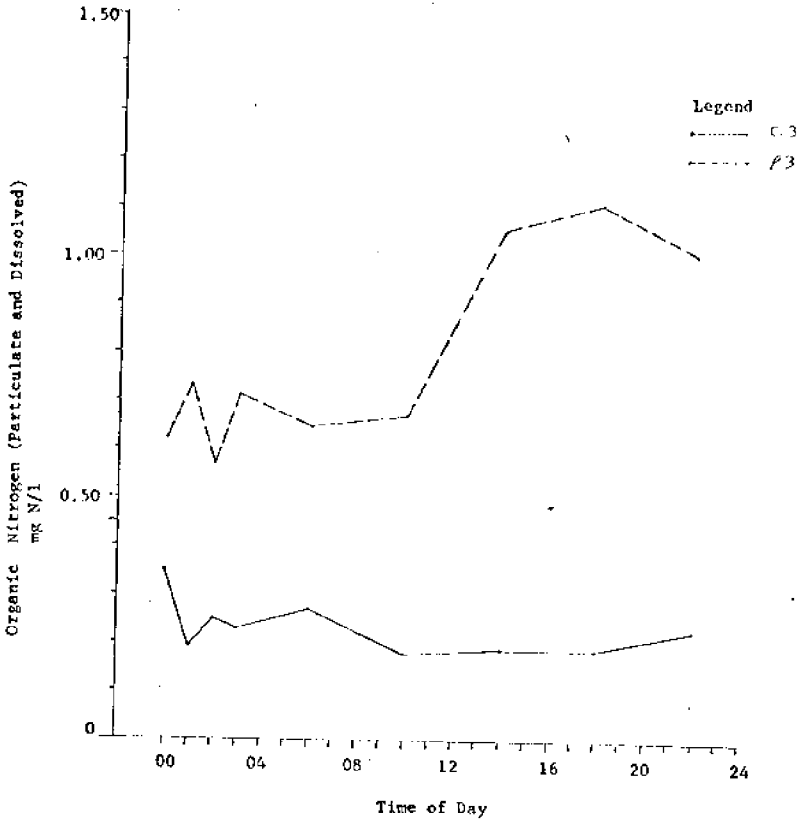

Figure 10. Diel study of bottom water total organic nitrogen C3 and P3, 18 August 1970.

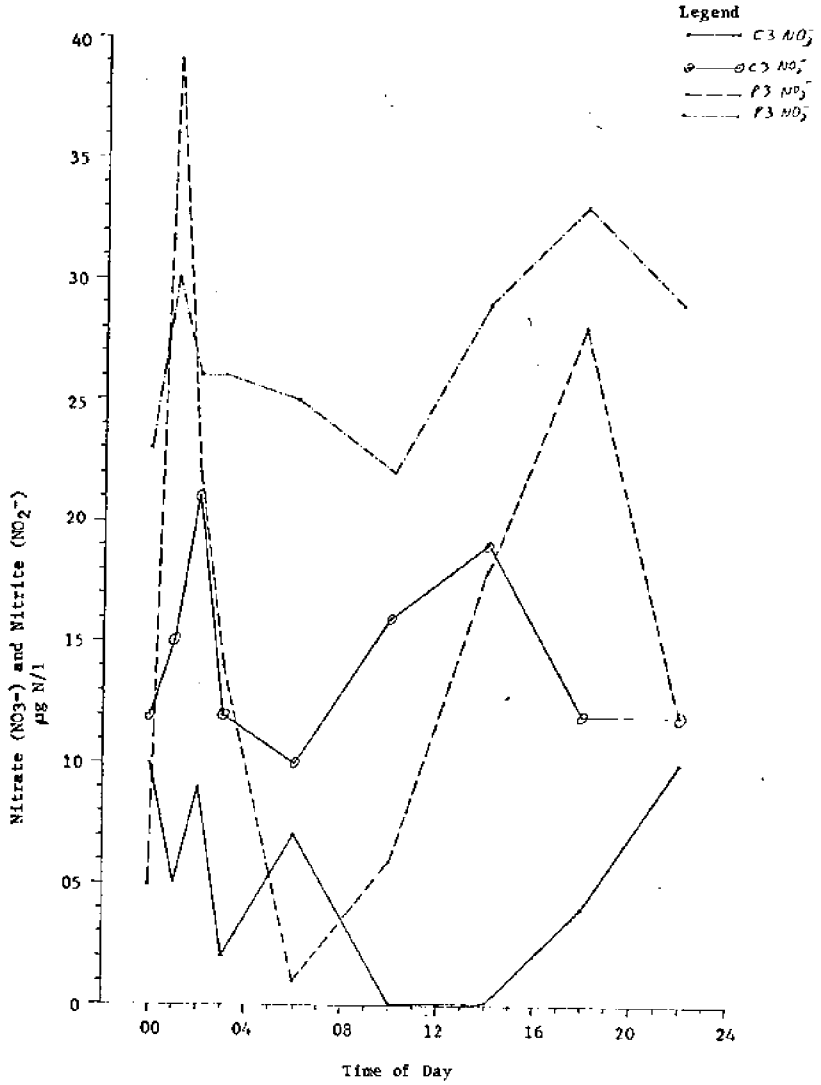

Figure 11. Diel study of bottom water nitrate and nitrite C3 and P3, 18 August 1970.

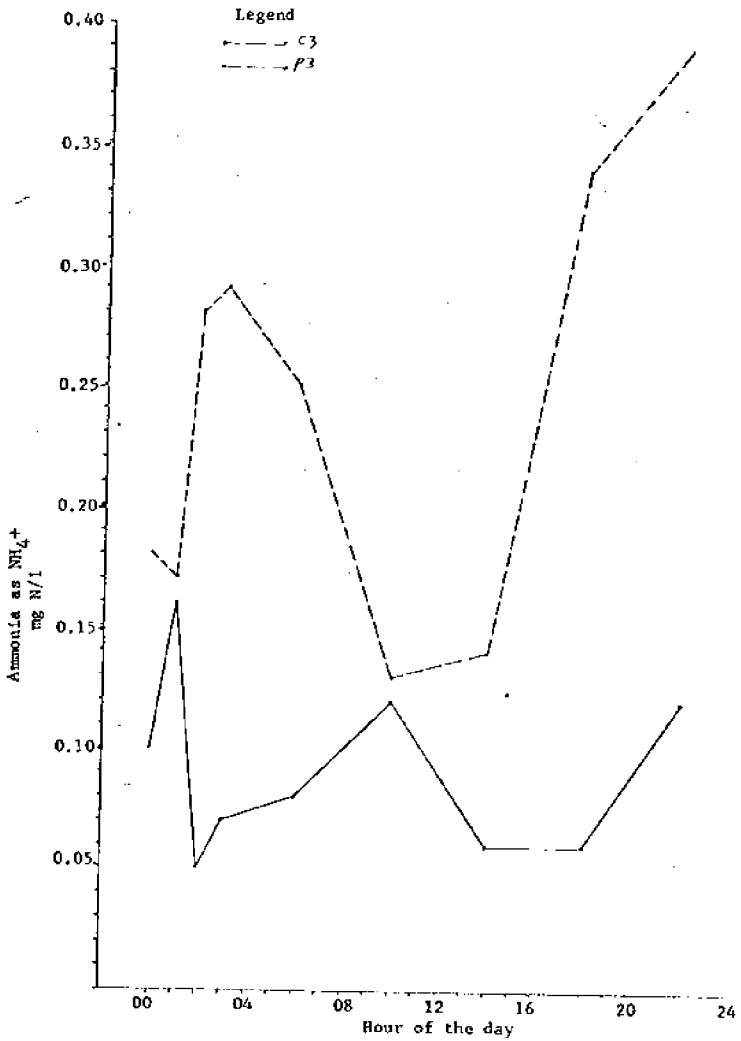

Figure 12. Diel study of bottom water ammonia nitrogen P3 and C3, 18 August 1970.

A STUDY OF AMMONIA DIFFUSION ACROSS THE AIR/WATER SURFACE OF THE SEA GRANT PONDS

Martin E. Raps*
Department of Environmental Science and Engineering

INTRODUCTION

The investigation of ammonia diffusion across the air/water surface of the sea ponds was conducted from 19 August 1970 to 5 December 1970. The purpose was to develop a method for determining the rate of nitrogen loss through ammonia diffusion which will be used in compiling a nitrogen budget for the sea ponds during 1971.

MATERIALS AND METHODS

The clear plastic dome system developed by Copeland and Duffer (1964) and more recently used by Day (Odum and Chestnut, 1970) was modified for the purpose of determining the rate of ammonia diffusion from the pond surface to the atmosphere. A recirculating, essentially closed system was run for various periods of time (Fig. 1a). An open system which pulled atmospheric air in addition to diffused ammonia through the dome and into the flask was also employed (Fig. 1b). When the open system was used, an "atmospheric blank" was run along with the experimental run. This set-up consisted of an HCl flask and a pump open to the atmosphere over the pond and throttled to the same flow rate as the experimental pump (Fig. 1c). Thus, atmospheric ammonia was in effect being measured.

In either system, ammonia diffusing from the water surface under the plastic dome was bubbled into the flask and trapped in the 0.1M hydrochloric acid sloution contained therein. Solutions were analyzed for ammonia in Dr. Wood's laboratory using the method described by Solorzano (1969). At first, a boric acid method of analysis was tried which used HCl to titrate the ammonium borate formed, with bromcresol green detecting the end-point. However, this method was discarded in favor of Solorzano's direct method because of its unreliability and non-specificity.

Experimental periods were first set at three hours duration but were lengthened to twelve, and in most cases, twenty-four hours. The twenty-four hour experiments were always started after sundown in order that an entire day of sunlight would be represented by that run. pH measurements were taken at the beginning and end of each experimental period with a Beckman pH meter.

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*Work phase on nitrogen budget with Dr. C. M. Weiss, Dept. of ESE.

RESULTS

The results are summarized in Table 1. A three-hour interval diurnal was begun at pond P-1 on 29 August, but after only two runs it was discontinued because very little ammonia was detected. Twelve-hour runs were then conducted on pond P-1 and the results indicated that greater diffusion (6.5 μg at N/m^2) occurred during the period from 900 to 2100, and the least (0.1 μg at N/m^2) occurred during the following 2100 to 900 period. The results of the twenty-four hour experiments showed that diffusion was substantially greater in the P-ponds than in the C-ponds. Pond P-3 greater than 30.0 ug at N/m^2 per day. On the other hand, pond C-2 showed no detectable diffusion.

The results of pond P-3 from 8 September to 5 December were obtained by using the open system method where an "atmospheric blank" was run along with the experimental flask and compared with a laboratory blank. The results of the atmospheric blank for the four dates showed an equivalent ammonia diffusion rate of approximately 0.1 μg - at N/m^2 per day.

DISCUSSION

The maximum amount to which a gas may be dissolved in sea water is a function of the partial pressure of the gas in the above atmosphere, and the temperature and salinity of the water (Raymont, 1963). However, in the case of ammonia, solubility is also affected somewhat by pH because, although it is a weak Bronsted base, it will react with water to accept a proton. Therefore, the relative availability of protons will influence the form of ammonia most prevalent, as shown in the following equation:

$$NH_3 + HOH \rightleftharpoons NH_4^+ + OH^- , \quad K = 1.8\ 10^{-5}$$

By solving the equilibrium equation for the relative amounts of ammonia forms, it is found that, at pH = 8 which is common for the C-ponds, the ammonium ion is about twenty times more prevalent than ammonia; at pH = 9.5 which is frequently recorded in the P-ponds, ammonia is twice as prevalent as the ammonium ion. In acid to moderately basic solutions ammonia is highly soluble. However at high pH, it is speculated that the hydrogen bonds that water continually makes and breaks with the nitrogen atom of ammonia (the fabled ammonium hydroxide) probably occur less frequently due to the electronegative environment created by hydroxide at this pH. With the oxygen of hydroxide quite ready to share any one if its free pairs of electrons with an electron acceptor, nitrogen's single pair is at a geometric as well as electronic disadvantage. Therefore, at high pH the solubility of "ammonia" is essentially decreased and, with relatively high levels of ammonia beforehand, conditions favor ammonia dif-

fusion from the surface of the pond into the atmosphere. The very low concentration of ammonia estimated to be in the ponds' ambient atmosphere (about 1 part per billion) would, of course encourage the diffusion process.

The pH which influences this diffusion, particularly during the daylight hours in the P-ponds, is primarily controlled by the process of photosynthesis. Because of the higher nutrient inflow and concommitant higher productivity in the P-ponds, photosynthesis substantially reduces carbon dioxide levels, thus elevating pH to as high as 10 and creating favorable conditions for ammonia diffusion. For example, the pH reading at 1000 on 22 August was 9.1; the effec- pH for the daytime was therefore high, resulting in a high rate of diffusion. It can also be noted that pH lags slightly behind daylight in their sinusoidal behavior. This, in part, may explain why higher diffusion rates occurred in the twelve-hour period which is shifted towards the evening hours (i.e. 900 to 2100).

Another factor which may account for the shift in the equilibrium favoring ammonia diffusion at high pH is the fact that carbon dioxide exists in the carbonate form at pH = 10, and phytoplankton, while taking up carbonate for photosynthesis, pump out hydroxide in order to maintain acid-base and ionic balance internally (Odum, 1970). And this added hydroxide favors the liberation of ammonia.

A reason to account for the low diffusion rates at night in the P-ponds is the drop in pH due to the increased respiration and carbon dioxide build-up. The low results in the C-ponds, however, are due to the lower prevailing pH which is partly due to the lower productivity, which is the result of lower inflow of nutrients.

The rate of diffusion in the P-ponds is on the order of 0.1 mg $N/m^2/day$. A night time rate of diffusion for carbon dioxide is about 1 g $C/m^2/hr$. Even considering differences in concentration ammonia diffusion rate appears to be negligible.

SUMMARY

Ammonia diffusion was found to occur in low but detectable rates in the P-ponds. It was essentially non-existent in the C-ponds. The major cause for ammonia diffusion appears to be due to the increased pH during the late afternoon hours. A second factor was probably due to the higher concentrations and greater biological sources found in the P-ponds. Loss of nitrogen through ammonia diffusion seems to be a minor factor in the over-all nitrogen budget.

FUTURE WORK

An index for the rate of nitrogen fixation may be obtained by utilizing the independent discoveries of Schollhorn and Burris (1966) and Dilworth (1966) that the nitrogen-fixing complex reduces acetylene to ethylene. The production of ethylene can be measured by gas chromatography.

In situ flasks containing pond water samples will be flushed with argon-oxygen-carbon dioxide gas mixture to eliminate nitrogen gas. A known ratio of acetylene:nitrogen gas will be introduced. The ratio is on the order of 1:1000. This better simulates natural conditions and should allow allosteric inhibition by products to occur, if indeed they do occur in nature. (The authors cited used 100% acetylene atmospheres for shorter periods of incubation.) The flasks will be incubated in situ for periods up to twelve hours.

Water-sediment systems will be temporarily set up in the ponds using rigid plastic tubing two inches in diameter and approximately 1.5 meters long. The amount of nitrate denitrified at the water-sediment interface can be determined by measuring nitrate levels before and after the experimental period. A nitrification inhibitor, 2-chloro-6-(trichloromethyl) pyridine, will be added to essentially eliminate the formation of additional nitrate from ammonia in the water column. Denitrification rates can be determined by running similar experiments with the sediments closed off from the water column (Goering and Dugdale, 1966).

Inflow and outflow analyses will be conducted. All of the above data will be compiled to determine a nitrogen budget for each pond. An analog model will be constructed to relate phytoplankton levels to the compiled data.

Table 1.

Ammonia Diffusion Data

Date	time	Δt hrs.	Pond	K*	pH start	pH end
19 Aug	0600 - 0900	3	P-1	0.0	8.7	8.7
19 Aug	0900 - 1200	3	P-1	0.1	8.7	8.8
21 Aug	0600 - 1800	12	P-1	3.8	8.8	9.5
21 Aug	1800 - 0600	12	P-1	0.4	9.5	8.6
22 Aug	0900 - 2100	12	P-1	6.5	8.6	9.3
22 Aug	2100 - 0900	12	P-1	0.1	9.3	8.7
23 Aug	1200 - 2400	12	P-1	4.2	8.7	8.9
24 Aug	0000 - 1200	12	P-1	0.2	8.9	9.4
24 Aug	2100 - 2100	24	C-1	0.4	8.0	8.2
25 Aug	2200 - 2200	24	C-2	0.0	8.1	8.2
26 Aug	2300 - 2300	24	C-3	<0.1	7.9	8.1
28 Aug	0000 - 2400	24	P-3	12.0	9.2	9.3
4 Sept	2100 - 2100	24	P-2	3.4	8.7	8.6
6 Sept	2200 - 2200	24	P-1	0.1	8.9	9.7
8 Sept	2100 - 2100	24	P-3	>30.0	--	--
3 Oct	0000 - 2400	24	P-3	3.4	8.4	8.6
7 Nov	0000 - 2400	24	P-3	4.4	8.3	8.5
5 Dec	0000 - 2400	24	P-3	5.2	--	--

*μg at $NH_3/m^2/\Delta t$

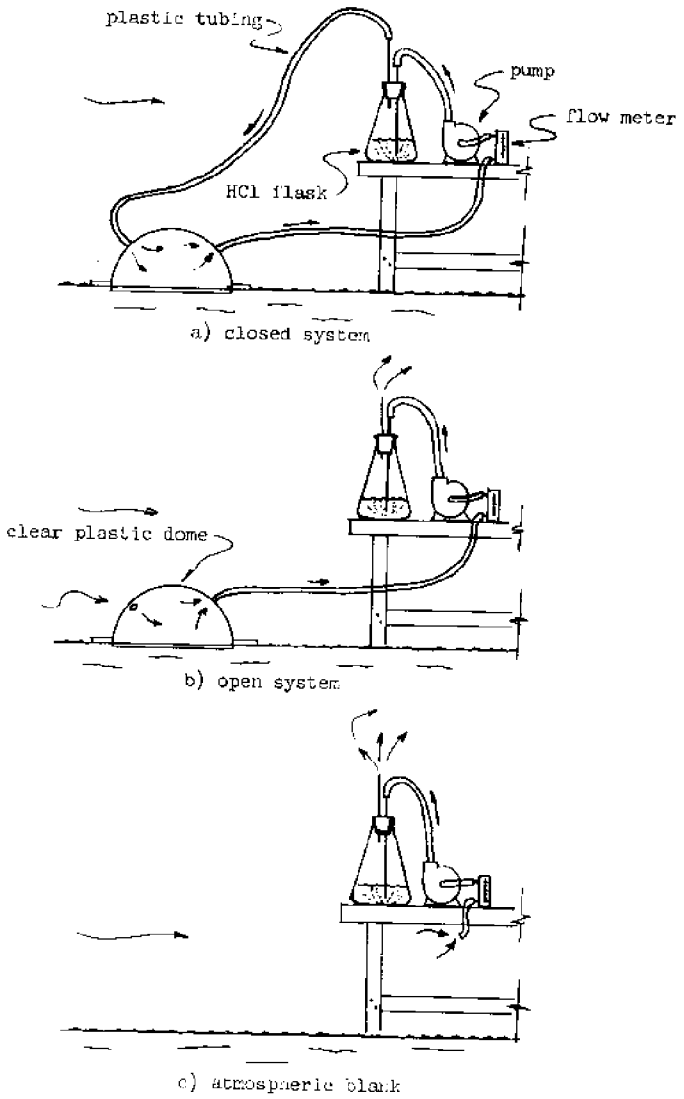

Figure 1. Ammonia Diffusion apparatus

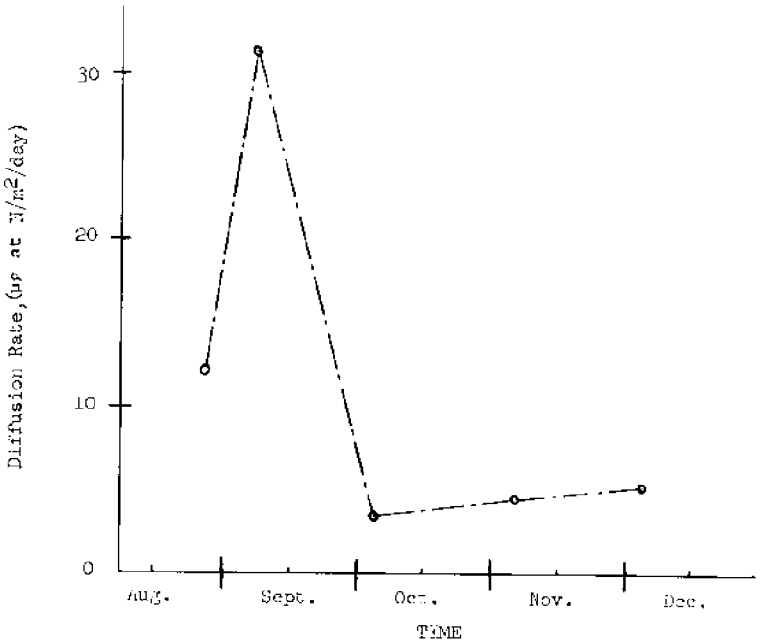

Figure 2. Pond P-3 Ammonia Diffusion Rates

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HETEROTROPHIC UPTAKE OF DISSOLVED ORGANIC MATTER

James A. Marsh, Jr., Post Doctoral Fellow*
Dept. of Environmental Sciences & Engineering
University of North Carolina
Chapel Hill, North Carolina

ABSTRACT

Heterotrophic uptake of dissolved, ^{14}C-labeled glucose was studied in Ponds C-1 and P-1 using methods of analysis based on the assumptions of active-transport kinetics. Uptake parameters determined were turnover time (T) of the natural substrate present, the maximum uptake velocity (V) attainable by the enzyme system of the suspended organisms, and the quantity (K + S), where S is the concentration of natural substrate in the water and K is the active-transport constant of the enzyme system. Various experiments indicated that uptake parameters at a given time could vary by 2-fold or more depending upon the length of incubation time and the time of day the experiments were conducted.

Water samples from both ponds were incubated with labeled glucose for various time intervals ranging from 5 to 90 minutes. Uptake of the labeled glucose, plotted simply as activity vs. time, was linear for at least 1 hour in P-1 and 1.5 hours in C-1. The percentage of glucose respired as CO_2 was not affected by incubation time for either pond.

Experiments indicated possible diurnal patterns for K + S and T in pond C-1 but not in P-1. All parameters in P-1, and V in pond C-1, showed almost 2-fold variations over the course of 24 hours but no distinct diurnal patterns. In both ponds, K + S and T varied together over the course of 24 hours.

T, V, and K + S all varied by as much as an order of magnitude in both ponds at different times over an 18-month period (ranges: V, .45 - 25 µg/l/hr; K + S, 2.9 - 75 µg/l; T, .8 - 109 hours). The variations were not correlated with seasonal changes in temperature or other obvious factors. There were no consistent differences between P-1 and C-1 for any of the parameters, nor were there any clear-cut distinctions between bloom and non-bloom situations in either pond.

In experiments utilizing high concentrations of added glucose (up to 3 mg/l), active-transport kinetics could apparently explain the results most of the time. There was no evidence for diffusion kinetics at these higher levels of added substrate, an observation contradictory to results reported by other workers. Experiments with sediments were not always successful, but there was some indication that the sediments were more active than pond waters in glucose turnover. Results from C-1 and P-1 were comparable

*Under direction of Dr. E. J. Kuenzler

INTRODUCTION

The work reported here was undertaken to contribute to our understanding of the carbon cycle in the Sea Grant ponds. The least-understood part of this cycle in most ecosystems concerns the transformations of dissolved organic carbon (DOC). It is not yet possible to measure the rates of production and utilization of all the DOC in a system, but various workers have had some success in dealing with certain aspects of the problem.

The greatest standing crops of organic carbon in most aquatic systems belong to the dissolved rather than the particulate fraction. Yet it is the particulate fraction that has been the more studied. Determinations of the flow rates of DOC to and from other compartments have been hampered by a lack of techniques for making quantitative measurements of these flows.

Standing crops of DOC in polluted systems might be expected to be even higher than in non-polluted systems. Of course, high standing crops do not necessarily indicate that the DOC is important in the energy flows within the ecosystem. Indeed, there has been some indication that the greatest portion of the DOC in deep-sea waters, for instance, is stable and does not participate in energy transformations within the system. Hence, it is not sufficient or even very informative in some cases to measure total amounts of DOC. What is needed is a dynamic, functional approach which measures rates at which DOC is produced, utilized, and transformed.

The use of radioactive tracers is an obvious approach to try in determining the trophic significance of DOC. Parsons and Strickland (1962) were the first to utilize this approach. They showed that labeled glucose and acetate were taken up by marine plankton at rates which fitted the assumptions of active-transport kinetics, and they proposed a formula for determining the velocity of uptake. They believed that by adding large amounts of labeled substrate to water samples and utilizing their formula to determine rates of uptake they could assess the "relative heterotrophic potential" of different waters. The amounts of substrate they added (250 µgC/liter) were far higher than the amounts naturally present in most waters.

Hobbie and Wright (1965) and Wright and Hobbie (1965, 1966) extended the methods of Parsons and Strickland and reported that the natural populations of suspended organisms in lake waters exhibited active-transport kinetics only at very low levels of added substrate (100 - 200 µg/l or less). They worked out equations for determining rates of uptake at these low levels of added substrate, where the concentrations of the labeled material

fall within the range normally found in natural waters. Results of such experiments are sometimes difficult to interpret, and a full assessment of the method has not yet been achieved. Information is provided only on rates of uptake of the labeled substrate by organisms suspended in the water, not on rates of excretion of DOC. However, these are the only methods presently available, and they are at least useful in comparing different ecosystems. I have therefore utilized this approach in the experimental ponds.

This report is concerned only with work done on the ponds since the 1970 annual report.

This work was supported mostly by Postdoctoral Research Fellowship 1-F2-WP-26, 435-01 from the Federal Water Pollution Control Administration; assistance from Grant No. GH-18 from National Science Foundation and Grant No. 180 and 232 from North Carolina Board of Science and Technology is also gratefully acknowledged.

METHODS

Water samples are taken from the ponds immediately before use. Ten ml of water is pipetted into 25-ml respiration flasks, and micro-pipets are used to add a series of different glucose concentrations from a standard solution of the radioactive substrate. Concentrations of added glucose in the flasks range up to 200 $\mu g/l$. All samples are done in duplicate. One duplicate set receives .3 ml of 2N H_2SO_4 before the addition of the labeled substrate. This set serves as a "control" which indicates the non-biological uptake of the substrate, and all other samples are corrected for this control when the data are processed. Immediately after the glucose addition the flasks are capped with syringe stoppers bearing plastic cups which extend into the flasks above the water samples. The plastic cups hold a folded piece of filter paper (Whatman #1). The samples are next placed in a dark incubator set at the temperature of the pond at the time of collection.

After incubation periods ranging from 15 minutes to 1 hour, organisms in the water samples are killed with H_2SO_4 injected into the flasks with a syringe. Then another syringe is used to add .2 ml of phenethylamine to the filter paper in the plastic cups. The flasks are then set back into the incubator for 1.5 hours to allow absorption of released CO_2 by the phenethylamine. Each water sample is filtered through .45 μ membrane filters which are then rinsed with filtered sea water. The filters are placed in scintillation vials and dried in an oven 75° C. Fifteen ml of scintillation fluid is then added to the vials. Immediately after CO_2 absorption the pieces of filter paper with the impregnated phenethylamine are placed in scintillation vials and scintillation fluid added. Scintillation fluid is prepared by dissolving 4 g of PPO and 50 mg of dimethyl POPOP in purified toluene and filling to 1 liter.

All samples were counted in a liquid scintillation counter before and after the addition of ^{14}C-toluene as an internal standard (Wolfe and Schelske, 1967). The data were then analyzed according to the techniques

worked out by Hobbie and Wright (1966). Figure 1 shows the results of a typical experiment on Pond C-1. $C\mu t/c$ was plotted against the concentration (A) of labeled substrate added to a water sample, where

C = disintegrations per minute (dpm/μl) of the standard substrate solution
μ = μl of substrate solution added
t = incubation time, in hours
c = dpm of organisms trapped on filters + dpm of CO_2 evolved

Wright and Hobbie have developed the calculations to indicate that, when the plot is linear, the intercept of the regression line with the ordinate is the turnover time (T), in hours, of the natural substrate in the original water sample. The slope of the line is 1/V, where V is the maximum uptake velocity of active transport attainable by the microbial enzyme system. The intercept of the regression line with the abscissa is -(K + S), where K represents the transport constant for the active-transport system. The smaller is K, the more effective is uptake at low substrate concentrations. S is the natural substrate concentration originally present in the water. This technique cannot be used to separate K and S.

RESULTS AND DISCUSSION

Diurnal Experiments

On two occasions experiments were conducted to test for possible diurnal patterns in the parameters K + S, T, and V. Water samples were collected from the ponds at approximate intervals of three hours, and replicate samples were incubated with 4 concentrations of added glucose for $\frac{1}{4}$ hour. This series was continued for 24 hours to get a complete daily pattern. On 16-17 June 1970 experiments were conducted on ponds P-1 and C-1. On 23-24 July 1970 experiments were conducted on C-1 only. Incubation temperature was varied through the course of the experiments to match the temperature in the ponds at the time of collection.

Figure 2 shows the results for pond C-1 on 16-17 June and suggests a diurnal pattern for K + S and T, with their values showing about a 2-fold difference for different times of day. The low values occurred during the night, and the high values tended to occur during the daylight hours. Until noon of the first morning K + S was above 50 ug/l but started dropping by 1600 hours and reached its minimum value of 18.6 μg/l at 1900 hours, or shortly before dark. It remained at 36 μg/l or less through the night but had risen to 60 μg/l at 0800 hours on the second morning. Turnover time, T, was 10 hours or greater during most of the daylight hours of the first day but also dropped to its lowest value (less than 4 hours) at 1900 hours. T then showed a gradual increase through the night and was again greater than 10 hours at 0800 hours on the second morning. V varied somewhat but did not show the 2-fold differences apparent in the other 2 parameters. The lowest V (3.57 μg/l/hr) occurred at 1600 hours on the afternoon of the first day, and the highest value (6.11 μg/l/hr) occurred at 0130 hours during the dark hours.

Figure 3 shows the results for pond P-1 on 16-17 June 1970. Again there were almost 2-fold differences in K + S (range: 13.9 - 27.6 µg/l) and in T (range: .73 - 1.16 hours), but these differences do not suggest any particular diurnal pattern. The greatest changes occurred during the 3 hours between adjacent sampling periods. V also varied by almost 2-fold at different times of day (range: 15.7 - 29.4 µg/l/hr), unlike the situation in pond C-1. Changes in V also do not appear to be correlated with time of day.

The diurnal experiment was repeated in pond C-1 on 23-24 July 1970, and the results are shown in Figure 4. There were 3-fold differences in K + S, with the lowest value (14.9 µg/l) coming at 2100 hours and the highest value (56.3 µg/l) coming at 0600 hours on the second morning. There were again 2-fold differences in T, with the lowest value (3.6 hours) at 2100 hours and the highest value (11.5 hours) at 0600 hours on the second morning. V varied by almost 2-fold (range: 3.21 - 6.26 µg/l/hr), but again there is no suggestion that it varied regularly with time of day. There was intermittent rainfall throughout the course of this second diurnal experiment on C-1, although the salinity at the sampling depth (40 cm) appeared to hold steady at about 19 ppt (as measured by a refractometer).

From the 3 figures discussed above it appears that K + S and T vary together on a diurnal basis. There is a very strong correlation between these 2 parameters for all samples except those taken from P-1 during the daylight hours of 16 June. Changes in V do not tend to be as strongly correlated with changes in the other parameters.

Turnover times for the natural substrate were always shorter (by a factor of 4 or more) in P-1 than in C-1. K + S was also generally lower in P-1, but V was higher (usually by a factor of 3 or more).

Figure 5 shows percentage respired (mean of all levels of glucose addition for a given water sample) plotted as a function of the time of day. It appears that % respiration was fairly constant for both dates in pond C-1 and tended to be somewhat higher in the morning hours for the single experiment with P-1 water.

Time Series

In work involving uptake of radioactive isotopes it is important to determine whether the uptake is linear with time. This was checked by collecting water from C-1 and P-1 and incubating with 2 concentrations of added radioactive glucose (29 and 300 µg/l) for various time intervals ranging from 5 to 90 minutes. The results are shown in Figures 6 through 8. The incubation temperature was 25° C.

Figure 6 shows that uptake in C-1 water (plotted as disintegrations per minute vs time) was linear for all time intervals up to 90 minutes for both concentrations of added glucose. Figure 6 shows that uptake in P-1 water was linear up to 90 minutes for a glucose concentration of 300 µg/l but was linear only up to 60 minutes for a glucose concentration of 29 µg/l.

Figure 7 shows that in C-1 water only 5% of the total activity added to the samples was taken up after 90 minutes for a glucose addition of 300 µg/l, and a plot of % uptake vs time was linear. For an added glucose concentration of 29 µg/l the plot of % uptake vs time was also linear, but 30% of the total added activity had been taken up by the cells after 90 minutes. In P-1 water (Figure 7) the plot of % uptake vs time was linear for a glucose addition of 300 µg/l, with almost 20% of the total label being taken up after 90 minutes. For the glucose addition of 29 µg/l, however, the plot of % uptake vs time was linear only up to 1 hour, with a leveling off at longer time intervals. This agrees with the plot of DPM vs time (Figure 6). After 1 hour 50% of the total label had been taken up by the cells, so it is perhaps not surprising that the uptake rate then declined.

Figure 8 shows the percentage of the total glucose uptake that was respired by the cells as a function of incubation time for C-1 and P-1 water. The glucose respired and released as CO_2 was between 15 and 20% of the total amount taken up for water from both ponds for both concentrations of added glucose and for all time intervals. It appears that the length of the incubation time has no effect on the apparent respiration for time intervals up to 90 minutes.

Effects of Incubation Time on Uptake Parameters

The decision on how long samples should be incubated with labeled glucose is primarily one of judgment. Presumably the length of incubation should not affect the essential information provided by this kind of experiment, but this has not been critically examined in the past. The experiments discussed in the preceding section indicate that uptake of the radioactive label was linear with time up to at least 1 hour, so it would seem that any incubation times shorter than this would be satisfactory. I conducted a set of experiments to determine the effects of incubation time on uptake parameters. Four concentrations of glucose were added to a series of water samples from both P-1 and C-1 according to the usual procedure. Various sets of samples were incubated for time intervals of .25, .5, .75, and 1 hour, and the samples were processed as usual. The incubation temperature was 25° for all samples.

Figure 9 shows the resulting plots of Cut/c vs A for C-1 water. Table 1 shows the various uptake parameters for the different time intervals. These parameters did not hold constant with different incubation times. The greatest calculated turnover time, T, was 80% greater than the lowest value. The greatest calculated K + S value was 52% greater than the lowest value. It is interesting that the greatest differences in these parameters were between the two shortest incubation times (.25 and .5 hr). The greatest calculated V value was 34% greater than the lowest value, and the greatest difference here was between the longest (1 hr) and the shortest (.25 hr) incubation times.

Figure 12 shows plots of Cμt/c vs A for P-1 water incubated with glucose for different time intervals. The calculated uptake parameters are shown in Table 2. The differences between the highest and lowest values of T and K + S were more than 2-fold. As with C-1 water, the greatest differences did not come between the longest and shortest incubation times, however. The highest value for V was 42% greater than the lowest value and the greatest difference was between the longest and shortest incubation times. This was similar to the pattern for C-1 with a longer incubation time resulting in a higher calculated V.

A similar experiment was conducted with C-1 water in January, 1970, with incubation temperatures of 9.5° and incubation times of .5, 1, 2, and 4 hours. The water had very little suspended material at that time. Even after 4 hours' incubation, there was little or no uptake of radioactive glucose and no indication that active-transport kinetics existed.

Experiments on Pure Cultures

A series of uptake experiments was conducted on several organisms isolated from the ponds by Dr. James Staley. At the same time uptake experiments were conducted on natural pond water in the usual manner for comparison with the pure cultures. Incubation temperature for all experiments was 28.5° C.

Figure 11 shows the results with a pure culture isolated from P-1 water and designated as "slow-growing isolates". Experiments were conducted with two concentrations of the pure culture and with freshly collected pond water. The K + S value was approximately the same for all 3 experiments. V calculated for the natural pond water (29.3 μg/l/hr) was much greater than for either cell concentration of the pure culture. The turnover time for the natural pond water (1.19 hrs) was also much shorter than for either cell concentration of the pure culture. Apparently, the particular organism isolated was not contributing greatly to the overall glucose turnover in P-1 water.

Figure 12 shows the results of experiments with another pure culture of organisms isolated from P-1 and designated as "fast-growing isolates." In this case experiments with the culture did not show glucose uptake consistent with active-transport kinetics.

Figures 13 and 14 show the results of experiments with C-1 water and both "slow-growing" and "fast-growing" isolates from C-1 water. Pure cultures of both isolates in 2 concentrations failed to exhibit active-transport kinetics.

Sediment Experiments

It is of interest to know whether organisms in the sediments are more active in turning over dissolved organic compounds than are organisms in the water itself. Attempts were made to answer this question. Surface

sediment from the edges of P-1 and C-1 was scraped up with a spatula and placed in a volumetric cylinder. Only the aerobic layer of the sediment was sampled in this way. The sediment was then diluted 100:1 in artificial sea water. Aliquots of the suspension were then pipetted into the incubation flasks where the sediment immediately settled to the bottom of each flask. Glucose additions were then made and the flasks treated in the usual fashion.

Figure 15 shows the results with P-1 sediment on 30 January 1970 with an incubation temperature of 8.5° C. Results of a similar experiment with P-1 water are given for comparison. The calculated turnover time for the 100:1 sediment suspension was 22.3 hrs, indicating that the turnover time for the sediment before dilution would be about .2 hrs. This is faster than the calculated turnover time of 3 hrs for P-1 water. Calculated V for the sediment suspension was 1.58 µg/l/hr, indicating a probable V for undiluted sediment of 158 µg/l/hr. This was far higher than the V of 3.65 µg/l/hr for P-1 water. K + S for the sediment suspension should give a minimum figure for undiluted sediment and was 35.2 µg/l/hr in this experiment, or some 3 times as high as the value (11 µg/l) for P-1 water. This suggests that the sediment should be as active as the water in turning over substrate.

Figure 15 shows the results of a similar experiment for sediment collected from C-1. T for the suspension was 15.9 hrs, indicating a T of about .2 hrs for undiluted sediment. This is very similar to the undiluted sediment figure for P-1. V was 1.59 µg/l/hr (comparable to V for the P-1 sediment suspension), indicating a V for undiluted sediment of about 159 ug/l/hr. K + S for the C-1 sediment suspension was 25.3 µg/l, somewhat lower than K + S for the P-1 sediment. A water sample from C-1 showed no uptake of radioactive glucose at all at this time. Again the implication is that glucose turnover is more active in the sediments than in the water.

These experiments were repeated in July, 1970, with incubation temperatures of 29° C, and uptake experiments were also conducted on sediment suspensions in filtered pond water (P-1 and C-1, respectively) as well as suspensions in artificial seawater. However, the results were inconclusive, with poor agreement between replicates; and there was no indication of active-transport kinetics in the sediment suspensions.

Effects of Ageing

Vaccaro and Jannasch (1967) showed that holding a water sample at room temperature for 24 hours or longer after collection may lead to the appearance of active-transport kinetics in water which originally showed no uptake or erratic uptake. Figure 16 shows results of an ageing experiment which I conducted in January, 1970, with incubation temperatures of 8-9°C. At the time of the initial collection there was little or no uptake of labeled glucose, even after 4 hours. The water was very clear, indicating that very little suspended material was present. After 2 liters of the water had been held for 24 hours with magnetic stirring at room temperature, there was an indication of active-transport kinetics. Such a pattern was

also found after the water had been held for 48, 72, and 96 hours. Table 3 shows the changes that occurred in uptake parameters during the time the water was held in the laboratory. The observed T, K + S, and V steadily decreased with time, with the most drastic decrease occurring in K + S and the least decrease, in T. If the K value of the bacterial population had not changed, it might have been expected that the sum K + S would have reached a stable point and shown no further decrease once S approached 0. The implication is that K was changing and the nature of the bacterial population was changing. The observed decrease in V might be explained merely by an increase in bacterial numbers.

Effects of Enrichment

Vaccaro (1965)tried enriching water samples with various dissolved organic compounds and observing the response of the bacterial populations with regard to various uptake parameters. I tried such an experiment in January, 1970, on water collected from P-1. An initial uptake experiment was run on a water sample and 2 liters of it was then held at room temperature with magnetic stirring and a glucose enrichment of 100 µg/l. After 24 hours suspended bacterial and algal cells were trapped on .45µ membrane filters and resuspended in an equal volume of filtered (.45µ filters) P-1 water. Aliquots of the new suspension were then pipetted into the incubation flasks and given a series of added glucose concentrations as usual.

Results of this experiment are shown in Figure 17. Vmax decreased from 3.65 to 1.80 µg/l/hr after the enrichment; K + S decreased from 11.0 to 7.70 µg/l. T showed an increase from 3.0 to 4.3 hr.

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TABLE 1

Uptake parameters in C-1 water for various incubation times, 12 May 1970

Incubation time (Hours)	T (Hours)	K + S (µg/1)	V_{max} (µg/1/hr)
.25	2.21	17.5	7.92
.5	1.23	11.5	9.30
.75	1.50	16.4	8.58
1.0	1.54	16.3	10.6

TABLE 2

Uptake parameters in P-1 water for various incubation times, 13 May 1970

Incubation time (Hours)	T (Hours)	K + S (µg/1)	V_{max} (µg/1/hr)
.25	3.74	49.4	13.2
.5	1.94	26.5	13.7
.75	1.38	21.0	15.2
1.0	1.55	28.9	18.7

TABLE 3

Changes in uptake parameters after C-1 water was held in the laboratory for various time periods.

No. of hours held	T (Hours)	K + S (µg/1)	V_{max} (µg/1/hr)
0	No uptake of radioisotope at initial collection time		
24	6.9	171	24.8
48	6.6	71.1	10.8
72	5.0	34.4	6.85
96	2.2	5.44	2.53

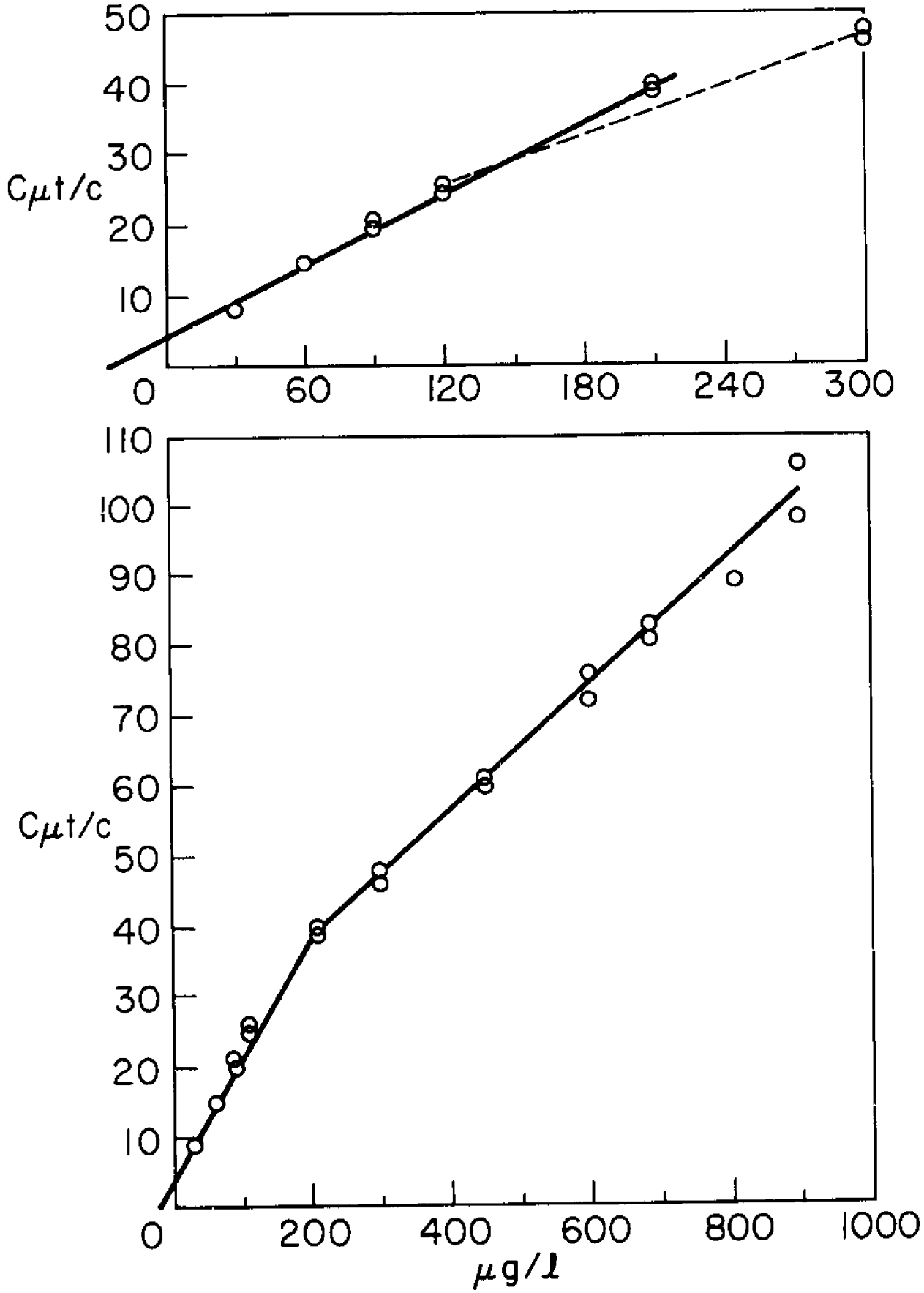

Fig. 1. Glucose uptake as a function of added substrate in C-1, 7 March 1969.

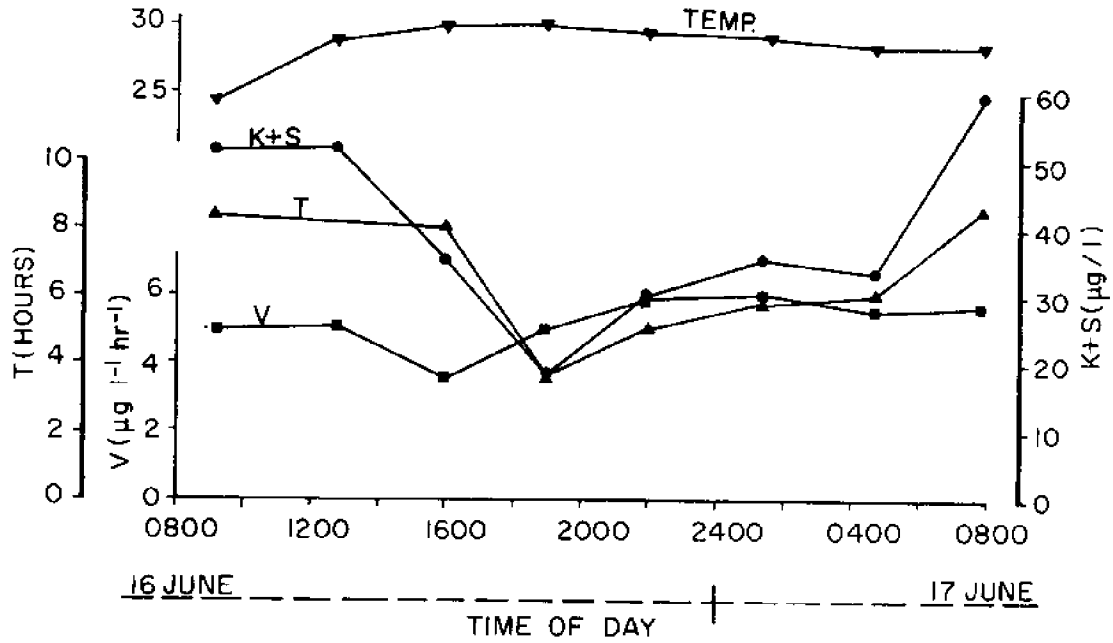

Fig. 2. Diurnal variation of uptake parameters in C-1, 16-17 June 1970.

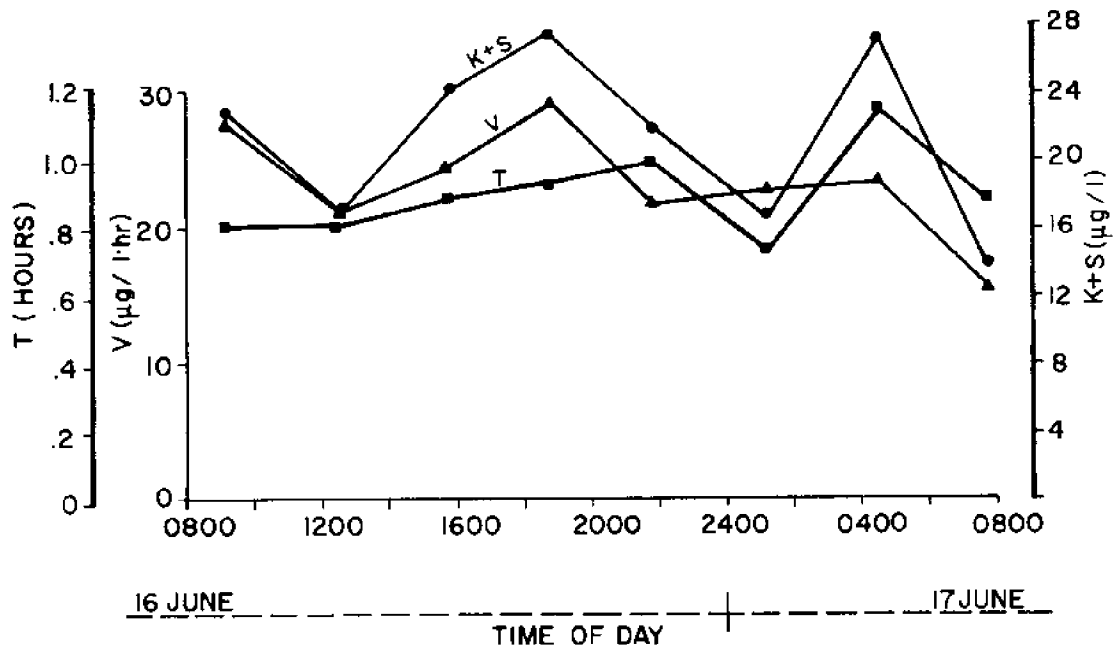

Fig. 3. Diurnal variation of uptake parameters in P-1, 16-17 June 1970.

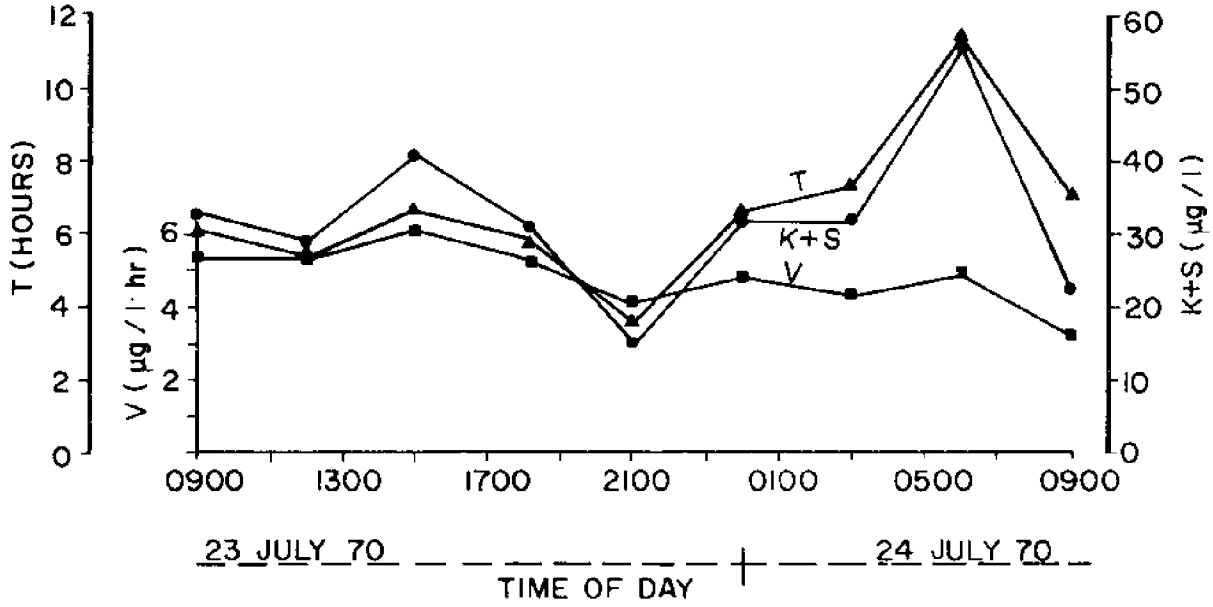

Fig. 4. Diurnal variation of uptake parameters in C-1, 23-24 July 1970.

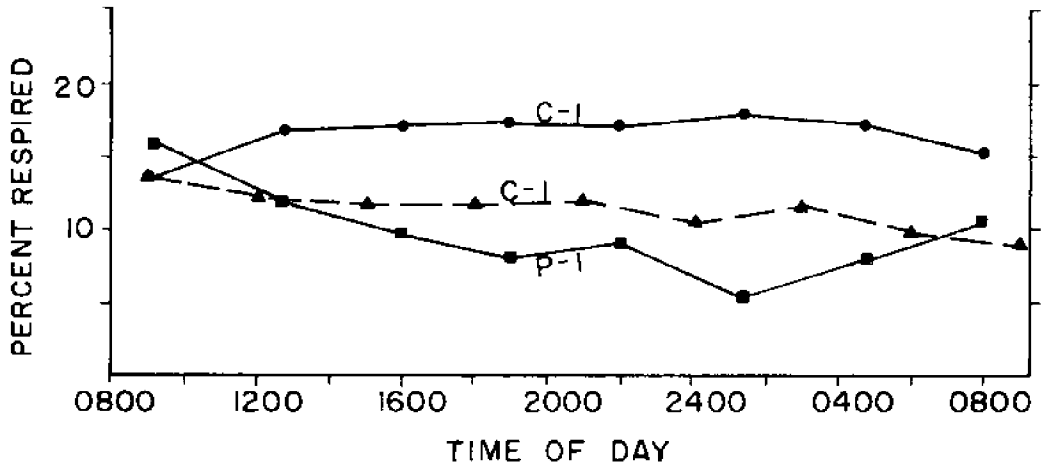

Fig. 5. Diurnal pattern of % respiration; % R determined as CO_2 evolution divided by total glucose uptake. Each point represents the mean % R for all concentrations of added glucose at a given time. Solid lines, 16-17 June 1970. Broken line, 23-24 July 1970.

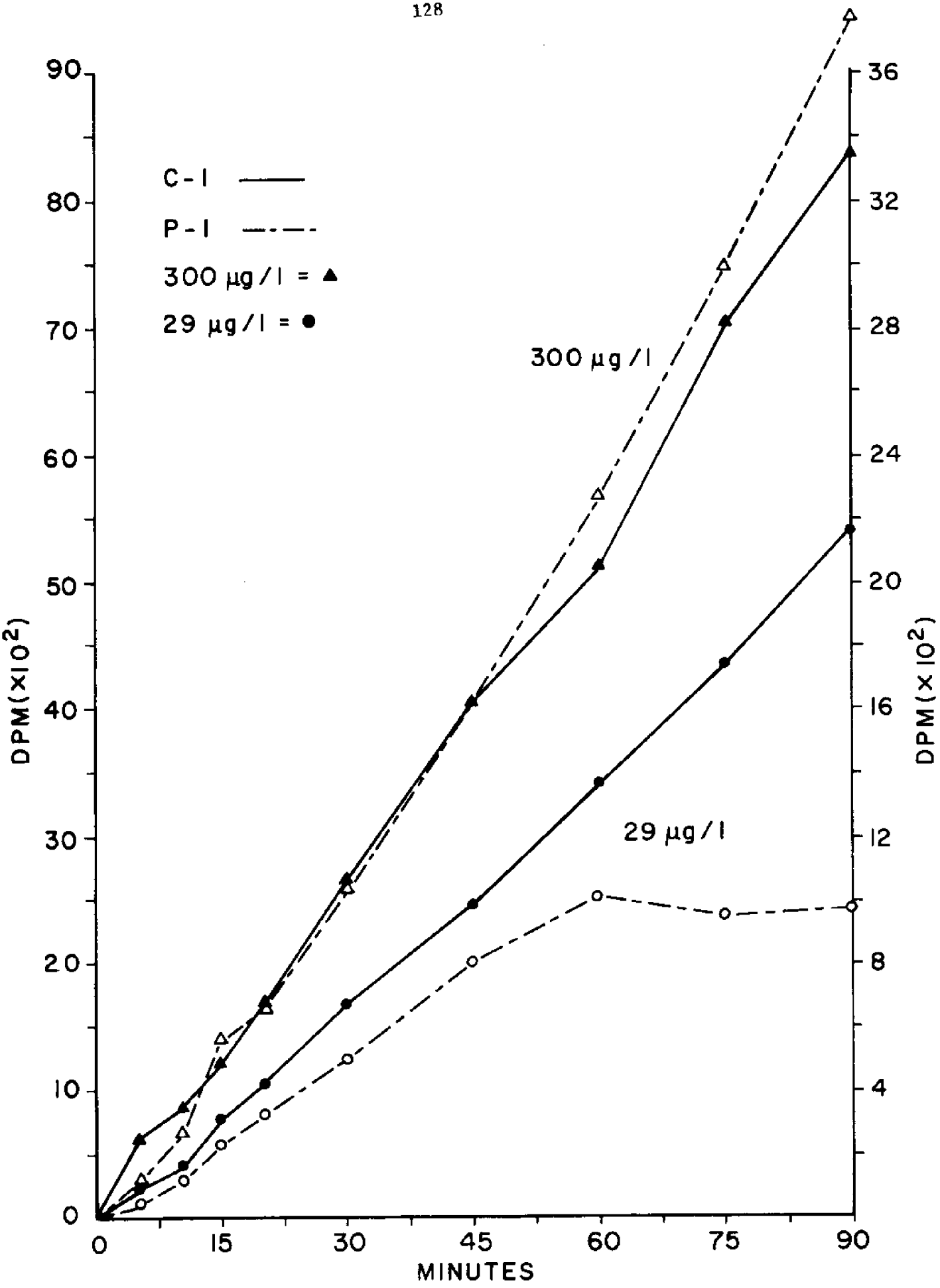

Fig. 6. Radioactive glucose uptake in C-1 and P-1 as a function of incubation time, 11 May 1970. Incubation temperature = 25°C.

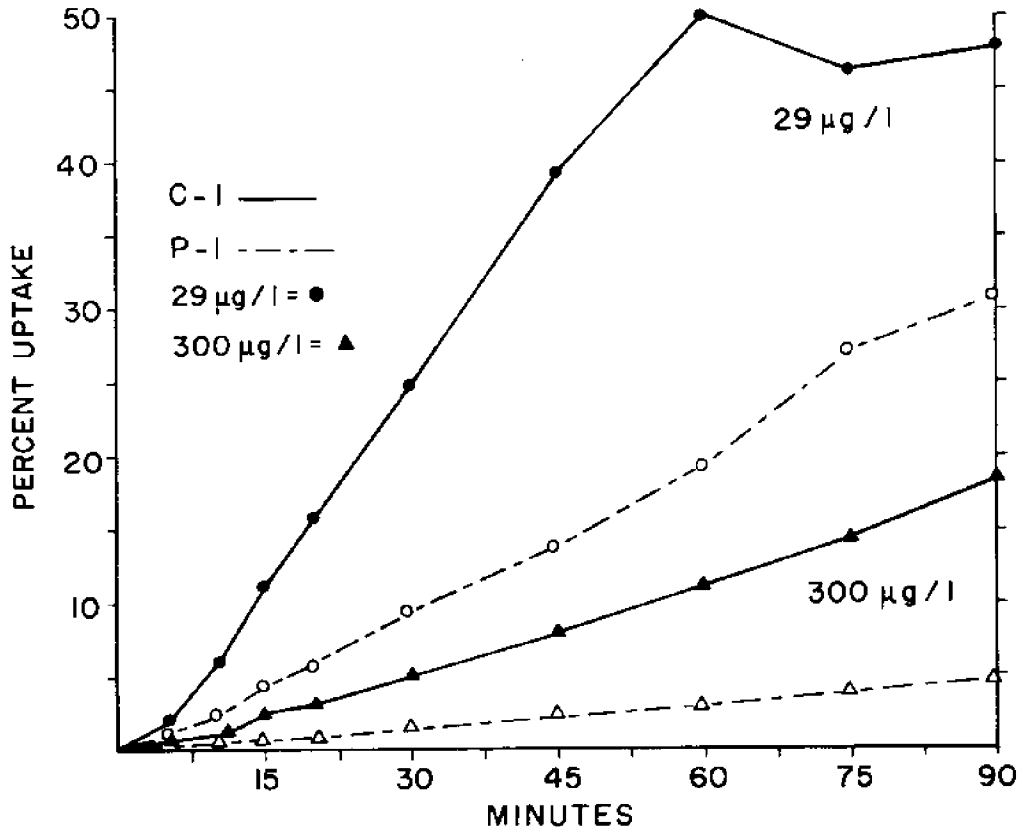

Fig. 7. Uptake of labeled glucose as percentage of total amount added to C-1 and P-1 waters, 11 May 1970: 2 concentrations of ^{14}C-glucose originally added to water samples, as indicated.

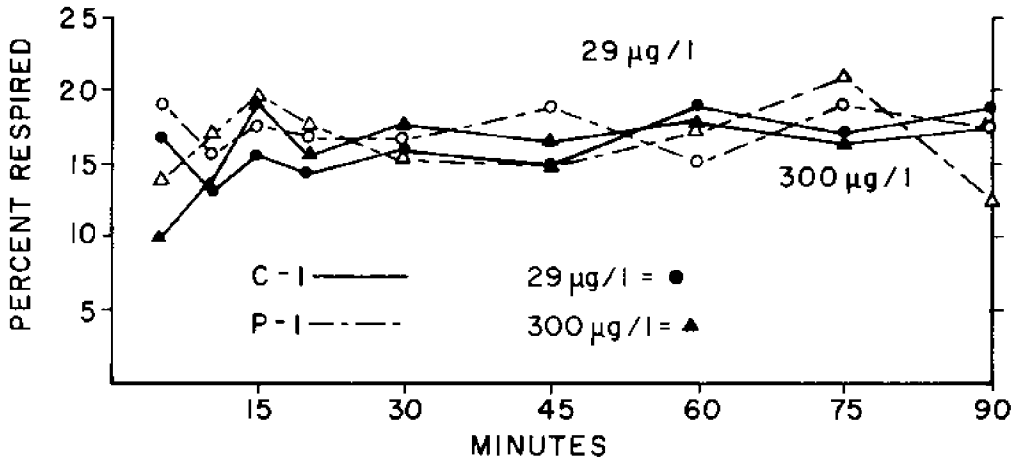

Fig. 8. Percent respiration as a function of incubation time, calculated as CO_2 evolved divided by total radioactivity taken up, for 2 concentrations of added glucose.

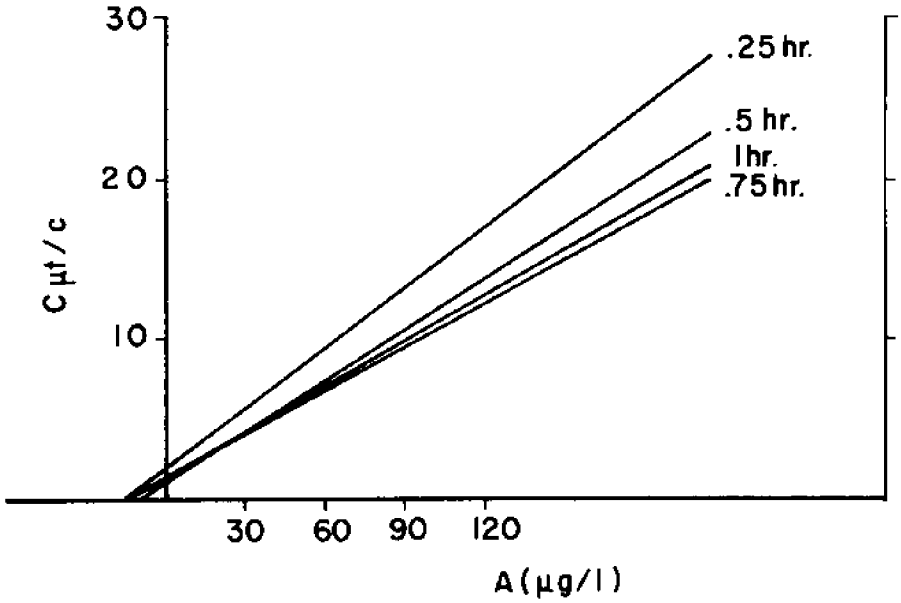

Fig. 9. Calculated linear regression lines for glucose uptake in C-1 water for different incubation times, 12 May 1970. Incubation temp. = 25°C.

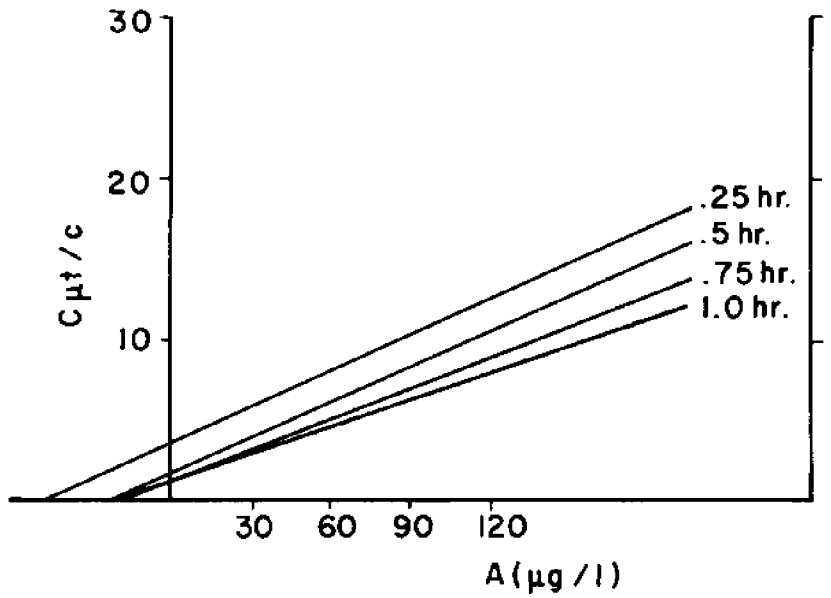

Fig. 10. Calculated linear regression lines for glucose uptake in P-1 water for different incubation times, 13 May 1970. Incubation temp. = 25°C.

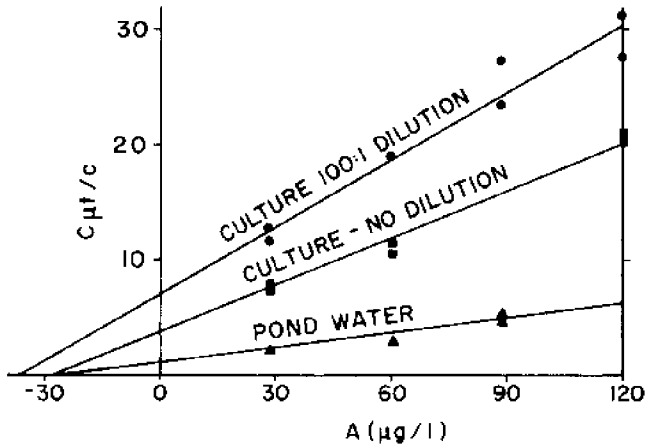

Fig. 11. Uptake experiments with P-1 water and "slow-growing isolates," 27 June 1970. Points and linear regression lines.

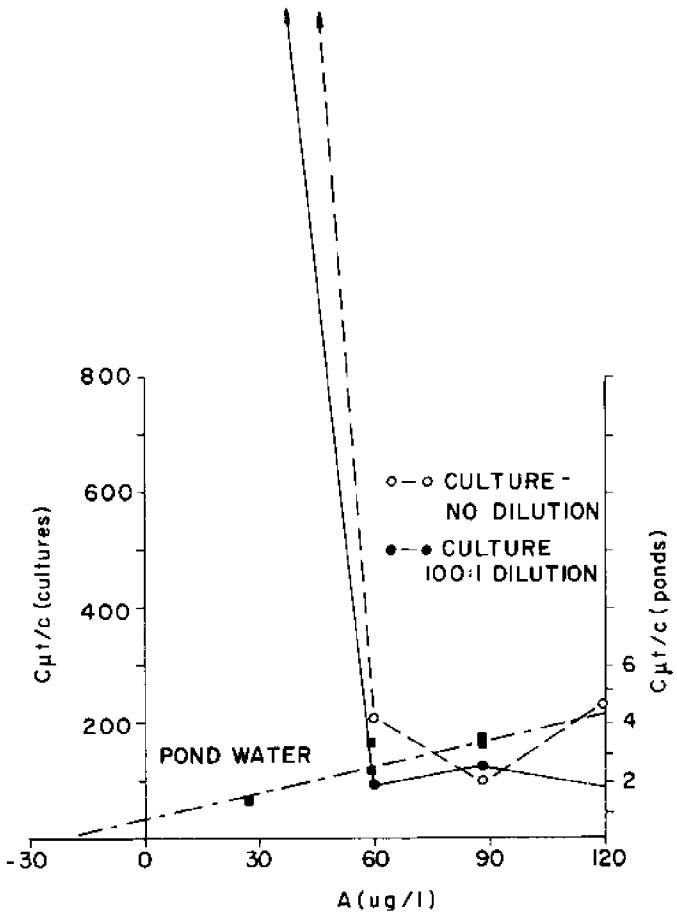

Fig. 12. Uptake experiments with P-1 water and "fast-growing isolates," 26 June 1970.

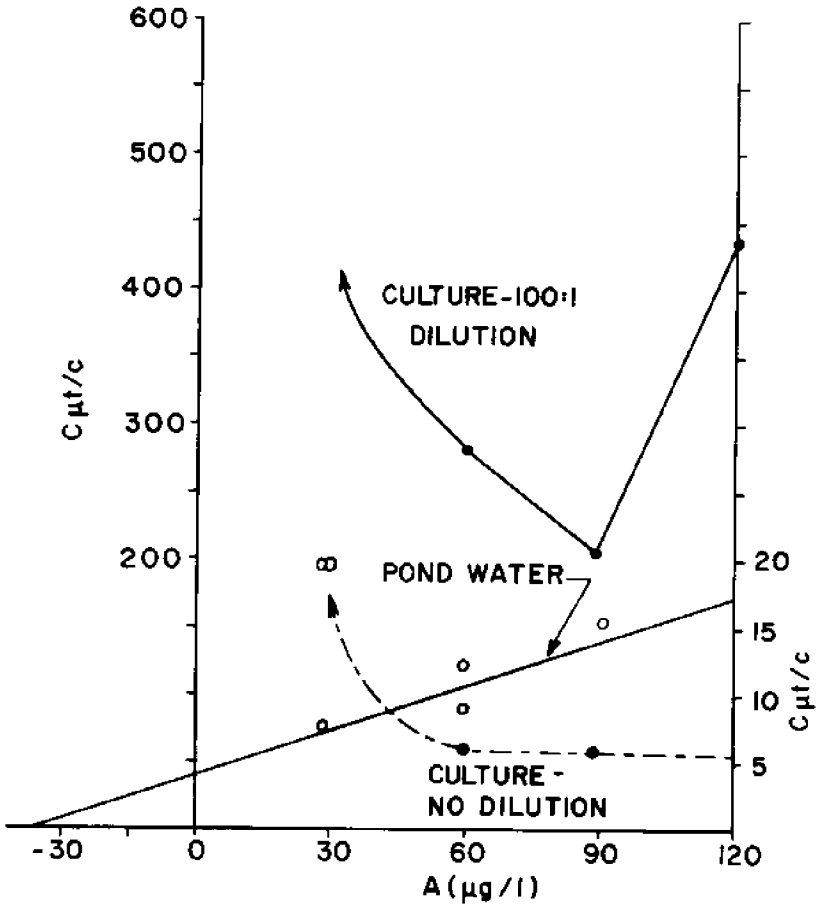

Fig. 13. Uptake experiments with C-1 water and "fast-growing isolates," 26 June 1970.

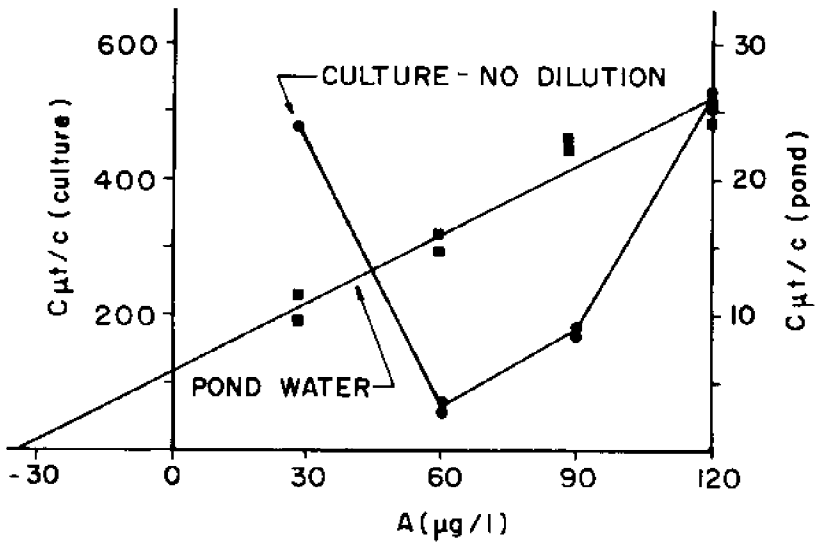

Fig. 14. Uptake experiments with C-1 water and "slow-growing isolates," 28 June 1970.

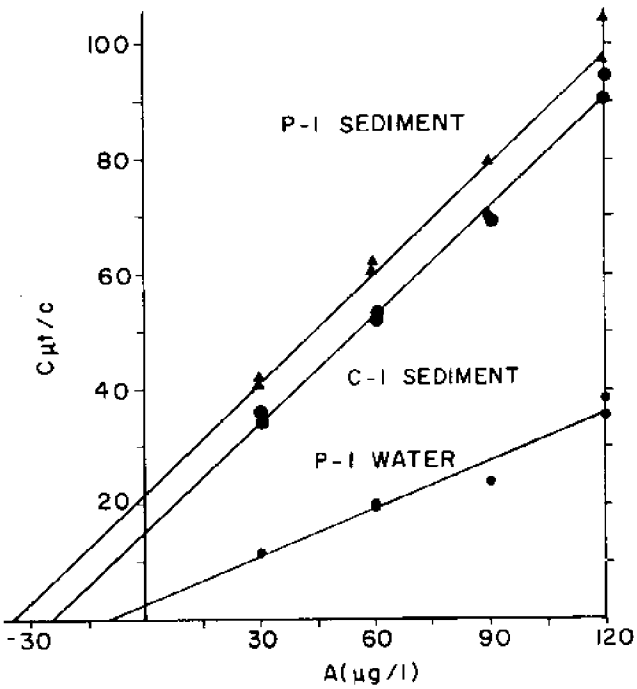

Fig. 15. Glucose uptake in sediment suspensions from C-1 and P-1 and in P-1 water, 30 January 1970. No uptake was observed in C-1 water at this time. Incubation temperature = 8.5°C.

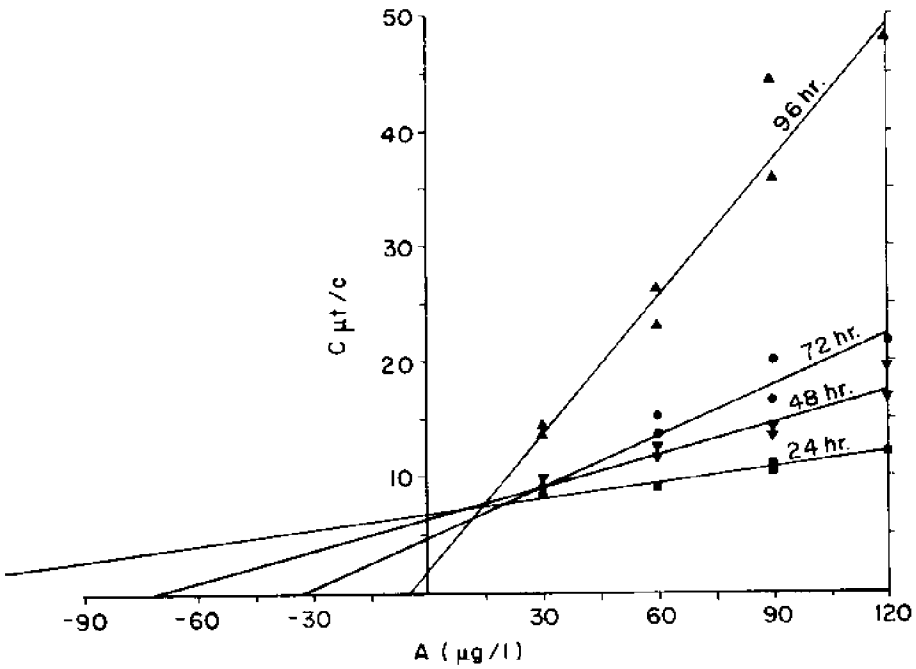

Fig. 16. Uptake experiments with C-1 water after holding at room temperature for various time intervals after collection. No uptake was observed at the time of collection. Incubation temperature for different experiments varied between 8 and 9°C but was constant for any given experiment.

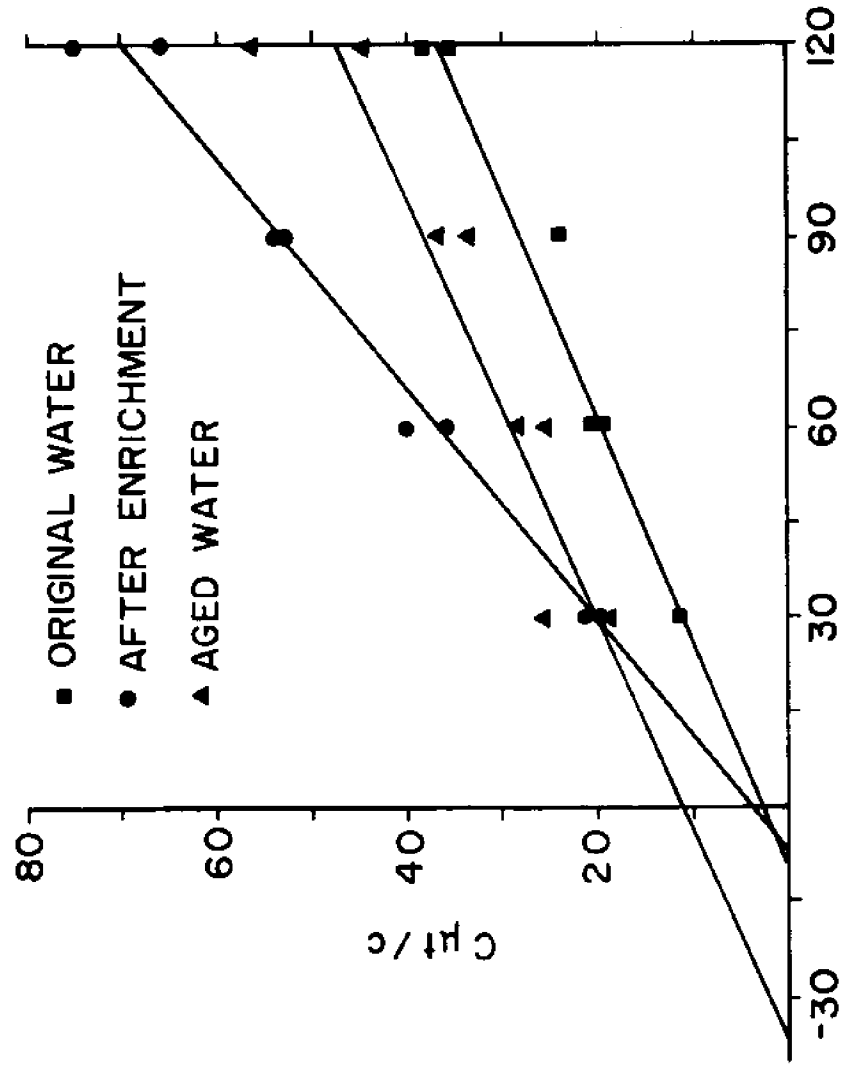

Fig. 17. Uptake experiments in P-1 water after enrichment and in original water, 27-28 January 1970. Incubation temp. = 8°C. Circles indicate resuspension of organisms in original P-1 water (filtered) after holding for 24 hours with glucose enrichment. Triangles indicate resuspension in aged filtered seawater.

PHYTOPLANKTON POPULATIONS IN BRACKISH WATER PONDS

by Peter M. Campbell*

INTRODUCTION

This report represents a continuation through the year 1970 of the 18 month study begun by Dr. Edward J. Kuenzler on effects of sewage plant effluent on phytoplankton populations in self-designing brackish water ecosystems. (21.) The ecosystems involved are six man-made ponds, each containing roughly 200 m^3 of water and with a mean depth around 0.4 m, located in Morehead City, N.C. Three of these ponds receive treated sewage from the Morehead City sewage treatment plant and estuarine water from adjacent Calico Creek and are designated polluted (P) ponds, while three other ponds at the Institute of Marine Sciences receive fresh water and estuarine water from adjacent Bogue Sound and are designated control (C) ponds.

Fresh and saline water inflow to the ponds is regulated to maintain brackish conditions throughout the year and to avoid salinity stresses. Seed populations from the local phytoplankton become introduced into the ponds with the pumped-in water, where because they are then no longer subject to the continual flow and mixing of different waters characteristic of the adjacent estuaries, the adaptable phytoplankton species are better able to develop toward climax communities during each season. The response of these phytoplankton populations to presence or absence of sewage plant effluent in the ponds, measured in this study by species composition, distribution and cell concentrations through the year, is not only an important problem in itself, but such detailed information on the phytoplankton community is also of interest to those working on the productivity and nutrient cycling phases of this project.

METHODS

At the end of each month, samples were collected from all ponds for study, a total of 36 C-pond and 36 P-pond samples for the year. Each of these was an integrated sample obtained by combining pond water collected at depths of 0.7 m, 0.4 m, and 0.1 m. All samples were examined within 5-6 hours of collection in the following manner: A measured portion from each well-shaken integrated sample, normally 10 ml, was placed in a 15 ml conical centrifuge tube and run at full speed in a clinical centrifuge for 10 minutes, after which all but a few drops of the centrifugate was carefully drawn off by pipette. The phytoplankton cells were then resuspended in the remaining water, about .05 ml, and these last drops were then transferred by pipette

*Under supervision of Dr. E. J. Kuenzler

to a clean slide. The drop was stirred, mixed and spread out on the slide to disperse the phytoplankton somewhat before placing a 22 mm^2 coverslip on top. With the right size drop, the sample then always spread just to the edge of the coverslip. This preparation was then examined live under an A. O. Spencer compound microscope with phase-contrast, surveying the slide at 200 X for large species, counting medium-sized species in selected transects across the slide at 450 X, and using oil-immersion at 1000 X for counting small species in selected transects and for careful identification and measurement of all species, using phase contrast for examination of diatom frustules. From knowing the number of fields-of-view across the coverslip for each objective, the number of transects counted, and the original volume of the sample concentrated under the coverslip, the number of cells/ml in the original sample was calculated. When heavy bloom concentrations provided too many cells in a field of view for comfortable counting, smaller volumes of the sample were then centrifuged, or counts were made directly from a buretted .02 ml volume of sample. An 8-gang mechanical counter was an invaluable aid in counting dominants in the samples. All phytoplankton measurements, free-hand drawings, and other taxonomic data were made on 3 x 5 cards for quick reference.

The traditional method of plankton counting centers around preserving the samples, settling them out in special chambers, and observing them with an inverted microscope. But because most phytoflagellates do not preserve well enough to be identifiable with any degree of certainty, live plankton examination becomes an absolute necessity when species other than diatoms and armored dinoflagellates are to be seriously studied. The problem, in the absence of successful preservatives, has been to develop methods which enable both live observation and enumeration at the same time. Methods utilizing special known-volume counting chambers such as haemocytometers, Sedgewick-Rafter cells or Palmer cells allow for accurate counts, but have the disadvantage of too great a chamber depth for critical use of Oil-immersion lenses in identification of small species.

The method developed in this study, involving taking a known aliquot volume and concentrating it into a drop spread out by a coverslip to its edges, enables both a reasonably accurate enumeration of cells per unit volume plus identification of these same cells with oil-immersion lenses, and takes advantage of the inevitable drying out of the slide to bring details of cells more sharply into focus and to gradually slow and immobilize motile flagellates for fine observation after their free-swimming normal character has been noted, thus enabling more confident identifications. By centrifuging two equal volumes of the same sample at a time, a second

is readily available should the first dry out too much before counting is completed. This method requires no special inverted microscope, and there is no problem with cleaning and preparing special counting chambers since simple microscope slides and coverslips are all one needs.

RESULTS AND DISCUSSION

Salinities of C-pond samples varied from 16.0 to 21.2 ‰ with the yearly average at 18.2 ‰, while salinities of P-pond samples ranged from 13.5 to 20.0 ‰ with the yearly average at 17.0 ‰. Temperatures in C-pond samples ranged from a January low of 5°C. to an August high of 31°C., and in P-pond samples from a January low of 6°C. to an August high of 33°C.

The yearly average for total phytoplankton abundance in the C-ponds was 3.0×10^4 cells/ml, while for the P-ponds it was 1.8×10^6 cells/ml. The seasonal distribution of this total phytoplankton abundance is presented in fig. 1, where it may be seen that in all seasons but spring the P-ponds contained 10 to 100 times the cell concentrations of the C-ponds. The development of these plankton concentrations, as high as 10^7 cells/ml in the P-ponds, was made possible by the combination of organic nutrient inflow from the sewage plant and the low flushing rates of the ponds, between 1/2 and 2 times a month, quite a difference from the adjacent estuaries subject to two tidal exchanges per day.

The ponds contained over 151 species of phytoplankton representing nine classes of algae, with a diversity from 15 to 38 (average of 25) species per sample occurring in C-ponds and from 12 to 35 (average of 21) species per sample in P-ponds. The systematic account of the species, with 99 figures, is presented at the end of this report.

Control Ponds

Those species which in any C-pond sample attained concentrations of 10^3 cells/ml or more are presented in table 1. The seasonal distribution of these eight dominants is shown in figure 2.

Many differences in response appear between the dominant phytoplankton of the C-ponds in 1970 compared to 1969, which suggests that the ponds are continuing to mature. Monallantus stichococcoides (Kuenzler's "small forms") continued its bloom concentrations from the fall of 1969 in ponds C-2 and C-3, dying off at the end of spring, but failed to return in the autumn of 1970 with any of the previous year's strength. In the summer bloom of 1969, tiny Nannochloris atomus achieved 10^5 cells/ml concentrations

in all C-ponds, but in 1970 it bloomed spottily in the spring and autumn, with only one spring sample reaching the previous year's peak densities. In 1970 the small pillbox cells of Cyclotella caspia showed scattered C-pond presence, and achieved a 10^4 cells/ml summer density only in C-1, while the previous year it did this only in C-3 and was abundant in each season in C-2. Cosmopolitan Nitzschia closterium, an autumn and winter species from 1969, also made a strong appearance in the summer in 1970 but did not reach the previous year's autumn peak of 10^3 cells/ml. The only rather close similarity in seasonal distribution was between Nitzschia frustulum (perhaps misidentified?) in 1969 and Nitzschia proxima in 1970, both abundant in summer and early autumn from C-1 and C-2.

The tiny blue-green bean-shaped motile cells of Hemiselmis virescens, unreported from 1969, were abundant in spring and summer from all C-ponds. But even here, as with the other dominants, the difference in seasonal presence and abundance in response to the three C-ponds, originally designed to provide for replication, suggests again, as Kuenzler noted the previous year, that there are large differences among the ponds. (Fig. 2) Ochromonas ? vallesiaca occurred in the summer in only C-1 and C-2, while Ochromonas ? minuscula achieved a winter bloom only in C-3, the only pond in which the spring bloom of Monochrysis lutheri also occurred. Fig. 1 hinted at such differences by revealing C-3 and C-2's greater cell concentrations in the first half of the year, and C-1's build-up in the latter half of the year while C-3 dropped to the levels C-1 had begun with.

Polluted Ponds

The nutrient richness of the P-ponds enabled twice as many species to achieve concentrations of at least 10^3 cells/ml as in the C-ponds. These dominants are presented in table 2, and their seasonal distribution is shown in figure 3.

From December 1969 to early May 1970 all P-ponds were pea-soup green with dense 10^6 cells/ml concentrations of the small xanthophyte Monodus guttula. On May 11th all the blooms suddenly crashed, the ponds turned gray, and the ensuing decomposition killed many fish and other organisms unable to tolerate the depleted oxygen levels. In August and September the Monodus began appearing in the plankton again and by December all ponds had returned to pea-soup bloom concentrations again.

After the spring crash the ponds rapidly recovered with a number of phytoplankton species vying for dominance through

the summer and autumn, as shown in figure 3 where at the same time some differences among the P-ponds are also suggested. Goniochloris pulchra, a xanthophyte with 10 times the cell volume of Monodus, as shown in table 3, assumed dominance in late spring in all P-ponds but dropped off as summer progressed. Oocystis parva also appeared in abundance at this time and continued through the summer and autumn, though not in the concentrations acheived the previous year. The small prasinophyte Pyramimonas plurioculata made a strong bid for dominance in all P-ponds in the summer, as did the centric diatoms Cyclotella striata var. ambigua and Coscinodiscus sublineatus, and the small flagellate Chroomonas amphioxeia. Nitzschia closterium's fluctuating abundance did not achieve the concentrations of the previous year, but did extend through summer and autumn in all P-ponds as it also did in the C-ponds. Tiny Nannochloris atomus appeared in spotty blooms from late spring to early autumn as it did in the C-ponds. Hemiselmis virescens and the minute diatom Navicula arvensis were weak in P-2 compared to the strong summer-early autumn abundance in the other two ponds. The small solitary centric diatom Chaetoceros muelleri appeared after the Monodus crash and then continued in all ponds through to autumn with stronger concentrations than had been achieved in the previous year. In P-2 an autumn bloom of 10^5 cells/ml colored the normally green water brownish. In P-2 and P-3 this Chaetoceros bloom was followed in November by 10^4 cells/ml concentrations of the cosmopolitan dinoflagellate Prorocentrum minimum. The importance of this biomass increase is indicated in table 3 where one sees that the protoplasmic volume of these cells is almost 60 times that for cells of Monodus guttula, which would put the Monodus-biomass-equivilant of the November Prorocentrum bloom in P-2 higher than the biomass achieved by Monodus the following month. (Fig. 3) Prorocentrum minimum is unusual in its size compared with the other dominants in table 3, all small cells with proportionately large surface areas that enable more rapid metabolism and growth when advantageous conditions appear in the rapidly fluctuating estuarine environment.

Incidentally, it was noted from observations on zooplankton grazing during the counts that the smaller species of phytoplankton appeared to become food sources for ciliates and rotifers, while the larger species seemed to be consumed by small crustacea, though this is based on few observations. It would be of interest to know what effect changes in the available food particle size has on the herbivore populations in the ponds.

Indicator Species

Those species of phytoplankton which were present in a number of C-pond samples, but found in only a few or no P-pond samples are indicated in table 1 as being potential

indicator species for unpolluted brackish waters in this area, while those species with high frequency in P-pond but low frequency in C-pond samples are indicated in table 2 as being potential indicator species of the presence of organic wastes in the estuaries. This is at present a tentative conclusion, being based on only one year's data from a special environment. There are of course other factors to be considered, such as the possible difference in seed sources, since the C-ponds are supplied plankton from broad shallow Bogue Sound, while the P-ponds receive plankton input from both the sewage plant and small marsh-bordered Calico Creek. The size of the seed population is probably an important factor for many of these species in determining whether or at what time a bloom develops in one pond compared to another, and could account for some of the variation apparent between ponds. It would be interesting to observe the effect on these possible indicator species of transferring concentrations of P-pond phytoplankton to one of the C-ponds, and vice versa, at regular intervals throughout the year.

Class Distribution

Figure 4 presents the seasonal distribution of the major taxonomic groups of phytoplankton averaged for C-ponds and P-ponds. Though generalizations are difficult to draw from these graphs, they do suggest some basic trends: the centric diatoms, pennate diatoms and dinoflagellates in polluted waters paralleled one another in their appearance in the spring and peaking in autumn; the cryptomonads, prasinophytes and chlorophytes were generally more abundant in the warmer months of the year with greater cell concentrations in polluted waters; haptophytes and chrysophytes were generally limited to unpolluted waters, where pennate diatoms were in steady abundance all year. The xanthophytes, normally a class of minor phytoplanktonic significance, stand out as the most important group in this study, with polluted waters containing pea-soup densities of cells in the colder months of the year and strong concentrations even through the summer, and with an abundance of cells in unpolluted waters all months of the year but June and July. It would be interesting to know whether as xanthophytes these dominant species exert selective pressure on pond food chains over and above the effect of small food particle size or the stress of being in dense concentrations.

It is hoped that the methods described in this paper, and the following systematic account of the species identified from the ponds, along with the associated plates and references, will form a strong basis for encouraging further examinations of phytoplankton populations from Calico Creek, Bogue Sound, and from other polluted and unpolluted estuaries along the Carolina coast.

SYSTEMATIC ACCOUNT OF THE SPECIES

References given (number from literature cited in parentheses) are generally those of value in the identification of the species, rather than older often unobtainable references of historical interest.

BACILLARIOPHYCEAE

1. Melosira moniliformis (Müll.) Ag.; Hustedt, 1930, (17.) Kieselalg. 1, p. 236, f. 98. Single cells 19μ x 10μ.
Present in two C-1 samples, July and November.

2. Melosira sulcata (Ehr.) Kutz.; Hustedt, 1930, (17.) Kieselalg. 1, p. 276, f. 118, 119. Cells 29μ x 13μ, in short filamentous colonies.
Present twice in summer, C and P ponds.

3. Skeletonema costatum (Grev.) Cl.; Hustedt, 1930, (17.) Kieselalg. 1, p. 311, f. 149. Cells 12-21μ x 2-5μ, in short filamentous colonies. Pl. 1, fig. 1.
Present in ten C-pond samples from winter and spring, with 10^2 cells/ml in January and February; one P-pond sample.

4. Cyclotella striata var. ambigua Grun.; Hustedt, 1930, (17.) Kieselalg. 1, p. 345, f. 176d-e. Cells 12-19μ in diameter, striae 7-8/10μ. Pl. 1, fig. 2.
From 22 P-pond samples, May to December, with peaks in summer up to 10^3 cells/ml; one C-pond presence. See fig. 3 for seasonal distribution.

5. Cyclotella caspia Grun.; Hustedt, 1930, (17.) Kieselalg. 1, p. 347, f. 177. Cells 4-5.5μ in diameter, with very fine striae. Pl. 1, fig. 3.
Present in 12 C-pond samples from all seasons, 10^4 cells/ml in August; one P-pond presence. See fig. 2 for seasonal distribution.

6. Coscinodiscus sublineatus Grun.; Hustedt, 1930, (17.) Kieselalg. 1, p. 394, f. 205. Cells 21-33μ in diameter, with 10 areolae/10μ. Pl. 1, fig. 4.
Present in 16 P-pond samples, from April to November, 10^3 concentration in June. See fig. 3 for seasonal distribution.

7. Coscinodiscus rothii (Ehr.) Grun.; Hustedt, 1930, (17.) Kieselalg. 1, p. 400, f. 211. Cells 46-66μ in diameter, 7-8 areolae/10μ.
Present in two P-pond samples, summer.

8. Leptocylindrus danicus Cl.; Hustedt, 1930, (17.) Kieselalg. 1, p. 558, f. 318. Cells 55-105μ x 8-9μ, in short filaments.
Present in four C-pond samples from autumn and winter.

9. Rhizosolenia fragilissima Bergon; Hustedt, 1930, (17.) Kieselalg. 1, p. 571, f. 324. Cell fragments 4.5-6.5μ in width.
Present in two C-2 pond samples, spring and autumn.

10. Rhizosolenia hebetata f. semispina (Hensen) Gran; Hustedt, 1930, (17.) Kieselalg. 1, p. 592, f. 338. Cell 134μ x 2μ with 43μ spines.
Autumn presence in one C-pond sample.

11. Rhizosolenia calcar-avis M. Schultze; Hustedt, 1930, (17.) Kieselalg. 1, p. 592, f. 339. Cell fragments 19μ in width with 38μ spine.
Single winter presence in C-pond.

12. Bacteriastrum delicatulum Cl.; Hustedt, 1930, (17.) Kieselalg. 1, p. 612, f. 353. 21μ diameter valve with terminally bifurcating spines.
Present in one winter C-pond sample.

13. Chaetoceros lorenzianus Grun.; Hustedt, 1930, (17.) Kieselalg. 1, p. 679, f. 385. Cell 13μ x 5μ.
Spring presence in one C-pond sample.

14. Chaetoceros costatus Pav.; Hustedt, 1930, (17.), Kieselalg. 1, p. 699, f. 399. Cells 21μ x 20μ, in short filamentous colony.
Single C-pond presence in winter.

15. Chaetoceros subtilis Cl.; Hustedt, 1930, (17.) Kieselalg. 1, p. 723, f. 413. Cells 10μ x 7μ, in short filamentous colony with 115u spines.
Winter presence in one C-pond.

16. Chaetoceros debilis Cl.; Hustedt, 1930, (17.) Kieselalg. 1, p. 730, f. 580. Cells 5μ x 4μ with 25μ spines, in filamentous colonies of 2-4 cells. Pl. 1, fig. 5.
Present in five C-pond, four P-pond samples from spring, autumn and winter.

17. Chaetoceros muelleri Lemm.; Hustedt, 1930, (17.) Kieselalg. 1, p. 750, f. 439. Cells solitary, normally 4-10μ x 3-9μ; resting spores in May 10μ x 8μ. In P-pond October blooms cells were thinly silicified with faint setae and were smaller in size: 2.5-6u x 2.5-4u. Pl. 1, fig. 6a-c.
Present in 10 C-pond samples from all seasons, up to 10^2 cells/ml, with a December bloom of 10^4 cells/ml in C-3. Abundant in 21 P-ponds from spring to autumn with densities each season reaching 10^4 cells/ml with dominance in October reaching 10^5 cells/ml helping to color the water a reddish-brown. See fig. 3 for seasonal distribution.

18. Triceratium reticulum Ehr.; Hustedt, 1930, (17.) Kieselalg. 1, p. 823, f. 465-486. 43μ cell, 5 areolae/10μ.
Present in one summer C-pond sample.

19. Cerataulina bergoni Per.; Hustedt, 1930, (17.) Kieselalg. 1, p. 869, f. 517. Cells 43-57μ x 7-10μ.
Present in six C-pond samples, autumn and winter.

20. Fragilaria brevistriata Grun.; Hustedt, 1959, (17.) Kieselalg. 2, p. 168, f. 676a-e. Cells 6-13μ x 2.5-3μ, with 14-18 marginal striae/10μ.
Present in two winter C-pond samples.

21. Synedra aff. tabulata (Ag.) Kutz.; Hustedt, 1959, (17.) Kieselalg. 2, p. 218, f. 710b. Cells 43-150μ x 4-4.5μ, but differing from the species in having finer marginal striae, 20-24 striae/10μ. Pl. 1, fig. 8.
Present in seven C-pond and two P-pond samples from all seasons.

22. Asterionella japonica Cl.; Hustedt, 1959, (17.) Kieselalg. 2, p. 254, f. 734. Cells 50-68μ x 7-11μ, single or in colonies. Pl. 1, fig. 9a-b.
Present in six C-pond samples from winter and autumn.

23. Cocconeis cf. placentula var. euglypta (Ehr.) Cl.; Hustedt, 1959, (17.) Kieselalg. 2, p. 349, f. 802e. Only rapheless valves observed: 13-14μ x 7-8μ, 16 striae/10μ.
Present in three C-pond samples from autumn and winter.

24. Achnanthes orientalis Hustedt, 1959, (17.) Kieselalg. 2, p. 390, f. 838. Cells 8-12μ x 4-4.5μ, rapheless valve with 20 striae/10μ, raphe valve with 28 striae/10μ. Pl. 1, fig. 10a-b.
Present in three C-pond and three P-pond samples from summer to winter.

25. Achnanthes clevei Grun.; Hustedt, 1959, (17.) Kieselalg. 2, p. 391, f. 839a. Only the rapheless valves observed, 11-16μ x 6-8μ, with 18 striations/10μ.
Present in one C-pond and one P-pond sample from spring.

26. Mastogloia pumila (Grun.) Cl.; Hustedt, 1959, (17.) Kieselalg. 2, p. 553, f. 983. Cells 25-30μ x 9-10μ, valve marked by a lyrate hyaline area, striae 20-25/10μ, loculiferous rim with two larger central chambers. Pl. 1, fig. 11a-b.
Presence in six C-pond samples, April to June, November.

27. Gyrosigma fasciola (Ehr.) Griff. & Henfr.; Patrick, 1966, (26.) p. 328, pl. 26, f. 4. Cells 94-106μ x 12-15μ, with transapical striae 25-26/10μ, finer than Patrick's description, and the narrow ends set off less sharply from the body. Pl. 1, fig. 12.
Present in 11 C-pond and 9 P-pond samples from all seasons.

28. Gyrosigma balticum (Ehr.) Rabh.; Hustedt, 1930, (16.) Bacill., p. 324, f. 331. Cells 196-200μ x 20-23μ, 14 transapical striae/10μ. Pl. 1, fig. 13.
Present in four P-pond samples from May to July.

29. Gyrosigma simile (Grun.) Boyer; Hustedt, 1955, (19.) p. 34, pl. 10, f. 3. Cell 120μ x 18μ, with 16 transapical striae per 10μ.
Present in one C-pond sample in December.

30. Gyrosigma beaufortianum Hustedt, 1955, (19.) p. 34, pl. 10, f. 7-8. Cells sigmoid, 57-60μ x 6-7μ, with stauroid central nodule.
Present in two C-2 pond samples from November and December.

31. Pleurosigma ? angulatum var. aestuarii (Breb.) V. H.; Stubby sigmoid cells with central sigmoid raphe 75-93μ x 19-21μ, with 16 transapical striae/10μ and 20 diagonal striae per 10μ. Whether the diagonal striae were absent from the ends of the valve was not observed.
Present in four C-pond samples, one P-pond sample from May and June.

32. Pleurosigma salinarum Grun.; Patrick, 1966, (26.) p. 333, pl. 27, f. 2. Valves 77-105μ x 14-18μ, transapical striae 28-30/10μ. Pl. 1, fig. 14.
Present in 11 C-pond samples and 11 P-pond samples, in all seasons. P-pond maximum of 10^2 cells/ml in August.

33. Pleurosigma strigosum W. Sm.; Patrick, 1966, (26.) p. 335, pl. 28, f. 2. Valves 185-360μ x 20-29μ, somewhat narrower than Patrick's description, with 16-18 transapical striae/10μ. Pl. 1, fig. 15.
Present in five C-pond samples only from April to June, but in 13 P-pond samples from May straight through to December.

34. Diploneis smithi (Breb.) V.H.; Hustedt, 1959, (17.) Kieselalg. 2, p. 647, f. 1051. Valves 28-38μ x 10-17μ, with 10 ribs/10μ. Pl. 1, fig. 16.
Present in 28 C-pond samples from all seasons, and from May to December was found in all C-ponds each month. Also present in three autumn P-pond samples.

35. Diploneis smithi var. pumila (Grun.) Hust.; Hustedt, 1959, (17.) Kieselalg. 2, p. 650, f. 1052d-e. Valve 11μ x 7μ, 14 ribs/10μ.
Single C-pond presence in autumn.

36. Diploneis gruendleri (A.S.) V.H.; Hustedt, 1959, (17.) Kieselalg. 2, p. 707, f. 1084. Valve 50μ x 39μ, with 7 ribs/10μ.
Single P-pond presence in summer.

37. Stauroneis salina W. Sm.; Hustedt, 1930, (16.) Bacill., p. 258, f. 414. Valve 45μ x 11μ, 20 striae/10μ.
Single P-pond presence in spring.

38. Navicula subforcipata Hustedt, 1964, (17.) Kieselalg. 2, p. 533, f. 1569. Valves 11-15μ x 7-8μ, with lyrate hyaline area, 18-20 rows of punctae/10μ.
Presence in one C-pond, one P-pond, spring.

39. Navicula pygmaea Kutz.; Hustedt, 1964, (17.) Kieselalg. 2, p. 538, f. 1574. Valves 18-42μ x 8-11 μ, lyrate hyaline area and 32 rows of punctae/10μ. Pl. 1, fig. 17.
Presence in 13 C-ponds from January to June, October to December; presence in three P-ponds.

40. Navicula mutica var. tropica Hust.; Patrick, 1966, (26.) p. 455, pl. 42, f. 4. Valve 26μ x 9μ, with stauroid central area, 20 rows of punctae/10μ.
Single C-pond presence from May.

41. Navicula granulata Bail.; Hustedt, 1955, (19.) p. 75; Hendey, 1951, (12.) p. 49, pl. 12, f. 2. Valves 54-82μ x 26-32μ, with 12 rows of punctae/10μ.
Present in two C-pond samples from summer.

42. Navicula cf. pseudosilicula f. olympica Sovereign; Hustedt, 1966, (17.) p. 786, f. 1762. Valve 41μ x 9μ, with 28 rows of punctae/10μ.
Single C-pond presence from spring.

43. Navicula arvensis Hust.; Patrick, 1966, (26.) p. 483, pl. 46, f. 1-2. Valves 5-8μ x 2-3μ, no striations visible. Pl. 1, fig. 18a-b.
Well presented in 13 P-pond samples in summer and autumn, with densities of 10^4 cells/ml in July and 10^3 in August; one C-pond presence. Large numbers of this species were also found in the estuary adjacent to the P-ponds in the summer. See fig. 3 for seasonal distribution.

44. Navicula cf. muralis f. agrestis (Hust.) Lund, 1946, (22.) p. 83, f. 3A-I. Valves 8-10μ x 3-3.5μ, striae 20/10μ in center, 25/10μ at ends. Pl. 1, fig. 19.
Presence only in C-ponds, 16 samples from January to July, with 10^2 cells/ml in February and March.

45. Navicula aff. friska Carter, 1966, (5.) p. 467, pl. 3, f. 7-10. Valves 10-13μ x 3-4μ, with 24-28 striae/10μ. Pl. 1, fig. 20.
Presence only in P-ponds, 16 samples from all seasons, with densities of 10^3 cells/ml in February and March.

46. Navicula aff. obsoleta Hustedt, 1942, (18.) p. 69, f. 1-16. Valves 8-12μ x 2-3.5μ, 23 striae/10μ.
One P-pond sample in January, one C-pond sample in March.

47. Navicula rogallii Hustedt, 1961, (17.) p. 32, f. 1190. Valves 41-70μ x 4.5-6μ, with 25 striae/10μ. Pl. 1, fig. 21.
Present only in C-ponds, 12 samples from all seasons.

48. Navicula sp. Valves 15-36μ x 5.5-9.5μ, with 25-28 striae/10μ. Pl. 1, fig. 22.
Present in 11 C-pond samples, from January to March, then October to December; present in 9 P-pond samples from October to December.

49. Navicula sp. Valves 19-30μ x 5-7μ, with 16-20 striae per 10μ. Pl. 1, fig. 23.
Present in 13 C-pond samples from spring, summer and winter; also from six summer P-pond samples.

50. Navicula salinarum Grun.; Hustedt, 1955, (19) p. 37, pl. 7, f. 25. Valves 24-36μ x 7-11μ, with 20 striae/10μ. Pl. 1, fig. 24.
Present in four C-pond and two P-pond samples from February to May.

51. Navicula lanceolata (Ag.) Kutz.; Hustedt, 1930, (16.) p. 305, f. 540. Valves 30-40μ x 6.5-8μ, with 14-16 striae/10μ, finely delineate. Pl. 1, fig. 25.
Present in 18 C-pond and 11 P-pond samples from all seasons.

52. Navicula cf abunda Hust.; Hustedt, 1955, (19.) p. 37, pl. 9, f. 10-12. Valves 30-40μ x 7-8μ, narrower than Hustedt's description, with 10-12 delineate striae/10μ.
Present in one C-pond and one P-pond sample from March.

53. Navicula cf. peregrina (Ehr.) Kutz.; Hendey, 1964, (13.) p. 201, pl. 30, f. 12-13. Valves 55-72μ x 12-14μ, half the size range given by Hendey, with 6 delineate striae/10μ in the center, 9/10μ at the ends. Pl. 1, fig. 26.
Present in 6 samples only from pond C-3, all seasons.

54. Navicula yarrensis Grun.; Hustedt, 1955, (19.) p. 32, pl. 9, f. 2. Valves 60-83μ x 13-24μ, with 7-8 ribs/10μ. Pl. 1, fig. 27.
Present in five P-pond samples from May to August, two C-pond samples from November and December.

55. Amphora cf. delicatissima Krasske; Hustedt, 1930, (16.) Bacill., p. 346, f. 635. Single valve 10.5-16μ x 4-6μ, delicate striae 22-25/10μ. Pl. 2, fig. 1a-b.
Presence in six P-pond samples from spring and summer.

56. Amphora tenerrima Ale. & Hust.; Hustedt, 1955, (19.) p. 39, pl. 14, f. 15. Single valve 19μ x 5μ, with 34 striae per 10μ.
Obtained in culture by Wm. Woods from ponds C-2 and P-2.

57. Amphora cf. tumida Hustedt, 1956, (**20.**) p. 120, f. 51-52. Single valves 14-27μ x 4.5-6μ, with 16-20 striae/10μ. Pl. 2, fig. 2.
Present in 25 C-pond samples from all seasons, and 17 P-pond samples from May to December.

58. Amphora granulata Greg.; Hustedt, 1955, (**19.**) p. 40, pl. 14, f. 8-10. Single valves 30-48μ x 8-11μ, with 12 striae per 10μ. Pl. 2, fig. 3.
Present in three C-pond and two P-pond samples from summer.

59. Amphora ovalis var. affinis Grun.; Peragallo, 1908, (**27.**) pl. 44, f. 18. Single valves 51-55μ x 8-12μ, with 12 rows of elongate pores/10μ. Pl. 2, fig. 4.
Present in 15 C-pond samples and 18 P-pond samples, from spring to autumn.

60. Amphora angusta Greg.; Peragallo, 1908, (**27.**) p. 231, pl. 50, f. 37. Single valve 25-64μ x 6-15μ, with 16-20 striae per 10μ. Pl. 2, fig. 5.
Present in 10 C-pond samples from all seasons, one P-pond sample from summer.

61. Amphora angusta var. ventricosa Greg.; Hustedt, 1955, (**19.**) p. 42, pl. 16, f. 6. Single valves 51-81μ x 9-10μ, with 9 striae/10μ. Pl. 2, fig. 6.
Present in six P-pond samples, one C-pond sample, spring to autumn.

62. Amphiprora paludosa var. duplex Donk.; Peragallo, 1908, (**27.**) p. 185, pl. 38, f. 16-19. Cells in girdle view 29-64μ x 23-30μ, with 36 delicate striae/10μ. Pl. 2, fig. 7.
Present in 22 C-pond samples from all seasons, 6 P-pond samples from May to September.

63. Amphiprora paludosa var. hyalina Eulenst.; Peragallo, 1908, (**27.**) p. 185, pl. 38, f. 20. Cells 12-17μ x 7-8μ, strongly twisted, no striations visible but 25 punctae/10μ along the keels. Pl. 2, fig. 8.
Present only in C-ponds, 11 samples from winter to summer, with 10^2 cells/ml concentrations from February to April.

64. Tropidoneis lepidoptera Greg.; Peragallo, 1908, (**27.**) p. 188, pl. 39, f. 3-7. Cells in girdle view 125-136μ x 35μ, with 16 rows of punctae/10μ. Pl. 2, fig. 9.
Present only in P-ponds, 16 samples from May to October, reaching 10^2 cells/ml concentration in September.

65. Tropidoneis pusilla (Greg.) Cl.; Hendey, 1964, (**13.**) p. 256, pl. 27, f. 1-2. Cells in girdle view 55-66μ x 18-27μ, with 18-20 striae/10μ.
Present in one P-pond and two C-pond samples, summer and autumn.

66. Denticula cf. subtilis Grun.; Hustedt, 1955, (19.) p. 43, pl. 9, f. 26. Valve 8μ x 3μ, with 8 costae/10μ.
Single P-pond presence in May.

67. Rhopalodia musculus var. producta Grun.; Peragallo, 1908, (27.) p. 303, pl. 77, f. 23-24. Valves 17-21μ x 7-9μ, with 16 striae/10μ. Pl. 2, fig. 10.
Present in pond C-3 from October to December.

68. Cylindrotheca gracilis (Breb.) Grun.; Hustedt, 1930, (16.) p. 393, f. 746. Cells 88-143μ x 3-4.5μ, with 22 fine keel punctae/10μ.
Presence in two C-ponds in February.

69. Bacillaria paradoxa Gmel.; Hustedt, 1930, (16.) Bacill., p. 396, f. 755. Cells 84-108μ x 4.5μ, with 10 keel punctae/10μ and 30 striae/10μ. Pl. 2, fig. 11.
Presence only in C-ponds, eight samples from winter to summer.

70. Nitzschia compressa (Bail.) Boyer; Wood, 1961, (32.) p. 694, pl. 55, f. 174. Valves 17-34μ x 11-14μ, with 7 rows of areolae/10μ. Pl. 2, fig. 12.
Present in three C-ponds from summer, four P-pond samples from summer and fall.

71. Nitzschia marginulata Grun.; Peragallo, 1908, (27.) p. 270, pl. 70, f. 14-17. Valve 88μ x 13μ, with 12 keel punctae and 24 striae/10μ.
Single winter C-pond sample.

72. Nitzschia apiculata (Greg.) Grun.; Hustedt, 1930, (16.) Bacill., p. 401, f. 765. Valves 37-59μ x 6-6.5μ, with 13 keel punctae and 24 striae/10μ. Pl. 2, fig. 13.
Presence in 19 C-pond samples from all seasons, four P-pond samples from May, November and December.

73. Nitzschia hybrida Grun.; Hustedt, 1930, (16.) Bacill., p. 406, f. 778. Valves 57μ x 8μ, with 12 keel punctae and 20 striae/10μ.
Single winter C-pond presence.

74. Nitzschia hybridaeformis Hustedt, 1955, (19.) p. 44, pl. 15, f. 9-11. Cells 25-36μ x 6-8μ, half the length given by Hustedt, with 10 keel punctae and 30 delicate striae per 10μ. Pl. 2, fig. 14.
Presence in four C-pond samples, from February to June.

75. Nitzschia panduriformis var. minor Grun.; Peragallo, 1908, (27.) p. 269, pl. 70, f. 5. Valves 16-33μ x 5-13μ, with 10 keel punctae and 24 rows of punctae/10μ. Pl. 2, fig. 15.
Presence in five C-pond samples from spring to autumn, one P-pond sample from May.

76. Nitzschia spathulata Breb.; Peragallo, 1908, (27.) p. 284, pl. 73, f. 4. Cells 41-61μ x 6-9μ, with 5 keel punctae/10μ. Pl. 2, fig. 16a-b.
Presence in 11 P-pond samples from August to December, two C-pond samples from November and December.

77. Nitzschia cf. angularis Sm.; Peragallo, 1908, (27.) p. 284, pl. 73, f. 6-7. Cells 52-56μ x 8-9μ, with 4-5 keel punctae/10μ. Pl. 2, fig. 17.
Presence in eight C-pond samples from March to June, and three P-pond samples from May to July.

78. Nitzschia cf. communis var. hyalina Lund, 1946, (22.) p. 104, f. 136K. Valves 10-14μ x 2-3μ, with 16-18 keel punctae/10μ, no striae visible. Pl. 2, fig. 18a-b.
Presence in 16 P-pond samples from spring to winter, with 10^3 cells/ml concentrations from July to September; presence in 9 C-pond samples, January to April and in September. See figs. 2 and 3 for seasonal distribution.

79. Nitzschia cf. laevis Hustedt, 1955, (19.) p. 46, pl. 15, f. 5. Valve 18μ x 5μ, with 16 keel punctae/10μ, no striae visible.
Present in pond C-3 in February and April.

80. Nitzschia proxima Hustedt, 1955, (19.) p. 46, pl. 16, f. 3. Cells 12-35μ x 1.5-3μ, with 10-13 keel punctae/10μ and 25 striae/10μ. Pl. 2, fig. 19.
January to September C-pond presence with densities reaching 10^2 cells/ml each season and a peak of 10^4 cells/ml in August; presence in five P-pond samples from spring to autumn with 10^3 cells/ml in May. See fig. 2 for seasonal distribution.

81. Nitzschia frustulum (Kutz.) Grun.; Hustedt, 1930, (16.) p. 414, f. 795. Valves 25-36μ x 4-5μ, with 11 keel punctae and 22-24 striae/10μ. Pl. 2, fig. 20.
Presence in 24 C-pond samples from all seasons, and three P-pond samples from July and December.

82. Nitzschia grossestriata Hustedt, 1955, (19.) p. 46, pl. 16, f. 8-10. Valves 33-42μ x 5-6μ, with 8 keel punctae and 16 rows of punctae/10μ. Pl. 2, fig. 21.
Four presences in pond C-3, summer and autumn.

83. Nitzschia cf. fonticola Grun.; Hustedt, 1930, (16.) Bacill., p. 415, f. 800. Valves 11μ x 2μ, with 12-16 keel punctae and 24 striae/10μ.
Presence in three C-pond samples from winter.

84. Nitzschia cf. serpenticula Cholnoky, 1968, (7.) p. 79, f. 148. Valves sigmoid, 30-31μ x 2.5-3μ, with 16-18 keel punctae/10μ. Pl. 2, fig. 22.
Presence in 12 C-pond samples from January to May, also July and October; one P-pond presence in August.

85. Nitzschia sigma (Kutz.) Sm.; Hustedt, 1930, (16) Bacill., p. 401, f. 813. Valves 110-335μ x 9-13μ, with 6 keel punctae and 30 striae/10μ. Pl. 2, fig. 23.
Presence in 11 C-pond samples from winter to summer, one P-pond presence in June.

86. Nitzschia sigma var. rigidula Grun.; Peragallo, 1908, (27) p. 291, pl. 74, f. 10-11. Sigmoid valves 48-120μ x 4.5-6.5μ, with 9 keel punctae and 32 striae/10μ. Pl. 2, fig. 24.
Presence in 14 C-pond samples from all seasons, one P-pond presence in December.

87. Nitzschia obtusa var. scalpelliformis Grun.; Hustedt, 1930, (16.) Bacill., p. 422, f. 817d. Valves 57-63μ x 4.5-5.5μ, with 8-9 keel punctae/10μ. Pl. 2, fig. 25.
Presence in pond C-3, April to June and September.

88. Nitzschia longissima (Breb.) Ralfs.; Cupp, 1943, (9.), p. 200, f. 154. Valves 135-225μ x 4.5-7μ, with 12 keel punctae/10μ. Pl. 2, fig. 26.
Presence in 20 C-pond samples from all seasons, three P-pond samples from fall and winter.

89. Nitzschia closterium W. Sm.; Hustedt, 1955, (19.) p. 48, pl. 16, f. 16-18. Cells 35-92μ x 2-6.5μ, with 12-16 keel punctae/10μ. Pl. 2, fig. 27a-c.
Presence in 26 C-pond samples from every month; presence in 24 P-pond samples from every season with peaks of 10^3 cells/ml in summer and 10^4 cells/ml in October. See figs. 2 and 3 for seasonal distribution.

90. Gen.? sp.? Somewhat lunate cells with bluntly rounded ends, containing one or two strap-shaped pale yellow-green plastids, the cell walls surviving treatment for clearing diatom frustules and bearing fine marginal striations, 12/10μ, appearing not to be keel punctae; no raphe present. Cells 34-36μ x 4-5μ. Pl. 2, fig. 28.
Present in three C-pond samples from summer.

CRYPTOPHYCEAE

91. Hemiselmis virescens Droop; Butcher, 1967, (4.) p. 17, pl. 1, f. 8. Bean-shaped cells 4-6μ x 2-4μ with turquoise chromatophore and spherical refractive body but no stigma. Pl. 2, fig. 29a-b.
Present in 21 C-pond samples from all seasons, numbers building from 10^2 cells/ml in late spring to 10^3 cells/ml levels in July and August; present in 21 P-pond samples from May to December, with 10^4 cells/ml levels reached in July and September. See figs. 2 and 3 for seasonal distribution.

92. Chroomonas diplococca Butcher, 1967, (4) p. 26, pl. 1, f. 14. Ovoid cells 7-8μ x 4-6μ, with single parietal turquoise green chromatophore and 1-2 refractive bodies but no pyrenoid or stigma. Pl. 2, fig. 30.
Present in six P-pond samples from spring to fall, two C-pond samples from summer.

93. Chroomonas minuta var. apyrenoidosa (Hulburt); Hulburt, 1965, (15.) p. 90, pl.3, f. 5-8. (Butcher, 1967, places all Rhodomonas species with only two rows of trichocysts in the genus Chroomonas) Cells 6-8μ x 3-5μ, with single dorsal golden brown chromatophore sometimes with a reddish tint, and a spherical refractive body near the apex. Pl. 2, fig. 31a-b.
Present in three P-pond samples in August and September, P-3 in August 10^4 cells/ml; presence in all C-ponds in September, reaching 10^2 cells/ml.

94. Chroomonas amphioxeia (Conr. & Kuff.) Butcher, 1967, (4.) p. 31; Hulburt, 1965, (15.) p. 92, pl. 3, f. 9-12. Cells irregularly oval but variable in shape, two rows of trichocysts, dorsal yellow-brown chromatophore and anterior refractive body, sometimes a pyrenoid-like bulge present in the chromatophore. Pl. 2, fig. 32a-c showing some of the variability in form. Cells 6-11μ x 4-7μ.
Present in four C-pond samples from spring to autumn; presence in 14 P-pond samples from May to October reaching abundances of 10^4 cells/ml in July and August. See fig. 3 for seasonal distribution.

95. Cryptomonas stigmaticum Wislouch; Carter, 1937, (6.) p. 53, pl. 6, f. 38-40. Generally ovoid cells 12-16μ x 6-10μ, golden-brown chromatophore parietal, with ventral red stigma, two starch-ensheathed pyrenoids in the middle of the cell.
Present in two P-pond samples, July and October.

96. Cryptomonas pseudobaltica Butcher, 1967, (4.) p. 44, pl. 6, f. 2. Ovoid cells 13-15μ x 7-9μ, with parietal reddish- to yellowish-brown chromatophore and dorsal pyrenoid with starch sheathe, four rows of trichocysts lining the gulet. Pl. 2, fig. 53.
Present in four P-pond samples from spring to autumn, two C-pond samples from September.

97. Cryptomonas ovata Ehr.; Butcher, 1967, (4.) p. 39. Ovate cells 18-21μ x 10-11μ, starch grains obscuring all internal detail but the two parietal olive green golden chromatophores and the large gullet outlined by many trichocyst rows.
One P-pond presence in November, one C-pond presence in December.

DINOPHYCEAE

98. Prorocentrum minimum (Pav.) Schiller, 1933, (29) p. 32, f. 33. Cells broadly ovoid and compressed, 14-20μ x 11-20μ x 8μ, theca of two valves with striate margins, apical tooth blunt and short, sometimes absent. Yellow-brown chromatophores irregularly lobed, nucleus basal. Pl. 3, fig. 1a-b.

Present in four C-pond samples from winter, abundant in 10 P-pond samples from fall and winter, reaching 10^4 cells/ml densities in November and December. See fig. 3 for seasonal distribution.

99. Exuviaella compressa (Stein) Ostenfeld; Schiller, 1933, (29) p. 17, f. 11. Elliptical cells slightly compressed, 20μ x 16μ, two yellow-brown parietal chromatophores with two central pyrenoids, basal nucleus.

Single winter C-pond presence.

100. Oxyrrhis marina Dujardin; Schiller, 1933, (29) p. 26 f. 255. Cells generally elongate-elliptical with unsymmetrical hypocone, 27μ x 18μ, no chromatophore, granular cytoplasm, nucleus in epicone.

Present in two P-pond samples in April, 10^2 cells/ml.

101. Gymnodinium sp. nov.#1; Orbicular cells with obliquely truncate antapex, 9-20μ x 8-18μ, dorsoventrally compressed; girdle sub-equatorial, wide and shallow, displaced 1/2 girdle width, sulcus on hypocone only, shallow; transverse flagellum encircling cell, longitudinal flagellum 1½ times body length; chromatophores irregularly elliptical, yellow-brown, peripheral, around five in number; spherical nucleus centrally placed; one to several red stigmatic granules adjacent to the sulcus. Pl. 3, fig. 2.

Present in three C-pond samples from spring and fall, and in three P-pond samples from summer and winter.

102. Gymnodinium sp.; Orbicular cells 9-13μ x 7-11μ, sub-equatorial girdle displaced 1/2 girdle width, wide and shallow, sulcus shallow on hypocone, longitudinal flagellum 1½ times body length, chromatophores absent, food body often in epicone, nucleus basal. Pl. 3, fig. 3.

Present in four C-pond samples from spring to autumn, and five P-pond samples from spring, autumn and winter.

103. Gymnodinium sp. nov.#2; Broadly elliptical cells somewhat dorsiventrally compressed, 8-15μ x 6-12μ; girdle sub-equatorial, wide and shallow, not displaced, sulcus very shallow on hypocone; transverse flagellum encircling the body, longitudinal flagellum somewhat longer than body length; chromatophores absent; large spherical nucleus in hypocone; elongate pale red stigma adjacent to the sulcus. Pl. 3, fig. 4.

Present in three P-pond samples from summer, with 10^2 cells/ml in July; one C-pond sample from autumn.

104. Katodinium asymmetricum (Massart) Pott, 1957, (11.) p. 288; Schiller, 1933, (29) p. 434, f. 460a-c. Arrowhead-shaped cells 17µ x 12µ, chromatophores absent, assimilate body often present in epicone, nucleus in hypocone. Pl. 3, fig. 5.

Present in three P-pond from summer, with 10^2 cells/ml in July.

105. Gyrodinium dominans Hulburt, 1957, (14.) p. 212, pl. 3, f. 1-3. Broadly fusiform cells 16-30µ x 11.5-20µ, longitudinal striations on body, girdle displaced 1/4 the body length, chromatophores absent, nucleus central, food body often in the hypocone. Pl. 3, fig. 6.

Present in 10 P-pond samples from late spring to early winter with 10^2 cells/ml concentrations from July to September; present in four C-pond samples, May and September.

106. Gyrodinium estuariale Hulburt, 1957, (14.) p. 209, pl. 1, f. 15-16. Ellipsoid cells 11-15µ x 7.5-9µ, dorsiventrally compressed, with somewhat obliquely truncate antapex, wide shallow girdle displaced one girdle width, sulcus shallow on hypocone, chromatophores brownish-yellow, one or two in hypocone and in epicone, central nucleus. Pl. 3, fig. 7.

Presence in two P-pond and two C-pond samples from August, with 10^2 cells/ml in C-1.

107. Gyrodinium metum Hulburt, 1957, (14.) p. 211, pl. 1, f. 11-12. Broadly fusoid cells 9µ x 7µ, with "chinaman's hat" epicone, chromatophores absent, nucleus sub-central. Pl. 3, fig. 8.

Abundant in three C-pond samples from July to September, reaching 10^3 cells/ml in August; 10^2 cells/ml in three P-ponds from August.

108. Heterocapsa triquetra (Ehr.) Stein; Conrad & Kufferath, 1954, (8.) p. 118, f. 6, pl. 7, f. 4; Schiller, 1937, ( ) p. 145, f. 147. Thecate spindle-shaped cells 23-25µ x 12-15µ, irregularly lobed yellow-brown chromatophores, large elliptical nucleus in the epitheca. Pl. 3, fig. 6.

Presence in three C-pond samples from winter, one P-pond sample from March.

109. Peridinium aciculiferum Lemm.; Schiller, 1937, (29.) p. 162, f. 160. Broadly ovoid cells 33-45µ x 27-38µ, theca with apical horn, 2-4 antapical spines, numerous elongate golden-brown chromatophores, large elongate red stigmatic body adjacent to sulcus. Pl. 3, fig. 10.

Present in pond P-3 in September, 10^2 cells/ml, and october; C-pond presence in September.

110. Peridinium achromaticum Lev.; Schiller, 1937, (29.) p. 209, f. 225. Rhomboid thecate cells 34-41µ x 29-36µ,

sulcus excavating the antapex, chromatophores absent, large elliptical nucleus in center of cell. Pl. 3, fig. 11a-b.

Present only in P-ponds, 15 samples from May to October, with 10^2 cells/ml levels in May and August.

111. Peridinium cf. trochoideum (Stein) Lemm.; Schiller, 1937, (29.) p. 137, f. 134. Pear-shaped cells 21-25μ x 17-18μ, epitheca with apical horn, hypotheca hemispherical, chromatophores deep golden-brown.

Presence in one C-pond and one P-pond in autumn.

HAPTOPHYCEAE

112. Aspidiophora viridissima Sjöstedt, 1924, (30.) p. 9, f. 19-26; Syracosphaera brandti in Carter, 1937, (6.) p. 37, text-fig. 3. Sjöstedt's name for this species takes precedence over Cricosphaera carterae (Braarud & Pagerl.) Braarud, 1960. Globular cells 6.5-13μ in diameter, covered with elliptical ring coccolith scales 1.3 x 2μ in size, two flagella, two golden-brown parietal chromatophores. Pl. 3, fig. 15.

Present in three C-1 pond samples from spring and winter, with 10^3 cells/ml in April.

113. Chrysochromulina sp. (species identification requires E.M. detail of body scales); Sub-orbicular cells 4-7μ x 3.5-6μ, two parietal brownish-yellow chromatophores and basal leucosin body, two flagella and a haptonema up to 20u long when extended. Pl. 3, fig. 16a-b.

Present in nine C-pond samples from spring to winter, with 10^3 cells/ml in August, 10^4 cells/ml in October; present with 10^3 cells/ml concentrations in three P-ponds from May.

114. Prymnesium parvum Carter, 1937, (6.) p. 40, pl. 3, f. 5-16. Elongate-elliptical cells 8-11μ x 3.5-4.5μ with obliquely truncate apex, two long parietal brownish- to greenish-yellow chromatophores, two flagella and a short haptonema. Pl. 3, fig. 17.

Present in five C-pond samples, winter to summer.

CHRYSOPHYCEAE

115. Ochromonas sp. (with affinities to O. minuscula Conrad, 1930); Cells 5-9μ x 4-8μ, basically orbicular but variable in shape, two flagella with the shorter 2/3 the length of the longer, a single parietal olive-yellow chromatophore with no stigma. Pl. 3, fig. 18a-d.

Present in three C-pond samples in winter, reaching a density of 10^4 cells/ml in January. See fig. 2 for seasonal distribution.

116. Ochromonas sp. (with simalarities to O. vallesiaca Skuja, 1948); Cells orbicular to elliptical, 4.5-8μ diameter,

cell surface rugose probably from presence of small scales, single parietal strap-shaped brownish-yellow chromatophore bearing an orange stigma on an anterior cornor; two flagella, the shorter less than 1/3 the length of the longer. Pl. 3, fig. 19.

Presence in eight C-pond samples from spring to autumn, with 10^3 cells/ml levels in April and July; single P-pond presence in July with 10^4 cells/ml. See fig. 2 for seasonal distribution.

117. Pavlova gyrans Butcher, 1952, (1.) p. 183, pl. 2, f. 35-38. Ovoid to obovoid cells 7-9μ x 4-4.5μ; thick sigmoid anteriorly directed flagellum, short fine laterally directed flagellum, and long fine trailing haptothrix all incerted anterioventrally; two parietal brownish- to greenish-yellow chromatophores, one bearing an anterior orange stigma. Pl. 3, fig. 20.

Present in five C-pond samples from February and March.

118. Monochrysis lutheri Droop, 1953, (10.) p. 34, f. 14-16. Sub-triangular cells strongly compressed, 5μ x 4μ x 2.5μ; a thick sigmoid flagellum anteriorly directed and a fine short flagellum laterally directed both incerted in the concave side of the cell; two olive green chromatophores, the more posterior one associated with a cluster of orange granules. Pl. 3, fig. 21a-b.

Present in three C-pond samples from spring, with 10^4 cells/ml in March and 10^3 cells/ml in April.

XANTHOPHYCEAE

119. Nephrochloris salina Carter, 1937, (6.) p. 16, pl. 2, f. 10-22. Sub-elliptical cells dorsiventrally compressed, 6-10μ x 5-7μ; thick sigmoid flagellum anteriorly directed, fine short flagellum laterally directed, both incerted anterioventrally; two parietal greenish-yellow chromatophores, two central disc-shaped refractive bodies. Pl. 3, fig. 22.

Presence only in P-ponds, eight samples from summer to winter, with 10^2 cells/ml in August and September.

120. Monallantus stichococcoides Pascher, 1939, (24.) Heterokont., p. 425, f. 292. Cylindrical cells 3-7μ x 1.5-2.5μ, with delicate walls, solitary, 1-2 pale green chromatophores with no pyrenoids. Pl. 3, fig. 23a-f.

Abundant in C-ponds only, 16 samples from January to May, October to December, with densities of 10^3 cells/ml in February and March, 10^4 cells/ml in January and May, and 10^5 cells/ml in April. See fig. 2 for seasonal distribution.

131. Nonodus guttula Pascher, 1939, (24.) Heterokont., p. 438, f. 301. Comma-shaped cells 3-5μ x 2-4μ, with mucronulately tipped delicate walls, 1-2 pale green chromatophores, several refractive globules in the cell ends. Pl. 3, fig. 24a-i.

Presence in three C-pond samples from winter and spring; abundance in seven P-pond samples from August to November with 10^3 to 10^4 cells/ml concentrations, and pea-soup dense dominance in 17 P-pond samples with 10^6 cells/ml densities from January to March building to 10^7 cells/ml in April, followed by a sharp and complete crash of the blooms on May 11, cells returning in August and attaining 10^6 cells/ml concentrations again from October to December. See Fig. 3 for seasonal distribution.

132. Goniochloris pulchra Pascher, 1939, (24.) Heterokont., p. 623, f. 483. Strongly compressed triangular cells 11μ x 10μ x 2.5-5μ, with a regularly warty patterned cell wall, 3-6 pale green chromatophores, sometimes a cluster of orange granules in the center. Pl. 3, fig. 25a-d.

Presence in two C-pond samples from summer and autumn; presence in 18 P-pond samples from May to December dropping from 10^4 cells/ml in May and June, 10^3 cells/ml in June and July, down through 10^2 cells/ml in August and September. See fig. 3 for seasonal distribution.

133. Centritractus aff. belonophorus Lemm.; Pascher, 1939, (24.) Heterokont., p. 853, f. 707, 709, 711. Cylindrical cells 5-9μ x 2-2.5μ, cell wall halves with acute ends extended into long setae. These cells are less than a third the size given by Pascher. Pl. 3, fig. 26.

Abundant in two C-pond samples from August and September, two P-pond samples from August, 10^3 to 10^2 cells/ml.

134. Gen.? sp.? Elongate-cylindrical cells 4-15μ x 1-1.5μ, with 1-2 elongate pale greenish-yellow plastids, pale bluish globules at the ends of the cell. Pl. 3, fig. 27a-c.

Presence in five C-pond samples from summer to winter with 10^3 cells/ml in July; presence in two summer P-pond samples.

EUGLENOPHYCEAE

135. Eutreptia cf. lanowii Steuer; Butcher, 1961, (3.) p. 4. Fusiform cells long pointed posteriorly, 21-55μ x 7-18μ, with two flagella, one the length of the cell, the other half this length; large red anterior stigma, numerous discoid green plastids, no pyrenoids, numerous elliptical pyramylum grains, central nucleus. Pl. 3, fig. 12.

Presence in two C-pond samples from spring and winter, four P-pond samples from autumn.

136. Euglena aff. proxima Dangeard; Butcher, 1961, (3.) p. 9, pl. 1, f. 7, pl. 2, f. 2. Metabolic cells generally fusiform with tapering posteriority, 23-45μ x 7-15μ, flagella about cell length, anterior red stigma, numerous discoid small green plastids and numerous discoid paramylum grains. Pl. 3, fig. 13

Present in two C-pond samples in May, up to 10^2 cells/ml; present in four P-pond samples, summer and fall.

137. Trachelomonas ? obovata Stobes; Pascher, 1913, (25.) p. 151, f. 287. Obovate orange theca 16-18μ x 9-10μ with rugose surface and a ringed apical pore, olive-green plastids inside. Pl. 3, fig. 14.

Presence in pond C-3 from March to July.

PRASINOPHYCEAE

138. Thalassomonas minima Butcher, 1959, (2.) p. 41, pl. 8, f. 8. Very small ovate cells 3μ x 2μ x 1μ, laterally compressed, with a longer fine flagellum and a shorter thicker flagellum curved around the cell, single green plastid with a red stigma on the side. Pl. 3, fig. 28.

Presence in six C-pond samples from late spring to autumn, with 10^3 cells/ml in June; Presence in four P-pond samples with 10^3 cells/ml in May, down to 10^2 cells/ml in June and October.

139. Heteromastix pyriformis (Carter) Manton; Carter, 1937, (6.) p. 12, pl. 1, f. 13-16. Pyriform compressed cells 4.5-6μ x 4-5μ x 2-3μ, with a thicker curved 9μ flagellum and thinner 16-27μ flagellum, parietal green plastid with basal pyrenoid and two anterior lobes, one bearing a red stigma. Pl. 3, fig. 29.

Present in 11 C-pond samples from all seasons with 10^2 cells/ml abundances in late spring and summer; present in five P-pond samples with some 10^2 cells/ml concentrations in summer and autumn.

140. Pyramimonas plurioculata Butcher, 1959, (2.) p. 31, pl. 2, f. 3, pl. 7, f. 8. Somewhat pyramidal cells 6-8μ x 4-5μ with bluntly rounded posterior and four-lobed anterior, four flagella, a four-lobed green plastid with basal pyrenoid, an anterior double red stigma between two lobes and posterior red granules. Pl. 3, fig. 30.

Presence in eight C-pond samples from spring to early fall; presence in 14 P-pond samples in the same time period with 10^4 cells/ml in June, 10^3 cells/ml in June and July. See figs. 2 and 3 for seasonal distribution.

141. Pyramimonas grossii Parke; Butcher, 1959, (2.) p. 30, pl. 8, f. 1-2. Obovoid cells with four-lobed anterior 8μ x 5μ, four-lobed plastid with basal pyrenoid, lateral red stigma, four flagella.

Single summer C-pond presence.

142. Pyramimonas nanella Conr. & Kuff., 1954, (8.) p. 231, pl. 3, f. 6. Sub-hemispherical cells 4-5μ x 4-5μ with four-lobed anterior, four flagella, four-lobed plastid with basal pyrenoid and red stigma at the tip of one of the lobes. Pl. 3, fig. 31.

Summer presence in one C-pond; presence in six P-pond samples with 10^4 cells/ml density in June, 10^3 cells/ml densities in July.

143. Tetraselmis maculata (Kylin) Butcher, 1959, (2.) p. 67, pl. 10, f. 12, pl. 11, f. 12. Ovate compressed cells 8-13.5μ x 5-9μ, two rounded anterior lobes, four flagella, two-lobed rugose green plastid with basal pyrenoid, large red stigma near the pyrenoid. Pl. 3, fig. 32.

Presence in five C-pond samples from all seasons, one P-pond sample from summer.

144. Tetraselmis contracta (Carter) Butcher, 1959, (2.) p. 63, pl. 12, f. 7. Broadly ovoid cell with two-lobed anterior 19μ x 14μ, compressed, protoplasm contracted away from the cell wall, two-lobed green plastid, large anterior stigma.

Single spring C-pond presence.

CHLOROPHYCEAE

145. Chlamydomonas sp. Ovoid cells 4-5μ x 3-4μ with cup-shaped green plastid filling the posterior half of the cell, large lateral orange-red stigma, two flagella, pyrenoid apparantly absent. Pl. 3, fig. 33.

Presence in pond C-3 in February and March, P-1 in July and August with 10^2 cells/ml.

146. Nannochloris atomus Butcher, 1952, (1.) p. 181, pl. 1, f. 27-29. Small spherical green cells 2.2- 3.5μ in diameter, with granular cytoplasm. Pl. 3, fig. 34a-b.

Presence in 15 C-pond samples from all seasons, 10^4 cells per ml in April increasing to 10^5 in May, 10^3 from August to October; abundance in six P-pond samples, 10^3 cells/ml in May, July and September. See figs. 2 and 3 for seasonal distribution.

147. Oocystis parva West & West; Whitford, 1969, (31.) p. 47, pl. 12, f. 14. Football-shaped cells 6-15μ x 5-12μ, cell wall not thickened to form tips, 1-4 parietal green plastids. Pl. 3, fig. 35a-b.

Single C-pond presence in June; 22 sample P-pond presence from all seasons, with 10^4 cells/ml levels in July and September and 10^3 cells/ml in May, June and August. See fig. 3 for seasonal distribution.

148. Gen.? sp. ? Small ovoid cells 4-5μ x 3-4μ, with a parietal green plastid covering the cell surface. Pl. 3,

fig. 36a-b.
Presence in seven C-pond samples from July to November, 10^2 cells/ml in summer, 10^3 cells/ml in September.

149. Gen.? sp.? Tiny sub-spherical cells 1.5-2μ in diameter, with a parietal cup-shaped green plastid filling less than half the cell. Pl. 3, fig. 37.
Presence in four C-pond samples from September and December, with 10^3 cells/ml in September; single September P-pond presence.

CYANOPHYCEAE

150. Merismopedia glauca (Ehr.) Nag.; Whitford, 1969, (31.) p. 132, pl. 60, f. 46. Monostromatic colonies of groups of tetrads of granular blue-green cells each 4-5μ in diameter.
Single C-pond and single P-pond presence in summer.

151. Spirulina subsalsa Oersted; Prescott, 1951, (28.) p. 480, pl. 103, f. 14. a 1u thick bluegreen filament tightly coiled into a 2.5μ spiral, with motility. Pl. 3, fig. 38.
Presence in pond C-3 from September to December, presence in four P-pond samples from November and December.

FLAGELLATA

152. Calycomonas ovalis Wulff; Conrad & Kufferath, 1954, (8.) p. 133, pl. 5, fig. 2. Ovoid orange lorica 5μ x 4μ with a small anterior opening and 5-6 annular thickenings. Pl. 3, fig. 39.
Present only in C-ponds, seven samples from August to October.

Appreciation is expressed to Dr. Edward Kuenzler and the staff at the Institute of Marine Sciences in Morehead City for making available the equipment, research space, accomodations and transportation needed for the study, and to Dr. Ruth Patrick and Dr. C. W. Reimer of the Academy of Natural Sciences, Philadelphia, for their valuable assistance and for the use of the literature collections of the Academy.

ERRATA: Missing reference for 31. Pleurosigma ? angulatum var. aestuarii (Breb.) V. H.: Patrick, 1966, (26.) p. 332, pl. 27, f. 3a-c.

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Table 1: 19 C-POND DOMINANT and/or POSSIBLE INDICATOR SPECIES

* = possible indicator species

DOMINANT SPECIES attaining concentrations of 10^3 cells/ml or more	SEASON OF DOMINANCE	MAXIMUM DENSITY cells/ml	C-pond/P-pond FREQUENCY RATIO
1. *Monallantus stichococcoides*	Winter, spring	7×10^5	16/0 *
2. *Nannochloris atomus*	Spring	9×10^5	15/6
3. *Ochromonas ? minuscula*	Winter	1×10^4	3/0
4. *Cyclotella caspia*	Summer	1×10^4	12/0 *
5. *Monochrysis lutheri*	Spring	1×10^4	3/0
6. *Nitzschia proxima*	Summer	1×10^4	22/5
7. *Hemiselmis virescens*	Summer	3×10^3	21/21
8. *Ochromonas ? vallesiaca*	Spring	1×10^3	8/1
SPECIES with concentrations less than 10^3 cells/ml showing high C-pond/P-pond frequency ratios			
9. *Navicula* cf. *muralis* f. *agrestis*			16/0 *
10. *Navicula* cf. *friska*			16/0 *
11. *Nitzschia sigma*			12/0 *
12. *Navicula rogallii*			12/0 *
13. *Amphiprora paludosa* var. *hyalina*			11/0 *
14. *Cacillaria paradoxa*			8/0 *
15. *Nitzschia sigma* var. *rigidula*			14/1 *
16. *Nitzschia* cf. *serpenticula*			12/1 *
17. *Skeletonema costatum*			10/1 *
18. *Amphora angusta*			10/1 *
19. *Diploneis smithii*			28/3 *

Table 2: 19 P-POND DOMINANT and/or POSSIBLE INDICATOR SPECIES

* = possible indicator species

DOMINANT SPECIES attaining concentrations of 10^3 cells/ml or more	SEASON OF DOMINANCE	MAXIMUM DENSITY cells/ml	P-pond/C-pond FREQUENCY RATIO	
1. Monodus guttula	Autumn, winter and spring	12×10^6	24/3	*
2. Chaetoceros muelleri	Summer, autumn	3×10^5	21/10	
3. Prorocentrum minimum	Autumn	9×10^4	10/4	
4. Hemiselmis virescens	Summer, autumn	7×10^4	21/21	
5. Nitzschia closterium	Autumn	4×10^4	24/26	
6. Oocystis parva	Summer, early fall	2×10^4	22/1	*
7. Chroomonas amphioxeia	Summer	2×10^4	14/4	
8. Goniochloris pulchra	Late spring	1×10^4	18/2	*
9. Pyramimonas plurioculata	Summer	1×10^4	14/8	
10. Navicula arvensis	Summer	1×10^4	13/1	*
11. Nannochloris atomus	Summer	1×10^4	6/15	
12. Ochromonas ? vallesiaca	Summer	2×10^4	1/3	
13. Nitzschia cf. communis var. hyalina	Summer	8×10^3	16/9	
14. Cyclotella striata var. ambigua	Summer	4×10^3	22/1	*
15. Coscinodiscus sublineatus	Summer	1×10^3	16/0	*
16. Nitzschia proxima	Spring	1×10^3	5/22	
SPECIES with concentrations less than 10^3 cells/ml showing high P-pond/C-pond frequency ratios				
17. Tropidoneis lepidoptera			16/0	*
18. Peridinium achromaticum			15/0	*
19. Nephrochloris salina			8/0	*

Table 3: AVERAGE PROTOPLASMIC VOLUMES FOR DOMINANT PHYTOPLANKTON SPECIES WITHOUT LARGE CENTRAL VACUOLES
measured in cubic microns

1.	Nannochloris atomus	12 μ^3
2.	Monodus guttula	20 μ^3
3.	Monallantus stichococcoides	25 μ^3
4.	Monochrysis lutheri	35 μ^3
5.	Hemiselmis virescens	45 μ^3
6.	Ochromonas spp.	60 μ^3
7.	Pyramimonas plurioculata	90 μ^3
8.	Chroomonas amphioxeia	100 μ^3
9.	Oocystis parva	130 μ^3
10.	Goniochloris pulchra	230 μ^3
11.	Prorocentrum minimum	1150 μ^3

Cell volumes determined by measuring the water displacement of clay scale models.

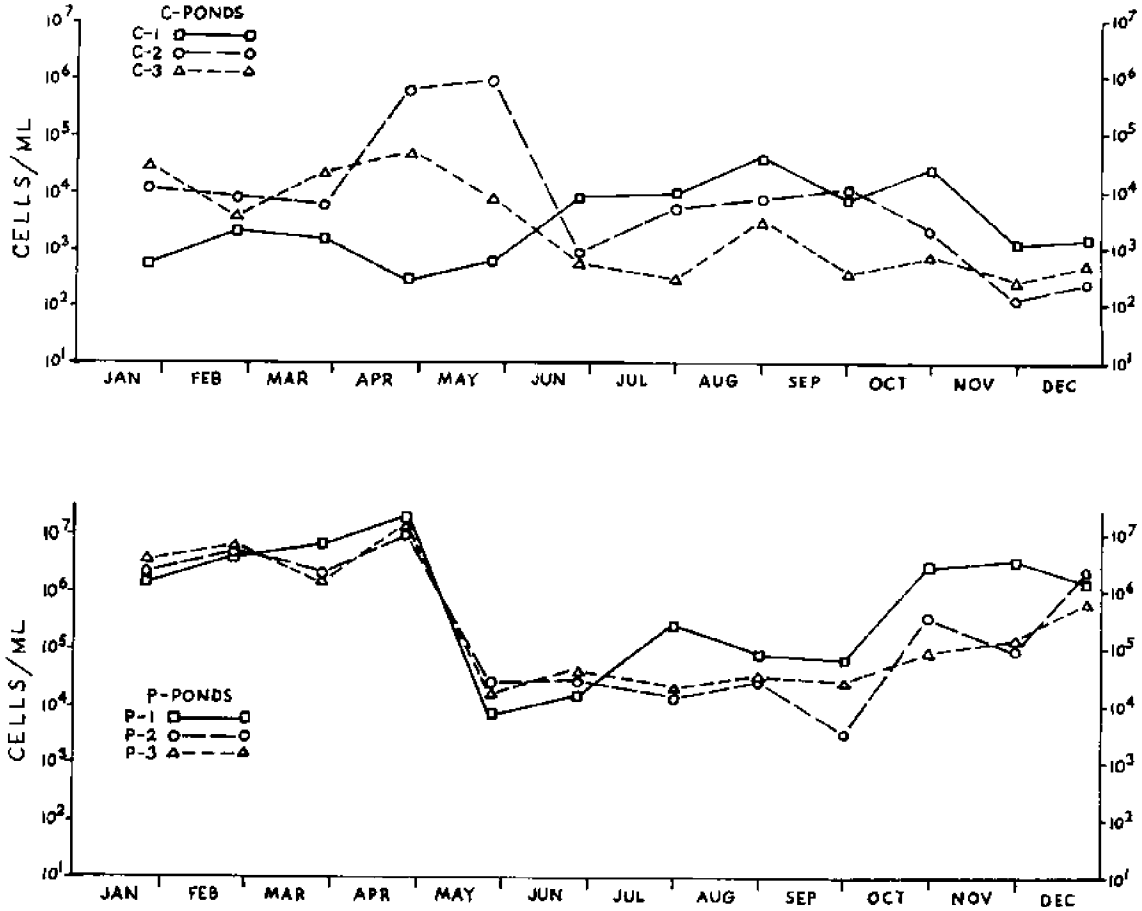

Fig. 1. Seasonal distribution of total phytoplankton abundance in each control and polluted pond. As in all figures, cell densities are plotted in cells/ml on a logarithmic scale to show greater detail of lower cell concentrations.

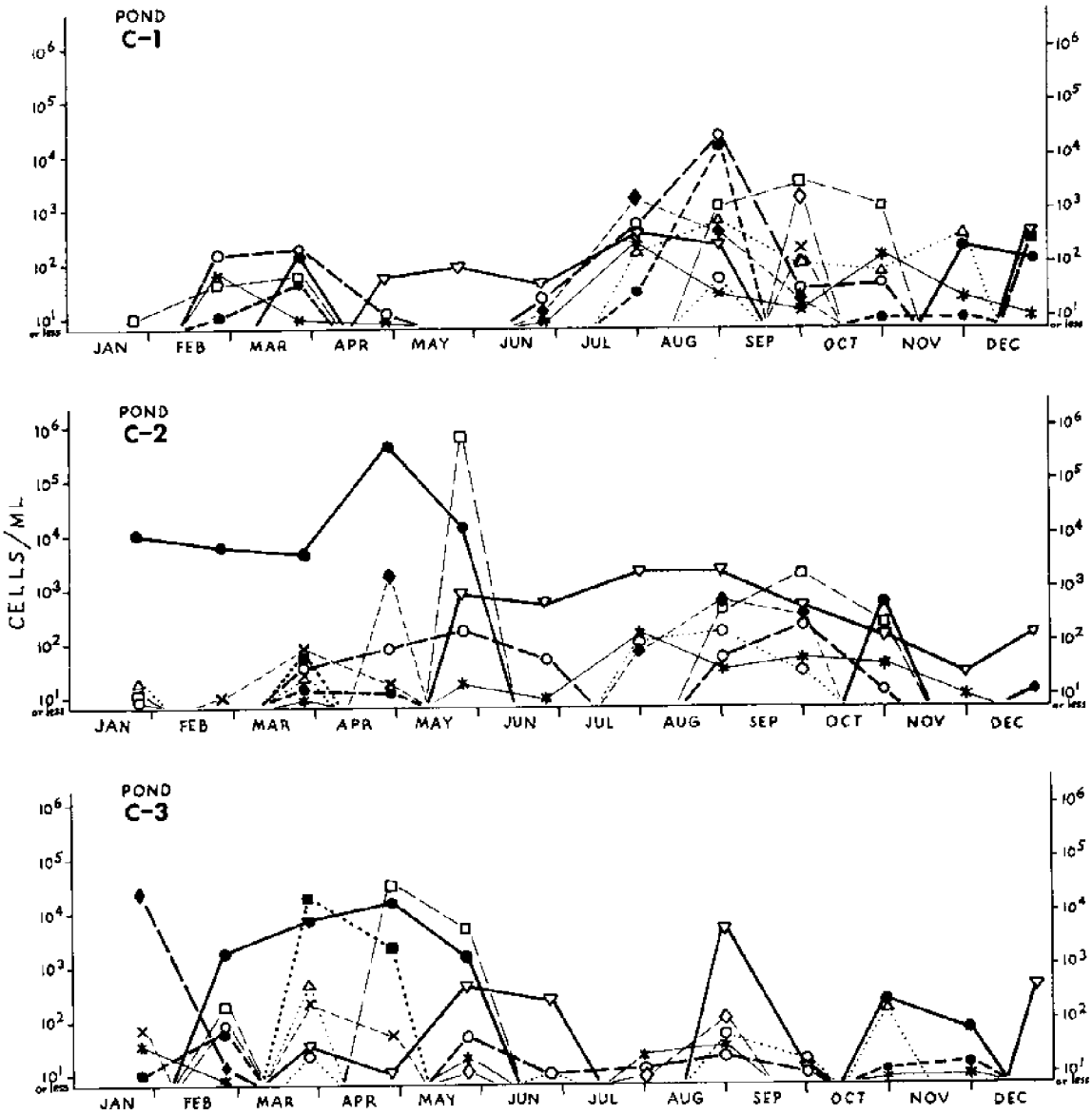

Fig. 7. Seasonal distribution of dominant phytoplankton species in the three central ponds.

- Monallantus stichococcoides
- Nannochloris atomus
- Ochromonas ? minuscula
- Cyclotella caspia
- Monochrysis lutheri
- Nitzschia proxima
- Hemiselmis virescens
- Ochromonas ? vallesiaca
- Chaetoceros muelleri
- Nitzschia closterium
- Chroomonas amphioxeia
- Pyramimonas plurioculata
- Nitzschia cf. communis var. hyalina

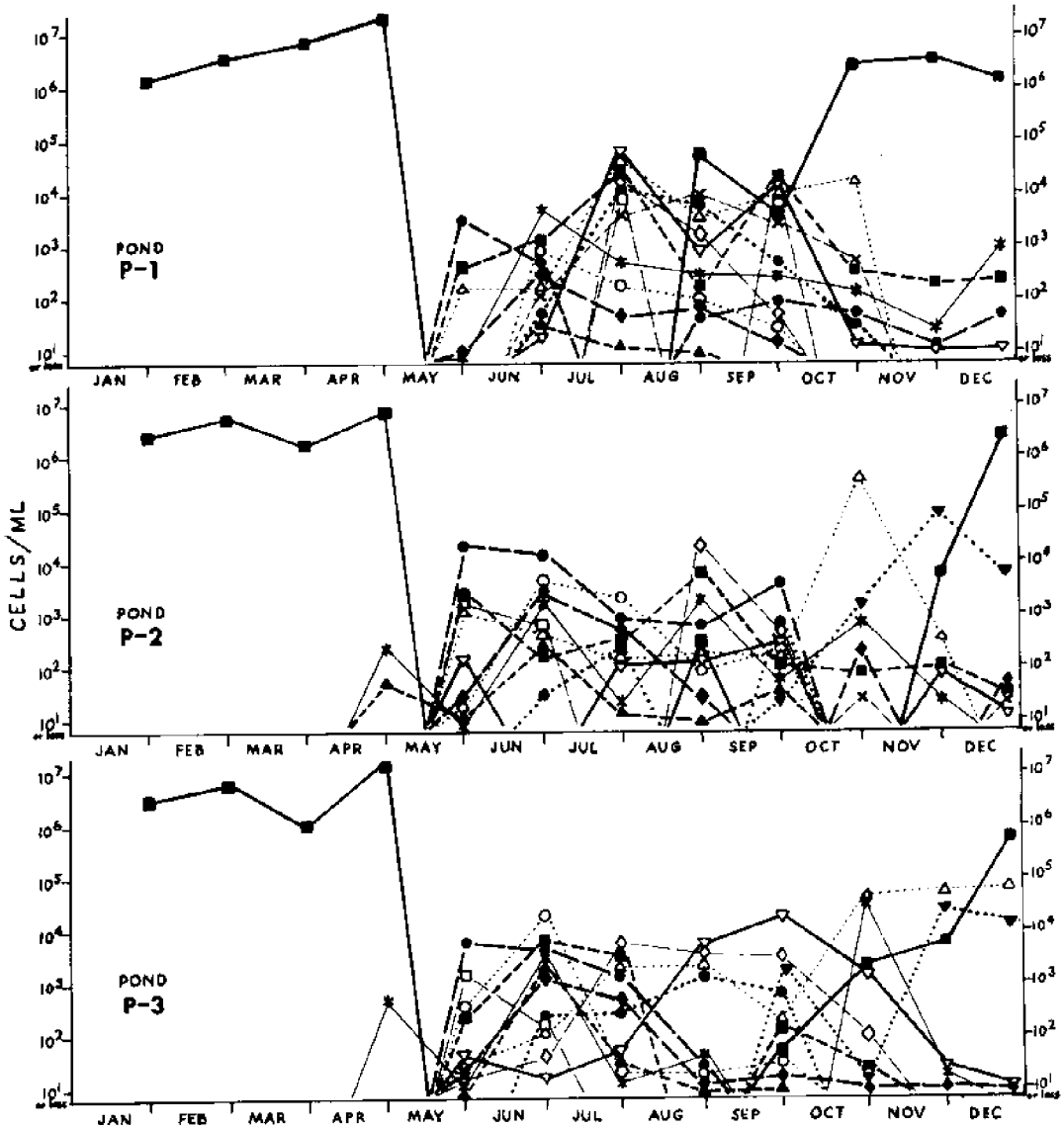

Fig. 3. Seasonal distribution of dominant phytoplankton species in the three polluted ponds.

- Monodus guttula
- Cheetoceros muelleri
- Prorocentrum minimum
- Hemiselmis virescens
- Nitzschia closterium
- Oocystis parva
- Chroomonas amphioxeia
- Goniochloris pulchra
- Pyramimonas plurioculata
- Navicula arvensis
- Nannochloris atomus
- Nitzschia cf. communis var. hyalina
- Cyclotella striata var. ambigua
- Coscinodiscus sublineatus

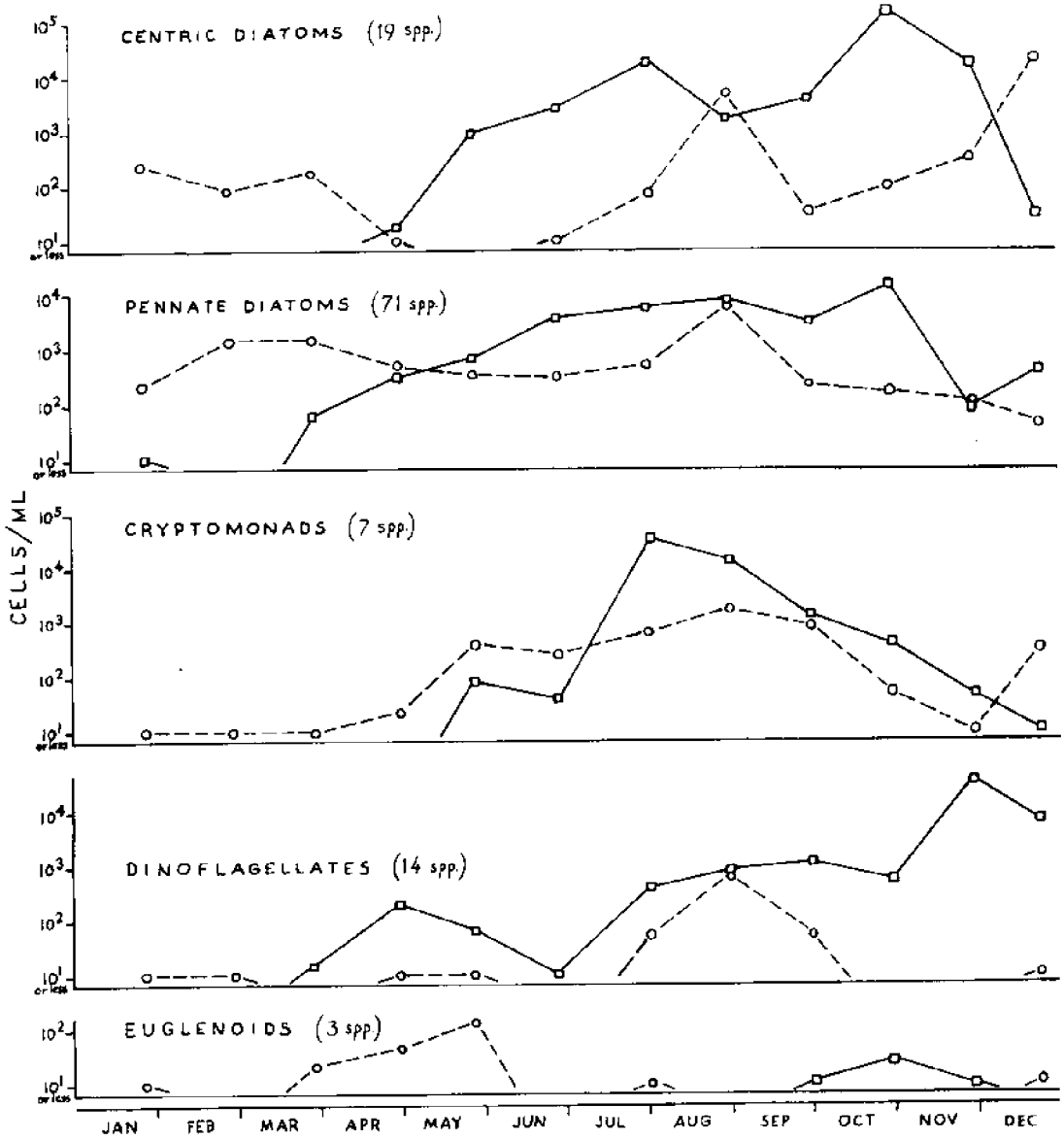

Fig. 5. Seasonal distribution of phytoplankton by major taxonomic groupings.

Average of three C-zones ○——○
Average of three U-zones □——□

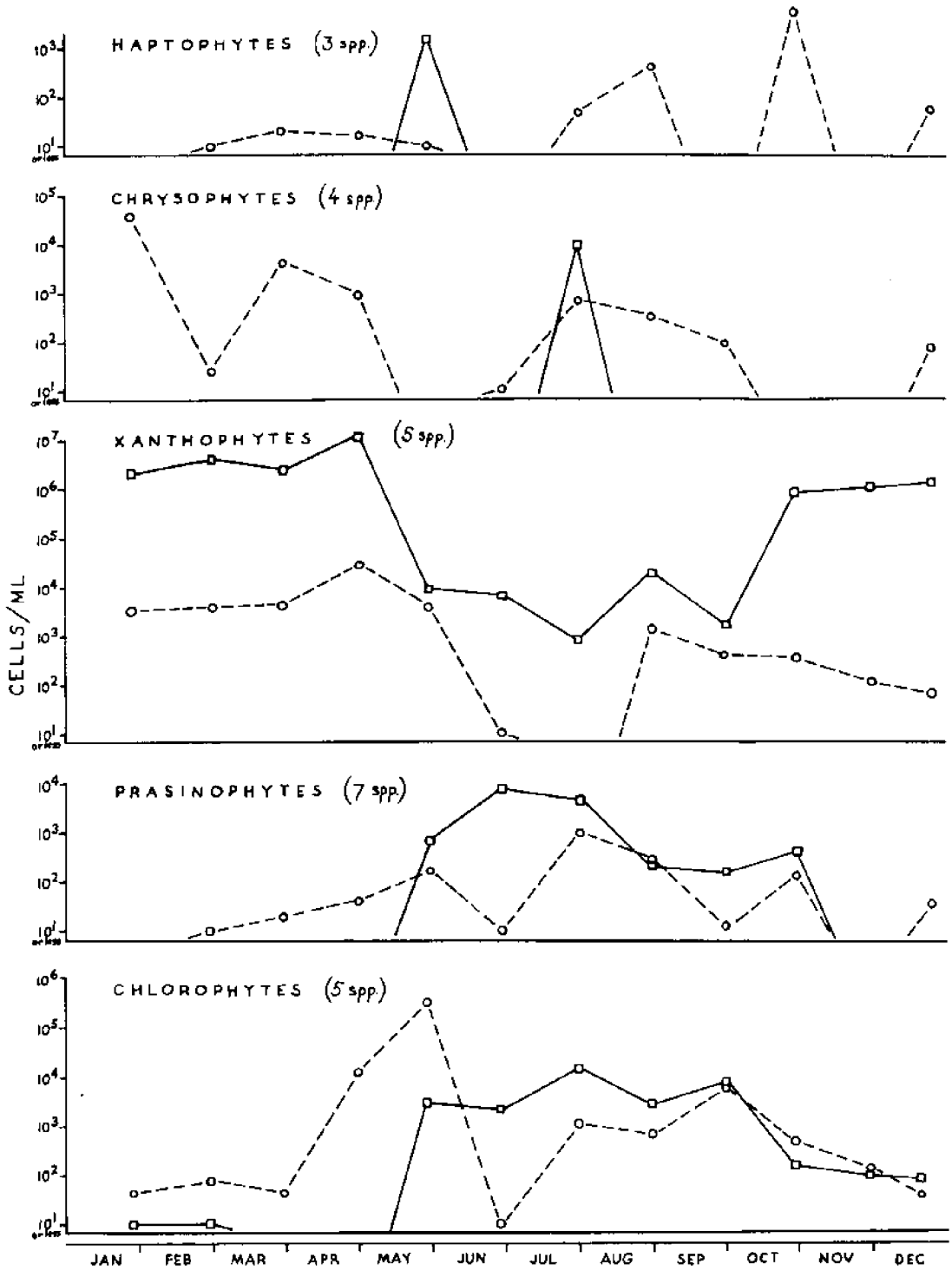

Fig. 4. (con't) Seasonal distribution of phytoplankton by major taxonomic groupings.

Average of three C-ponds ○———○
Average of three E-ponds □———□

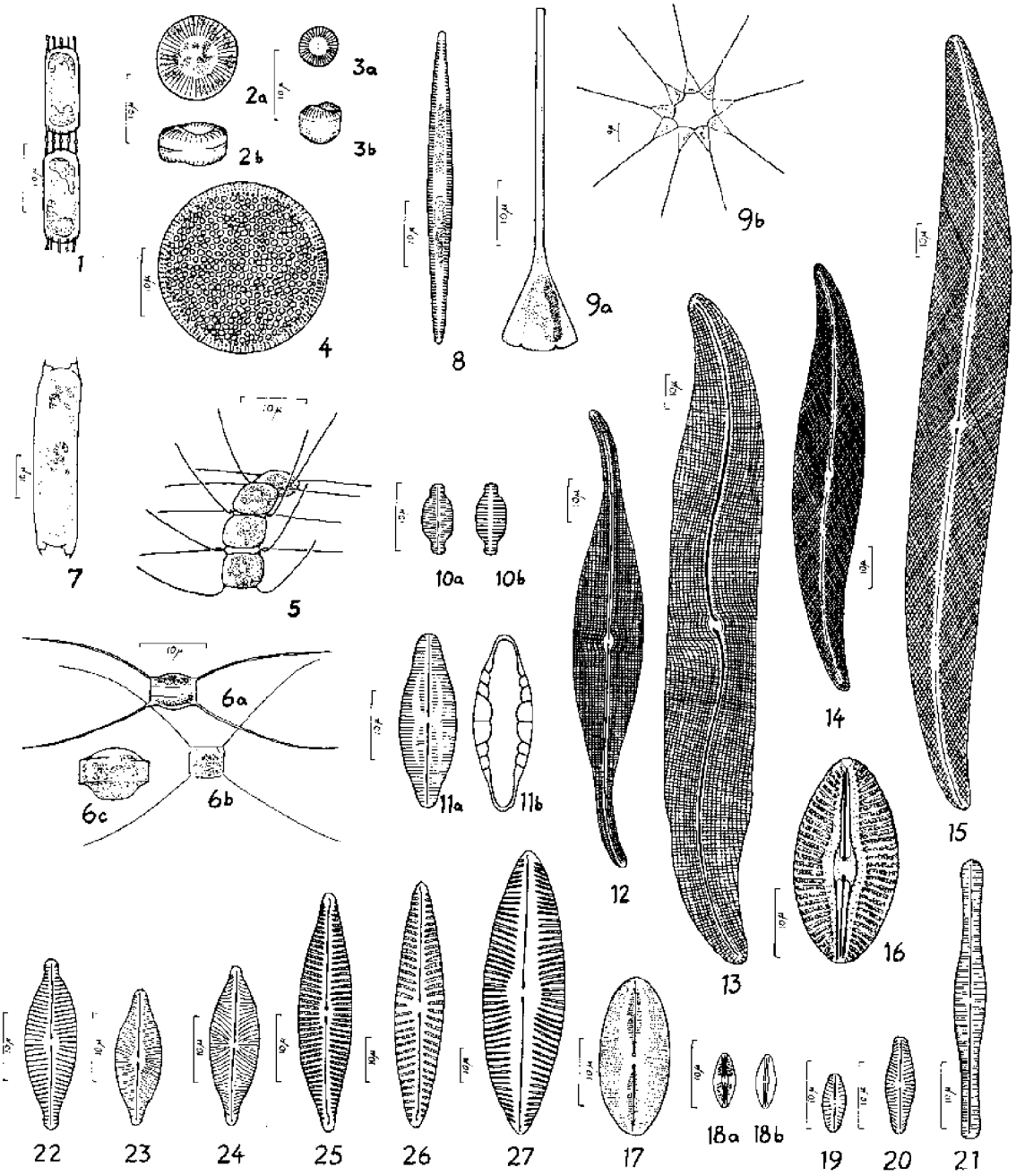

PLATE 1

1. Skeletonema costatum (Grev.) Cl.
2. Cyclotella striata var. ambigua Grun.
3. Cyclotella caspia Grun.
4. Coscinodiscus subincatus Grun.
5. Chaetoceros debilis Cl.
6. Chaetoceros muelleri Lemm., 6b thinly silicified cell from October, 6c resting spore.
7. Cerataulina bergonii Per.
8. Synedra tabulata (Ag.) Kutz.
9. Asterionella japonica Cl., 9b colony.
10. Achnanthes orientalis Must.
11. Rhoicosphenia curvata (Kütz.) Grun. Cl.
12. Gyrosigma fasciola (Ehr.) Griff. & Henfr.
13. Gyrosigma balticum (Ehr.) Rabh.
14. Pleurosigma salinarum Grun.
15. Pleurosigma strigosum W. Sm.
16. Gyrosigma spencerii (Quek.) Cl.
17. Navicula pygmaea Kutz.
18. Navicula arenaria Donk.
19. Navicula cf. muralis f. accrotis (Bust.) Lund
20. Navicula cf. friske Carter
21. Navicula rosellii Bust.
22. Navicula sp.
23. Navicula sp.
24. Navicula salinarum Grun.
25. Navicula lanceolata (Ag.) Kutz.
26. Navicula cf. peregrina (Ehr.) Kutz.
27. Navicula yarrensis Grun.

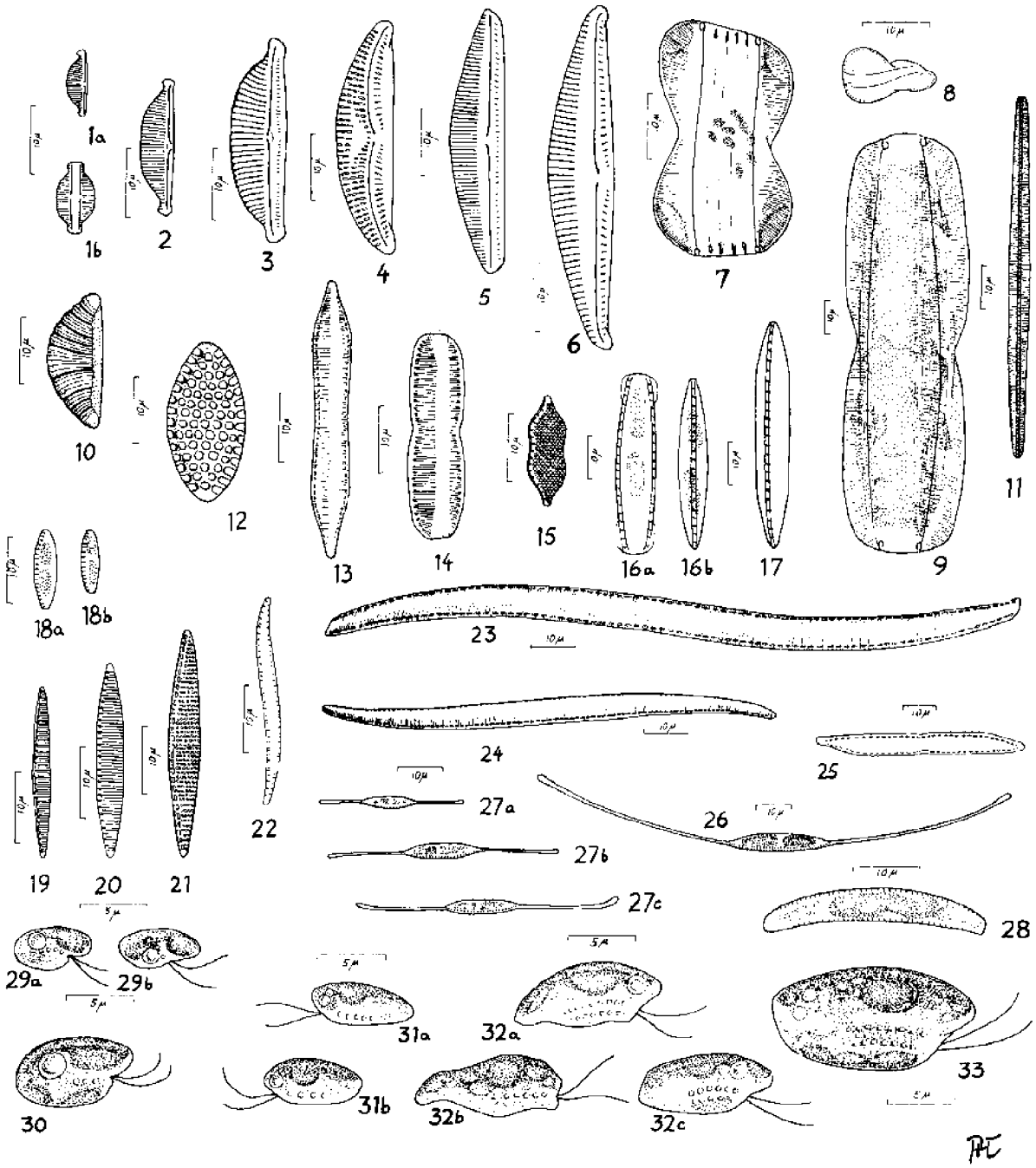

PLATE 2

1. Amphora cf. delicatissima Krasske
2. Amphora cf. tumida Hust.
3. Amphora granulata Grev.
4. Amphora ovalis var. affinis Grun.
5. Amphora angusta Grev.
6. Amphora angusta var. ventricosa Grev.
7. Amphiprora paludosa var. duplex Donk.
8. Amphiprora paludosa var. hyalina Kulenst.
9. Tropidon is lepidoptera Grev.
10. Rhopalodia gibberula var. producta Grun.
11. Epithemia zebra var. porcellus (Kutz.) Grun.
12. Nitzschia compressa (Bail.) Boyer
13. Nitzschia obliquata (Sturm.) Grun.
14. Nitzschia hybridaeformis Hust.
15. Nitzschia pandurriformis var. minor Grun.
16. Nitzschia sanguinea Ehrb.
17. Nitzschia cf. angularis Sm.
18. Nitzschia cf. communis var. hyalina Lund
19. Nitzschia proxima Hust.
20. Nitzschia frustulum (Kutz.) Grun.
21. Nitzschia grunowiana Hust.
22. Nitzschia cf. serpentiraia Cholnoky
23. Nitzschia sigma (Kutz.) Sm.
24. Nitzschia sigma var. rigidula Grun.
25. Nitzschia obtusa var. scalpelliformis Grun.
26. Nitzschia longissima (Ehrb.) Ralfs.
27. Nitzschia closterium W. Sm.
28. Gen.? sp.?
29. Gemiselmis virescens Droop
30. Chroomonas drulococca Butcher
31. Chroomonas minuta var. apyrenoidosa (Hulburt.
32. Chroomonas acrbioxcia (Conr. & Kuff.) Butcher
33. Cryptomonas pseudobaltica Butcher

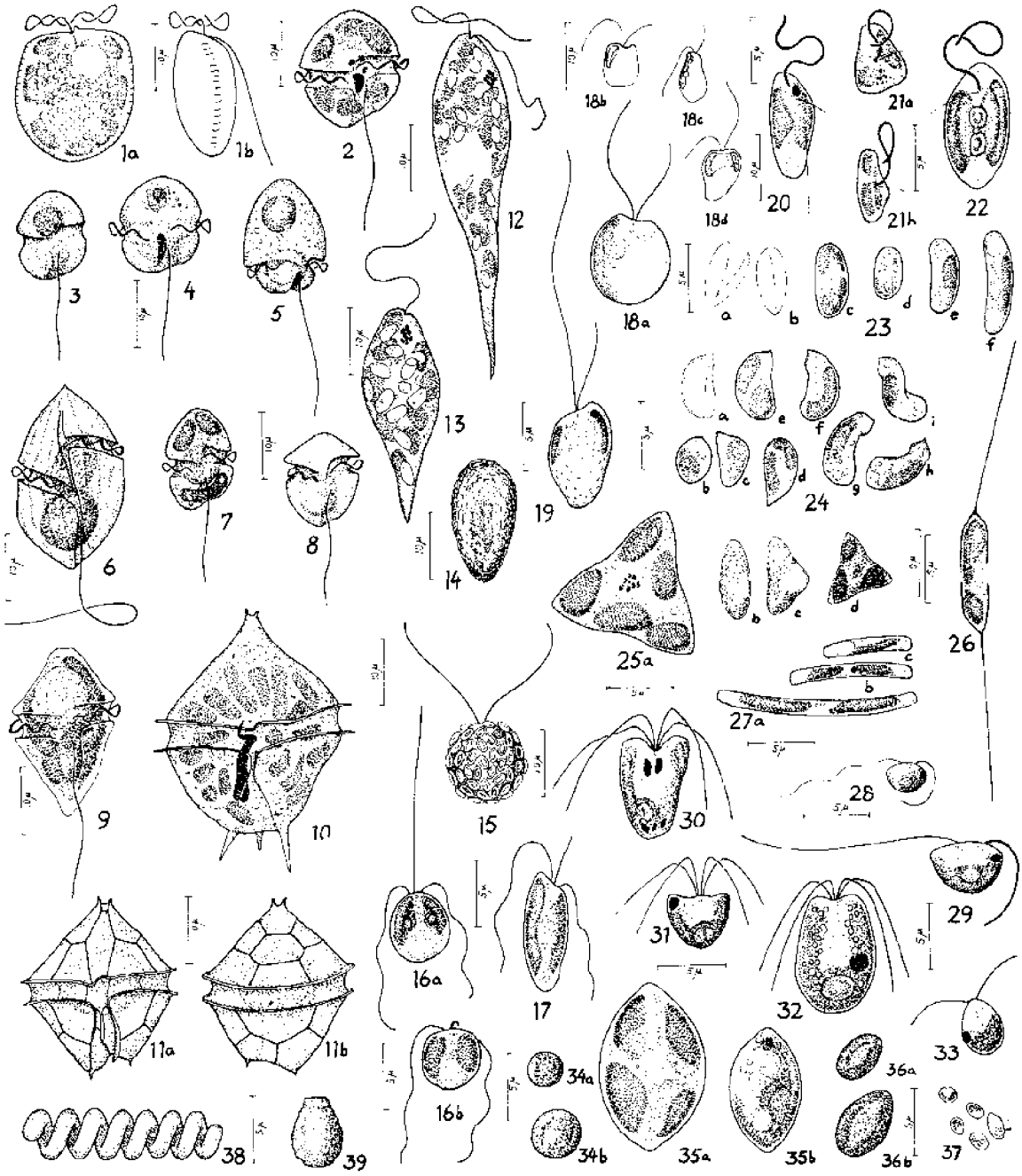

PLATE 3

1. Prorocentrum minimum (Pav.) Schiller
2. Gymnodinium sp. nov.
3. Gymnodinium sp.
4. Gymnodinium sp. nov.
5. Katodinium asymmetricum (Massart) Fott
6. Gyrodinium dominans Hulburt
7. Gyrodinium estuariale Hulburt
8. Gyrodinium mctum Hulburt
9. Heterocapsa triquetra (Ehr.) Stein
10. Peridinium aciculiferum Lemm.
11. Peridinium achromaticum Lev.
12. Eutreptia cf. lanowii Steuer
13. Euglena aff. oxyuris Schmarda
14. Trachelomonas ? ovovata Stokes
15. Scrippsiella trochoidea (Stein) Loeblich III
16. Chrysochromulina sp., 16a gastrozooid extended.
17. Prymnesium parvum Carter
18. Chlamomonas ? minuscula Dangeard
19. Ochromonas ? vallesiaca Skuja
20. Pylaiella grana Butcher
21. Monochrysis lutheri Droop
22. Nephrochloris salina Carter
23. Monallantus stichococcoides Pascher, 23a,b cast off walls.
24. Gonadus guttula Pascher. 24a cast off wall, d-f from Bathman, i-i from winter.
25. Ioniochloris eulonga Pascher, 25b-d cell wall surfaces.
26. Cryptomonas aff. erosa Ehrenb.
27. Gen.? sp.?
28. Tetraselmis minima Butcher
29. Heterocapsa pyriformis (Carter) Manton
30. Pyramimonas plurioculata Butcher
31. Pyramimonas nanella Conr. & Kuff.
32. Tetraselmis marulata (Nylin) Butcher
33. Chlamydomonas sp.
34. Xanthochloris atomus Butcher
35. Oocystis parva West & West
36. Gen.? sp.?
37. Gen.? sp.?
38. Spirulina subsalsa Oerstedt
39. Calycomonas ovalis Wulff.

LABORATORY STUDIES ON THE GROWTH AND NUTRITION OF MONODUS FROM THE TREATED SEWAGE PONDS IN MOREHEAD CITY

Max H. Hommersand and David M. Talbert

Department of Botany

University of North Carolina at Chapel Hill

INTRODUCTION

During the months from November until April a small unicellular member of the genus Monodus (Xanthophyceae) is usually the dominant algal species in the waste-ponds. Its density in the ponds reaches 12,000,000 cells/ml by April and then it largely disappears as the average temperature of the ponds rises above 20°C in May. A number of the physical parameters of the ponds are probably determined by Monodus. 1) Light penetration of the ponds is limited and wavelength dependent according to the density of the Monodus blooms. 2) Absorption of radiant heat is affected. 3) The pH tends to rise to pH 10-10.5 in response to the utilization of HCO_3^- ions by Monodus during its photosynthesis. 4) NH_4^+ ion tends to be depleted selectively due to absorption by Monodus and to be lost into the atmosphere at high pH. 5) NO_3^- ion also tends to be depleted from the ponds late in a Monodus bloom.

Because of the obvious importance of this species to the over-all ecology of the ponds we began studies on the nutrition, growth and physiology of Monodus when money became available last June. Miss Joy Morrill worked on the project during the summer, 1970, and Mr. David Talbert has continued the study beginning in September 1970. In these preliminary experiments our principal effort has been to measure growth as a function of the parameters of salinity, temperature, light intensity, pH, CO_2, organic load, and nitrate and ammonium ion concentration.

MATERIALS AND METHODS

Most experiments were carried out on a platform shaker in Roux flasks containing 100 ml of culture solution (fig. 1). Control conditions were 13‰ salinity (½ sewage, ½ Calico Creek water), 17°C temperature, 750 ft. candles light intensity and gassing with air. Cells were inoculated at densities of 0.5 to 1.0 x 10^6 cells/ml and growth was followed by counting cell numbers in a Neubauer-Levy haemacytometer at daily intervals for 4-6 days. Typically a single factor (salinity, pH etc.) is varied and all other experimental parameters are maintained as in the controls. The specific conditions used in each experiment are described in the Results section.

Studies on the interaction of light intensity and temperature in regulating growth were carried out in a crossed gradient apparatus (fig. 2) that gave 20 combinations of light and temperature ranging from 5°C to 25°C and 100 ft. candles to 700 ft. candles. Temperature control was achieved by heating one end of a sand-filled tray to 30°C and cooling the other end to 0°C. Light was provided by cool white fluorescent lamps that were clustered toward one side of the tray. Fine control of light intensity was achieved by screening with stipple-tone paper.

RESULTS

Initial experiments were carried out to determine the degree of specificity in the requirement for water from the Morehead City ponds. It was observed (fig. 3 a,b) that water taken from the open ocean and diluted to 26‰ salinity with distilled water could substitute for the Calico Creek water normally provided in the ponds. Sewage from the treatment plant in Chapel Hill was as effective in promoting the growth of Monodus as was Morehead City sewage (fig. 3 b,c). A comparative analysis of the effluent from the Chapel Hill and Morehead City filter plants revealed some important differences. Chapel Hill sewage contained more than twice the total organic carbon (74 mg/1 compared to 29 mg/1), 3 times the total nitrogen (34 mg /1 compared to 13.0 mg/1), and about the same level of total phosphate (10 mg/1) in an analysis based on samples taken in April 1970. Although the growth rate was the same with sewage from both trickling filter plants, the cells maintained in Chapel Hill sewage and Calico Creek water were much greener and grew to about 2½ times the maximum density reached by cell populations grown in Morehead City sewage (37×10^6 cells/ml compared to 15×10^6 cells/ml).

A standard inorganic nutrient medium commonly used for growing algae (von Stosch 1963), was supplied in addition to and in place of Morehead City sewage (fig. 3 a,b). (von Stosch medium is primarily NO_3 and PO_4 salts, Fe, Mn and EDTA biotin, thiamin and B_{12} vitamins.) It should be noted that nitrogen is available as NO_3^- rather than NH_4^+. While this medium sustained growth of Monodus the growth rate was about 50% of controls in the absence of the treated sewage.

Temperature-Light.--In experiments in which both temperature and light were varied in some 20 combinations in the crossed gradient apparatus the maximum growth rate was obtained at 20°C and 700 ft. candles (fig. 4). Higher light intensities tended to bleach the cells. Higher temperatures (25°C) could be sustained only if the cells were gassed with air and shaken at high rates (fig. 3g). The light requirement necessary to sustain growth at 25°C rose to 1,500 ft. candles and the cell population tended to "crash" and settle out at 25°C much as it does in the ponds, unless all parameters were kept optimal. Gassing with 5% CO_2 in air tended to inhibit growth at 25°C (fig. 3h).

Salinity.--Salinity had surprisingly little effect on the growth of Monodus. Cells were generally grown at a salinity of 13‰ in either Calico Creek water or diluted sea water. In an experiment (fig. 4) in which the cells were transferred to salinities ranging from ~0 to ~30‰, nearly optimum

growth rates (one division per day) were obtained at all salinities tested. There was usually a lag in the onset of growth upon transfer from one salinity to another. The lag is especially long above 25‰ salinity, but neither the ultimate growth rate nor the maximum cell density was affected. Highest growth rates were obtained at ~0‰ salinity, suggesting that this particular species of Monodus normally inhabits freshwater. High growth rates were also obtained in undiluted Chapel Hill sewage, although this organism has never been recorded for Orange County.

pH.--The effects of pH were investigated using inorganic phosphate buffers and the organic buffers citrate, MES, TES, HEPES and TRIS. Natural buffering by substances present in the treated sewage occurs between pH 9.5 and 10.5. Titration studies showed that the buffering material was particulate in nature and was filtered out by millipore filters of 0.45μ average porosity. High loads of organic phosphate used as a buffer was not tolerated by the cells. The organic buffers however did not appear to interfere with growth. Using organic buffers as indicated in figure 5 it was observed that neither the growth rate nor the maximum density of the standing crop were particularly affected by pH in the range from 7.0 to 10.5. Under no conditions could growth be sustained at a pH below 6.0. A sharp cut off undoubtedly occurs between pH 6.0 and 7.0. We have reason to suspect that Monodus lacks carbonic anhydrase activity and that inorganic carbon must be supplied as HCO_3^-. Experiments are under way to test this hypothesis by controlling pH and CO_2 tensions independently. A rise in pH always accompanies photosynthesis and cell growth up to pH 11.0. This is clear evidence that HCO_3^- is being taken up directly.

Axenic cultures.--In September, 1970, we were successful in getting our Monodus stocks into unialgal and bacteria-free culture. The method used was to filter cells repeatedly on millipore filters having a porosity of 1.2μ and 3.0μ. (The cell dimensions average 3μ wide x 5μ long). Smaller bacteria are washed out by this procedure. The cell suspension was diluted and aspirated onto 0.7% agar plates containing sterilized Morehead City sewage Calico Creek water 1:1. Clones of 8-16 cells were transferred to tubes containing the isolation medium and were grown for 2 months. About 40 single-cell isolates were grown up in this fashion and tested to determine if they were bacteria free. Three of the fastest growing axenic cultures were selected and maintained and the others were discarded.

Nutritional requirements.--With axenic cultures in hand it was possible to carry out nutritional studies. Neither glucose nor acetate supported growth of Monodus in the dark. Furthermore, neither organic substrate improved growth in the light. The yield in Morehead sewage/ Calico Creek water (1:1)was not increased by the addition of trace elements: iron, zinc, manganese, cobalt, copper or boron. In Monodus the growth rate appears to be limited by the rate of cell division which averages one doubling per day under optimal conditions. The maximum population density in stationary phase was found limited by the nitrogen concentration in the medium. Maximum yields obtained with Morehead sewage/ Calico Creek water varied somewhat depending on the time of year the

sewage was collected, but never exceeded 25 x 10^6 cells/ml. With supplemental nitrogen supplied as KNO_3 at 100 mg/l (14 mgN/l) the yield rose to 40 x 10^6 cells/ml. With nitrogen supplied as NH_4Cl at 50 mg/l + 74.6 mg KCl (14 mgN/l) cell densities exceeded 55 x 10^6 cells/ml. The growth rate was also slightly stimulated with NH_3-N.

Growth on a defined medium.--On the basis of the experiments just described, we attempted to grow Monodus on a completely defined medium. Provasoli's ASP-2 medium (Provasoli et al. 1957) appeared to meet expected nutritional requirements with minor modifications. The medium contained the following substances in 100 ml solution: 500 mg $MgSO_4 \cdot 7H_2O$, 1,110 mg NaCl, 60 mg KCl, 27.7 mg $CaCl_2$, 5 mg $NaNO_3$, 3 mg $K_2HPO_4 \cdot 3H_2O$, 15 mg $Na_2SiO_3 \cdot 9H_2O$, 100 mg TRIS, 0.2µg B_{12}, 5.0 mg NH_4Cl, plus trace amounts of iron, zinc, manganese, copper, cobalt and boron. Growth rates were normal in modified ASP-2 and cell densities reached 40 x 10^6 cells/ml. Stationary cultures remained healthy and the cells were normal in appearance. No vitamin requirement could be demonstrated following three serial transfers in a vitamin-free medium. Other experiments showed that TRIS buffer is not used as a nitrogen source by the cells. We expect to find a much simpler defined medium that will support the growth of Monodus.

CONCLUSIONS

The behavior of Monodus under experimental conditions in the laboratory parallels its behavior in the ponds at Morehead City:

1) salinity in the waste ponds has varied from 6‰ to 23‰ with no apparent effect on Monodus. None would be expected on the basis of our studies.

2) the Monodus bloom commences in November, may damp-down somewhat in mid-winter, and expands to a maximum population density in April. The mid-winter temperatures in the ponds average between 5° and 15°C, temperatures which sustain the growth of Monodus. The major bloom occurs in April as the temperature rises to 20°C, and crashes early in May as the temperature exceeds 25°C. The same behavior with respect to temperature is obtained in the laboratory. The rapid settling out and death of Monodus and the associated drop to zero in the oxygen tension of the ponds is explainable in terms of the intermediary metabolism of this species. Monodus accumulates oils as a photosynthetic reserve. Its uniform suspension in the ponds is partly due to this property and partly dependent on the tendency of the cells to repell one another and be randomly dispersed. As the temperature rises respiration exceeds photosynthesis in the dense populations of the bloom and the oil reserve is used up. The cells settle rapidly, oxygen consumption goes up as photosynthesis exceeds respiration and the cells die. The same behavior can be seen in pure culture in a 5-gallon carboy.

3) pH rises to 10.5 in the waste ponds in response to the photosynthetic activity of the Monodus cells just as it does with pure cultures in the laboratory.

4) the maximum population density of Monodus at ca. 17°C in the ponds is about 12 x 10^6 cells/ml which approaches the value of 15 x 10^6 cells/ml obtained under the same conditions in the laboratory. These data suggest that the size of the Monodus bloom is nitrogen-limited in the ponds as it is under our experimental conditions.

On the basis of the data at hand it should be possible to re-design the ponds to obtain optimum yields of Monodus or to minimize its presence. We are proceeding with further laboratory studies and hope to undertake a pilot project in the field designed to control the growth and yield of this alga.

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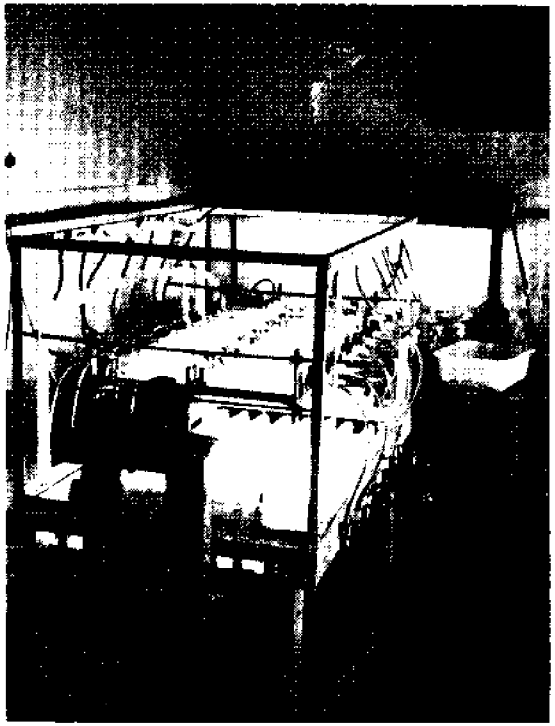
von Stosch, H. A. 1963. Wirkungen von Jod und Arsenit auf Meeresalgen in Kultur. Proc. 4th Int. Seaweed Symp., Pergamon Press, Oxford, pp. 142-150.



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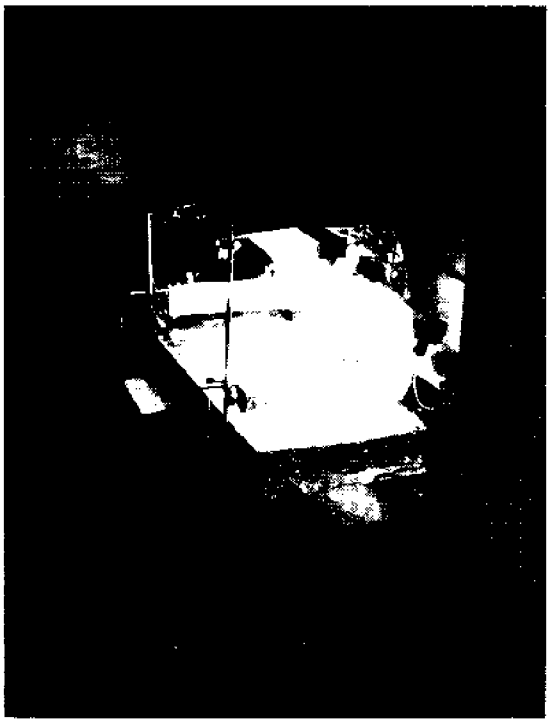

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Figure 1. Platform shaker for culturing Monodus showing the arrangement of the lights and gassing.

Figure 2. Cross-gradient apparatus.

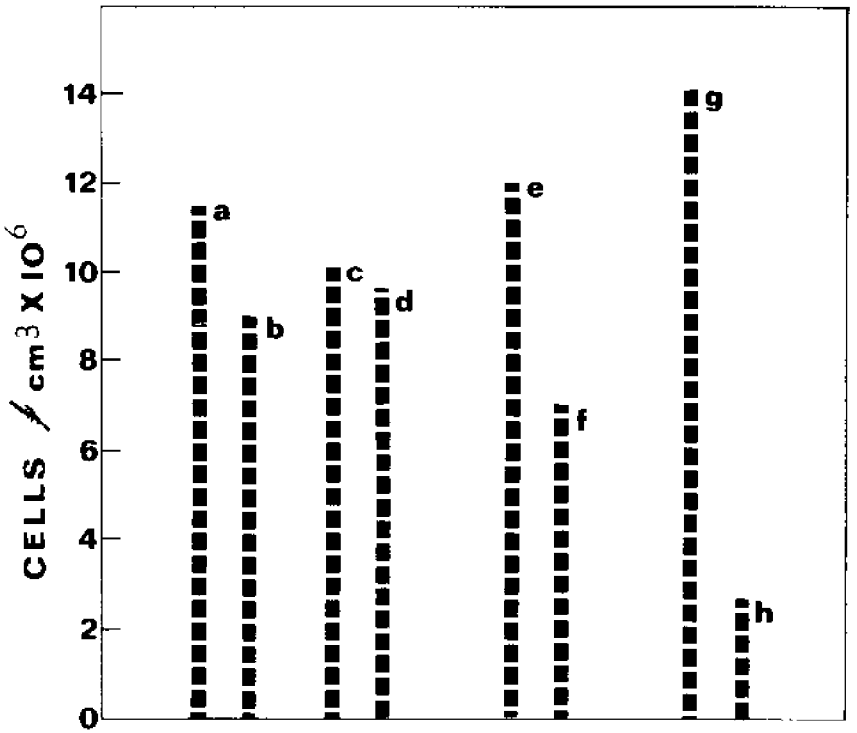

Figure 3. Growth of cells in different media in Roux flasks, volume 100 ml, final salinity 13‰, inoculum 1.0 x 10^6 cells/ml, gassed with air, other parameters as specified.

a. Morehead sewage/Calico Creek water 1:1, 17°C, 750 ft. candles, 4 days.
b. Morehead sewage/diluted ocean water 1:1, 26% , 17°C, 700 ft. candles, 4 days.
c. Chapel Hill sewage/Calico Creek water 1:1, 17°C, 700 ft. candles, 4 days.
d. Chapel Hill sewage/diluted ocean water 26%.1:1, 17°C, 700 ft. candles, 4 days.
e. Morehead sewage/diluted ocean water 26% 1:1, 17°C, 700 ft. candles, von Stosch nutrient supplement (1.0 ml/l), 5 days.
f. seawater 13‰, 17°C, 700 ft. candles, von Stosch nutrient supplement (1.0 ml/l), 5 days.
g. Morehead sewage/Calico Creek water 1:1, 25°C, 1500 ft. candles, gassed with air, 4 days.
h. Morehead sewage/Calico Creek water 1:1, 25°C, 1500 ft. candles, gassed with 5% CO_2 in air, 4 days.

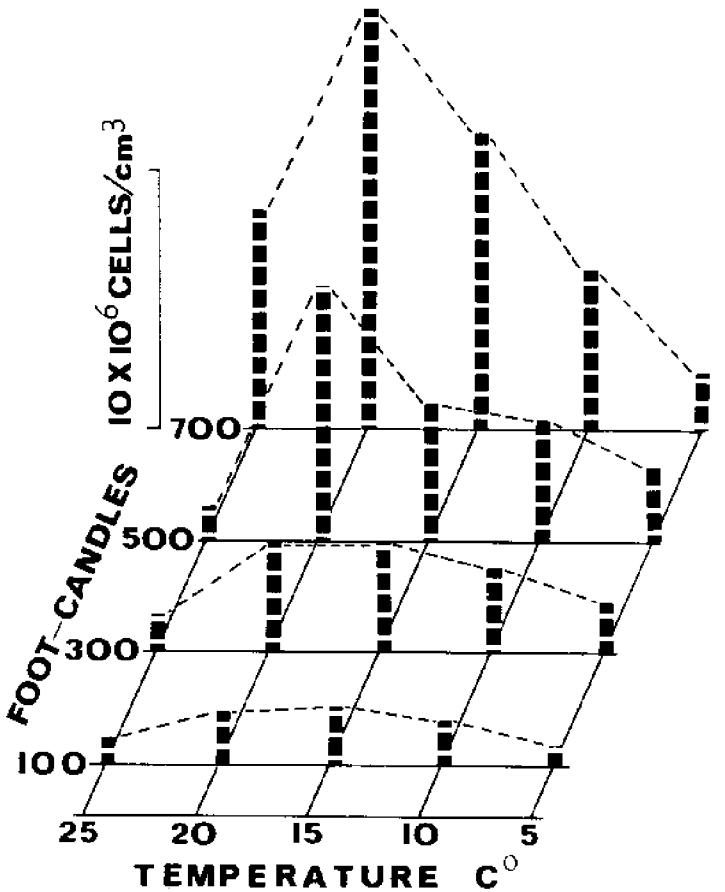

Figure 4. Growth in 6 days in a crossed gradient apparatus. Sterilized Morehead sewage/Calico Creek water 1:1, 13‰ salinity, temperatures and light intensities as indicated. Inoculum 0.5 x 10^6 cells/ml, volume 25 ml.

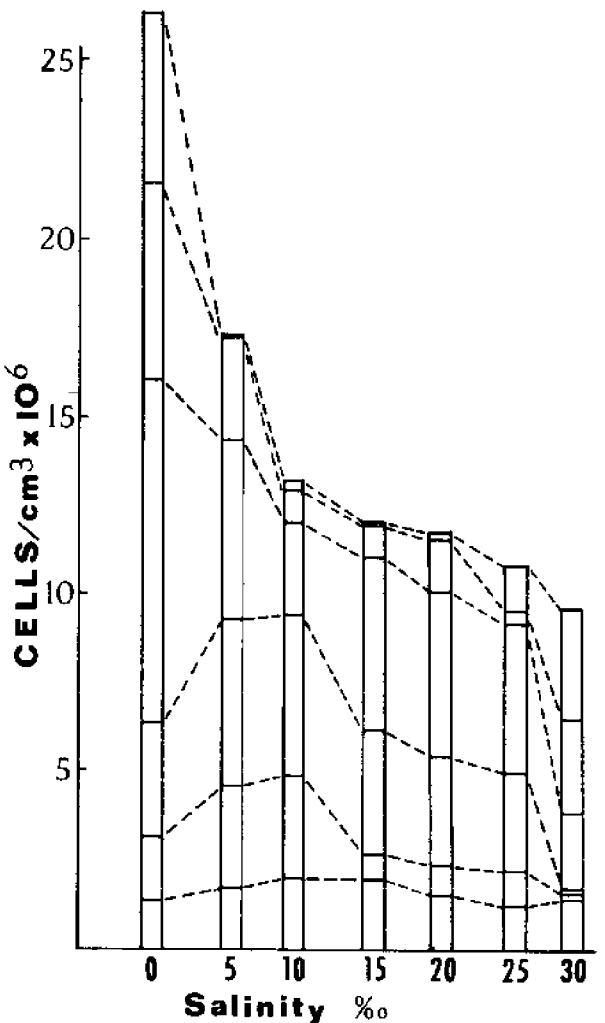

Figure 5. Daily growth of cells for 6 days in Roux flasks as a function of salinity. 100 ml sterilized Morehead/Calico Creek water 1:1, 17°C, 700 ft. candles. Inoculum 1.0 x 10^6 cells/ml, gassed with air.

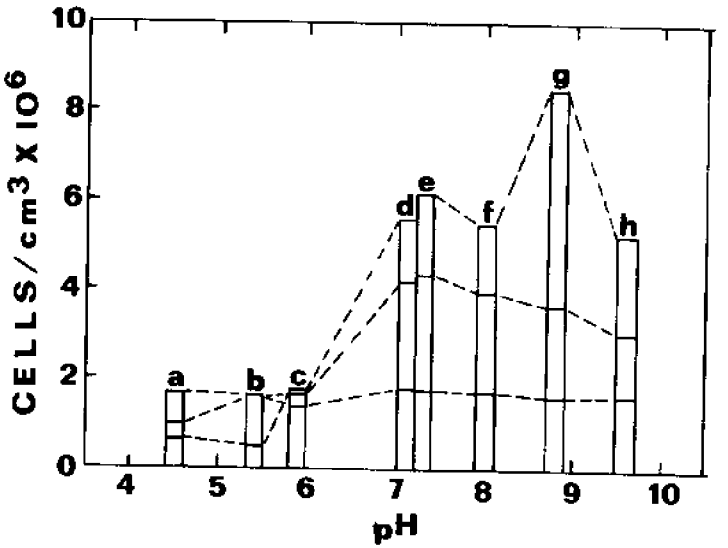

Figure 6. Daily growth of cells for 4 days in Roux flasks as a function of pH with counts taken beginning on the second day. 100 ml sterilized Morehead sewage/Calico Creek water 1:1, 13‰salinity, 17°C, 700 ft. candles. Inoculum 1.0 x 10^6 cells/ml, gassed with air.

a. citrate I. pKa = 4.7
b. citrate II. pKa = 5.5
c. MES pKa = 6.2
d. HEPES pKa = 7.5
e. TES pKa = 7.5
f. TRIS pKa = 8.2
g. Control pH 8.8
h. Control pH 9.6

DECAPOD CRUSTACEAN AND FISH POPULATIONS IN EXPERIMENTAL MARINE PONDS RECEIVING TREATED SEWAGE WASTES*

Michael David Beeston

INTRODUCTION

Growing urbanization of coastal areas of the United States, accompanied by increasing disposal of municipal wastes in the marine environment, is influencing recipient waters to an unknown extent. Some effects, such as eutrophication of certain estuaries by uncontrolled dumping of phosphate-rich waste water or run-off from livestock feedlots have been documented (Barlow, Lorenzen and Myren, 1963; Ryther, 1954; NcNulty, 1961). Larger questions concerning the nature of pathways that may recycle man's wastes in estuaries remain unanswered. Will altered aquatic ecosystems falter into undesirable "dead" waters, or, perhaps with management, will new systems emerge that might even increase production of harvestable food? Should the latter obtain, waste disposal could be turned to man's advantage.

Seeking answers to these and other questions, a model estuarine system was proposed which would demonstrate responses to disposal of treated municipal waste in marine waters. Six ponds were constructed in the summer of 1968 under grants from the North Carolina Board of Science and Technology and the National Science Foundation, Sea Grants Project Division. Three ponds were located adjacent to the Morehead City, North Carolina, sewage treatment plant where they could receive a mixture of treated effluent and salt water from Calico Creek, a small marsh-bordered estuary which is flushed twice daily and receives discharge from the sewage plant. These were designated as waste or "P" ponds (see Odum and Chestnut, 1970) and labelled P-1, P-2, P-3 for experiments. The other three ponds were located at the University of North Carolina Institute of Marine Sciences, Morehead City, and designed to receive a mixture of city tap water and salt water from nearby Bogue Sound. These were designated as control or "C" ponds and labelled C-1, C-2, C-3. Appropriate pumps, mixing chambers, and regulators were installed at both sites to deliver adjustable rates of flow and salinities (Odum and Chestnut, 1970) simulating average estuaries in North Carolina.

Once filled by pumping and back flooding through standpipe drains, the ponds were seeded in autumn with plankton from tows made in Bogue Sound and with an assortment of larger estuarine animals and plants common in the area. Another seeding of plankton was accomplished in early spring, 1969, and a third in spring, 1970. With the exception of these seedings and introduction of material that passed through the pumps, the pond life was allowed to develop as it would--a self design. Following initial adjustments, salinity

*From a thesis for the degree of Master of Science from the Curriculum in Marine Sciences under the direction of Dr. Austin B. Williams.

since January 1969 has been maintained between approximately 10 and 20 ppt and all of the ponds have developed vigorous and diverse populations of plants and animals (Odum and Chestnut, 1970).

Different populations of the pond and surrounding marsh environment have been studied by various workers: phytoplankton, benthic algae, submergent and emergent spermatophytes, bacteria, fungi, zooplankton, molluscs, fishes and larger crustaceans; carbon, nitrogen, phosphorus and calcium budgets; and total metabolism. Eventually an energy network will be simulated for the two sets of ponds.

This paper deals chiefly with the opulation structure, biomass, and growth rates of some of the decapod crustaceans in the ponds. Specifically, blue crabs, Callinectes sapidus Rathbun, grass shrimps, Palaemonetes vulgaris Say and P. pugio Holthuis, and commercial shrimps, Penaeus aztecus Ives and P. duorarum Burkenroad were examined. Since no one was working on the fish populations during 1969, and because fishes were caught while sampling for penaeids, populations and biomass determinations were accomplished for the fishes also.

Shrimps and prawns are highly esteemed and cultured in ponds in many of the countries of the Far East. In Singapore, ponds are constructed in mangrove swamps in such a manner that they are flooded through sluice gates daily on high spring tides (Kow, 1969). The inflowing water brings larval and post-larval prawns along with food for the prawns into the ponds. Prawns which have grown to maturity in the ponds are caught in nets attached to the sluice gates when the tide ebbs at night. Thus the prawns are caught daily. Management includes regulation of the sluice gates in conjunction with tides and occasional poisoning of predatory fish. In the Philippines, shrimp post-larvae or fry are caught commercially and sold to pond operators (Caces-Borja, 1969). The fry are placed in specially prepared nursery ponds for 1 to 1 1/2 months and then transferred to rearing ponds. Predators are eliminated from culture ponds and sometimes fertilizer or supplementary feeds composed of fish, crabs, or rice are added. The shrimp are harvested by means of bamboo screen traps or bag nets attached to the sluice gates. Still other methods of pond cultivation are used in other countries.

Penaeid shrimps comprise the most valuable fishery of the east coast of North America and their life history and biology, similar to those of the Asian species, have been studied by numerous investigators (Burkenroad, 1934; Pearson, 1939; Williams, 1955a, 1965; Gunter, 1961; Eldred, Ingle, Woodburn, Hutton and Jones, 1961; Perez Farfante, 1969). The general pattern of spawning at sea, migration of larvae and post-larvae to estuarine "nursery" grounds, growth, and migration of young adults back to near offshore waters is nearly the same for all three species of commercial shrimps in North Carolina, varying only in the time of spawning and migration (Williams, 1955a, b, 1959 1969).

The annual catch of blue crabs in the eastern United States is worth millions of dollars. Biology of the species has been described by numerous workers (Churchhill, 1919; Gray and Newcombe, 1938; Newcombe, 1945; Newcombe, Sandoz and Rogers-Talbert, 1949; Van Engle, 1958; Fischler, 1965; Williams, 1965; and others). Life history and ecology of this brachyuran species are analogous in many ways to those of the penaeid species, involving migration of transforming larval stages from spawning areas near or in the sea to estuarine "nursery" grounds for the young and subsequent migration of females back to mouths of estuaries and adjacent ocean for spawning.

In contrast to penaeid shrimps and blue crabs, the smaller shrimps, Palaemonetes pugio and P. vulgaris, spend their entire lives in estuarine environments similar to those found in the ponds. The life history and ecology of grass shrimps has been described by Knowlton (1970), Wood (1967), and Williams (1965).

The general life pattern of the foregoing crustaceans may be affected by life in sewage-enriched waters and especially in ponds. Experimentation should give some indications of how these crustaceans are affected.

Several special conditions are inherent in the self design experiment. Although ponds in each series (P and C) are not identical in contents and evolutionary state, they do in most respects constitute three replicates of each situation. Sampling for population estimates of the larger animals is simpler than in open water because the opulations are captive--there can be no migration in or out. The only factors involved in forming estimates of population structure from samples are standing crop, recruitment of larvae, and mortality.

Throughout this period of study (1969-1970) one limit placed on sampling was that none of the animals be harvested. With the exception of animals used for dry weight determinations, all samples were returned unharmed to the ponds from which they were taken. Particularly fragile forms such as anchovies were counted and returned immediately without weighing.

ACKNOWLEDGMENTS

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I wish to express appreciation to my major advisor, Dr. Austin B. Williams for his inspiration, help and patience. Dr. Williams and Dr. Frank J. Schwartz helped with identification of animals. Special thanks are due Greg Rice for help with seining and trapping, and Rosemond Baldree who typed the manuscript.

MATERIALS AND METHODS

The blue crab population was estimated using a multiple mark-recapture method. During 1969, crabs were caught in commercial chicken wire crabpots which had been covered with 1/4-in. hardware cloth and baited with dead fish. The sex and weight of the crabs were recorded, and a plastic numbered tag of the Nesbit-Fiedler type (Cronin, 1949) was attached between the lateral spines of the carapace of medium and large sized individuals with stainless steel wire. Very small crabs were marked with waterproof ink. Both of these types of marks are lost when crabs molt. All six traps were placed in one pond at a time, each pond being sampled about once a week. The population in each pond was estimated on the basis of three samples taken over a two-week period, calculations being based on a method given by Robson and Regier (1968) for multiple mark-recapture experiments in which the proportion of marked fish appearing in a random sample provides an estimate of the proportion marked in the population. Biomass was calculated as the product of estimated population and mean weight of each sample population and converted to dry weight per unit area for each pond.

The length of time during which samples were taken may have introduced error due to mortality and tag loss. Therefore in March 1970, six additional traps were obtained and two traps were placed in each pond every day for seven consecutive days. This increased the number of samples and shortened the length of the sampling period. The Nesbit-Fiedler tag was still used, but in addition, a notch was cut in the edge of the broad dactylus (paddle) of the last pereiopod. Although the notch was largely lost at the next molt, it was recognizable on crabs 8 cm wide or larger. Notching the paddle apparently has no effect on the crab and is useful for population estimates. Biomass as dry weight per unit area was calculated as before. Because of low nighttime oxygen values observed in the waste ponds during midsummer (Odum, Hall and Masarachia, 1970) and the death of six crabs and several pounds of killifish in a trap which was left in P-3 overnight on July 1, 1970, the traps were thereafter set in the morning and removed at evening of the same day.

Sampling of the grass shrimps, Palaemonetes pugio and P. vulgaris, was done at night when distribution of the shrimps throughout each pond appeared to be uniform. A 1 x 3.5 m seine of 1/8-in stretched mesh was pulled across each pond with a measured line held taut between the poles and the captured grass shrimp were removed and weighed wet. Weight per unit area was calculated by assuming that

the grass shrimp were distributed uniformly in the pond and that the amount in the area seined was proportional to that in the entire pond. Dry weight of grass shrimp (g/m^2) was estimated for each pond. Beginning in spring, 1970, samples of 100 from each pond were preserved periodically for determination of species, sex, length, and egg bearing condition of females. Length-frequency histograms were constructed from these data.

Samples of commercial shrimps, Penaeus aztecus and P. duorarum, were captured at night with a seine. To insure reasonably accurate samples of these active animals, a small-meshed seine of sufficient size to span the width of the ponds was employed (5 x 60 ft. of 1/2-in stretched mesh). The seine was emplaced by pulling one end across the width of the pond between two permanently marked points with a line. Once the seine was set, two men could sweep the whole end of a pond with one tow. At the end of a tow the net was pulled up on the bank, any shrimp caught were collected and their length and weight recorded. Dry weight per unit area (g/m^2) was calculated by assuming that the shrimp were uniformly distributed during darkness and that the amount caught in the area seined was proportional to the amount in the pond. Beginning in spring, 1970, it was decided to do a mark-recapture estimate on the population of Penaeus. Following a method described by Costello (1959), McCoy (1968), and Neal (1969), a biological stain, Fast Green FCF, was injected into the abdomen of captured shrimp. The stain, carried by the blood stream, concentrates in the gill area and is easily recognized. The stain is reported to cause little or no mortality and may be expected to last up to eight months (Neal, 1969). Between 0.15 and 0.20 ml of a 0.5% solution of Fast Green FCF in distilled water was injected into shrimp of 110-120 mm total length. Length and weight were recorded and the marked shrimp held overnight to insure that only healthy individuals were released.

The staining method of marking worked well; however, so few shrimp were caught (four in C-2 and three in C-1) that the population could not be estimated by this method. Instead, estimates were based on assumption that number caught in the area seined was proportional to number in the whole pond.

The sampling technique used to capture penaeids also worked well for sampling fishes. Populations of fishes in the control ponds were characterized by small spot, Leiostomus xanthurus Lacepede, pinfish, Lagodon rhomboides (Linnaeus), flounder, Paralichthys dentatus (Linnaeus), and miscellaneous others, each having only one size class. These populations were studied using a multiple mark-recapture method. Fish caught were identified, counted, weighed, branded with a wire dipped in liquid nitrogen (Fujihara, 1967; Mighell, 1969), and released. In subsequent catches, recaptures were counted and unmarked fish counted, weighed and branded. Populations were estimated using the method described by Robson and

Regier (1968). Biomass was estimated as the product of population size and average weight. Populations in the waste ponds were characterized by large numbers of small killifishes, principally Fundulus heteroclitus (Linnaeus) and Cyprinodon variegatus Lacepede, which were 8 cm and smaller in length. As with the shrimps, biomass was estimated by assuming that amount caught in the seine from a known area was proportional to amount in the entire pond.

Samples of all of the above organisms were first sought with the aid of a weighted lift net (12 x 60 ft) emplaced on a pond bottom for 24 hours before being raised. A quick lift of the net would capture all overlying animals. The technique was abandoned because it required too much manpower, blue crabs trapped under the net at time of emplacement tore ragged holes in the net each time it was used, and no killifishes were caught in the waste ponds although they were observed in the crab pots there.

RESULTS

All species of crustaceans and fishes caught during July and August 1969, using seining and trapping procedures previously described, are listed in Table 1. The control ponds contained twice as many species as the waste ponds.

Baited Traps

Crabs

Diversity of crabs was about the same in both sets of ponds. Callinectes sapidus was abundant in both sets of ponds while only one individual of Callinectes similis Williams, a smaller and more oceanic member of the genus (Williams, 1966), was captured in C-2. A single specimen of Panopeus herbstii H. Milne Edwards was found in a crab trap in P-3 during August 1969.

By weighing a sample of crabs, drying to constant weight at 90°C, and reweighing them, the ratio of dry weight to wet weight was found to be 0.233:1.

Population and biomass estimates for Callinectes sapidus in July 1969 and 1970 are shown in Table 2. Both population level and biomass of blue crabs in g/m^2 dry weight were higher in the first year of the ponds' existence than in the second. Mean biomass of Callinectes in g/m^2 dry weight, variance, standard deviation, and standard error of the mean were calculated for each set of three ponds. Mean biomass, range, and two standard errors on either side of the mean (the interval within which we are 95% confident that the true population mean lies) are shown in Figure 1

to provide a visual estimate of statistical significance. Weight-frequency distributions of blue crabs (Figures 3a and b) also reflect reduction of numbers from 1969 to 1970.

A distinct size class of small blue crabs (less than 50 g) was present in C-1 in July 1969 (Fig. 2a). In August the number of crabs caught from this size class increased but that from larger size classes decreased. By March 1970, crabs from larger size classes had disappeared from C-1 leaving only crabs from the 0-50 g class of the previous summer; this class remained evident in June and July 1970 samples (Fig. 2a). The mean weight of crabs in the 0-50 g class was 32 g on August 20, 1969, 61.2g on March 28, 1970, 109.6 g on June 12, and 135 g on July 21. The growth rate producing this increase was 0.13 g/day from August 1969 to March 1970 and nearly constant from March to July 1970 being 0.63 g/day from March 28 to June 12 and 0.65 g/day from June 12 to July 21.

Small 1/8-in Mesh Seine

Grass shrimps

Diversity of grass shrimps was greater in the control ponds than in the waste ponds (Table 1). Palaemonetes pugio was abundant in both control and waste ponds, P. vulgaris less abundant in control ponds and absent from waste ponds.

The ratio of dry weight to wet weight for grass shrimps was 0.234:1. Mean biomass of Palaemonetes in g/m^2 dry weight, variance, standard deviation, and standard error of the mean were calculated for each set of three ponds. Mean biomass, range, and two standard errors on either side of the mean (Figure 3) provide a visual estimate of statistical significance. During late summer, and at least through October 1969, biomass of grass shrimps was low in the control and high in the waste ponds. By early April 1970, biomass was low in both sets of ponds. During summer, 1970, biomass increased only slightly in the control series, but in the waste ponds it reached levels similar to those of the previous August. Biomass of Palaemonetes in P-2 was 5.3 g/m^2 dry weight on August 30, 1969, and 9.1 g/m^2 dry weight on October 25, 1969. These were by far the highest values observed for grass shrimp.

Length-frequency histograms for Palaemonetes occurring in the control ponds are shown in Figures (4a and b) and for the waste ponds in Figures (4c and d). Overwintering populations resume growth in spring, ovigerous females being observed as early as March 30 in the control ponds. Males reach a maximum carapace length of 14 mm while females reach 18 mm. Sex of individuals with carapace length less than 4-5 mm could not be determined and these were arbitrarily classed as females. Females in the waste ponds reached a maximum length of 16 mm in July and August and those in the control ponds 18 mm.

Percentages of females in the samples of Palaemonetes identified, sexed and measured for preparation of length-frequency histograms are shown in Table 3. During spring, populations in the waste ponds had higher percentages of females than those in the control ponds.

Large 1/4-in Mesh Seine

Penaeid shrimps

Penaeids occurred only in the C ponds. In summer, 1969, Penaeus aztecus occurred in all three control ponds and several P. duorarum were found in C-2 and C-3. Although penaeids were caught as late as mid-November 1969, none were found the following spring. Only a few P. aztecus were caught in summer, 1970.

The ratio of dry weight to wet weight for penaeids was 0.273:1. Estimated population and biomass of these shrimps in g/m^2 dry weight for July 1969 and August 1970 are shown in Table 4, both estimates being lower in 1970 than in 1969. In both years, penaeids attained an average weight of 28 g and length of 140 mm by August 1.

Fishes

Results of sampling for fishes pertain only to 1969. Diversity of fishes was lower in waste ponds than in control ponds (Table 1). Fundulus heteroclitus was the most abundant fish in all waste ponds, and a few were observed in C-1 and C-3. The ratio of dry weight to wet weight for fish was 0.248:1. Estimated population and biomass in g/m^2 dry weight of Fundulus in the waste ponds is given for September 1 and October 25, 1969, in Table 5. The low value observed in P-2 on September 1 and the great increase from September 1 to October 25 in all three waste ponds stand out clearly.

Cyprinodon variegatus was abundant in P-1 and observed in P-2. Estimated population and biomass for P-1 was 292 individuals and 0.26 g/m^2 dry weight on September 1, and 2320 individuals and 2.4 g/m^2 dry weight for October 18.

Jumping mullet, Mugil cephalus Linnaeus, was common in P-3 and observed in C-3. Estimated population and biomass for P-3 was 18 individuals and 0.5 g/m^2 dry weight in October, 1969.

Gambusia holbrooki (Baird and Girard) was observed in C-3, P-2, and P-3, and Gobiosoma bosci (Lacepede) found in ponds C-1, P-1, and P-2. Anguilla rostrata (La Sueur) was found in C-2, P-1, and P-3.

In the control ponds, spot, Leiostomus xanthurus, was the most abundant fish and the only large fish occurring in great enough numbers to permit a mark-recapture population estimate. Estimated population and biomass of this species in September was 190 individuals and 1.4 g/m^2 dry weight in C-2, and 200 individuals and 1.3 g/m^2 dry weight in C-3.

Pinfish, Lagodon rhomboides, was observed only in the control ponds, and croaker, Micropogon undulatus Linnaeus, was found in C-1 and C-2. Paralichthys dentatus was present in C-1, nine individuals being observed when the experimental lift net was tried, and also observed in C-3. Membras martinica (Valenciennes) was found in C-1. The bay anchovy, Anchoa mitchilli (Valenciennes), was observed in C-1 and abundant in C-2 and C-3. Aegothea oculata, (Richardson, 1905) common in pond C-3, is an external parasitic isopod on anchovies. (The form is thought to be actually a developmental stage of Lironeca ovalis (Say) [Williams and Deubler, 1968 on authority of Dr. T. E. Bowman, Smithsonian Institution].)

DISCUSSION

Diversity and Environmental Stress

Low diversity of species observed in the waste ponds is characteristic of aquatic environments stressed by low dissolved oxygen concentrations (Odum, 1959). Although low oxygen concentration was never a general property, the waste ponds yielded high daytime and low nighttime oxygen values, (greater than 24 and less than 1 ppm in May 1970; Martha Smith, personal communication), while the control ponds generally underwent a gentle diurnal variation above and below 100 percent saturation (Odum and Chestnut, 1970).

Penaeid shrimp and fishes were apparently most drastically affected by low dissolved oxygen levels, there being no penaeids and only certain species of fishes found in the waste ponds. Eighteen dead spot, Leiostomus xanthurus were found in the shallows of P-2 on July 7, 1969, before seining began, indicating that they were introduced but failed to survive.

Blue crabs also responded to oxygen stress in the waste ponds; crabs and small fishes were observed in the shallows during early morning hours, and the crabs crawled up on the pond banks at times.

Grass shrimps developed greater biomass in the waste than in the control ponds. They were apparently not effected by low dissolved oxygen levels, but only Palaemonetes pugio was found in the waste ponds. The waste ponds experienced a lower salinity (about 5 ppt) than the control ponds (Odum and Chestnut, 1970) and P. pugio prefers water of lower salinity than P. vulgaris (Holthuis, 1949). While competitive exclusion of one species by another frequently occurs in nutrient or food enriched systems, the importance of lower salinity in the waste ponds cannot be ruled out as a major factor in the absence of P. vulgaris from those ponds.

Biomass

The small number of biomass estimates for blue crabs show no statistically significant difference between control ponds and waste ponds.

The decrease in population level and biomass of Callinectes from 1969 to 1970 (Table 2) probably occurred because blue crab populations in the ponds are dependent upon hand seeding for recruitment. The larval stages are too large and fragile to pass through the pumps and they require water of near oceanic salinity for spawning, hatching, and transformation of numerous larval stages. Ovigerous, female crabs have been observed in the ponds but the egg "sponges" failed to develop normally.

Amount of seeding accomplished in the two years differed considerably. For several months after filling, the ponds were seeded with plankton, small shrimp, crabs and fish, benthic plants and other estuarine forms. In March and April, 1969, extensive plankton tows supplied seed material for the ponds including thumbnail-size crabs and small fishes. The only seeding after that time was material from two plankton tows introduced in late March, 1970.

Because penaeid shrimps have breeding habits and developmental sequences of fragile larval stages similar to Callinectes, they were also dependent upon artificial seeding for repopulation in the ponds. Repopulation is even more important in the case of penaeids since winter cold may be fatal. Seining in March 1970 indicated that none had survived winter in the ponds even though they had been caught there in mid-November 1969. Reduced populations of these forms reflect reduced seeding effort.

The low fish population observed in pond P-2 on September 1, 1969, was probably a result of irregularity in seeding. The tremendous increase in numbers and biomass of Fundulus and Cyprinodon in all the waste ponds from September 1 to October 18, 1969, (Table 5) was probably caused by growth of larval fish to catchable size as well as recruitment through flooding of the waste ponds by high spring tides on October 13.

Biomass of Palaemonetes in summer and fall reached levels comparable to those of fishes and blue crabs in the waste ponds indicating that grass shrimp play an important role in that system following an annual cycle of intensive growth in summer and decline in winter. In the control ponds, biomass of Palaemonetes remained at a lower, nearly constant level throughout the year.

Biomass of Palaemonetes was especially high in P-2 in September and October when biomass of fish, Fundulus and Cyprinodon, was low. This may be an indication of ability of one species to perform the role of a second when the second is absent; or absence

of predation on grass shrimp by fish allowed Palaemonetes to increase to high population levels.

Life History of Palaemonetes

Working in North Carolina estuaries, Knowlton (1970) used length-frequency data to trace the life history of Palaemonetes vulgaris. He found ovarian development in February and March, egg deposition beginning in early April, peak egg production in May, and a high percentage of ovigerous females were observed through September. Juvenile recruitment began in early June producing a bimodal length-frequency distribution. Sexual maturity was attained at about 5 mm carapace length. By late summer large females disappeared and females from the spring-hatched generation were spawning, but at a smaller size than females which overwintered. Adult females were larger than adult males. Growth was rapid in summer, stopped in winter and resumed in spring. Working in Galveston Bay, Texas, Wood (1967) found a similar pattern for P. pugio but two spawning peaks (July and October).

Length-frequency distributions of populations in both sets of ponds (Fig. 4) show a life history almost identical to that found by Knowlton. Egg deposition by large, overwintering females began in late March and peak egg production occurred in May. Juvenile recruitment began in early June as indicated by the bimodal character of frequency distribution. Steady progression of the peaks indicates that Palaemonetes grew to adult size in two to three months in summer. Reduction in size of smallest ovigerous females through summer indicates that spring-hatched females were entering the breeding population.

Female Palaemonetes tended to be larger than males, females reaching a maximum size of 18 mm and males 14 mm. The size difference was less pronounced during young stages. Knowlton (1970) found that in almost all populations sampled, females slightly outnumbered males, but Wood (1967) found that males were more abundant in summer and females in winter. My sampling (Table 3) indicates that, while ratios varied from pond to pond, females comprised a large percentage (85-90%) of the population in the waste ponds during early spring, but males were more abundant in late summer. Percentages of males and females were more even in the control ponds. One can speculate that high percentage of females provides a means of achieving high biomass levels by early summer as was the case for the waste ponds.

Aquaculture

Blue crabs grew as well in the waste ponds as in the control ponds and were the largest animals in the waste ponds. They perhaps offer the best possibility for combination of food harvest and waste amelioration in ponded estuarine environments receiving treated sewage wastes under conditions paralleling those in this experiment.

SUMMARY

1) Population, biomass, and life history of some decapod crustaceans and fishes were studied in six estuarine ponds, half of which received treated sewage wastes.

2) Diversity was lower in ponds receiving treated wastes.

3) Population levels of blue crabs, Callinectes sapidus, and commercial shrimps, Penaeus aztecus and P. duorarum, were dependent on artificial seeding.

4) Palaemonetes pugio and P. vulgaris were able to breed in the ponds and P. pugio reached high (up to 9.1 g/m^2 dry weight) biomass levels in summer in ponds receiving treated wastes.

5) Life history of Palaemonetes was found to be essentially the same as that described by other workers.

6) Blue crabs offer a possibility for harvestable food from estuarine ponds receiving treated sewage wastes.

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Table 1. Species found in the ponds during summer, 1969, using seining and trapping techniques.

	C-1	C-2	C-3	P-1	P-2	P-3
Aegathoa oculata (parasitic isopod)			+			
Callinectes sapidus (blue crab)	+	+	+	+	+	+
Callinectes similis		-				
Palaemonetes pugio (grass shrimp)	+	+	+	+	+	+
Palaemonetes vulgaris (grass shrimp)	+	+	+			
Panopeus herbstii (mud crab)						-
Penaeus aztecus (brown shrimp)	+	+	+			
Penaeus duorarum (spotted shrimp)		-	-			
Anchoa mitchilli (bay anchovy)	-	+	+			
Anguilla rostrata (American eel)		-		-		-
Cyprinodon variegatus (sheepshead minnow)				+	-	
Fundulus heteroclitus (killifish)	-		-	+	+	+
Gambusia holbrooki (top minnow)			-		-	-
Gobiosoma bosci (naked gobi)	-			-	-	
Lagodon rhomboides (pinfish)	-	-	-			
Leiostomus xanthurus (spot)	+	+	+			
Membras martinica (silverside)	-					
Micropogon undulatus (croaker)	-	-				
Mugil cephalus (mullet)			-			+
Paralichthys dentatus (flounder)	+		-			

+ indicates relatively abundant population.

- indicates only one or several individuals observed.

Table 2. Population and biomass estimates for Callinectes sapidus in control (C) and waste (P) ponds during a summer month in two years.

	July 1969		July 1970	
Pond	Estimated population	Biomass g/m^2 dry weight	Estimated population	Biomass g/m^2 dry weight
C-1	65	4.1	40	2.5
C-2	43	2.7	14	1.4
C-3	39	2.0	15	1.3
P-1	53	4.5	30	2.3
P-2	43	3.5	21	1.8
P-3	37	2.7	26	1.5

Table 3. Percentage of female Palaemonetes in control (C) and waste (P) ponds during 1970.

Date	Pond	Percent		Pond	Percent	
3-3	C-1	47				
4-10				P-1	85	
5-5	C-2	25		P-2	91	
6-22	C-1	47	54*	P-1	62	45*
	C-2	78		P-2	46	
	C-3	36		P-3	27	
7-15	C-1	51	38*	P-1	71	52*
	C-2	44		P-2	53	
	C-3	18		P-3	32	
8-1	Control ponds		42*	Waste ponds		34*
9-1	Control ponds		57*	Waste ponds		32*

* mean for three ponds.

Table 4. Population and biomass estimates for Penaeus aztecus in control (C) ponds during selected summer months in two years.

	July 1969		August 1970	
Pond	Estimated population	Biomass g/m^2 dry weight	Estimated population	Biomass g/m^2 dry weight
C-1	29	0.47	(3)*	0.05
C-2	52	0.75	10	0.15
C-3	21	0.26	--	--

* Three found in Roy Hyle's fish trap August 27, 1970.

Table 5. Population and biomass estimates for Fundulus heteroclitus in waste (P) ponds during late summer-early fall, 1969.

	September 1, 1969		October 18, 1969	
Pond	Estimated population	Biomass g/m^2 dry weight	Estimated population	Biomass g/m^2 dry weight
P-1	466	0.80	3320	4.2
P-2	34	0.06	1050	1.3
P-3	725	1.9	1235	1.6

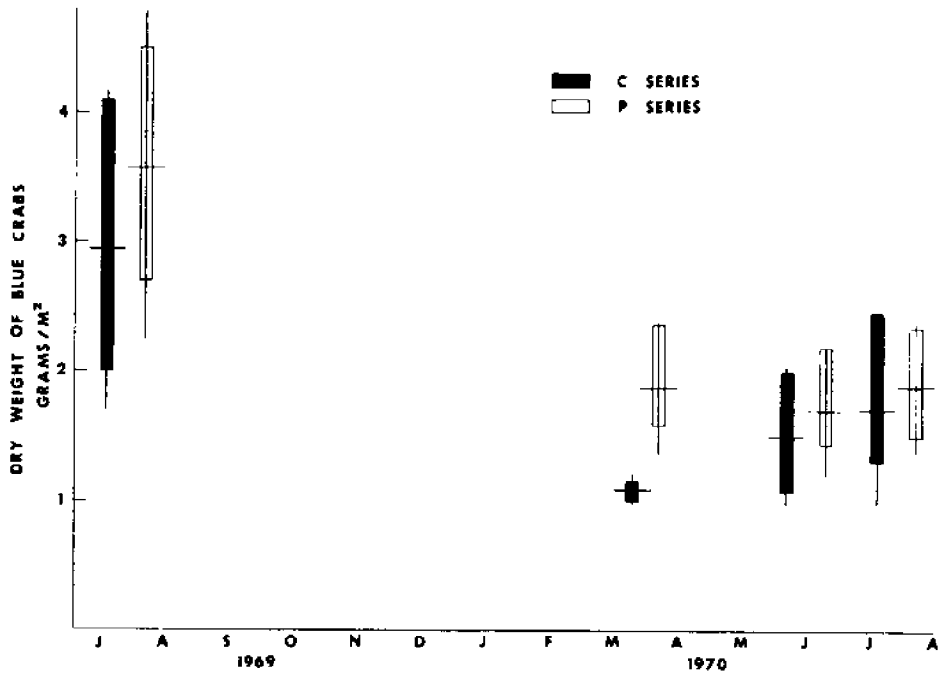

Fig. 1. Biomass of Callinectes sapidus (dry weight g/m²). Horizontal line indicates mean for each series, vertical bar indicates range of three samples, and vertical line indicates two standard errors on either side of the mean.

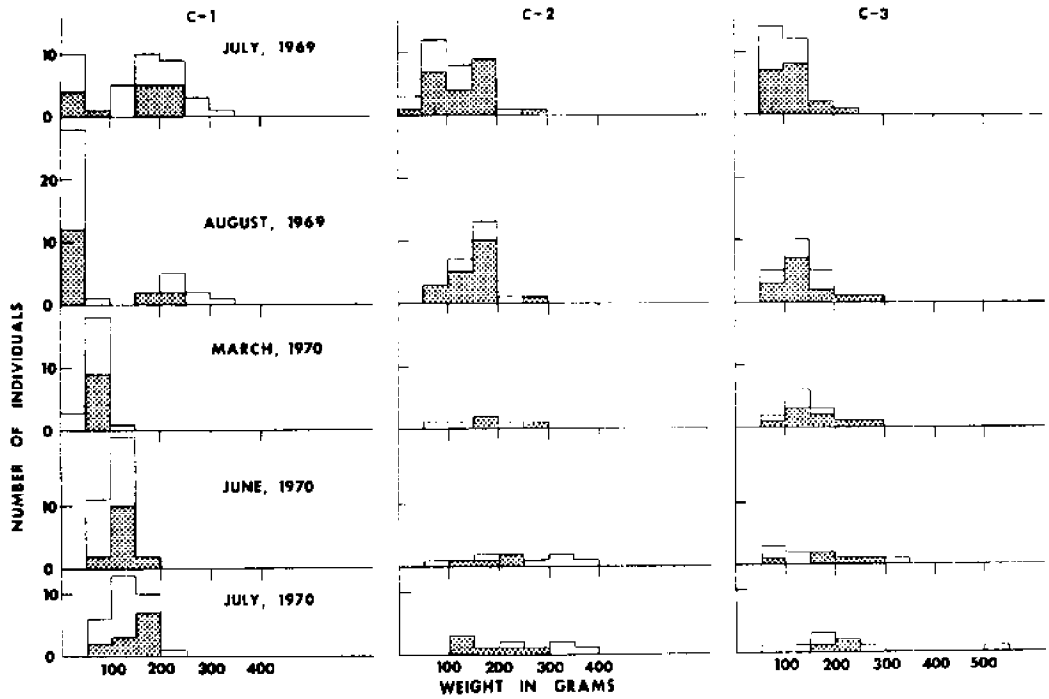

Fig. 2a. Weight-frequency distributions of Callinectes sapidus in control ponds; . . . males, ▨ females.

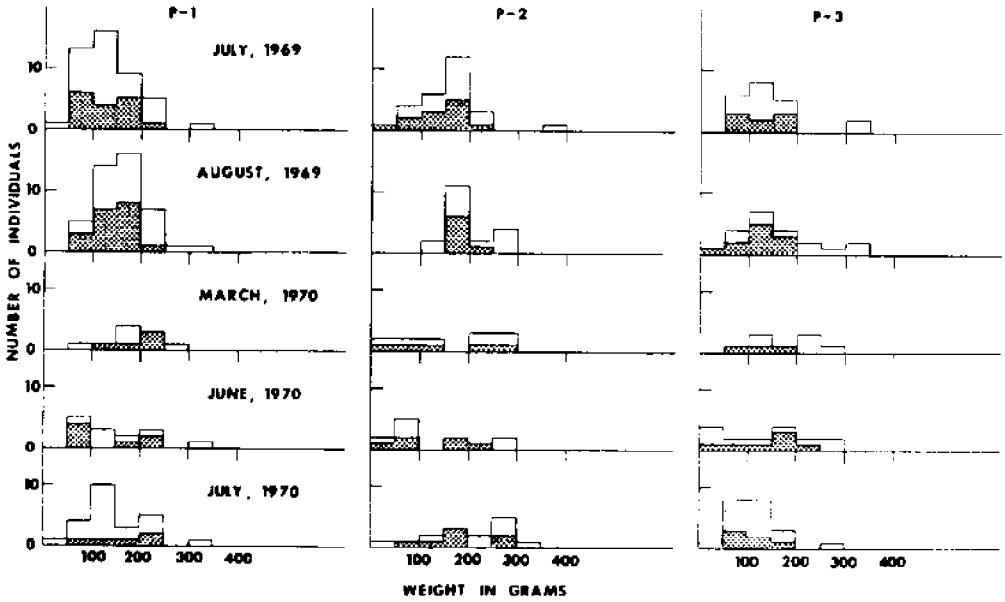

Fig. 2b. Weight-frequency distributions of Callinectes sapidus in waste ponds; ▭ males, ▨ females.

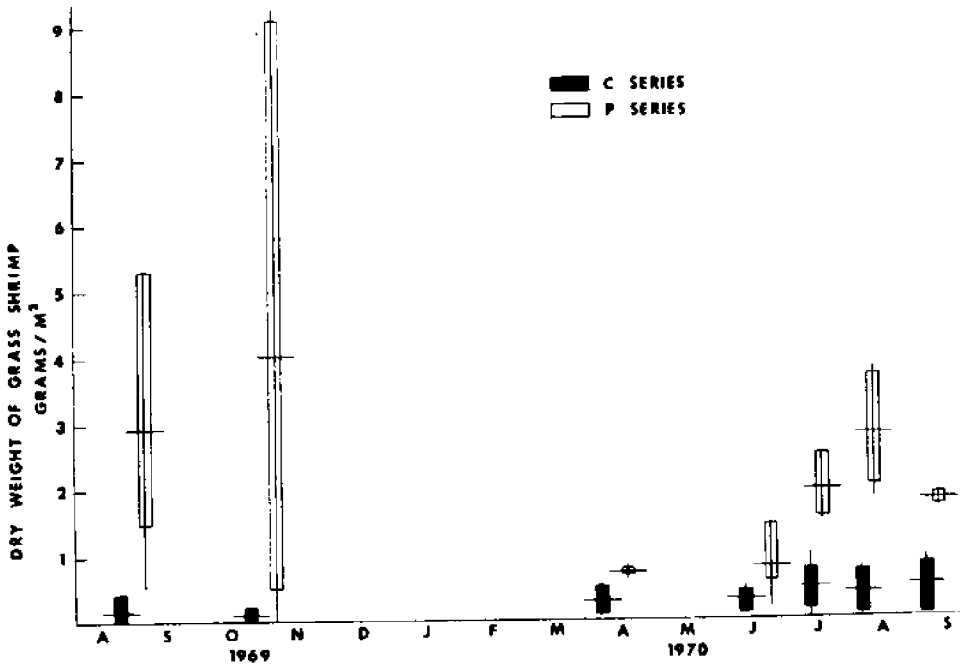

Fig. 3. Biomass of Palaemonetes pugio and P. vulgaris (g/m^2 dry weight). Horizontal line indicates mean for each series, vertical bar indicates range of three samples and vertical line indicates two standard errors on either side of its mean.

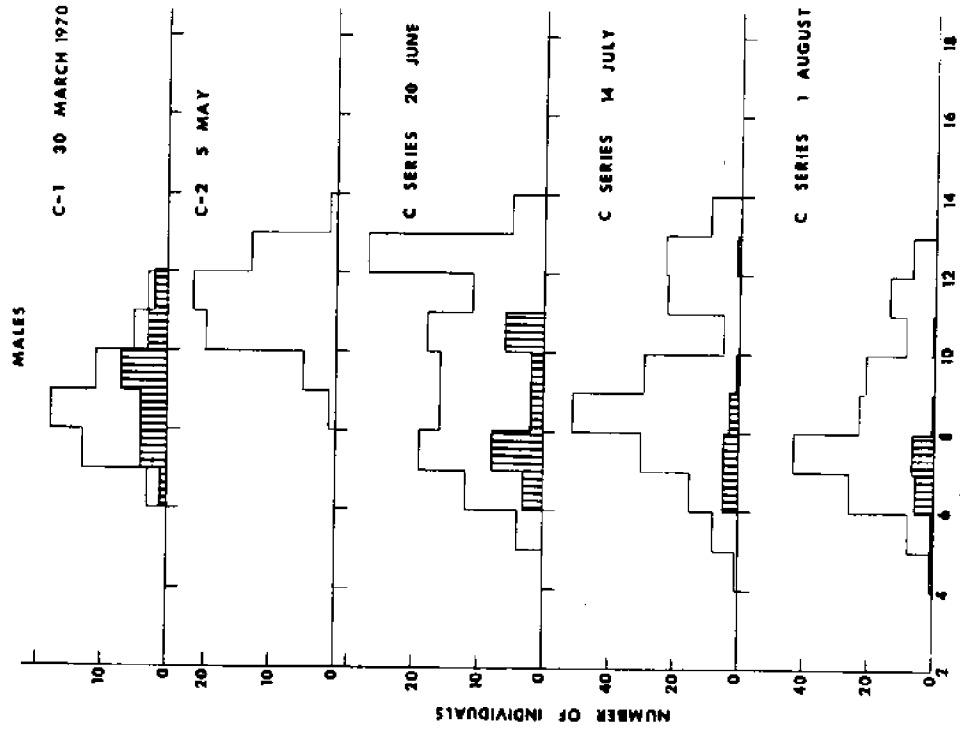

Fig. 4b. Length-frequency histogram of male Palaemonetes in control ponds;
P. pugio, ▨ P. vulgaris.

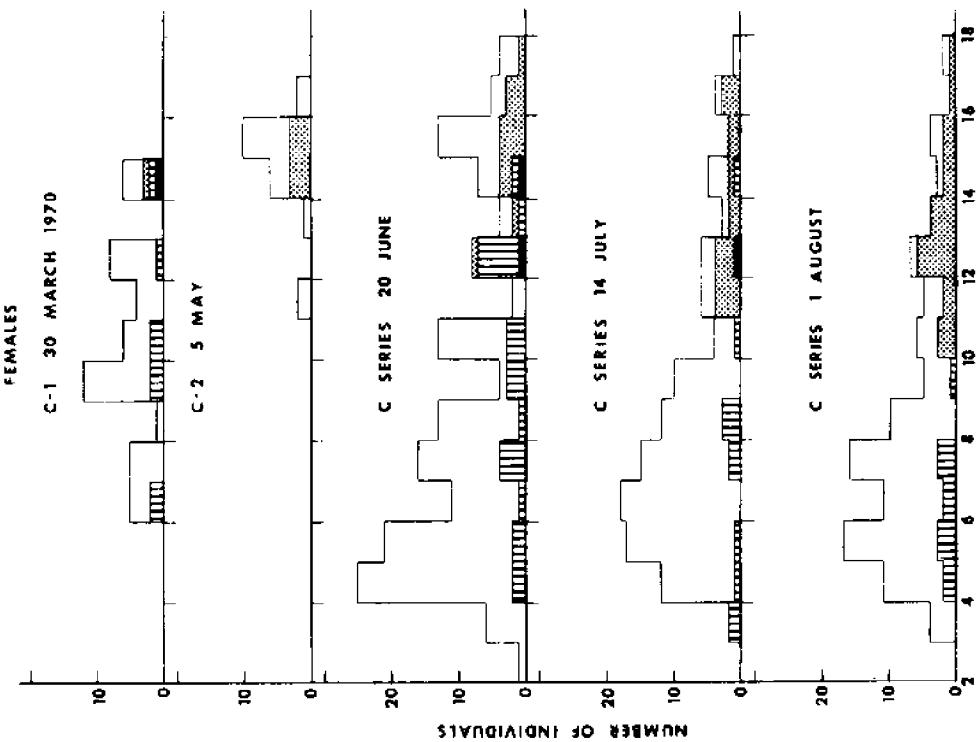

Fig. 4a. Length-frequency histograms of female Palaemonetes in control ponds;
non-ovigerous P. pugio, ▨ ovigerous P. pugio,
▨ non-ovigerous P. vulgaris, ■ ovigerous P. vulgaris.

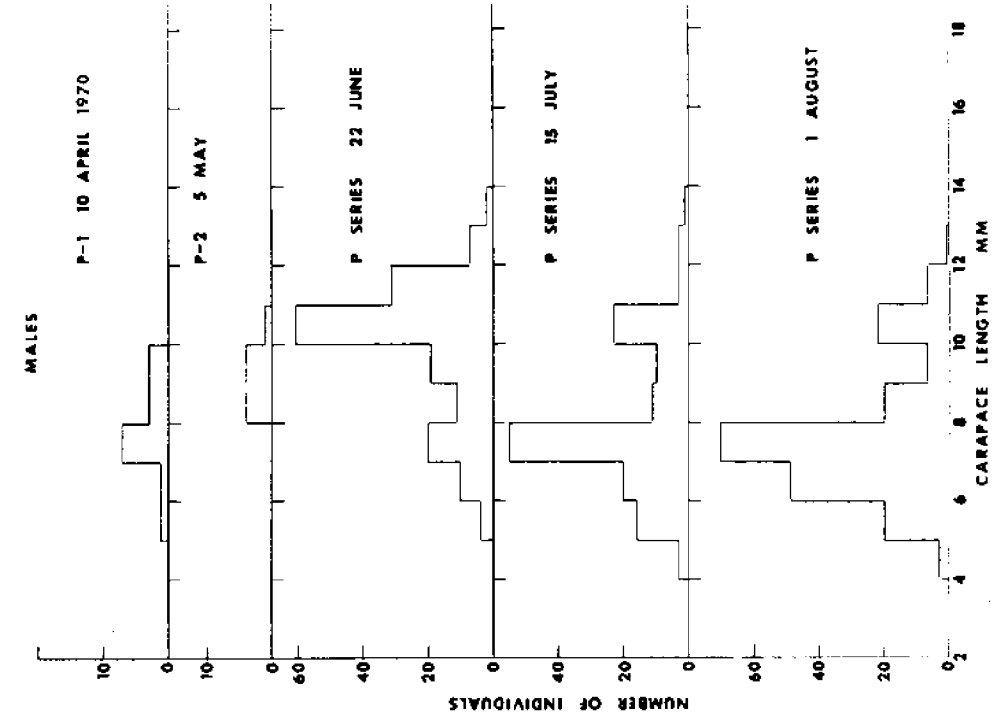

Fig. 4d. Length-frequency histogram of male Palaemonetes pugio in waste ponds.

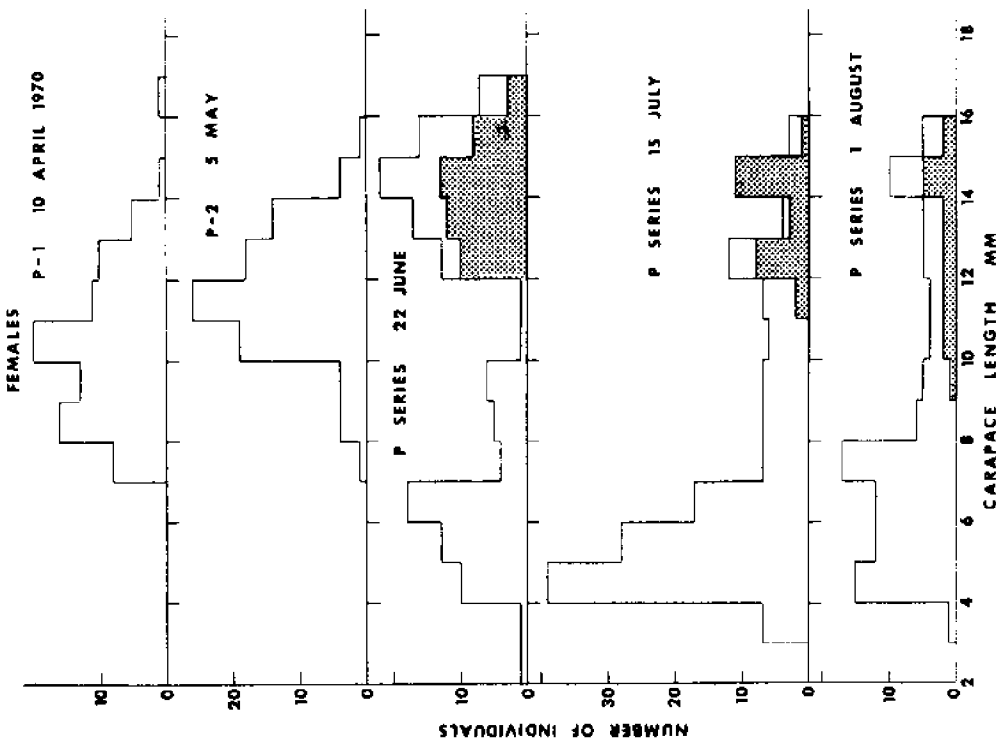

Fig. 4e. Length-frequency histogram of female Palaemonetes pugio in waste ponds; ▒ non-ovigerous, ▒ ovigerous.

REEF POPULATIONS OF MUD CRABS AND SNAPPING SHRIMP

by Eugene Walton and Austin B. Williams
Department of Zoology and Institute of Marine Sciences
University of North Carolina

INTRODUCTION

During autumn 1968, concrete block and scallop shell reefs were constructed in a set of six artificial estuarine ponds at Morehead City, North Carolina (Odum and Chestnut 1970). Three experimental ponds (designated P-1, P-2, and P-3) received water from Calico Creek mixed with effluent from the Morehead City Sewage Treatment Plant, and three control ponds (designated C-1, C-2, and C-3) received water from Bogue Sound diluted with Morehead City tap water. Rates of flow and salinities, maintained by means of regulated pumps, were set to simulate average estuaries in North Carolina (Odum and Chestnut, 1970). By summer, 1970, associations in the reef environments had been developing for nearly two years. This study, conducted from June to Oct. 1970, was a qualitative and quantitative investigation of the decapod crustacean populations existing primarily in the reefs. Block and shell reef populations were compared within each pond, within each subset of three ponds (C and P series), between the two subsets, and with populations living in similar habitats in Bogue Sound and Calico Creek for the following: species diversity, population density, biomass, age composition, dispersal activity, and life history. Influences on the populations resulting from seeding procedures prior to and during the study (Odum and Chestnut, 1970) and dissolved oxygen fluctuations observed during the study are assessed.

Sampling of the shell and block reefs in the ponds produced significant numbers of three species of decapod crustaceans, the xanthid mud crab Eurypanopeus depressus (Smith), the snapping shrimp Alpheus heterochaelis Say, and the grass shrimp Palaemonetes sp. Palaemonetes occur elsewhere in the ponds and have been investigated separately (Beeston, 1970; and in this Annual Report). Thus, this was essentially a study of E. depressus and A. heterochaelis populations.

MATERIALS AND METHODS

The shell and block reefs are arranged from shore outward for approximately 25 feet in each of the ponds (Odum and Chestnut, 1970). They lie in water from a few inches to three feet in depth. The decapod crustaceans living in the reefs could not be effectively captured for population studies by seining or use of baited minnow and crab traps because of small size, feeding behavior, and propensity for living in interstices. Hand sampling was satisfactory for the block reefs but not for the shell reefs where a modified lift net proved effective.

Screen Sampling

Expanded metal screening of 15 mm mesh overlayed by plastic screening of 1 mm mesh stapled to a wooden frame of 3/8 X 1 1/2-inch lumber was fabricated into a rectangular screen measuring 49.0 X 59.5 cm giving an area of 1/4 m^2. The screen could be fitted with wire handles and a marking float, positioned in an area raked clean of shells, then recovered with shells. Screens, with contents, were sampled later by grasping their handles and lifting them to the surface. Mud crabs and snapping shrimp that had migrated into shells overlying the screens

were then hand picked from the exposed shells and screen for study. Higer (1967) used a similar technique to sample shallow-water fishes quantitatively in Everglades National Park, Florida. A preliminary sample taken with the aid of such a screen on June 20 was satisfactory, eight were constructed, and sampling began on June 27.

For the purpose of this study, one week was found to be the optimum interval between taking samples. F. J. Schwartz (personal communication) used oyster baskets filled with shells in a study of xanthid crabs at the Chesapeake Biological Laboratory on the Patuxent River, Maryland. He found no statistically significant differences in species composition or number of xanthid crabs from samples taken at 7, 14, or 21 day intervals. Shell-reef samples in the ponds were taken at nearly weekly intervals from June 27 until Oct 6. Screens were occasionally moved about on the reef for a measure of distribution. A second screen was placed in C-2 on July 9 for crab dispersal and distribution studies and examined weekly thereafter Screens in all ponds were always sampled on the same day, but order of sampling varied. The regular weekly sampling was conducted at night on Sept. 15. Screens were removed from P-1 and P-3 on July 27 after samplings failed to produce any organisms of interest to this study.

All xanthid crabs were identified using a key from Williams (1965). They were sexed, measured for maximum carapace width, weighed wet after being blotted with paper towels, and returned to the shell reefs. After width-weight relationships were established, recording of individual wet weights was discontinued.

Individual snapping shrimp from each sampling of the shell reef in C-1 (the only shell reef with an abundant population of snapping shrimp) were measured from rostral tip to telson tip (considered as total length). The total sample of shrimp was weighed wet after blotting with paper towels, and the shrimp were then released on the shell reef. Not every small snapping shrimp from pond or comparison area outside the ponds was identified to species, but they were visually checked during handling and appeared to be Alpheus heterochaelis.

Biomass, Dispersal, and Hydrophone Studies

Selected E. depressus collected from shell reefs of the C ponds were oven dried at 80°C until weights stabilized. Average dry weights were then determined.

Marking experiments for population estimates and dispersal studies were begun in late June. Trials of paints, nail polishes, and ink from marking pens proved unsatisfactory, and the crabs were too small for metal or plastic tags to be used A method of anterolateral tooth clipping used by Edward (1958) on a shore crab was employed successfully. Wooden stakes were driven at the center of each shell reef of the C ponds on June 29. Releasing of crabs with certain teeth clipped at the stakes provided a basis for measuring dispersal. Thereafter, all crabs from pond samples were checked for marks.

Areas of shell reefs in the C ponds were determined by walking the perimeter of the reefs in soft-soled shoes and using a stretched cord to measure lengths and widths. Estimates of total population number and biomass were calculated by multiplying the population number and weight for each $1/4m^2$ sample by 4, and this figure by the total area of the reef in square meters.

The six ponds were investigated with the aid of a hydrophone on Aug. 12-15, as were areas of Calico Creek and Bogue Sound, to determine presence of snapping shrimp by listening for their characteristic noise produced by cheliped manipulation.

Block Reef Sampling

The block reefs in C-2 and P-3 were hand sampled on July 24 and 25 respectively A seine of 1/4-inch mesh was stretched around each reef and left staked in place while each block was lifted out of the water and the crabs picked off. When all blocks were removed, a smaller seine of 1/8-inch mesh was pulled through the area enclosed by the larger seine and xanthid crabs were picked from it. Finally, the larger seine was pulled ashore and examined for contents. The block reef was then reconstructed. All crabs taken were keyed to species, sexed, measured, and the total sample wet weight was recorded. The block-reef sample from C-2 was also checked for presence of marked crabs from the shell reef in C-2. All were then marked by anterolateral tooth clipping and scattered over the block reef. On July 27 the block reef in C-2 was again hand sampled, but this time without seines The blocks were lifted and xanthid crabs removed. These were treated as before (keyed, sexed, etc.) and the number of recaptures recorded. Using this information, a Petersen mark-recapture estimate of the total size and wet weight of the E. depressus population was made.

A sampling screen was covered with shells from the shell reef in C-2 on Aug. 6, emplaced in the block reef, and removed later to determine whether the block reef contained populations of small crabs that may have been missed in hand sampling This check was used for each of the block reefs in the C and P ponds after they were sampled by hand. Block reefs were further hand sampled as follows: C-1, Aug. 6; C-3, Aug. 13; C-3 for population estimate, Aug. 16; P-1 and P-2, Sept. 15; C-2, Sept. 16, with recapture sampling for population estimate on Sept. 21.

In addition to the above samples, snapping shrimp from the block reef in C-1 (Aug. 6) and from the screen sample planted there (Aug. 13) were measured for total length, weighed wet, and returned to the block reef.

Comparison Samples

Suitably shelly comparison areas were found at the end of the small pier immediately behind the Institute of Marine Sciences, behind Dewey's Motel (about one mile west of the Institute on Bogue Sound), and at the northern end of Fifth Street near the mouth of Calico Creek in Morehead City. A screen was placed in discarded scallop shells immediately off the small pier and examined on Aug. 3, and in like manner the Fifth Street oyster reef on Aug. 19 and Oct. 1. A shelly area behind Dewey's (intact and broken scallop and oyster shells) was surveyed on Sept. 15. All comparison screens were implanted in water deep enough to insure coverage at normal low tide and sampled for mud crabs and snapping shrimp as they were in shell reefs of the ponds.

Plankton Studies

A number 20 plankton net was hand towed in C-2 on July 9 in a search for xanthid zoeae.

A series of monthly plankton samples made by R. E. Dowds from Mar. to Aug. 1970, in the C and P ponds, Bogue Sound, and Calico Creek were checked for presence of crab zoeae.

A number 20 plankton net was suspended under the supply pipe to C-2 on Sept. 16 and Oct. 1, and P-1 on Sept. 22 and 30. The samples obtained were investigated for content and viability of zooplankton.

Bottom Water Oxygen

Dissolved oxygen concentration, pH, and temperature were measured for a diurnal series of bottom water samples taken from the center of the shell reef in C-3 and P-3 on Aug. 17 beginning at 12:00 midnight. Samples were taken every hour for 24 hours using a Hale water sampler modified by W. J. Woods (personal communication) so that water immediately above the bottom would be driven by hydrostatic pressure differences through two 300 ml BOD bottles into a 4-l plastic bottle. Dissolved oxygen content of the samples was determined by Winkler titrations, pH measured with an automatic pH meter, and temperature taken with a standard thermometer.

A bottom water sample was taken directly over the center of the shell reef in C-3 and P-3 at dawn on Sept. 17 and 24 using Wood's device. Disolved oxygen in these samples was determined by Winkler titrations.

Oxygen Depletion Experiment

Effects of oxygen depletion on 8 E. depressus (2♂, 2♀ experimentals and 2♂, 2♀ controls) were investigated by modifying a method used by Deubler and Posner (1963) on flounder postlarvae. Dense salt water depleted of oxygen by nitrogen saturation was overlaid by less dense surface water saturated with oxygen in an inclined aquarium, allowing animals a choice of oxygen level. The method did not produce sufficiently low dissolved oxygen concentrations for present purposes. Therefore, a closed oxygen depletion system was employed. Approximately 1500 ml of oxygen-saturated water were placed in a 200 ml Erlenmeyer flask along with pieces of wood arranged to provide shelter and climbing surfaces for six E. depressus (4♀, 2♂). A two-holed rubber stopper tightly fitted to the flask was arranged with tubing and clamps so that nitrogen could be alternately bubbled through an air stone in the flask or used to drive water out of the flask for dissolved oxygen analysis. As dissolved oxygen was reduced in the flask by bubbling nitrogen into the water, crab behavior was observed and recorded. Inflow of nitrogen was terminated after four hours, though the flask was kept tightly sealed for another 17 hours. Water samples were periodically withdrawn from the flask for Winkler titrations.

RESULTS

Xanthid Crab Species Found

Williams (1965) indicated that nine xanthid crab species could be expected to occur in habitats in the Bogue Sound area similar to shell and block reef habitats of the ponds (Table 1). Sampling produced only three of these species in the ponds and in Bogue Sound and Calico Creek: Eurypanopeus depressus, Panopeus herbstii H. Milne Edwards, and Neopanope texana sayi (Smith).

Most xanthid crabs taken in the ponds were E. depressus. Estimated total populations and wet weights of this species are given in Tables 2-5 based on determined shell reef areas of 11.25 m^2 for C-1, 9.86 m^2 for C-2, and 13.8 m^2 for C-3. The same information for the block-reef samples in C-2 and C-3 is given in Table 6 along with Petersen mark-recapture estimates of total population size and wet weight. Table 7 summarizes results for samples of E. depressus from the block reef in C-1, P-1, and P-3. Figures 1-8 represent width frequency plots for E. depressus from all screen and hand samples in the ponds.

Shell reef areas were not determined in the P ponds. Two E. depressus were taken on the shell reef in P-2 on June 7. One was a gravid female with a maximum carapace width of 17 mm and a wet weight of 1.7 g, the other was a male of 14 mm width and 0.7 g wet weight. These were the only xanthid crabs found in shell reefs of the P ponds during the study.

Two Panopeus herbstii were found on July 24 in the block reef in C-2, having maximum carapace widths of 40 and 29 mm and respective wet weights of 22.0 and 9.1 g. The larger crab was recaptured on July 27. One 24-mm wide male P. herbstii weighing 3.8 g was hand picked from the C-1 block reef on Aug. 6. Two P. herbstii were found on Aug. 13 in the block reef on C-3, one a 40-mm male and the other a 27-mm gravid female. These crabs had a total wet weight of 40.0 g, and the female was recaptured on Aug. 16. One juvenile P. herbstii was captured in the shell reef of C-2 on Aug. 28, and two Neopanope texana sayi were found there, one a 3-mm juvenile on Aug. 20 and the other a 7-mm male on Sept. 9.

Relative numbers of the three species of xanthids were more evenly distributed in comparison samples from Calico Creek and Bogue Sound than in the ponds (Table 10). Width-frequency distributions for E. depressus taken in these comparison samples are given in Figure 8.

Dry Weight and Dispersal Studies

The relationship of maximum carapace width to wet weight of E. depressus is given in Figure 11. The same crabs were used in determining dry weights, which are presented in Table 11. Average weight loss per crab after drying was 58.2%.

Two E. depressus captured on the block reef in C-2, July 24, had been marked and released a week earlier on the shell reef in C-2. The two reefs are about 10 m apart. Crabs were repeatedly recaptured within a week 1 1/2 m from where they had been released after marking.

Snapping Shrimp

Snapping shrimp in the ponds were found only in the shell and block reef of C-1 in abundance. A few were taken from the block and shell reef of C-2. None were found in the other ponds. The hydrophone study of Aug. 12 to 15 confirmed presence of substantial numbers of snapping shrimp in C-1 and indicated a smaller though still significant population of snapping shrimp in C-2. No snapping shrimp noise was heard in C-3. A continual crackling caused by snapping shrimp was heard on the hydrophone in Bogue Sound. No snapping shrimp noise was heard in the P ponds or in Calico Creek.

Table 8 gives measured and calculated data for snapping shrimp from screen samples of the shell reef in C-1. Estimated total populations and wet weights based on the reefs calculated area are included. Similar data for the Aug. hand sampling of the block reef in C-1, the Aug. 13 screen sampling of this block reef, and the Aug. 3 comparison sample off the small IMS pier is presented in Table 9. No other comparison sample yielded snapping shrimp.

Length-frequency distribution for snapping shrimp from the regular samples in the shell reef in C-1 are given in Figure 9. Figure 10 shows length-frequency distributions for snapping shrimp from the Aug. 6 block reef hand sample and Aug. 13 screen sample C-1, and the Aug. 3 small IMS pier sample.

Plankton Studies

Numerous copepods but no crab zoeae were found in plankton samples from C-2 on the night of July 9. However, R. E. Dowd's monthly samples contained numbers of crab zoeae beginning with the samples of May 29. Two xanthid zoeae were found in the May 29 C-pond samples, but the rest were grapsids, mostly fiddler crab, Uca sp. Most of the biomass from the sample taken in Calico Creek on the night of July 31 was composed of zoeae of Uca sp.

Only one living copepod was seen in a 100 ml suspension of plankton taken from the net suspended all night under the C-2 inflow pipe on Sept. 16. The sample contained much sand and detritus. However, similar samplings in P-1 on Sept. 22 and 30, and in C-2 on Oct. 1, produced significant quantities of living material, mostly copepods. A living Uca sp. zoea was identified from the Sept. 22, P-1 sample.

Bottom Water Oxygen Samples

Dissolved oxygen, temperature, and pH data for the Aug. 17 bottom water diurnal study are given in Figure 12. Similar information is recorded in Table 12 for the C-3 and P-3 bottom-water samples taken at dawn on Sept. 17 and 24.

Oxygen Depletion Experiment

Dissolved oxygen concentration reached a minimum of 1.42 mg/l in the oxygen depletion experiment using Deubler and Posner's (1963) technique. Two hours of exposure to this condition produced no abnormal behavior in three of the four experimental crabs. A gravid female began to "fan" her abdomen when dissolved

oxygen concentration was lowered to 1.9 mg/l, and continued to "fan" occasionally until dissolved oxygen concentration rose above 1.9 mg/l three hours later. No abnormal behavior was observed in the control crabs.

Data resulting from the sealed flask experiment are summarized in Table 13 along with observations of crab behavior during the experiment. Dissolved oxygen concentration was driven below 0.3 mg/l and held for more than four hours.

DISCUSSION

This brief investigation was exploratory in nature, and the discussion of results should be read with this reservation in mind.

Species Diversity

There was an obvious difference in decapod crustacean reef fauna in each of the ponds, but only four species occurred. The C series had a denser and more varied population than the P series. Eurypanopeus depressus predominated over other crab species. Only seven other mud crabs (five Panopeus herbstii, and two Neopanope texana sayi) were taken in the ponds, all in the C series. The snapping shrimp, Alpheus heterochaelis, was confined unequally to two ponds in the C series. Moreover, the mud crab and snapping shrimp populations in the C series were different in each pond and should be considered as separate entities, not as similar parts of a general block or shell reef fauna All of these species were found in comparison samples from nearby natural waters in Bogue Sound and Calico Creek but in different proportions than in samples from the ponds.

Differences in species composition of the reef populations indicate that seeding was not uniform. Sources of crustaceans seeded, both by pumping and distribution of materials from plankton towing, were Bogue Sound and Calico Creek. The ponds were heavily hand seeded with plankton from tows in spring, 1969 (Odum and Chestnut, 1970), but not in 1970. M. D. Beeston (personal communication) attributed a change in populations of crabs and shrimp from 1969 to 1970 to lack of plankton seeding program in 1970, and to failure of the pond pumps to deliver an adequate supply of viable shrimp and crab larvae. Relatively large numbers of snapping shrimp in C-1 and E. depressus in all the C ponds possibly indicate breeding populations established early in the history of the ponds.

A plankton study was directed toward determining if xanthid zoeae could be found in the ponds, Bogue Sound, or Calico Creek in abundance. The few xanthid zoeae found in R. E. Dowd's plankton samples, and their absence from night-long plankton samples taken at outfalls of supply pipes to P-1 and C-2, indicated that zoeae have not been added effectively to the ponds by regular pumping Zooplanktonic crustacea can withstand passage through the pumps, but most of the survivors are copepods and nauplii, smaller and perhaps less fragile than larvae of larger forms.

Population Analysis of Pond Reefs

Crabs

When sampling began in late June, the shell reef in C-2 had a population of E. depressus that was more numerous and smaller in mean carapace width than populations in C-1 or C-3 (Tables 2-5). Only a few individuals with carapace widths of less than 10 mm were found during the summer in the shell reefs of C-1 or C-3, but the mean width of those in the shell reef of C-2 was generally near 10 mm. Mean widths increased slowly during summer in C-1 and C-3, but not in C-2.

Among E. depressus from block reefs, mean width of two samples in C-2 was considerably lower than that of the sample in C-3 (Table 6). The second sample from C-2 had a slightly lower mean width than the first.

The population and biomass of E. depressus in the shell reefs of all C ponds experienced a slow decline until a sharp drop began in mid August (Tables 2-5). The Sept. 16-21 mark-recapture sample from the block reef in C-2 yielded a population estimate approximately 50% as large as the population estimate of July 24-27 (Table 6). This drop was not as severe as that in the shell reefs of C-2.

F. J. Schwartz (personal communication) noted sharp drops in a population of xanthid crab, Rhithropanopeus harrisii (Gould), from the last week in July to the second week in August during each of four year's study in Chesapeake Bay. Schwartz hypothesized migration into deeper water as the reason for this decline. In contrast, E. depressus was most abundant during winter in his samples. The E. depressus population in shell reefs of the C ponds probably fell for some reason other than migration.

Sex ratios (Tables 2-5) indicate that the population of E. depressus in the shell reef of C-2 during July had a higher percentage of females than shell reefs in the other C ponds. As populations began to drop, wide fluctuations occurred in sex ratios because of the smaller number of crabs sampled, and value of comparisons among ponds diminished. Populations in the block reefs in C-2 and C-3 had similar sex ratios (Table 6).

The percentage of gravid female E. depressus in shell reefs of C ponds was quite the lowest in C-2 (Tables 2-5). Cycles of egg bearing in each reef seemed independent.

Snapping shrimp

Alpheus heterochaelis contributed importantly to biomass of the shell and block reefs in C-1. Data from hand sampling of the C-1 block reef on Aug. 6 suggested that snapping shrimp composed a larger percentage of the total biomass than did E. depressus (Tables 7 and 9). In July, biomass of snapping shrimp samples from the shell reef in C-2 constituted 1/3 to 1/2 that of E. depressus samples (Tables 2 and 8), and by late Sept. were approximately 2/3 that of the crab. Two hand surveys of the block reef in C-2 produced some snapping shrimp, and the hydrophone study indicated a sizeable population; however, the population in C-1 was undoubtedly larger

The population of snapping shrimp in the shell reef in C-2 increased in number and biomass during July while the mean total body length was decreasing (Table 8), caused by recruitment of small individuals which lasted until Sept. (Fig. 7). A distinct increase in mean total length was observed during Sept.

Mean length of the hand sample of snapping shrimp from the block reef of C-1 on Aug. 6 was larger than the screen sample of Aug. 13 (Tables 8-9, Figs. 9-10), reflecting difficulties encountered in sampling the smaller shrimp by hand; the screen undoubtedly gave a more representative picture of the population. Presence of a few very large individuals in the hand sample increased its wet weight 7 to 10 times over that of comparable numbers from screen samples taken on the shell reef.

Snapping shrimp in the shell reef in C-1 did not experience the late summer decline in population size (Table 8) noted for E. depressus. Knowlton (1970) reported that A. heterochaelis could be found year round in Bogue Sound and that there is no evidence for mass mortality or of decline in population during the coldest or warmest months.

Mark-recapture

Mark-recapture experiments with E. depressus in the C ponds indicated that crabs migrate about on the shell reefs, but the methods employed could not give good estimates of distances traversed per unit time. A screen placed in the shell reef of C-1 was cleared of crabs on Aug. 15 and resampled on Aug. 16; the number of crabs taken from the screen on the second day (8) was only one less than the day before (Table 2) indicating rather rapid migration into a cleared area in one day. Similarly, 34 Alpheus heterochaelis were cleared from an emplanted screen on Aug. 15 and 32 recovered from it on Aug. 16, showing that snapping shrimp also move about over the shell reef. The movement in one week of two E. depressus from a mark-release point on the shell reef of C-2 to recapture on the block reef 10 m away (Jul. 24) suggests some movement between reefs. However, these two crabs composed only a small fraction of the total marked in the shell reef.

Comparison Areas

One goal of this study was to compare decapod crustacean populations of similar habitats in Bogue Sound and Calico Creek to those of the ponds. This goal was never fully realized because of the lack of truly similar habitats in Bogue Sound or Calico Creek. The ponds by design have no significant water level changes or currents (Odum and Chestnut, 1970). Shelly or rocky areas along the shoreline of Bogue Sound or Calico Creek are exposed to tidal changes and currents. Salinities in the ponds generally are held between 15-20 $^{o}/oo$ and are thus lower than salinities usually found in Bogue Sound or Calico Creek (Odum and Chestnut, 1970). The comparison areas chosen were all shelly areas; a suitable rocky area for comparison with block reefs of the ponds was not found. All comparison areas chosen except the small IMS pier had living oysters in abundance, while the shell reefs in the ponds were basically composed of single valves of scallop shells. These are important environmental differences and could be major contributors to population dissimilarities observed between the ponds and comparison areas.

Densities of xanthid crabs and snapping shrimp in the comparison screens were within the range of densities found in the ponds. However, mean carapace widths of E. depressus were significantly smaller (Table 10, Fig. 8) than in the ponds; the largest E. depressus taken was a 13 mm male. No gravid female E. depressus were taken in comparison screens.

Accuracy of Population Estimates

Population size and biomass estimates of E. depressus based on the two screens placed in the shell reef of C-2 differ markedly for some weeks and agree favorably for others, indicating uneven distribution of the species over the reef. Population trends, however, were similar for the two screens, indicating that the samples allowed reasonable population size and biomass estimates, and reliable indication of population changes.

Environmental Factors

Difference in reef populations in the two pond series may be attributed to a number of environmental factors and exploratory attention was given to three of these: salinity, cover, and dissolved oxygen concentration.

Salinity

Low salinity may exclude Alpheus heterochaelis from the P ponds. Knowlton (1970) reported the approximate salinity range for survival of A. heterochaelis zoeae to be 15-30 °/oo; salinities in the P ponds have remained below 15°/oo for as long as five months (Odum and Chestnut, 1970). Xanthids, however, were expected to be more abundant in the P ponds, for several species commonly found in Bogue Sound are capable of tolerating low salinity levels (Williams, 1965).

Cover

Lack of suitable cover is one explanation for the small population of E. depressus found in the P ponds. The substrate of these ponds is soft mud unfitted for supporting any heavy object and considerably softer than the substrate of the C ponds. Consequently, the block reefs have settled deeply into this mud leaving less surface area exposed than in the C ponds. The shell reefs in the P ponds have also settled more deeply into the substrate than they have in the C ponds. Reduction of available crevices for hiding could be a limiting factor.

Dissolved oxygen concentration

The P ponds have been characterized by wide diurnal ranges of dissolved oxygen concentration, often supersaturated during the day but having extremely low concentrations at night (Odum, et al., 1970). Such fluctuation could exert stress on many organisms. Beeston (1970) reported that fish and crabs were killed in June 1969, by low oxygen concentrations when left overnight in a crab trap in P-3. Dissolved oxygen has not fluctuated as widely in the C ponds, and nighttime levels are uniformly higher than in the P ponds (Odum, et al., 1970). Level of dissolved oxygen is therefore a significant environmental difference between the two sets of ponds.

Xanthids present might be vulnerable to low oxygen concentrations. The highest part of shell reefs in the P ponds is only a few cm above the bottom where nighttime dissolved oxygen concentrations could reach lowest levels because of distance from the air-water interface. Portions of block reefs in the P ponds stand somewhat higher in the water column and are not subject to such great stress; however, the entire block reef was periodically exposed to low oxygen levels.

In the 24-hour study conducted to measure dissolved oxygen levels immediately above the bottom over the shell reef in P-3 and C-3 at hourly intervals, oxygen levels in P-3 were lower at night than in C-3, concentrations in both ponds remained rather high through the morning, and heavy cloud cover was responsible for a drop in oxygen production during mid-afternoon (Fig. 12). Care was exercised not to disturb the bottom in either pond, for large amounts of black, anaerobic sediments were easily stirred into suspension above the reef in P-3, which explains the drop in dissolved oxygen concentration to 0 mg/l in the second hourly sample and at 9:00 PM, when all oxygen in water of these samples was consumed in chemical reactions with compounds in the sediment.

Two single bottom water samples in P-3 and C-3 were taken subsequently at dawn when dissolved oxygen should have been near the daily minimum (Table 12). These samples were sediment free, and followed the general pattern established for the ponds, with lower readings in the P series. Dissolved oxygen concentration of 0.12 mg/l over the shell reef in P-3 on Sept. 24 confirmed that organisms in the reef must at least occasionally be subjected to near zero concentrations of dissolved oxygen. Segments of the block reef would be exposed to similar conditions.

In light of these findings, two experiments were conducted to seek information on ability of E. depressus to detect and tolerate low concentrations of dissolved oxygen. In the first, modified from Deubler and Posner (1963), the crabs survived two-hour exposure to oxygen concentrations of 1.42 mg/l, and the only visible reaction was "fanning" of the pleopods by a gravid female.

Results of a second "sealed system" experiment suggested that burrowing might be a response of E. depressus to dropping oxygen levels (Table 13), an understandable behavior for crabs living where low oxygen conditions might be improved by the next tide change. Continued exposure to lowered oxygen was associated with climbing out of the water. The crabs finally began to succumb after being exposed for seven hours to dissolved oxygen concentrations of less than 1.0 mg/l, and all eventually died.

The experiment indicated that several hours of exposure to oxygen levels lower than those periodically reached in the P ponds would be fatal to E. depressus. However, the crabs were still alive after exposure periods that greatly exceeded those usually occurring in the P ponds. A more thorough investigation into the oxygen question is needed to determine importance of low dissolved oxygen levels as an influence on xanthid distribution in the ponds.

Thus, no explanation can be given now for differences in density of E. depressus in the two series of ponds.

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TABLE 1.

LIST OF XANTHID CRAB SPECIES KNOWN TO OCCUR IN AREA COMPARED WITH THOSE CAPTURED DURING STUDY.

Species known from area.

1. Pilumnus sayi
2. Pilumnus dasypodus
3. Menippe mercenaria
4. Rhithropanopeus harrisii
5. Hexapanopeus angustifrons
6. Neopanope texana sayi
7. Eurypanopeus depressus
8. Panopeus herbstii
9. Panopeus occidentalis ?

Species found in samples from Bogue Sound and Calico Creek.

1. Eurypanopeus depressus
2. Panopeus herbstii
3. Neopanope texana sayi

Species found in samples from ponds.

1. Eurypanopeus depressus
2. Panopeus herbstii
3. Neopanope texana sayi

TABLE 2.

RESULTS OF SAMPLING SHELL REEF IN C-1; *E. DEPRESSUS.*

Date	Total N	Mean width carapace; mm ♂	♀	♂+♀	Total wet weight, g	Estimated pop.	Calculated total wet weight, g	Ratio ♂/♀	% ♀ gravid
6-30	12	15.8	12.3	14.1	-	540.0	-	1.0	50
7-7	21	14.5	11.9	13.4	18.0	945.0	811.4	1.3	67
7-14	17	15.5	12.3	14.4	18.4	765.0	828.0	1.8	16.7
7-22	12	16.4	13.6	15.3	14.2	540.0	639.0	1.4	40
7-28	16	16.0	13.0	14.3	15.5	720.0	697.5	.8	33
8-5	17	17.6	13.1	15.8	21.0	765.0	945.9	1.4	42.9
8-11	13	17.8	12.8	16.2	17.2	585.0	774.0	2.3	0
8-15	8	19.0	13.0	16.0	10.1	360.0	454.5	1.0	25
8-16	7	17.5	13.7	15.9	8.0	315.0	360.0	1.3	0
8-20	6	17.5	13.5	14.8	6.6	270.0	297.0	.5	50
8-28	8	19.0	13.3	14.8	12.2	360.0	549.0	.3	50
9-7	8	20	13.4	14.5	6.2	360.0	279.0	.2	80
9-15	7	18.0	14.5	16.0	7.0	315.0	315.0	.7	25
9-22	6	17.7	14.3	16.0	6.5	270.0	292.5	1.0	0
9-29	6	17.5	14.3	15.3	6.2	270.0	270.0	.5	.25
10-6	4	18.7	13.0	17.3	5.8	180.0	261.0	3.0	0

TABLE 3.

RESULTS OF SAMPLING SHELL REEF IN C-3; *E. DEPRESSUS*.

Date	Total N	Mean width carapace, mm ♂	♀	♂+♀	Total wet weight, g	Estimated pop.	Calculated total wet wt., g	Ratio ♂/♀	% ♀ gravid
6-30	4	-	9.7	9.7	-	220.8	-	0	0
7-7	14	16.0	12.8	12.8	9.7	772.8	535.4	.4	40
7-14	6	14.3	12.3	13.3	3.8	331.2	209.8	1.0	33
7-22	10	17.0	12.0	14.5	11.0	552.0	607.2	1.0	80
7-28	7	12.7	14.5	13.7	5.4	386.4	298.1	.7	75
8-5	7	14.6	15.0	14.3	7.4	386.4	408.5	6.0	100
8-11	11	17.5	13.9	15.2	12.5	607.2	690.0	.6	14.3
8-20	10	18.0	13.9	15.1	11.8	552.0	651.4	.4	86
8-28	8	16.0	14.3	15.1	10.5	441.6	579.6	1.0	75
9-7	5	14.5	14.3	14.4	4.8	276.0	264.9	.7	67
9-15	5	13.0	13.8	13.6	3.8	276.0	209.8	.3	100
9-22	4	-	16.0	16.0	3.6	220.8	198.7	-	25
9-29	6	17.5	14.3	15.3	6.2	270.0	279.0	.5	25
10-6	1	20.0	-	20.0	1.8	55.2	99.4	-	-

TABLE 4

RESULTS OF SAMPLING SHELL REEF IN C-2, SCREEN A; *E. DEPRESSUS*

Date	Total N	Mean width carapace, mm ♂	♀	♂+♀	Total wet weight, g	Estimated pop.	Calculated total wet weight, g	Ratio ♂/♀	% ♀ gravid
6-20	101	11.1	9.1	9.8	-	3,983.4	-	.6	19
6-30	61	11.5	9.0	9.9	-	2,405.8	-	.5	15.4
7-7	62	12.2	9.4	10.4	21.8	2,445.3	858.2	.4	6.9
7-14	36	12.3	10.0	10.4	12.3	1,419.8	483.5	.2	7.5
7-22	56	11.9	9.0	9.7	16.3	2,208.6	641.3	.2	16.3
7-28	41	12.0	9.1	10.0	18.7	1,617.0	739.5	.5	0
8-5	55	12.1	9.6	10.4	21.3	2,169.2	840.1	.5	2.7
8-11	27	15.4	9.6	9.9	9.3	1,063.8	366.4	.9	14.3
8-20	10	13.0	9.1	10.3	4.1	394.0	161.2	.4	16.7
8-28	7	8.0	9.7	8.2	2.4	275.8	94.6	.7	50
9-7	11	11.4	9.0	10.1	4.5	433.4	179.3	1.2	0
9-15	8	10.2	12.5	10.9	2.8	315.2	110.3	3.0	50
9-22	6	11.8	13.0	12.0	2.4	264.0	94.6	5.0	0
9-29	1	-	8.0	8.0	.2	39.4	7.9	-	0
10-6	1	8.0	-	8.0	.2	39.4	5.9	-	-

TABLE 5

RESULTS OF SAMPLING SHELL REEF IN C-2; SCREEN B; *E. DEPRESSUS*.

Date	Total N	Mean width carapace, mm ♂	♀	♂+♀	Total wet weight, g	Estimated pop.	Calculated total wet weight, g	Ratio ♂/♀	% gravid
7-16	64	10.3	9.4	9.7	20.6	2,542.2	811.3	.6	17
7-22	30	11.9	9.0	10.8	13.2	1,183.2	519.8	.8	0
7-28	34	11.5	9.3	9.7	11.3	1,340.9	443.7	.4	12.5
8-5	33	11.4	9.9	10.3	13.6	1,301.5	536.4	.5	18.2
8-11	33	11.5	9.9	10.6	15.1	1,300.2	594.9	.8	11.1
8-20	10	12.0	9.2	10.3	6.0	394.0	236.4	.7	16.7
9-7	2	12.5	-	12.5	2.1	78.8	81.9	-	-
9-15	5	9.7	9.0	9.4	2.1	197.0	82.7	1.3	0
9-22	1	-	8.0	8.0	.2	39.4	6.3	-	-
9-29	4	11.0	10.0	10.7	1.6	157.6	63.0	1.0	0
10-6	0	-	-	-	-	-	-		-

TABLE 6.

RESULTS OF MARK-RECAPTURE AND SCREEN SAMPLING OF BLOCK REEFS IN C-2 AND C-3; E. DEPRESSUS.

Location	Type of sample	Date	Total N	Mean width carapace, mm ♂	♀	♂+♀	Total wet wt., g.	Ratio ♂/♀	% of ♀ gravid
C-2	hand marking	7-24	203	16.7	12.6	14.0	202.2	.8	2.7
C-2	hand recapture	7-27	36	13.2	13.2	13.2	17.0	.4	0
C-2	screen	8-10	12	14.0	11.1	12.1	8.0	.7	0
C-3	hand marking	8-13	66	20.6	16.4	18.0	125.7	.6	56.4
C-3	hand recapture	8-16	27	19.8	17.5	18.3	41.3	.5	47
C-3	screen	9-15	2	15.0	17.0	16.0	-	1.0	100
C-2	hand marking	9-16	64	14.5	13.6	13.9	49.6	.5	19
C-2	hand recapture	9-21	66	15.0	13.6	12.6	49.5	.7	9.5

	July 24-27 mark-recapture in C-2	August 13-16 mark-recapture in C-3	September 16-21 mark recapture in C-2
N marked and released	191	61	64
N recaptured	36	27	66
N recaptured marked	23	14	22
Estimated pop.	298.9	117.6	192
Calculated total wet wt., g.	297.8	230.9	144.0

TABLE 7.

RESULTS OF SAMPLING BLOCK REEFS IN P-3, C-1, AND P-1; *E. DEPRESSUS*.

Location	Type of sample	Date	Total N	Mean width carapace, mm ♂	♀	♂+♀	Total wet wt., g.	Ratio ♂+♀	% of ♀ gravid
P-3	hand	7-25	4	20.5	17.5	19.0	10.4	1.0	50
C-1	hand	8-6	6	21.0	15.5	18.3	7.6	1.0	0
C-1	screen	8-10	1	18.0	-	18.0	-	-	-
P-1	hand	9-16	7	22.0	17.6	18.9	14.6	.4	60

TABLE 8.

RESULTS OF SAMPLING SHELL REEF IN C-1; SNAPPING SHRIMP.

Date	Total N	Mean total length, mm	Total wet wt., g	Estimated Pop.	Calculated total wet wt., g
6-30	24	18.9	-	1,080	-
7-7	32	18.8	-	1,440	-
7-14	47	16.5	-	2,115	-
7-22	39	15.1	4.5	1,755	202.5
7-28	61	14.4	4.9	2,745	220.5
8-5	45	15.9	4.7	2,025	211.5
8-11	40	15.1	3.7	1,800	166.5
8-15	34	14.5	3.2	1,530	144.0
8-16	32	12.8	2.4	1,440	108.0
8-20	42	15.5	5.1	1,890	229.5
8-28	59	14.3	-	2,655	-
9-7	35	15.5	3.7	1,575	165.6
9-15	55	15.6	5.7	2,475	256.5
9-22	45	16.0	5.2	2,025	234.0
9-29	28	15.9	4.1	1,260	184.5
10-6	36	14.3	3.2	1,620	144.0

TABLE 9.

RESULTS OF SAMPLING BLOCK REEF AND SCREEN IN BLOCK REEF OF C-1 AND SMALL INS PIER; SNAPPING SHRIMP.

Location	Type of sample	Date	Total N	Mean total length, mm	Total wet weight, g
Small INS pier	Screen	8-3	36	14.8	4.0
C-1	Hand	8-6	38	22.5	20.1
C-1	Screen	8-13	17	18.2	2.9

TABLE 10.

XANTHID CRABS TAKEN IN COMPARISON SAMPLES FROM BOGUE SOUND AND CALICO CREEK

Location	Calico Creek bridge	Small IME pier	5th St. oyster reef	Calico Creek	Dewey's Motel	5th St. oyster reef
Date	7-6	8-3	8-19	8-20	9-15	10-1
Method	Screen	Screen	Screen	Hand sampled	Screen	Screen
Total N	2	62	24	5	50	31
Total *E. depressus*	1	8	5	1	14	20
Total *P. herbstii*	1	19	15	4	30	4
Total *N. texana sayi*	--	35	4	0	6	1
Ratio ♂*E. depressus*: ♀*E. depressus*	--	6.0	.67	--	7.0	1.2
Mean width ♂*E. depressus* in mm	13.0	6.4	4.5	--	5.3	6.2
Mean width ♀*E. depressus* in mm	--	7.0	5.0	14.0	5.2	7.3
Total wet weight sample in g	13.4	23.6	18.9	29.0	31.0	8.8

TABLE 11.

DETERMINED DRY WEIGHTS OF E. DEPRESSUS SAMPLES (23 and 26 JULY). NUMBERS OF EACH SIZE FROM SAMPLES GIVEN IN PARENTHESES.

Carapace width mm, and number	Total dry weight, g	Weight per crab, g	Percentage dry weight / wet weight
5 (2)	.027	.014	45.3
6 (1)	.027	.027	53
7 (5)	.224	.045	44.8
8 (5)	.308	.062	36.3
9 (5)	.493	.098	41
10 (5)	.554	.111	34.6
11 (5)	.676	.135	30.7
12 (5)	1.106	.221	43.4
13 (7)	1.958	.280	44.4
14 (5)	1.758	.351	46.9
15 (5)	2.312	.462	45.3
16 (5)	2.688	.538	43.3
17 (5)	3.174	.635	41.8
18 (7)	4.739	.677	37.6
19 (6)	5.738	.956	43.5
20 (6)	6.238	1.040	41.6
21 (5)	6.009	1.202	38.8
22 (3)	3.698	1.233	37.4
23 (2)	3.214	1.607	44.6
		Average	41.8

TABLE 12.

DISSOLVED O_2 IN BOTTOM WATER TAKEN WITH W. J. WOOD's BOTTOM WATER SAMPLER AT DAWN.

Date	Location	Time	Temperature	Dissolved O_2 Concentration in mg/l
9-17	P-3	6:45	28°C	1.7
"	C-3	7:15	32°C	4.5
9-24	P-3	6:15	-	.12
"	C-3	6:45	-	4.3

TABLE 13.

RESULTS OF EXPERIMENT WITH E. DEPRESSUS (4♂, 2♀) SEALED IN FLASK CONTAINING WATER BEING DEOXYGENATED WITH BUBBLING NITROGEN.

Time in minutes	Dissolved O_2 Concentration in mg/l	Significant behavior
0	8.0	Crabs quiet and hidden
5	5.9	Increased activity
15	4.3	Burrowing behavior
25	3.3	
35	2.2	
45	1.2	All crabs burrowed
60	.6	
85	.6	Two crabs climbing air stone
120	.28	Crabs capable of strong movement
200	.28	
220	.28	One crab climbed to near water line
345	.83	Another crab climbing
350	--	One crab out of water
480	--	Two crabs out of water, two more near surface
500	.55	One dead crab, one moving, three out of water
505	--	One crab in distress
635	--	Four crabs in distress
640	.97	Three crabs dead
1301	2.5	All crabs dead, one above water

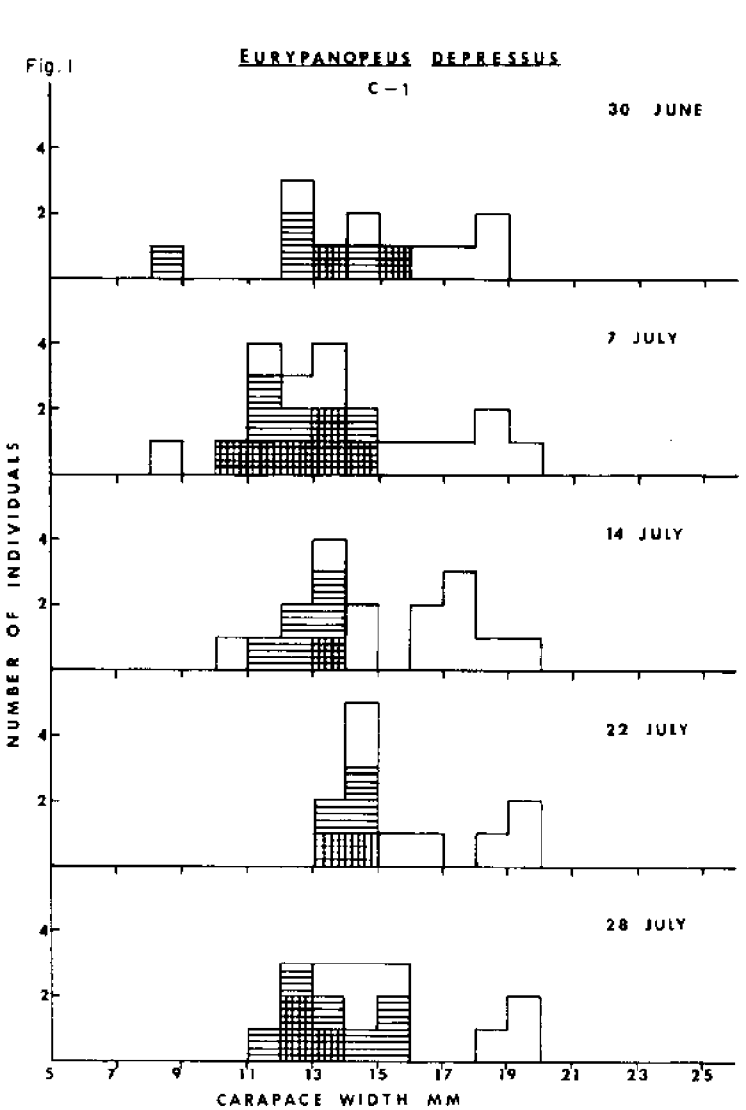
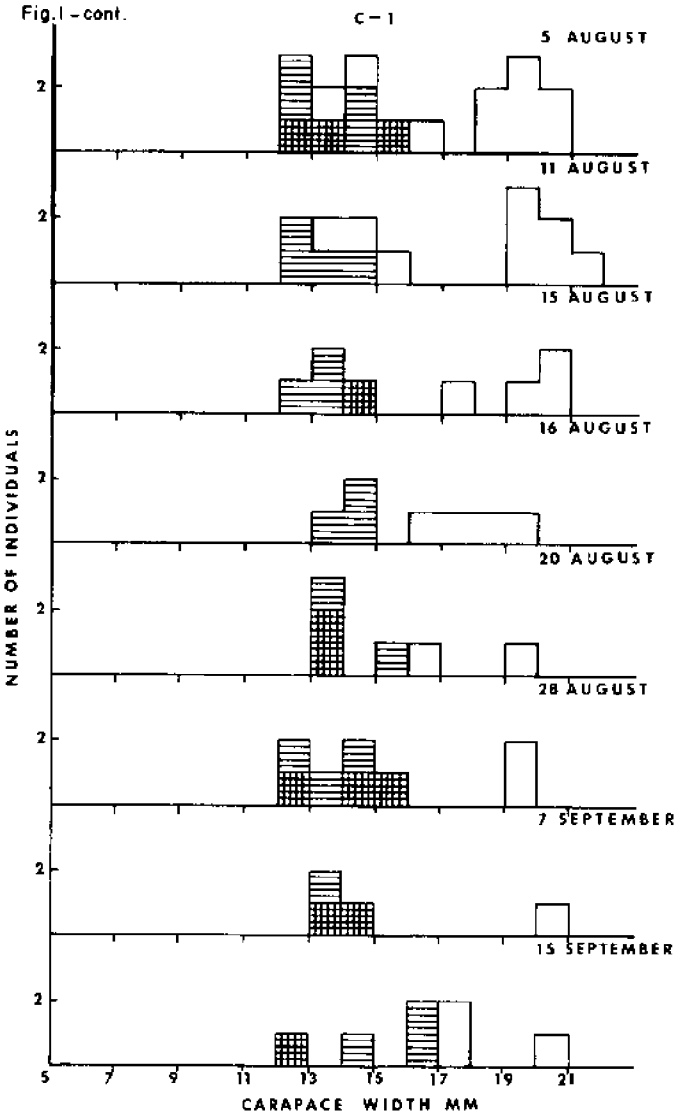
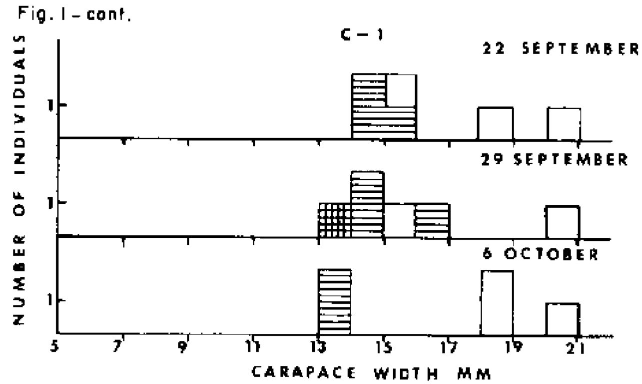


Fig. 1. Width-frequency distribution for E. depressus in screen samples taken from shell reef in C-1. Open bars = males; horizontal lines = females; cross-hatching = gravid females.





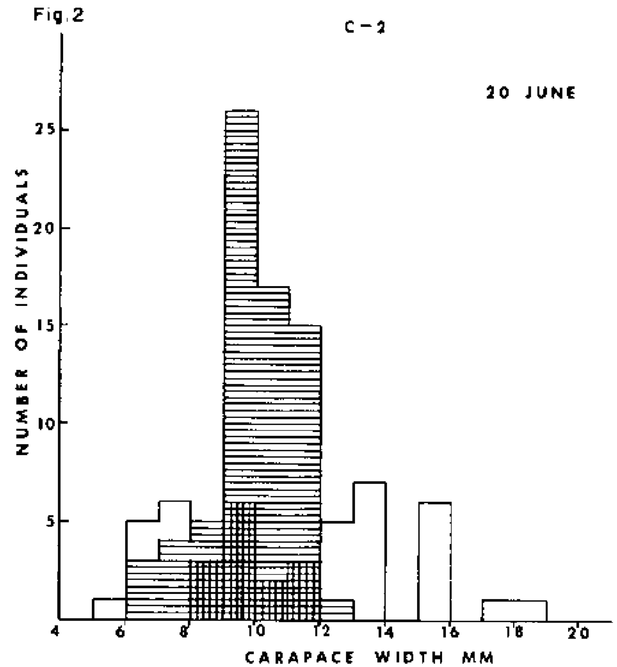
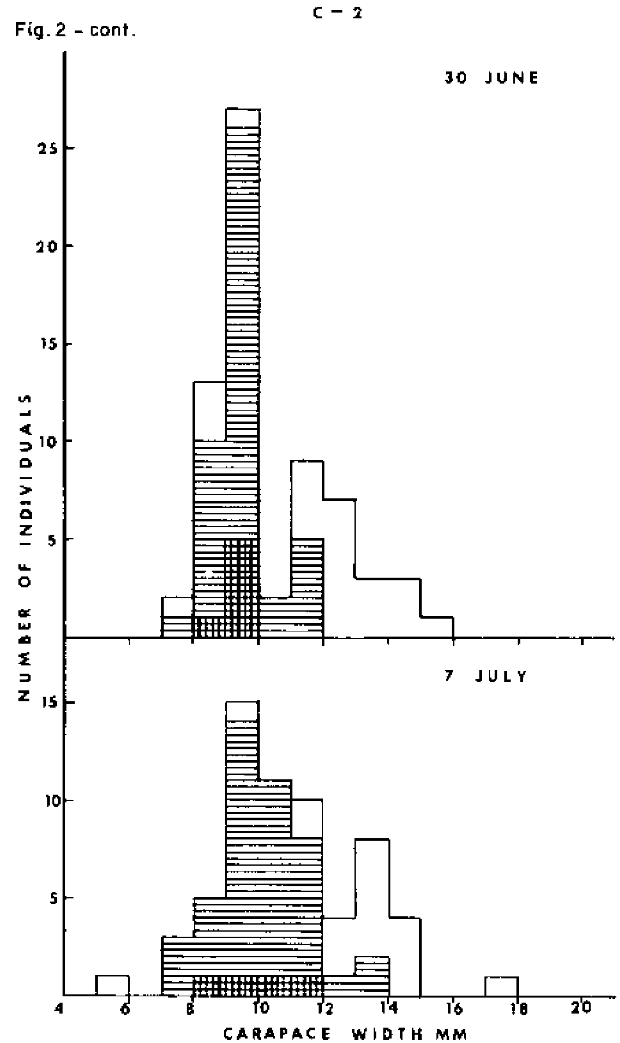


Fig. 2. Width-frequency distribution for E. depressus in screen samples taken from shell reef in C-2. Second screen (a) implanted 14 July. No E. depressus taken on October 6, C-2-a sample. Open bars = males; horizontal lines = females; cross-hatching = gravid females.



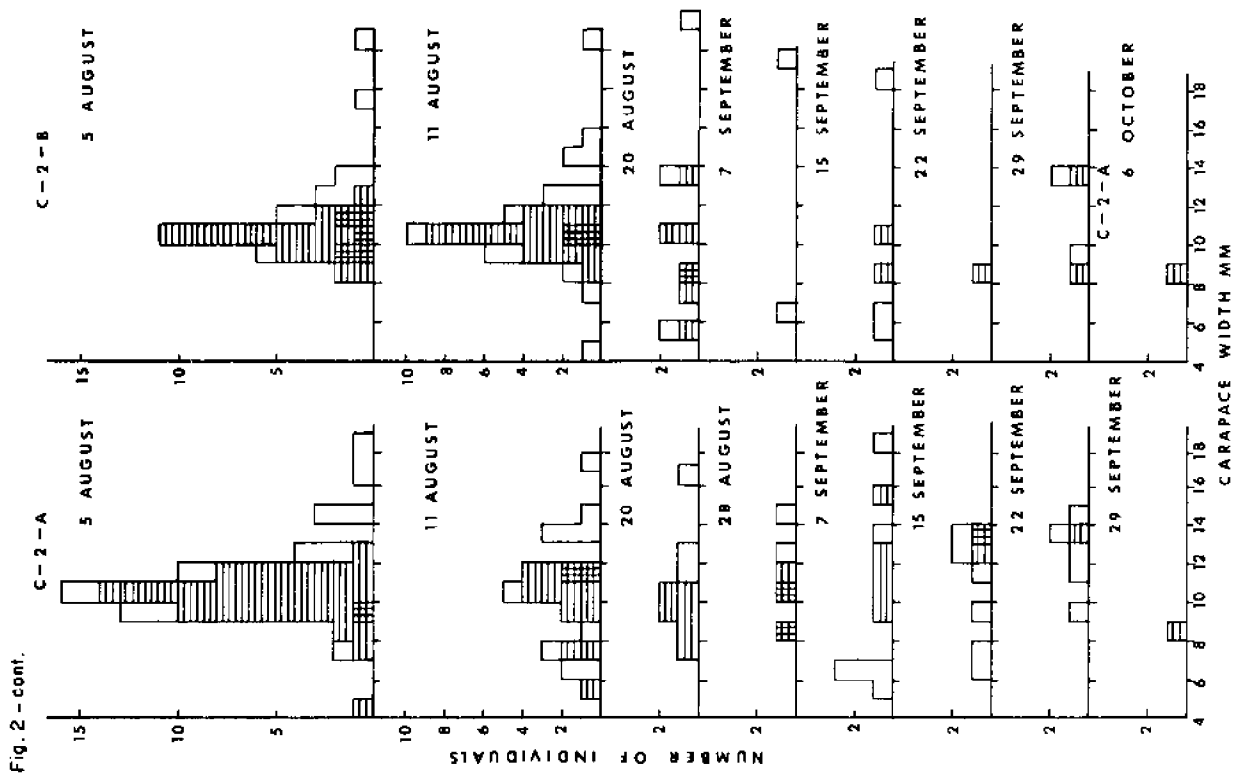


Fig. 2 - cont.

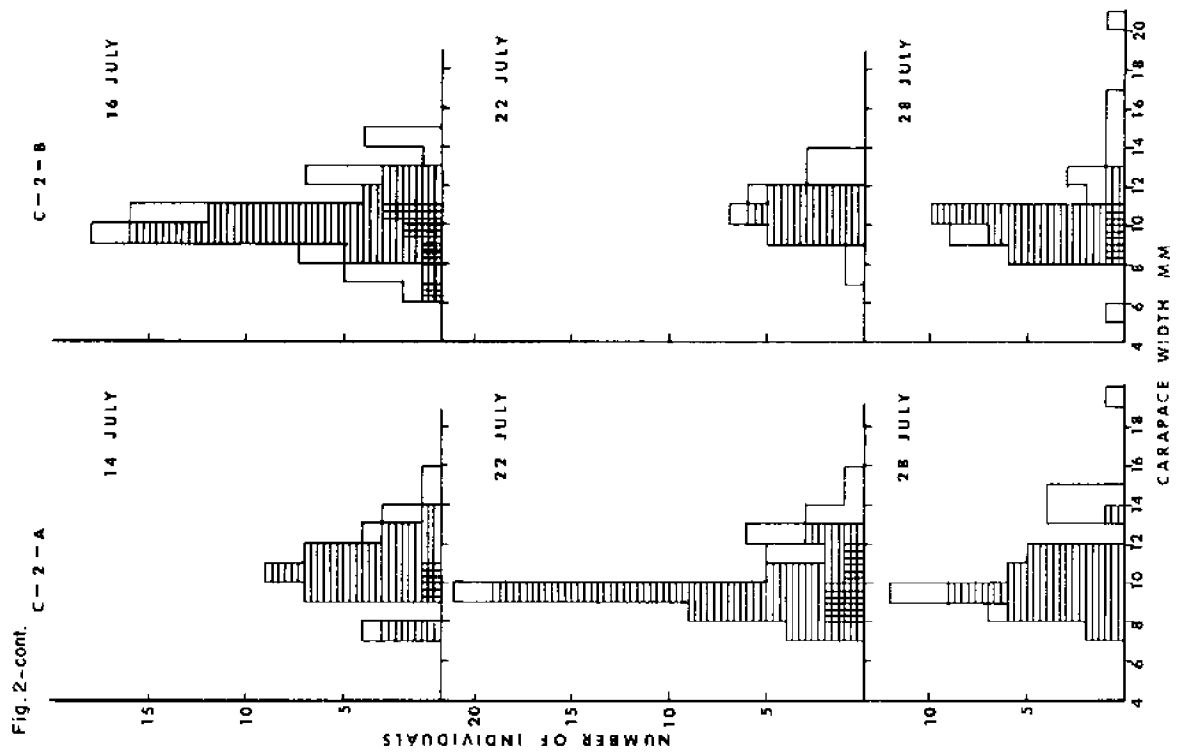


Fig. 2-cont.

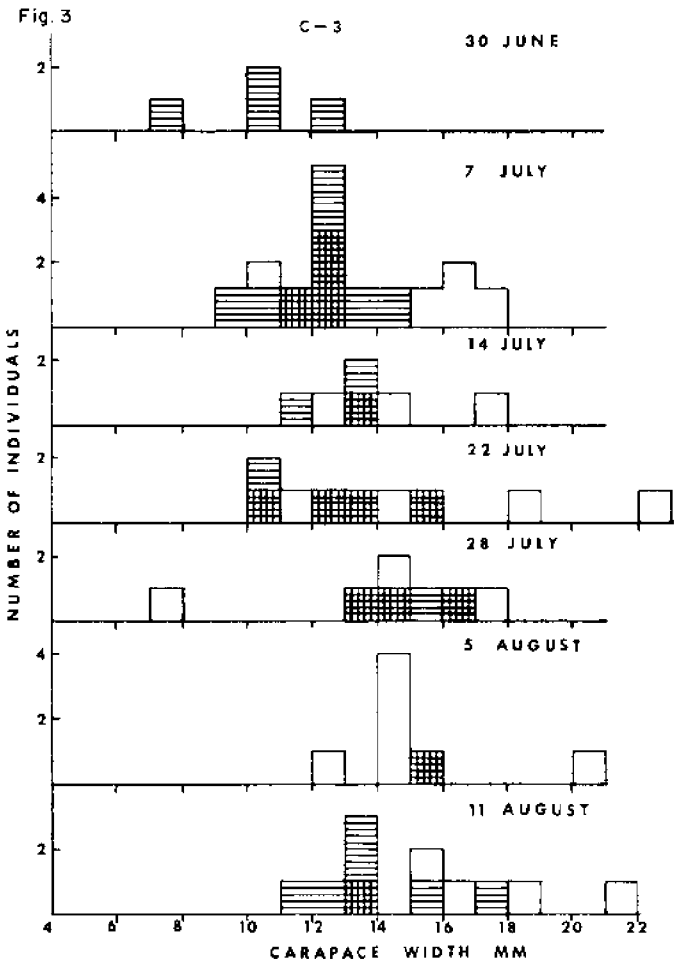


Fig. 3. Width-frequency distribution for L. dorrocsus in screen samples taken from shell reef in C-2. Open bars = males; horizontal lines = females; cross-hatching = gravid females.

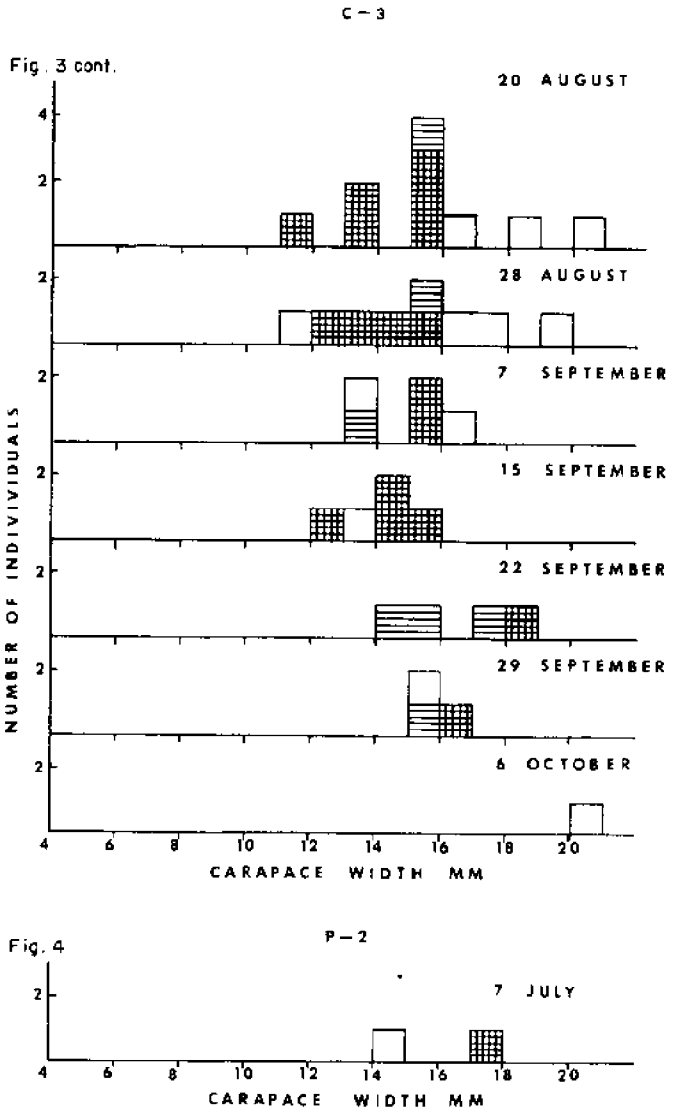


Fig. 4. Width-frequency distribution for L. dorrocsus on July 7 screen sample taken from shell reef in P-2. No other mud crabs taken in P series. Open bar = males; cross-hatching = gravid females.

Fig. 5

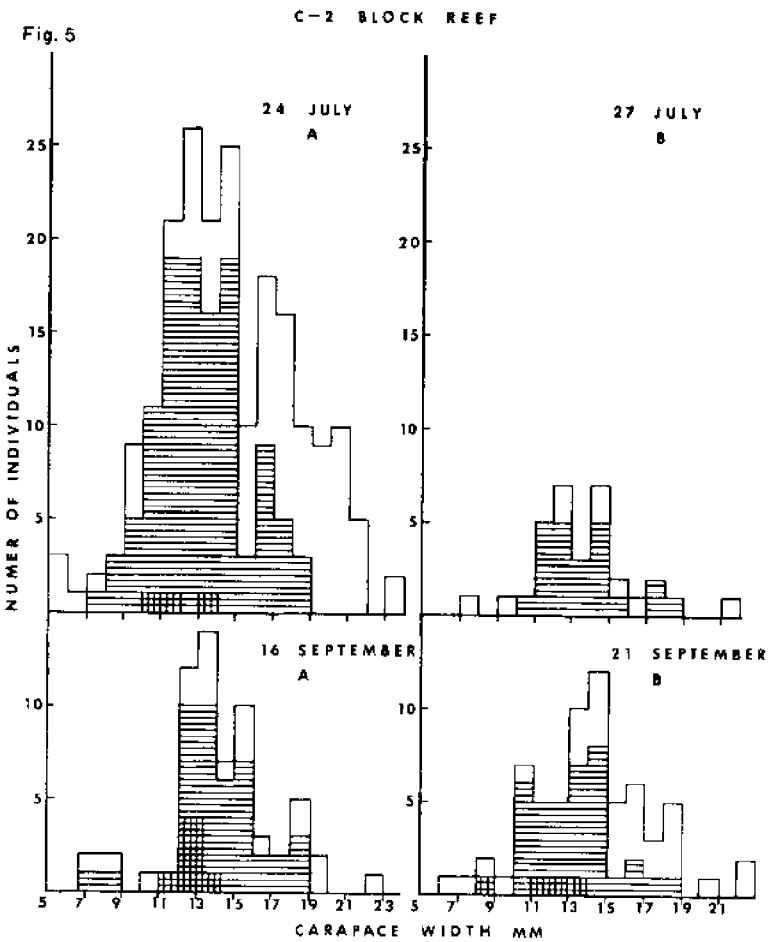


Fig. 5. Width-frequency distribution for C. opilio in sand samples for mark-recapture population estimates taken from block reef in C-2. A = marking sample; B = recapture sample; open bars = males; horizontal lines = females; cross-hatching = gravid females.

Fig. 6

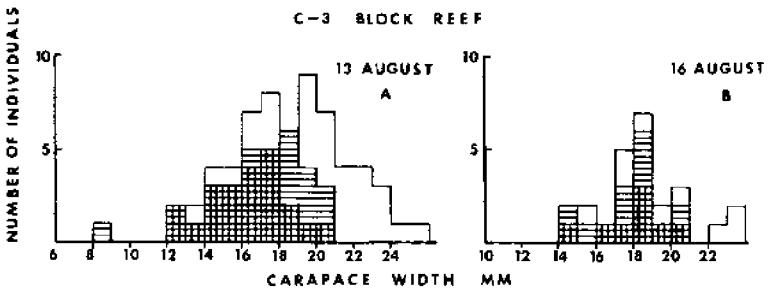


Fig. 6. Width-frequency distribution for C. opilio in hard samples for mark-recapture population estimate taken from block reef in C-3. A = marking sample; B = recapture sample; open bars = males; horizontal lines = females; cross-hatching = gravid females.

Fig. 7

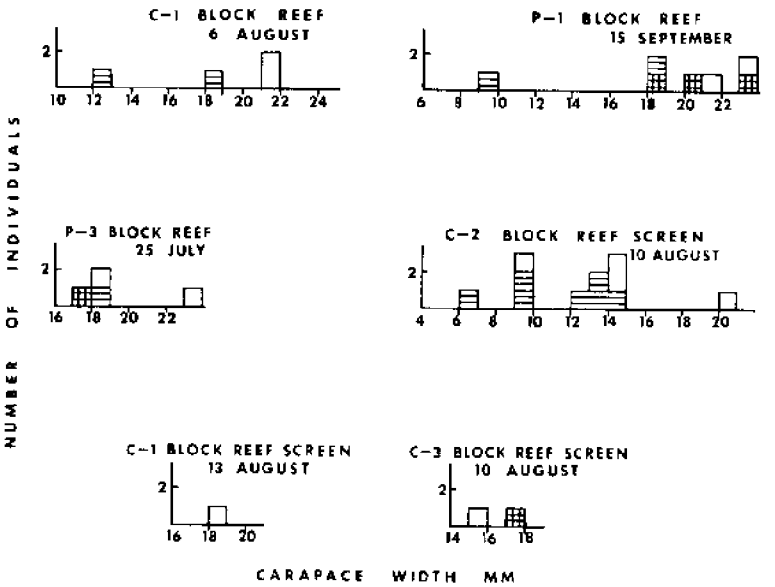


Fig. 7. Width-frequency distribution for C. opilio taken in hard and screen samples taken from block reef of five areas. Open bars = males; horizontal lines = females; cross-hatching = gravid females.

Fig. 8

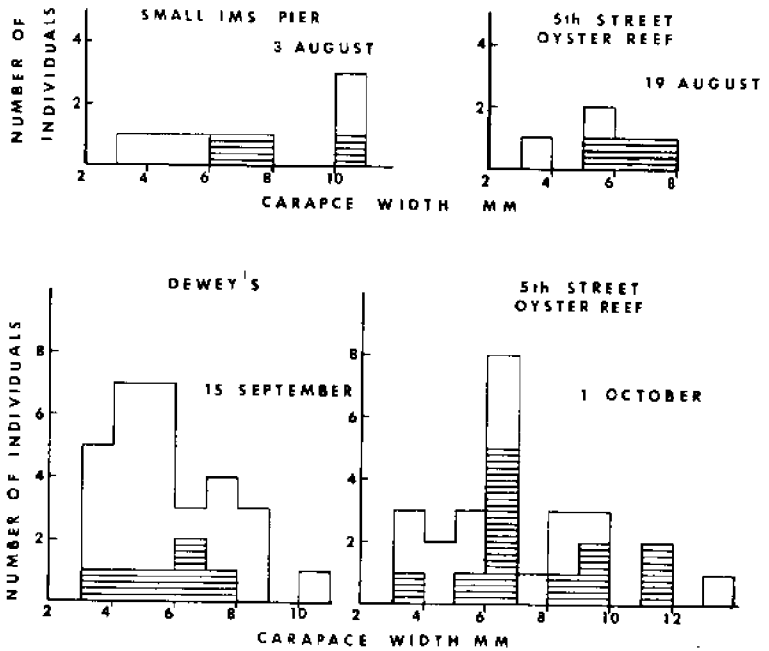


Fig. 8. Width-frequency distribution for [illegible] taken in comparison common samples from Lapu Lapu Count and Dollar Creek. Open bars - males; horizontal lines - females.

Fig. 9

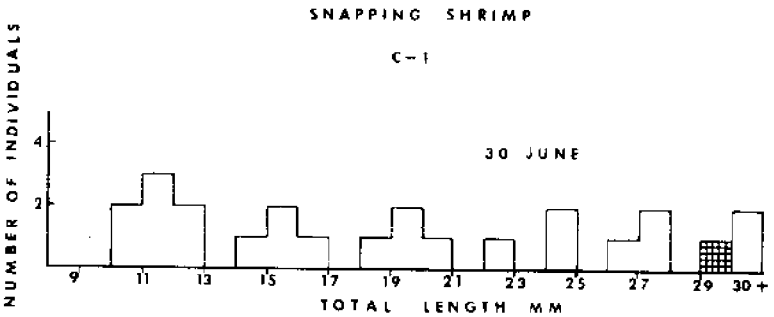
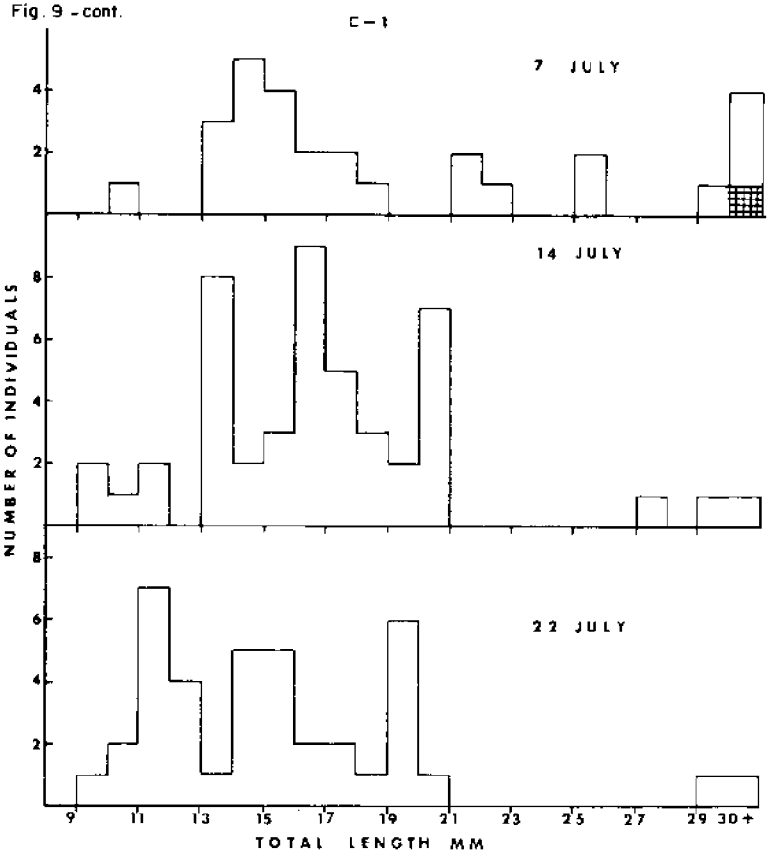


Fig. 9. Length-frequency distribution for snapping shrimp [illegible] in samples taken from shell reef in [illegible]. Cross-hatching [illegible] of color.

Fig. 9 - cont.



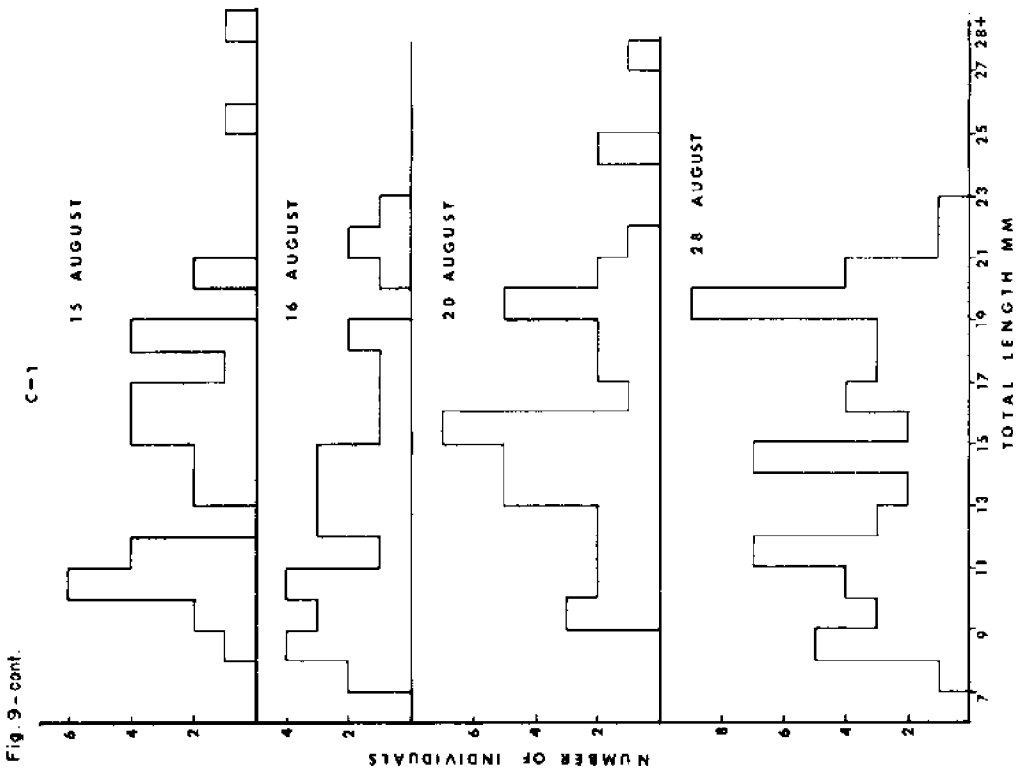


Fig. 9 - cont.

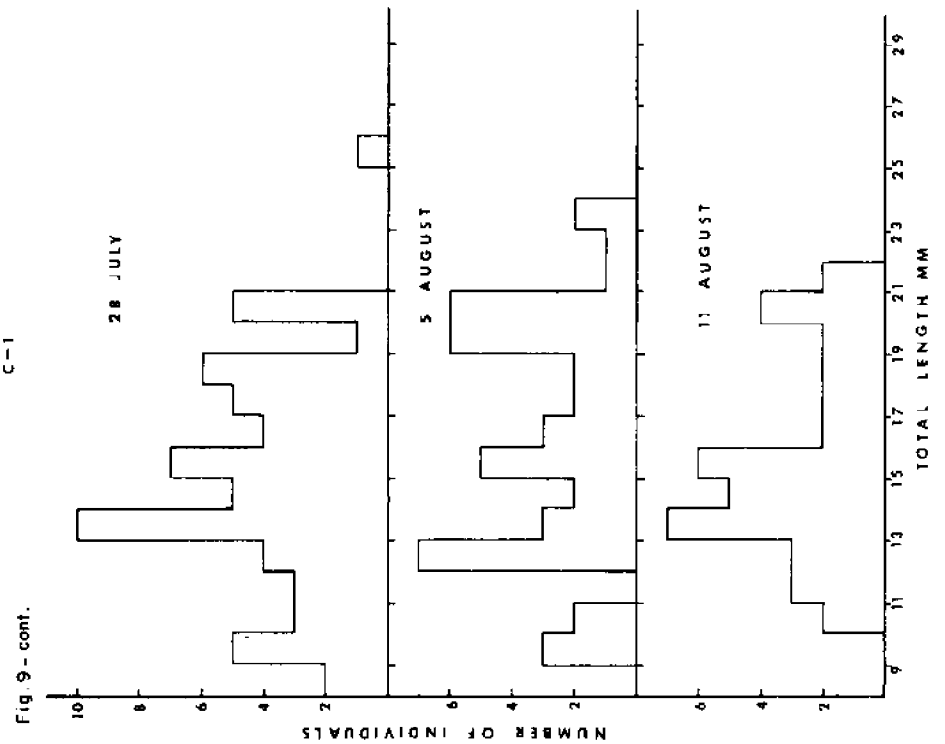
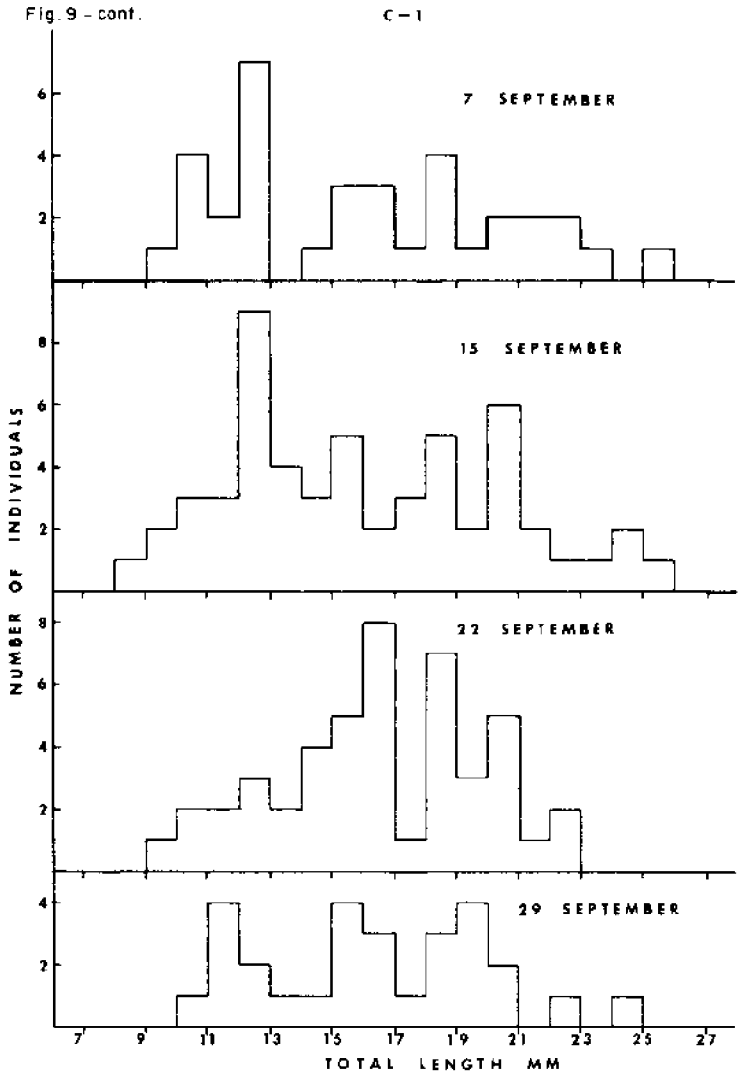
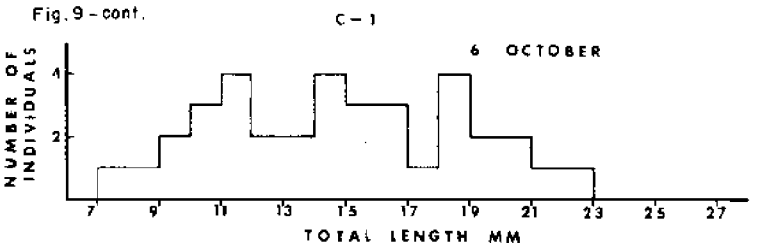


Fig. 9 - cont.





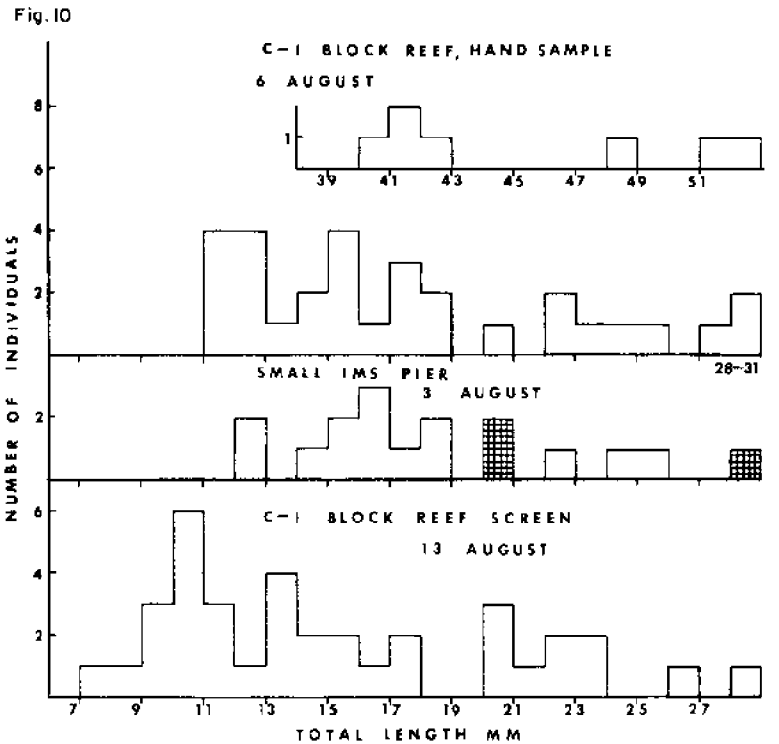


Fig. 10. Length-frequency distribution for snapping shrimp in hand and screen samples of C-1 block reef and in screen sample of small IMS pier. Cross-hatching = gravid females.

Fig. 11

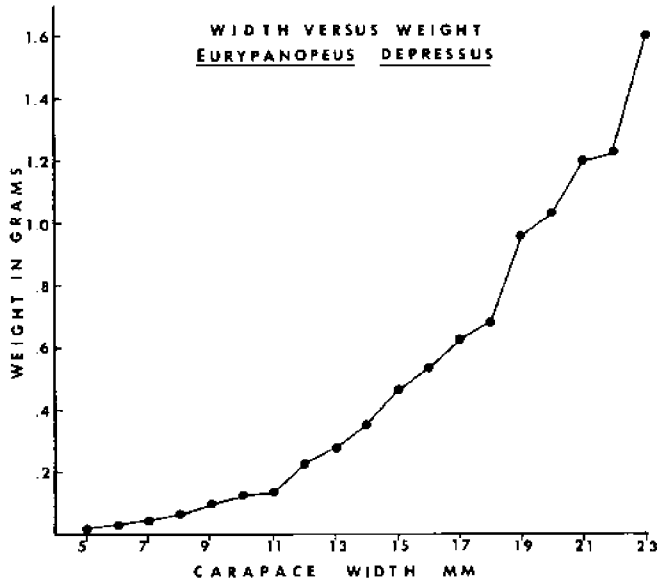


Fig. 11. [illegible]-weight relationships for [illegible] taken on July 22-July 26 hand-picked and [illegible] in [illegible].

Fig. 12

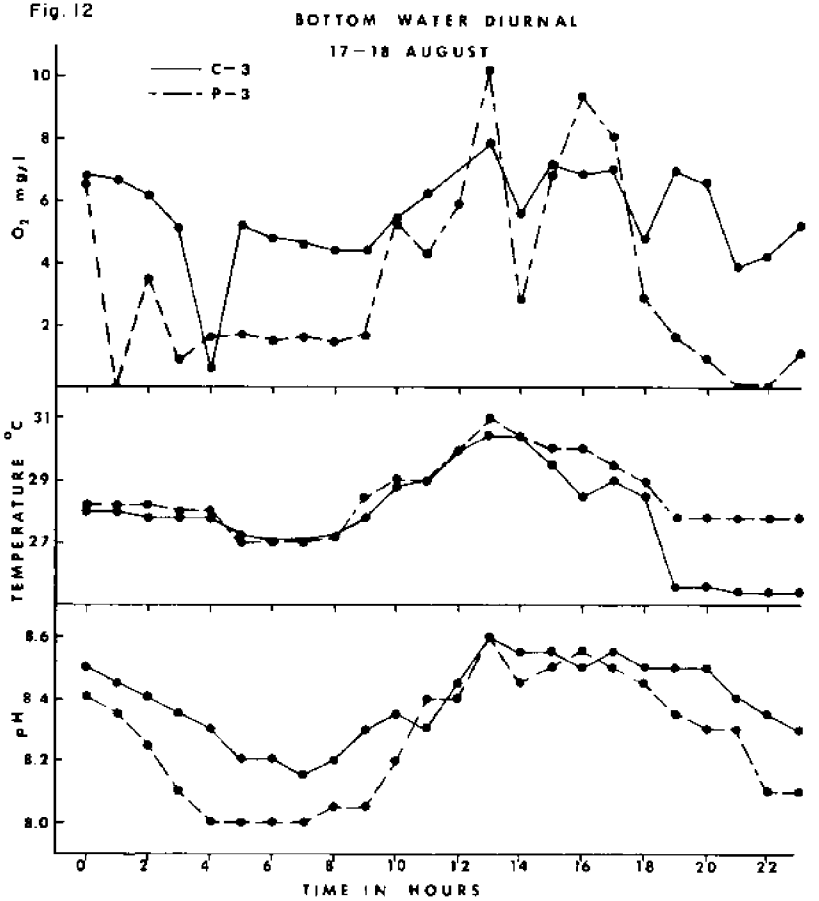


Fig. 12. Dissolved oxygen, temperature, and pH of August 17 bottom water diurnal from C-3 and P-3. 0 hour = 10:00 midnight.

POND MEIOFAUNAL STUDIES by J. R. Hall*, Dept. of Zoology, UNC

INTRODUCTION

This is a report on a composite study working with various aspects of meiofaunal-sediment interactions. First, a survey of the meiobenthic infauna of the ponds is being made to provide information for the modeling phase of the project. Second, the effect and effectiveness of sandy beaches as sea water filter for the removal of particulate and soluble organic carbon is being investigated. Third, recognising that the redox pattern of marine sediments is in intimate interaction with the meiofauna (Fenchel, 1969; Fenchel and Riedl, 1970), Studies have been started attempting to find out what processes are important in producing and regulating sediment redox conditions.

MEIOFAUNAL SURVEY

Methods

The survey has been limited to ponds C-2 and P-2. Triplicate cores of 10 cm^2 cross sectional area were taken monthly close to the ends of the finger piers (depth about one meter). Care was taken not to puncture the clay lining of the ponds. Various methods were tried to separate the meiofauna from the sediment. These included preservation of the sample followed by seiving through a 64 um mesh, flotation using saturated magnesium sulfate solutions and centrifugation of live samples through a sea water-glycerine (or Kayro syrup) interface (Teal, 1960). Extracted samples were stained with Rose Bengal and picked under a dissecting michroscope (40x).

Results and Discussion

Great difficulties were encountered in trying to get realistic quantitative determinations of abundance. The bottom sediments of both ponds contain large quantities of plant debris. None of the extraction techniques used gave good separations of animals from this debris. This presented two difficulties in quantative work. First, picking animals frome the samples becomes very tedious and time consuming. Second, some animals will be hidden in the plant debris and never be seen, resulting in variable underestimations depending on the amount of debris.

Accepting these limitations, the best estimates of animal density in both ponds are about 150-200 individuals/ 10 cm^2 (1/1000 m^2). Abundance (inferred from ease of picking) varied slightly seasonally. Nematodes dominated in both C-2 and P-2, comprising about 98% of the total meiofauna. Families encountered included; Monohysteridae, Oncholaimidae, Cyatholaimidae, Linhomoeidae and Chromadoridae (identified by J. Ott). Harpacticoid copepods were next in abundance with representatives of only two families found; Laophontidae and Tachidiidae (identified by E. Lindgren). Representatives of the Turbellaria and Annelida were occasionally found. Periodically, species of epibenthic cyclopoid copepods occurred in both ponds. More species were found in the experimental pond, but more taxa occurred in the control pond.

*Work phase on meiofauna with Dr. R. J. Riedl

These abundance estimates are one third to one half those reported by others from similar environments. Krogh and Sparck (1935; from McIntyre, 1969) found 59-147 individuals/10 cm^2 (5.5m depth, 20‰ salinity) in the Øresund. Working in the Baltic, near Stockholm, Fenchel and Jansson (1966) found a mean number of 376 individuals/ 10 cm^2 in seven shallow (0.5m) subtidal cores (6‰ salinity). Recently, in an extensive survey of shallow subtidal sediments in eastern Denmark, Muus (1967) found a mean density of 365 individuals/10 cm^2 (0.5-2.0 m depth, 5-18‰ salinity). In all these studies, nematodes dominated among the metazoans with the crustacea next in abundance.

Biomass determinations were not attempted in this study. Best estimates from the literature (Wieser, 1960) would suggest a dry weight biomass somewhere in the range 0.1-1.0 mg/10 cm^2.

SAND FILTERS

Methods

Next to the mixing tank at the C pond site, three containers (96 gallon livestock watering tanks) were filled with beach sand from the mid-tide region of high energy beaches (Atlantic Beach). Water from the mixing tank is allowed to flow by gravity into the three sand filters at rates of 2, 1 and 0.5 l/min. Depth distribution and abundance of meiofauna in the sands has been checked at two week intervals for over three months (November, 1970- January, 1971)by taking cores (15.8 cm^2 cross sectional area) and sectioning the cores at 5 cm intervals. Samples were immediately preserved (5% buffered formalin) and extracted in the lab by repeated washing with tap water which was then passed through a 64 um seive. Staining and picking proceedures were identical to those of the previous section.

Results and Discussion

This preliminary study has shown that the meiofauna can easily survive in this artificial environment. Animals are most abundant in the upper ten cm of the sand. This is comparable to the metazoan distribution in artificial sand filters reported by Brink (1967) and McIntyre et al. (1969). Abundance has so far remained constant. Some difficulty has occurred in maintaining a constant water flow into the tanks. When this problem is solved, tanks will also be set up at the P pond site. Eventual plans call for monitoring dissolved and particulate carbon and total nitrogen of both inflow and outflow of all tanks to see how effectivly organic carbon is used and removed by sand filters.

SEDIMENT REDOX EXPERIMENTS

Methods

The basic assumption has been made in this series of experiments that in nature water circulates through many types of marine sediments

(Riedl et al., in preparation; Webb and Theodor, 1968). Sandy sediment with moderate organic and silt-clay content (redox discontinuity at 10-15 cm) was placed in plexiglass columns (4.5 cm diameter) closed with rubber stoppers. Outlets were provided at each end of the columns through the stoppers. The lower outlet was provided with a porous filter (aquarium bubbling stone). Platinum electrodes (10) were inserted through holes (on 10 cm centers) sealed with silicone rubber cement. Sea water in a 20 l glass carboy was pumped into a constant head reservoir and then allowed to flow by gravity through a sediment column. Flow rate was controlled with a hose clamp at 50 um/sec. Column outflow waw returned to the carboy. The carboy was provided with a gas inlet to allow flushing with air or nitrogen gas.

A fluctuating water flow through the sediment column was simulated by attaching to the bottom outflow a small sea water reservoir. This reservoir was moved relative to the upper sediment surface in the experimental column by attaching it to a rotating arm driven through a gearbox by a syncronous electric clock motor. The resulting cycle had a period of six hours. Sea water passing through the upper sediment surface in the experimental column was aerated.

All redox measurements were made with a Keithly 600A electrometer using a standard calomel reference electrode.

Results and Discussion

The results of three experiments are reported here. In the first experiment (Fig. 1a) sediment was placed in the column (upper sediment surface between electrodes two and three; electrodes numbered one through ten from the top) and the redox pattern allowed to stabilize for 23 hours. A flow of oxygenated sea water was started, run for 34 hours, stopped for 16 hours and then restarted. The results clearly show that flowing, oxygenated sea water can influence the redox profile in sediments.

The second experiment (Fig. 1b) was similar to the first with the exception that oxygen was first removed from the sea water in the reservoir by a 24 hour nitrogen gas flush. After the flow was started, an initial change in the redox pattern was observed which rapidly returned to the preflow pattern. Two hours and fifteen minutes after the flow was started, the sea water reservoir was flushed with air. The redox pattern changed rapidly with the redox discontinuity moving deeper into the sediments.

To see if a tidally induced water fluctuation in intertidal sediments might cause corresponding fluctuations in the redox pattern, the third experiment was performed (Fig. 2). The sediment level in this experiment was 0.5 cm below electrode 2. Fluctuations in water level in sediments obviously can produce fluctuating redox patterns.

These three experiments are admittedly rather crude simulations of natural events. Certain effects such as poisoning of the platinum electrodes are overlooked. In spite of the recognised limitations of the approach, the results do convincingly show that water movement undoubtedly plays an important role in regulating redox patterns in sediments. Diurnal fluctuations in redox patterns have been observed in nature (Fenchel, 1969) and explained as resulting from the daily dark-light cycle. Thus short time fluctuations in redox patterns have

been observed, and knowledge to date suggests these fluctuations result from complex interactions of environmental variables.

SUMMARY

Various aspects of meiofaunal-sediment interactions have been studied. A meiofaunal survey of ponds C-2 and P-2 showed that meiofaunal abundance was about 150-200 individuals/10 cm^2, somewhat lower than reported from similar environments by other workers. Experiments are in progress looking at the effectiveness of sand filters in removing organic carbon from sea water. Finally, water flow through sediments was shown to influence redox patterns in marine sediments.

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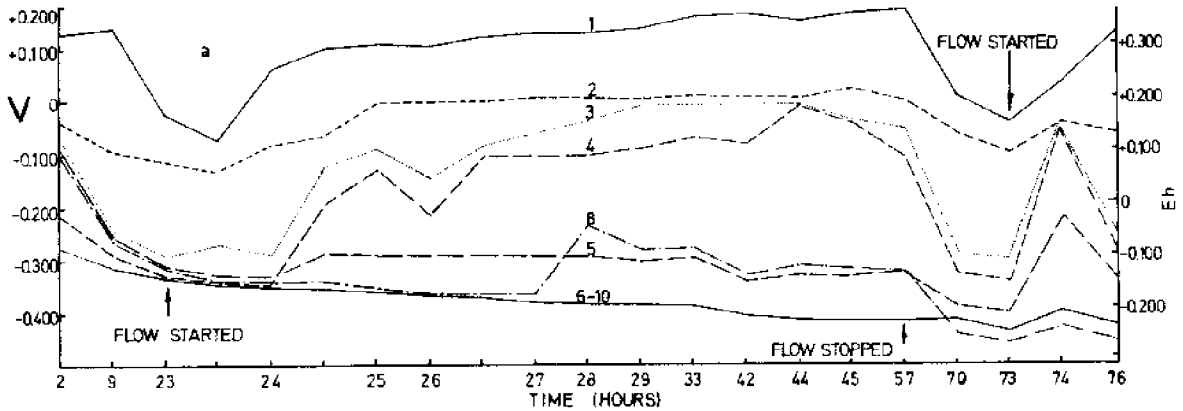

Figure 1a. Changes in the redox patterns in marine sediment resulting from a flow of oxygen saturated sea water through the sediment. Electrodes (on 1 cm centers) numbered 1 through 10 from the top. Sediment water interface between electrodes 2 and 3. Trend of electrodes 6, 7, 9 & 10 shown.

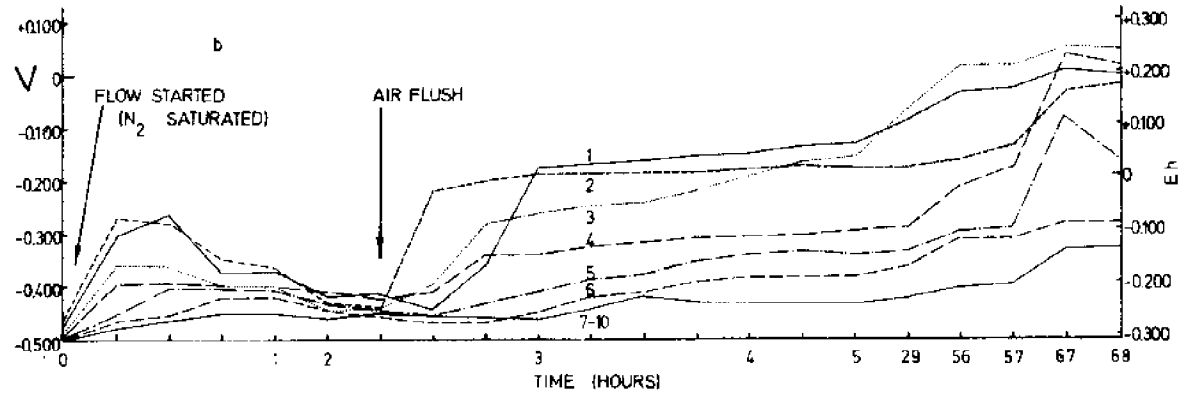

Figure 1b. Changes in the redox patterns in marine sediment resulting from a flow of nitrogen gas flushed sea water through the sediment. Electrode numbers, positions and sediment water interface as in Figure 1a. Trend of electrodes 7-10 shown.

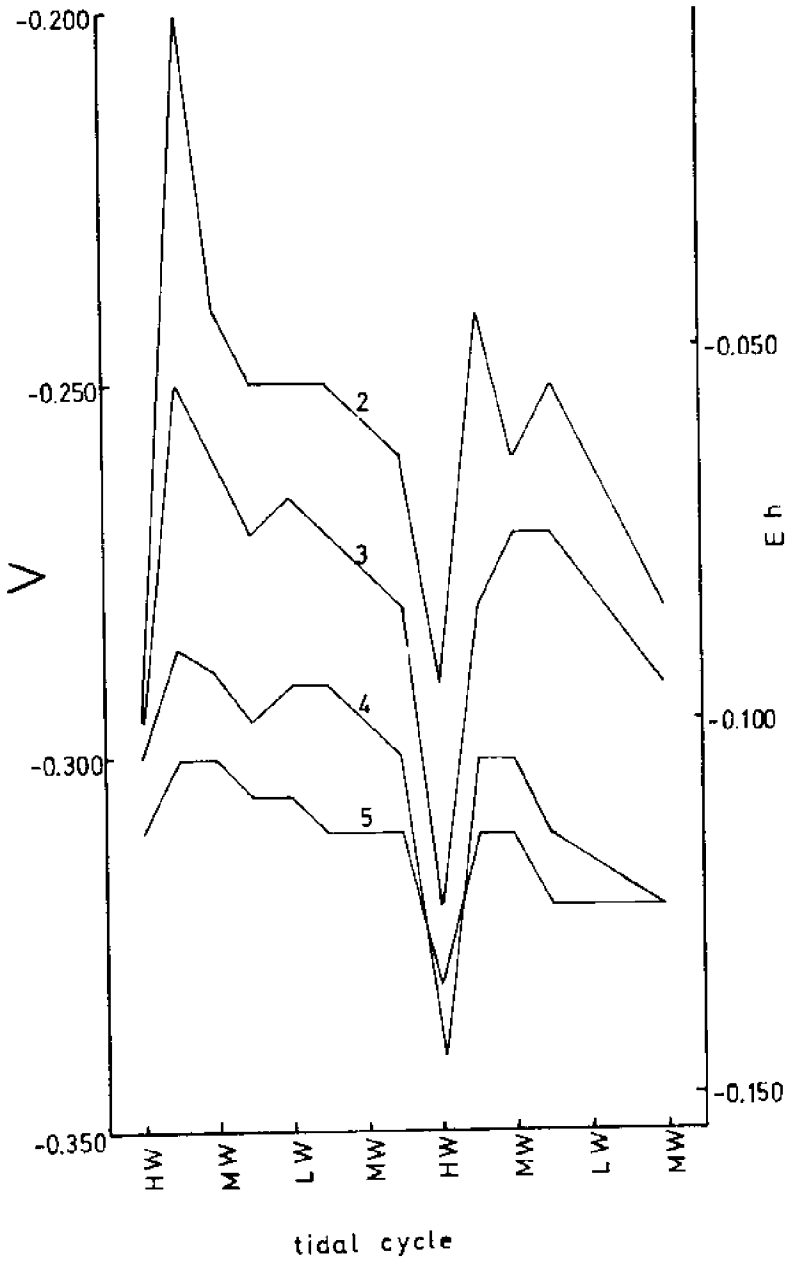

Figure 2. Changes in the redox patterns in marine sediment resulting from fluctuating water movement in the sediment. Electrodes as in Figure 1a. The sediment level was 0.5 cm above electrode 2.

THE EFFECTS OF WASTE EFFLUENT ON SHALLOW WATER FORAMINIFERAL POPULATIONS

by Ann LeFurgey*
Curriculum in Marine Sciences

Part I: Foraminiferal Populations

Any population of Foraminifera, protozoans with calcium carbonate or agglutinated arenaceous tests, reflects the conditions of the environment where the population occurs. For example, Foraminifera may be more abundant in fine-grained sediments which contain a larger amount of organic matter, and thus more potential food, than in a coarse-grained sediment (Phleger, 1960, p. 117). Bandy, Ingle, and Resig (1964a, b, 1965) have reported that abundances of planktonic and benthonic Foraminifera are modified by sewage outfalls off the coast of California. A depressed area with few Foraminifera occurs at discharge points; an aureole of large numbers of Foraminifera occurs beyond this zone, about 500 meters from the effluent source. The waters of the abundance aureoles are characterized by high phosphate concentrations and increased phytoplankton populations. A concurrent increase in populations of planktonic Foraminifera supposedly reflects the part these organisms play in the nutrient cycle as phytoplankton consumers.

In this investigation shallow water benthonic Foraminifera are proposed as sensors for the benthic environment in estuarine ecosystems developing with treated sewage waste. Although adequate quantitative data has not been compiled during the initial three-month sampling, the present report includes trends observed in foraminiferal populations and the future direction of the study.

METHODS

The entire sediment area of each pond is sampled from a small skiff or from the access pier in each pond. The top 1 to 2 centimeters of surface sediment is carefully removed from cores made with a two-inch diameter plastic coring tube and preserved in isopropyl alcohol. Rose bengal, a protein specific stain, is added to the alcohol at the time of collection. The stain gives a rosy color to living protoplasm, and permits subsequent separation of living from dead species (Walton, 1952). After being washed over a seive with 62-μ apertures, the samples are examined wet at low magnifications, 7-30X, with a dissecting microscope.

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* Study directed by Dr. Joseph St. Jean, Department of Geology, Principle Investigator.

DISCUSSION

In the control ponds tentative generic identifications include the Foraminifera Ammotium, Ammonia, Ammobaculites, Elphidium, Haplophragmoides, Miliamina, and Nonion. Ammonia and Nonion are most abundant in the faunas; Ammotium, Ammobaculites, and Elphidium are less abundant but common; Miliamina and Haplophragmoides are rare, occurring in only two or three samples. No specimens have been observed in the waste pond samples to date. Both living and dead tests occur in the control ponds. In the future, total population counts, living plus dead, per milliliter of wet sediment will be made for monthly samples. Estimations of biomass calculated as volume according to the method of Murray (1968, p. 440-442) will also be made. To determine foraminiferal populations at source areas, sampling at the water intake pipes in Calico Creek and Bogue Sound is required.

Since Foraminifera appear to be rare in the waste ponds, samples will be taken in Calico Creek at the point of effluent discharge and downstream from the discharge. A similar abundance aureole phenomenon may be exhibited by benthonic Foraminifera in estuarine areas as that described by Sandy, Ingle, and Resig (op. cit.) for deep water benthonic and planktonic species of California coastal waters. These authors also note a decrease in species diversity with increase in abundance of species. Decrease in species diversity of other organisms such as shrimp and fishes has been reported in the waste ponds as compared to the control ponds (Beeston in Odum, et al.,1969, p. 272-279). The level of diversity in zooplankton is low in both pond series (McCrary in Odum, et al., 1969, p. 259-271). In these ponds unusual concentrations of nutrients such as phosphates parallel the concentrations of nutrients reported in the outfall areas of the California coast.

Other workers (Lipps and Valentine, 1970) record that Foraminifera feed upon bacteria, diatoms, and nannoplankton, all usually below 50 microns in size. Foraminiferal predators include microcarnivorous species feeding totally on the protozoans and general feeders which process Foraminifera along with much other material. In their pioneer work Lipps and Valentine (op. cit.) propose that Foraminifera form a key link in the trophic structure of marine communities, as primary consumers of minute organisms and detritus. Their assimilated energy flows through other organisms to higher trophic levels. Figure 1 is the generalized diagram of Lipps and Valentine (op. cit.) showing the position of Foraminifera in marine community trophics.

The rarity of Foraminifera in the waste ponds automatically questions the proposed food chain. Is

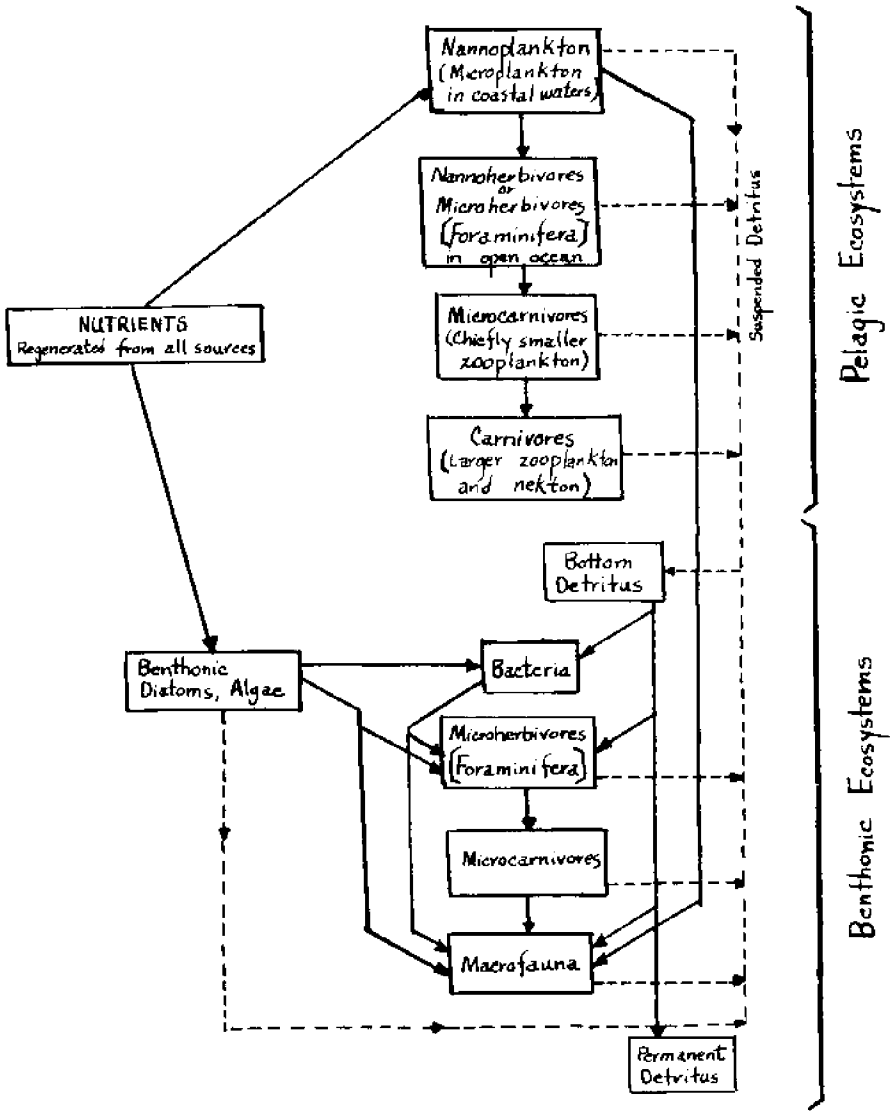

FIGURE 1: The position of Foraminifera in the trophic structure of marine communities, from Lipps and Valentine, 1970.

phytoplankton directly consumed by zooplankton and bottom filter feeders? Is the intermediate microherbivore level non-essential and therefore by-passed in a system where the microherbivores do not survive? Cultures of flourishing control pond species will provide some data necessary to clarify the questioned position of Foraminifera in the trophic structure. Purchase of a 25-gallon Instant Ocean Culture System has been approved; this system will facilitate laboratory observation of foraminiferal life cycles and provide controls for environmental variables, such as oxygen, temperature, salinity, substrate, and nutrient levels, affecting the life cycles.

Although taxonomic and stratigraphic studies of fossil Foraminifera are extensive, the entire life cycles of less than 25 foraminiferal species have been experimentally observed. The foraminiferal populations in the ponds near Bogue Sound and near Calico Creek are valuable to investigations of foraminiferal biology in general and for further clarification of the effects of sewage effluent in estuarine ecosystems at the microfauna level.

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Part II: Clay Mineralogy of the Substrate

Benson (1965), in a comparison of marsh and non-marsh sediments, demonstrated similarities in clays of the Neuse River estuary in marshes exposed to continual drying and rewetting and in underwater clays of Beaufort Inlet and Bogue Sound. The present investigation involves these same marsh clays transported into artificial ponds which were designed to simulate an estuary receiving sewage effluent. Here, rather than being exposed to the stresses of drying and rewetting, the minerals are in an environment stressed by fluctuating pH, high amounts of suspended organic solids, unusual anion and cation concentrations, and increased bacteriological activity. A survey-comparison of the clays present in ponds receiving effluent and in ponds receiving no effluent should reveal any differences in mineralogy. In the isolated pond systems these differences can then be traced to chemical or organic factors which favor diagnentic modification, to physical factors, such as flocculation and settling, or to variations in the mineral sources.

METHODS

Sampling Procedure

To determine if the clay mineral composition of the sediments within each pond is uniform as well as to compare the waste and control ponds, three samples were taken from each pond: A, water depth 6 inches; B, water depth 3 feet; C, water depth 1.5 feet. A 2-inch diameter plastic tube fitted with rubber stoppers was used to make the sediment core. The top 2 centimeters from the core, representing sediments deposited after construction, was carefully removed and reserved for subsequent analysis.

Sample Preparation

Pretreatment and Dispersal: All samples were dried at

40°C. The clay size fraction was separated using the Ingram (1970) methods for dispersal, preceeded by removal of substances that hinder dispersal: carbonates, removal with dilute HCl, and heat; soluble salts, removal by washing with deionized water and centrifuging.
Chemical Treatments: Samples were treated with magnesium acetate, magnesium acetate plus ethylene glycol, potassium acetate, and potassium acetate plus heat (Ingram, 1970).

Slide Preparation

Random Powders: Random powders were prepared from Mg-treated samples, by passing the treated and dried sample through a 325-micron seive. The powder was placed in an aluminum and glass holder and x-rayed from 4° 2θ to 60° 2θ to record both basal and secondary reflections.
Oriented Aggregates: Oriented aggregates were prepared from suspensions of clays treated with magnesium or potassium; the suspension was dropped onto a glass or ceramic slide, air dried, and x-rayed from 4° 2θ to 35° 2θ to record important basal reflections. Grams of clay per ml of suspension was determined for the Mg-treated samples by weighing a portion of the suspension before and after drying. Each slide was prepared with approximately equal weights of clay, so that x-ray results could be quantitatively compared.

X-Ray Procedure

All samples were x-rayed with a Norelco-Phillips Diffractometer using Copper K-alpha radiation. Strong peaks from the ceramic slide mounts were noted but were ignored since they did not interfere with any major mineral lines and did not add significantly to the background. Glass slide mounts produced some background "swells" which were taken into account in calculations of peak height. Identification of lines was made with the ASTM Index to Inorganic Compounds and syllabus literature from Ingram (1970).

Quantitative Procedure

Pierce and Seigel (1969) compare several methods of determining percentage composition for clay mineral assemblages and point out that any of the methods used is a reliable estimate rather than an absolute measure of composition. Since slight variations in sample preparation, slide preparation and mount, x-ray intensities, etc., cause large variations in x-ray patterns produced, in this analysis percentage composition is arbitrarily considered accurate to the nearest 10%.

The estimates of percentages of clay minerals present were made on the premise that area under the x-ray peak

is proportional to intensity, and relative intensities are proportional to amounts present. Since a 3-layer structure at 10A gives an intensity considerably less than an equal amount of a 2-layer at 7A or a 3-layer structure at 14A, the area of illite, a 3-layer mineral with a 10A basal reflection, must multiplied by a factor of 3 for comparison with 2- or 3-layer structures such as kaolinite or the 14A hydroxy-interlayer mineral (Freas, 1962). For example, the actual area under the 10A peak of illite equals the width at half the peak height times the peak height. The corrected area equals actual area times the correction factor 3.

RESULTS

Qualitative

Kaolinite, illite, and 14A hydroxy-interlayer mineral occur in all samples from both control and waste ponds; quartz is an accessory mineral also identified.

Kaolinite: Kaolinite is identified by the 7A 001 reflection which remains constant through all Mg, Mg-glycol, and K treatments, and disappears on heating the K-treated sample to 500°C.

Illite: The 001 reflection at 10A which remains unchanged through all treatments identifies illite. The 060 reflection at 1.498A classifies it as dioctahedral; the almost equal intensities of the 001 and 002 reflections indicate it is a highly aluminous member with little substitution of iron in the octahedral layer (Grim, 1968).

14A hydroxy-interlayer mineral: This mineral has one characteristic basal reflection at 14A, which changes after treatment with K plus heat. At 150°C, the peak broadens to 13-14A, and between 250-300°C the 14A peak has shifted to 10A.

Smectite: The identification of an expanding-layer smectite is subject to question. In one sample treated with Mg-glycol, the 14A peak persists, decreasing slightly in intensity, and a shoulder appears on this peak from 15-16A. This band may be interpreted as a trace amount of montmorillonite, which expands from 14A to approximately 17A with ethylene glycol treatment. Further experimentation is necessary to positively identify it.

Quantitative

Table 1 shows percentages of clays in the ponds; each percentage is calculated from Mg-treated samples taken at 1.5-foot and 3-foot depths respectively.

Sample	Pond	Percentages 7A	10A	14A
B	Control 1	30	40	30
C	Control 1	30	35	35
B	Waste 1	40	35	25
C	Waste 1	40	25	35

TABLE 1: Percentages of clays in the pond sediments.

Sample	Locality	Percentages Non-Marsh 7A	10A	14A	Marsh 7A	10A	14A
I	Beaufort	38	39	23	35	34	31
II	Bogue Sound	37	39	24	49	34	17
III	Beaufort	40	41	19	47	47	6

TABLE 2: Percentages of clays in the Newport River estuary, modified from Benson, 1965.

DISCUSSION

Visual examination of sediment samples from the six ponds shows that coarse size sand is concentrated around the ponds' outer perimeters and that fine-silt size grains are concentrated in the deeper central portions. There is no corresponding segregation of clay minerals; the species appear consistent for both perimeter and center of all ponds, in water depths from 0.5 feet to 3 feet.

The major mineral components are the same for both waste and control ponds: kaolinite, illite, and 14A hydroxy-interlayer; quartz also appears. Benson (1965) reported that these same species dominate the mineral assemblages of marsh and non-marsh areas in Beaufort-Bogue Sound. The marsh area cut by Calico Creek is a source of suspended sediments for the polluted ponds; both marsh and non-marsh areas from Bogue Sound, a source for the control ponds. Brett (1964) made similar identifications of constituent minerals in Bogue Sound; Benson (1965) also identifies them in the Neuse and Newport Rivers which feed the Calico Creek estuary and Bogue Sound. Thus, the minerals in the ponds parallel those of the source areas in species.

Percentage composition of any species varies ±10% from waste to control ponds. Table 2 shows average percentages of the postulated major source areas. The general trends in composition of ponds (Table 1) and source are similar, although the 14A hydroxy-interlayer is consistently high in all pond samples.

If the high percentage does indicate increase in the amount of 14A hydroxy-interlayer mineral, is the increase due to diagenetic modification of some other clay mineral or due to percentage variations in the source areas?

Factors favoring diagenesis include pH. Pawluk (1963) attributed formation of a 14A clay mineral to migration of hydroxyl and/or hydrated aluminum ions into the interlayer positions of illite and/or montmorillonite. An acid pH releases alumina ions from sediment constituents; an alkaline pH (8-9) releases silica; at pH values above 9, both silica and alumina are soluble (Grim, 1968). When pond waters are extremely basic, both silica and alumina can be dissolved from the minerals, which consequently decrease in crystallinity. The pH in the ponds varies as much as pH 5 to pH 9 in a 24-hour period, and in the two years since construction, pH has varied from 3 to 10. These unstable conditions perhaps generate a chemical environment producing the 14A mineral from illite and simultaneously degrading the 14A hydroxy-

interlayer mineral transported into the ponds as suspended material. Other hydroxy-interlayer minerals have been characterized by reduction of the 14A line to 12A on heating to 300° or 500°C (Ingram, et al., 1959). In the pond mineral the 14A peak reduces to a broad band at 150°C. At 250°C the scatter increases in the 14A to 10A region; at 300°C the 14A line shifts completely to 10A. The lower temperature for structural alteration may indicate incomplete substitution of hydroxy aluminum ions in the interlayer positions.

Precipitation of carbonates obviously might be expected at the higher pH levels in the ponds. In a study of carbon flows within the ponds (Day in Odum, et al., 1969, p.183-213), it was determined that carbonate alkalinities are alkalinities are replaced in part by hydroxide alkalinities, thus preventing excess carbonate precipitation and shifting concentrations of other inorganic constituents. Little carbonate was present in these samples; preliminary treatment with HCl revealed minimal amounts which dissolved immediately on heating.

Some cation and anion concentrations are known for the ponds. Total ionic calcium is above that expected for normal marine waters diluted to 15‰ (Fred Davis, personal communication). The excess calcium is attributed to the limestone aquifer which provides water for the city system. Although no data is available for magnesium or potassium, concurrent high concentrations of these ions may significantly affect ion replacement in the clay minerals, as reported by Dibbins, Nagland, and Johnson (1970) in minerals of the Pamlico estuary.

The level of suspended solids in the ponds reaches a summer high of 300 mg/l, 15-30% of which is organic (Woods in Odum, et al., 1969, p. 144-173). Whitehouse and McCarter (1958) have reported that organic matter slows down or hinders diagenetic changes of montmorillonite, chlorite, or illite during initial flocculation and settling of the clay materials. Grim (1968) also notes that humic matter inhibits flocculation in kaolinitic clays. The concentration of organics may exclude the possible diagenetic modification of illite to the 14A hydroxy-interlayer mineral. Lack of time for diagenesis to take place may be the limiting factor in the ponds, which are only two years old.

An alternative explanation for the high concentration of 14A mineral might also be source area variations. In the waste and control ponds either the estuarine area contributing the most sediment has a high percentage of the 14A mineral or in the waste ponds effluent from the secondary sewage treatment plant adds significant amounts of the 14A mineral.

Modification of minerals by filter feeding invertebrates has been reported (Anderson et al., 1958), but illite and kaolinite, dominant in the pond sediments, pass through the digestive system unaltered.

CONCLUSIONS

Kaolinite, illite, and 14A hydroxy-interlayer mineral occur in approximately equal amounts within a single pond and throughout the entire series of ponds, both control and waste. The trends in percentage composition parallel those of the proposed source areas. The generally higher percentage of 14A hydroxy-interlayer mineral possibly is due to diagenetic alteration of illite; however, differences in source areas more probably account for its high concentrations. Additional analyses to pinpoint source areas are requisite to complete evaluation of the pond mineralogy.

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THE EPIFAUNA AND WOOD-BORING FAUNA

by Richard E. Dowds and Austin B. Williams
Department of Zoology
and
Institute of Marine Sciences

INTRODUCTION

Two groups of brackish water impoundments, maintained by the University of North Carolina Institute of Marine Sciences in Morehead City, N.C., provide convenient sites for the study of estuarine organisms under controlled environmental conditions (Odum and Chestnut, 1970). The ponds differ from adjacent estuarine waters with respect to depth, salinity, tides, currents and freedom of exchange with neighboring waters. One group of ponds receives treated sewage effluent.

This study was concerned with the epifaunal and wood-borer communities of the ponds, as compared to those of adjacent natural waters. The first objective was to obtain an overview of these communities and to make broad comparisons. Since it was not possible in the time available to study in detail all of the organisms seen in this overview, certain groups were selected for more extensive study.

Sessile barnacles (Thoracica, Balanomorpha) were given special consideration because they were prominent in the pond epifaunas. Attempts were made to determine (1) characteristics of the established populations and (2) the supply and settling success of larvae. The wood-boring isopod, Limnoria tripunctata Menzies (Flabellifera), was given special attention because earlier work (Eric Lindgren, pers. commun.) suggested that it was absent from the ponds. Search for Limnoria was accomplished by (1) looking for established populations, (2) observing artificially introduced populations, and (3) assaying the availability of dispersal stages.

The period of study extended from March to August, 1970; however data from as far back as July, 1968 were available for analysis. The project was supported by grants from the National Science Foundation, Sea Grants Division and the North Carolina Board of Science and Technology. Special thanks is extended to Dr. Charles E. Jenner for assistance and advice.

PHYSICAL AND CHEMICAL CHARACTERISTICS

There were four study sites, each with a characteristic physico-chemical regime: (1) Bogue Sound, (2) Calico Creek, (3) the C ponds and (4) the P ponds. The first two sites are designated as natural waters in this paper (even though they both experience marked human influence) in order to distinguish them from the second two which are entirely man-made and maintained structures.

Bogue Sound is a shallow brackish lagoon separated from the open ocean by a long barrier island. The Bogue Sound study site is located around two piers at the Institute of Marine Sciences, and has a depth at mean low water of up to two meters. The muddy sand bottom is subjected to moderate surf action. Strong tidal currents come from a nearby connection with the ocean (Beaufort Inlet), and freshwater run-off enters seasonally from inland. Williams, et. al. (1967) reported monthly mean temperatures and salinities for this site, based on data accumulated over a 17-year period. Mean surface temperatures ranged from 10.8°C. (December) to 28.6°C. (July); mean surface salinities from 29.7°/oo (March) to 33.6°/oo (June). The salinity regime recorded in Bogue Sound during this study period is shown in Figure 1; the temperature regime was also recorded (Laughinghouse, unpublished data) but is not figured here.

Calico Creek is a small brackish stream which runs through salt marshes into the Newport River estuary and thence to the sea (Odum and Chestnut, 1970). Sampling was done at a small pier located near the P ponds, except for salinity and temperature measurements which were made at a highway bridge about a half mile downstream. At mean low water the channel depth is everywhere less than one meter and broad areas of mud flat are exposed. The Creek receives considerable freshwater input and strong tidal flow. Salinities during the study period are shown in Figure 1; they were lower and fluctuated more widely than those in Bogue Sound. Temperatures varied little from those in the Sound (Laughinghouse, unpublished data). Calico Creek has a very muddy bottom and carries a large silt load. It receives treated municipal sewage near the study site.

The experimental ponds are small (about 20 x 30 m) shallow (maximum depth 1 m) earthen basins, each with a single inflow and outflow pipe and a constant water height. The rate of inflow varies from pond to pond ranging between one and three times the volume of a pond per month (Odum and Chestnut, 1970).

The three C ponds receive sea water from the area in Bogue Sound described above mixed with fresh water from the municipal water supply. Temperatures during the study period (Laughinghouse, unpublished data) differed little from those in the Sound and Creek, but an important difference occurred in winter when freezing was more extensive and prolonged in the ponds than in the natural waters. Salinities * were markedly lower than those in the natural waters being maintained by design between 12 and 22°/oo.

Sea water from the three P ponds comes from the Calico Creek study site, and is diluted with sewage effluent rather than tap water. Both temperatures (Laughinghouse, unpublished data) and salinities there * agree closely with those observed in the C ponds except for slightly lower salinities in the spring.

*See Log of Activities and General Notes (above)

METHODS AND MATERIALS

Two types of sampling techniques were employed: (1) collecting surfaces -- to assay numbers of attached adults present, and (2) plankton samples -- to determine what dispersal forms were entering the ponds. The collecting surfaces included plexiglass plates, wooden surfaces and concrete blocks. Plexiglass plates submerged for short periods (up to four months) were particularly well suited for detailed observation of small stages. They consisted of two pieces of 4x4x1/8-inch plexiglass held tightly together with brass bolts; these could be separated in order to examine attached organisms through the clean inner surfaces with aid of a dissecting microscope. Graham and Gay (1945) indicated that 4x4-inch panels gave as reliable a sample of the epifauna as did larger panels. Plates were positioned in each of the ponds at 25, 50 and 75 cm from the bottom. At the Creek site the depth at low water was less than one meter, and the plates were spaced closer together; the uppermost plate was out of water at low tide. In the Sound the depth was greater than one meter at low water, therefore the plates were spaced at 40, 80 and 120 cm from the bottom.

Several types of wooden objects were suspended for various periods of time. Untreated pine stakes (4x4x50 cm) were suspended fully submerged at all of the sites and samples were cut off at intervals. They were used to test specifically for establishment of Limnoria tripunctata populations but also served as substrate for epifauna. Boards previously infected with Limnoria were submerged in all areas and portions were cut from them periodically to monitor the success of the species. Wooden stakes and pilings which had been in place for about two years were used to assess success of previous settings, particularly of barnacles. Some of this wood had been treated with creosote, some with pentachlorophenol, and some was untreated. Artificial concrete block reefs and concrete tiles submerged for two years were also examined for species present.

Quantitative samples of plankton entering the ponds via the seawater pumping systems were taken periodically with a nylon Wisconsin net (73 micron mesh). The samples were preserved in buffered 5% formalin, and a 5% aliquot of each sample was later examined.

RESULTS AND CONCLUSIONS

I. An Overview of the Communities

Bogue Sound

The epifaunal and wood-borer community observed in Bogue Sound was similar to that which McDougall (1943) described from nearby Beaufort Channel. The Sound community had a greater specific diversity and biomass per unit surface area than the other areas studied; and it also exhibited a marked seasonal progression of species. On clean surfaces placed in the water in spring, barnacles were initially dominant but were gradually overgrown by foliaceous and encrusting bryozoans in May. Ascidian tunicates tended to displace the bryozoans in June but then sloughed off in July, and bryozoans again predominated. The wood-borers, Limnoria tripunctata

and Bankia sp. (Pelecypoda, Teredinidae), made steady progress during the summer and fall toward completely riddling untreated wooden objects.

Three species of barnacles were found in the area: Balanus amphitrite subsp., Balanus eburneus Gould and Chthamalus fragilis Darwin. McDougall (1943) found a fourth species, Balanus improvisus Darwin, in Beaufort Channel, and Marshall (1969 and pers. commun.) found this species to be common on oysters in low salinity areas and some high salinity areas of Brunswick County, N.C. However, B. improvisus was not seen at the Sound site or any other site during this study. It is possible that some unidentifiable individuals were B. improvisus, but all of these were small immatures and most were dead; they could not be considered a successful adult population.

Among the other animals observed in the Sound community, amphipods, decapods, polychaetes, hydrozoans, protists and nematodes were common.

Calico Creek

The Calico Creek epifaunal and wood-borer community contrasted sharply with that seen in Bogue Sound, having far fewer species and a lower biomass. Stresses present in the Creek -- including highly variable salinity, heavy silt load, sewage and scarcity of suitable substrate -- must be at least partly responsible for this contrast. Notably present here were the same three species of barnacles seen in the Sound and heavy fouling by filamentous green algae. No obvious seasonal variations were observed.

C Ponds

The C ponds exhibited species diversity intermediate between that of Bogue Sound and Calico Creek. Two species of barnacles, Balanus eburneus and B. amphitrite, were present. Encrusting bryozoan colonies were overwhelmingly dominant on the undersides of many of the plexiglass plates. Small anemones and tubiculous amphipods of the family Corophiidae were also conspicuous.

P Ponds

The epifauna of the P ponds resembled that of the C ponds in terms of diversity, but many of the species were different. Encrusting bryozoans were scarce and Corophiid amphipods absent. Green and blue-green algae grew extensively. Rotifers and nematodes were common, and the same species of barnacles and anemones seen in the C ponds were found.

General

Notably absent from Calico Creek and all of the ponds were Limnoria, Bankia, foliaceous bryozoans, ascidians and hydrozoans. However, Limnoria which were artificially introduced to ponds C2, P2 and the Creek in infected wood survived there (Table 5); Bankia introduced in the same wood survived in the Creek and C2.

II. Barnacles

Adult Populations

Table 1 shows the results of a census carried out by counting and measuring all of the adult barnacles on a representative sample of the fouling surfaces. The density of barnacles was similar in Bogue Sound and Calico Creek. Mean diameter of barnacles was less in the Sound than in the Creek, but the difference was small when compared to the mean diameter of individuals in ponds C2 or P2.

Sampling surfaces in Bogue Sound and Calico Creek were wooden and were chosen so as to extend about one half meter (approximately the mean depth of the ponds) below high water. After the data were tabulated it became apparent that this choice of surfaces did not accurately reflect species composition in these areas. For example, Balanus eburneus was commonly seen in the Sound, yet it was not found on the surfaces sampled there; it may only be found at depths deeper than the deepest one sampled. In the Creek, Chthamalus fragilis was more dense on a PVC pipe than on the wooden surface sampled.

Adult barnacle populations in ponds C2 and P2 stood in marked contrast to those of the Sound and Creek. Density of barnacles was markedly lower in the ponds, and the mean diameter of individuals much greater. Moore (1935) found that in a population of Balanus balanoides, all of whose members had set at the same time, those individuals which were widely separated from others had a mean volume over six times that of crowded ones after two years of growth. The attainment of large size by barnacles in the ponds may have been favored by the sparse populations and lack of crowding there.

Species composition data for the ponds are unhampered by uncertainties applying to the data from the Sound and Creek, since all of the available types of fouling surfaces at all depths throughout the ponds were sampled. Chthamalus fragilis was completely absent from the ponds. This was expectable since Chthamalus is found high in the intertidal zone where it is daily exposed to the air (McDougall, 1943); it requires a strong current for feeding (Riedl, 1966; Southward and Crisp, 1959), and is not especially tolerant of low salinities (Wells, 1961). None of these conditions are met in the ponds.

Balanus amphitrite was found in the ponds, but in small numbers, suggesting a marginal existence. The species is primarily intertidal in occurrence (McDougall, 1943) and intolerant of low salinities (Wells, 1961). There was a large population of B. amphitrite on the walls of a nearby concrete impoundment which received a constant flow of undiluted water from Bogue Sound.

Balanus eburneus was highly successful in all of the ponds, being the predominant barnacle. The species occurs in low intertidal and subtidal areas (U.S. Naval Inst., 1952; McDougall, 1943) and is euryhaline (Wells, 1961; Moore and Frue, 1959), being found in nearly fresh water

in some estuaries (Broch, 1927). Thus it seems to be admirably adapted for conditions in the ponds.

During the census an interesting phenomenon was observed on the square pilings which support piers in the ponds. There was a consistent difference in the amount of barnacle fouling on the four faces of each piling (Table 2). Regardless which side of the pier the piling was on, the innermost face (which faced the underside of the pier) had the highest density of barnacles, the outermost face (which faced away from the pier) had the lowest density, and the densities on the lateral faces were intermediate between those of the other two. This distribution was not observed on free-standing square stakes.

The probable explanation is that barnacles show a preference for areas which are shaded, in this case the surface facing under the pier. McDougall (1943) observed this preference and found evidence for two mechanisms which could explain it: (1) the preference of cyprid larvae for settling on shaded surfaces, and (2) a higher mortality rate among young adults exposed to strong sunlight than among those not exposed.

II. Barnacles

Recruitment

The difference in density of barnacles between the natural waters and ponds suggests that a similar difference in recruitment rate must also have existed between them. That is, fewer larvae per unit area must have successfully settled in the ponds than in the natural waters. Table 3 indicates that this was the case during spring and summer of 1970. During that period large sets occurred in both the Sound and Creek, but the P ponds had very small sets and the C ponds none at all.

These small sets or absence of sets could be attributable to (1) a low density of larvae entering the ponds, (2) an extraordinarily low survival rate between the time of entry and maturity or (3) a combination of these occurrences. To date it is impossible to determine which of these alternatives is responsible, however there are data which bear on the problem.

McCrary (1970) found barnacle nauplii in densities up to two individuals per liter in several of the ponds and none in the remainder during spring and summer, 1969. She towed a plankton net with a 240 u mesh size alongside the piers in the ponds. One cannot assume that densities would be the same in parts of the pond farther away from the pier, since clustering of barnacle larvae near the shade of piers is a distinct possibility. Furthermore it is doubtful that she captured all of the larvae present; several of the early stages of nauplii of both Balanus amphitrite (Costlow and Bookhout, 1958) and Balanus eburneus (Costlow and

Bookhout, 1957) could easily pass through 240 u meshes.

Even if one were confident that all of the nauplii present had been captured and that densities were uniform throughout the ponds, these data would not be sufficient to establish one of the above alternatives as the correct one. One would also need to have detailed information on the sets occurring over the same period and parallel data from comparable areas in natural waters.

There are two possible sources from which barnacle nauplii could enter the ponds: (1) the natural waters via the sea water pumps, and (2) reproductively active adults living in the ponds. The number of larvae contributed by each of these sources has not yet been determined, but some pertinent information has been obtained.

Table 4 shows the density of barnacle larvae in plankton samples taken from seawater entering the mixing tanks of both series of ponds. The net used had a mesh size of 78 u which is small enough to capture even the smallest naupliar stages of barnacles. Although there are gaps in the data recorded, it is evident that considerable numbers of larvae were entering the mixing tanks. The density and viability of larvae delivered to the individual ponds from the mixing tanks was not determined.

The fecundity of adult barnacles in the ponds was not thoroughly investigated. Most adults were close enough to each other to copulate. Individuals should be examined during the breeding season for the presence of embryos in the mantle cavity.

III. Limnoria

Limnoria tripunctata and many of its burrows were found in wood in Bogue Sound; wood placed in the Sound in the spring became infested after three months. No Limnoria or evidences of it were found in the ponds or Calico Creek. No migrating adults (the common dispersal mechanism -- Johnson and Menzies, 1956) were observed in any of the plankton samples from sea water entering the ponds, even though sampling included the period of most active migration.

This last fact introduces questions concerning whether Limnoria was absent from the ponds due to intolerance of conditions there or because of unavailability of migrating adults. To answer these questions, boards with known concentrations of Limnoria tripunctata were suspended in all of the test areas. Estimates of Limnoria density in those boards at the time of placement and after one and four months in place are shown in Table 5. Populations survived in all of the areas, suggesting that the absence of dispersal forms prevented establishment of populations in the Creek and ponds.

SUGGESTIONS FOR FURTHER STUDY

A number of species which were excluded from detailed consideration here could be studied. Particularly interesting are the Corophiid amphipods found in the C ponds and the Sound. Casual observations indicate that they can live on a variety of substrates, being capable of burrowing into mud, building tubes on hard surfaces or even burrowing into wood to a certain extent.

The puzzling absence of Balanus improvisus from all of the study areas would be worth investigating. It, like B. eburneus, is euryhaline (it can live in less than 5°/oo salinity water -- Kuhl, 1967; Broch, 1927) and primarily subtidal (Bousfield, 1955; Kuhl, 1967). Both species would seem to be well suited to life in the ponds. It is possible that the absence of B. improvisus from the ponds is due to lack of access to them rather than intolerance of conditions there. One could test this hypothesis by artificially introducing adults and monitoring their survival.

A rewarding project could be undertaken to answer unresolved questions posed in the barnacle recruitment section. Study would require a carefully coordinated program of thorough plankton sampling and detailed observation of setting rates both in the ponds and natural waters. It would be useful to distinguish species when nauplii in the plankton samples are counted. Finally, adults in the ponds should be observed for reproductive condition.

SUMMARY

Bogue Sound, Calico Creek and two sets of experimental ponds in Morehead City, N.C. were studied with respect to their epifauna and wood-boring fauna. Depth, salinity, sewage input were carefully controlled in the ponds. Various collecting surfaces were used to assay adult populations, and plankton samples were taken to monitor dispersal forms entering the ponds.

Bogue Sound was found to have the highest specific diversity and greatest biomass of all the areas, and paralleled closely the community McDougall (1943) found in Beaufort Channel. Each of the other areas had its distinctive combination of species. Balanus eburneus, B. amphitrite, and Chthamalus fragilis were commonly found in the Sound and Creek; B. improvisus, previously known from the area, was unaccountably absent. Balanus eburneus and B. amphitrite were found in all of the ponds, but the former was overwhelmingly numerous. Barnacles in the ponds were much more sparsely distributed but larger than those in the natural waters. An apparent preference of barnacles for shaded surfaces was evident on the pier pilings in the ponds.

The investigation of larval barnacles demonstrated that larvae were present in the ponds and that some metamorphosed there. Unanswered questions include whether the adults in the pond reproduce, whether extraneous larvae survive passage into the ponds and to what extent each of these sources is responsible for the larvae seen in the pond. Also unknown is the reason for the small sets in the ponds.

Limnoria tripunctata was found in Bogue Sound but in none of the other areas. However, artificially introduced populations survived in all areas.

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TABLE 1.

BARNACLE FOULING ON WOOD AND CONCRETE SURFACES
SUBMERGED FOR TWO YEARS.

Location	Potential Fouling Surface (cm^2)	Number of Barnacles Per 100 cm^2	Mean Diameter of Barnacles (cm)	% Barnacles Live	% of Total			
					B. eburneus	*B. amphitrite*	*C. fragilis*	unidentifiable
Bogue Sound (at Pump Pier)	–	59.5	0.42	–*	0	52.8	44.5	2.6
Calico Creek (at Pump Pier)	–	61.4	0.75	55.2	43.8	40.5	1.0	14.8
C2	182,300	0.83	1.80	57.5	88.5	5.7	0	6.8
P2	175,228	1.33	1.61	77.4	96.3	0.8	0	2.8

* Indeterminate due extremely small size of some individuals.

TABLE 2.

DIFFERENTIAL BARNACLE FOULING ON
SIDES OF PIER PILINGS (MEAN VALUES)

	Barnacles Per 100 cm^2	% Live	Mean Diam. (cm)
POND C2			
Outermost	0.2	0	1.4
Lateral	0.3	66.7	1.8
Innermost	1.7	60.9	2.1
Lateral	1.3	64.3	2.2
POND P2			
Outermost	1.2	50.0	1.7
Lateral	3.2	68.6	1.9
Innermost	3.8	64.3	1.9
Lateral	3.0	65.6	1.7

TABLE 3.

BARNACLE FOULING ON STAKES BETWEEN
MARCH 14 and JULY 21, 1970

Location	Barnacles Per 100 cm^2		Mean Diam. (cm)
Sound	92.6		0.6
Creek	53.2		0.5
C1	0		-
C2	0		-
C3	0		-
P1	0.52	(Approx.)	1.1
P2	0.65	(Approx.)	1.2
P3	0.52	(Approx.)	1.3

TABLE 4.

MEAN NUMBER OF BARNACLE LARVAE PER LITER
PASSING INTO MIXING TANKS

	14 Mar	15 Mar	10 Apr	5 May	30 May	27 June
C Ponds	238.5	36.0	4.8	0.8	3.5	0.0
P Ponds	No Data	No Data	1.5	No Data	0.3	0.5

TABLE 5.

LIMNORIA TRIPUNCTATA PER CM^3 IN
INFECTED BOARDS PLACED IN FOUR HABITATS

	Initial 6 Aug	7 Sept	14 Dec
Sound	5.3	20.1	4.9*
C2	7.6	0.8	2.6
Creek	9.2	1.9	0.6
P2	8.5	9.2	3.1

* This sample heavily infested with Bankia sp. - therefore not all the volume sampled was available for Limnoria infestation - consequently this figure may be an underestimate.

MOLLUSCAN STUDIES

by Barbara Muse*

INTRODUCTION

Marked differences in shell increment and gross weight in growth studies of young oysters Crassostrea virginica were noted in experimental and control ponds from July 1969 to July 1970. Rate of growth in control ponds was nearly twice that in the experimental ponds which contained profuse plankton blooms (Chestnut, 1970). Several factors were undoubtedly inhibiting feeding of oysters in the experimental ponds. Studies are in progress to determine differences between oysters in the C-1 and P-1 ponds. These include: 1) analysis of the chlorophyll content of the water, feces, pseudofeces, stomach, digestive gland, and crystalline style 2) identification of algal species in the water, feces, pseudofeces, and stomach of the oysters, Rangia cuneata, and Modiolus demissus 3) wet weights and dry weights of the oysters 4) glycogen analysis of homogenized oyster tissue 5) kymograph recordings of the shell movement in response to various stimuli. The wet weights and dry weights and the glycogen analysis give an indication of the condition of the oysters, and the other three are an attempt to determine the amount of food intake. These studies may explain why growth was less in the P ponds even though more food was available.

MATERIALS AND METHODS

Chlorophyll analyses

Analysis of the chlorophyll content of the feces, pseudofeces, stomach, digestive gland, and crystalline style of C-1 and P-1 oysters as well as the water the oysters were in was according to the methods of Strickland and Parsons (1965). In this method chlorophyll is extracted from the cells with acetone. Each sample was weighed, ground in a tissue grinder with 90% acetone, and transferred to a 15 ml. centrifuge tube. The mixture was made

*Under supervision of Dr. A. F. Chestnut

up to the 10 ml. mark and left to extract for one hour in the refrigerator. At the end of this time the tubes were centrifuged and the clear supernatant liquid decanted into a 10 cm., 10 ml. cuvet (a 1 cm. cuvet was used if the extinction of light was too strong). The extinction of light was measured at 750 mμ, 665 mμ, 645 mμ, 630 mμ, and 480 mμ in the Hitachi-Perkins-Elmer Spectrophotometer. Then it was acidified with two drops of HCl and measured again at 750 mμ and 665 mμ. Cell-to-cell blanks and the turbidity blank, the reading at 750 mμ, were subtracted from the other readings (the 750 mμ reading was multiplied by 3 before subtracting from the 480 mμ reading). The amount of chlorophyll and carotenoids in ug can be calculated with the following formulas:

$$\text{Chl a} = 11.6\ E_{665} - 1.31\ E_{645} - 0.14\ E_{630}$$
$$\text{Chl b} = 20.7\ E_{645} - 4.34\ E_{665} - 4.42\ E_{630}$$
$$\text{Chl c} = 55\ E_{630} - 4.64\ E_{665} - 16.3\ E_{645}$$

plant carotenoids $= 4.0\ E_{480}$ where E stands for extinction.

These pigment values are divided by the weight of the tissue samples. Breakdown products of the chlorophylls, the pheophytins can interfere with the extinction at the various wavelengths. This can be checked by computing the amount of pheophytin and chlorophyll a according to the formulas:

$$\text{Chl a} = \frac{(A)(K)\ (E_o - E_a)\ (v)}{(W)\ (L)}$$

$$\text{Pheo} = \frac{(A)\ (K)\ \left[(RE_a) - E_o\right]\ (v)}{(W)\ (L)}$$

where,

A = absorption coefficient of chloropyll a
= 11.0
K = factor to equate the reduction in absorbency to initial chlorophyll concentration
= 2.43

E_o = absorbency before acidification
E_a = absorbency after acidification
v = volume of acetone used for extraction (ml.)
W = weight of sample in grams
L = cuvet path length (cm.)
R = maximum ratio of $E_o:E_a$ in the absence of pheo-pigments = 1.7

All concentrations are expressed in units of ug/gm which is equal to mg/kg. Concentrations in the water, by comparison, are expressed in mg./m^3. See tables 1 through 4 and figures 1 through 4.

Identification of algae

An attempt was made to identify the algae in the stomach, feces, and pseudofeces of C-1 and P-1 oysters and also in C-1 and P-1 water. Identification of algae in the stomach, feces, and pseudofeces of Rangia cuneata and Modiolus demissus was done for comparison. The sample to be studied was placed on a slide and covered with a cover slip. Then it was observed under the microscope.

Wet weights and dry weights of oysters

Oysters were first homogenized in a tissue grinder, weighed, and then dried in a drying oven in 10 ml. beakers at about 80° for at least 72 hours. At the end of this time the dry weight was taken, and the percent dry weight was calculated from the formula:

$$\% \text{ dry weight} = \frac{\text{dry weight}}{\text{wet weight}} \times 100$$

(Walne, 1970). The percent dry weight in each pond was averaged. The dry weight was found to vary less than 1% whether the oysters were left in 72 hours or several weeks, and, therefore, drying time was not critical. See Table 5.

Glycogen analysis

The method used for separating the tissue and glycogen was Pfluger's with modifications by Good, Somogyi and Kramer (1933). Oyster tissue was homogenized in a tissue grinder, and a small sample obtained for analysis with a capillary dropper (about 0.1 gm. or less). The glycogen was separated from the tissue by heating the tissue in a 5 ml. pyrex test tube with 1 ml. of 30% KOH. The test tube was loosely stoppered and placed in a boiling water bath. After the tissue was in solution, the glycogen was precipitated with 1.1 ml. of 95% ethanol. It was then heated till the mixture boiled, cooled to room temperature, and centrifuged. The mother liquor was decanted, and the test tube was allowed to drain. The remaining alcohol was expelled by rapid heating in a hot water bath. Glycogen standards of 0.100 mg., 0.050 mg., 0.025 mg., 0.010 mg., and 0.005 mg. were made up. The standards and tissue samples and reagent blanks were diluted up to 2 cc., with distilled water, and 5 cc. of diphenylamine reagent were added according to the method of Boettiger (1946). This was heated exactly 40 minutes in a boiling water bath, and then plunged in cold water for at least 3 minutes to halt the action. At this point dilution of the samples is necessary. This can be done by adding 0.1 ml. of the sample to 6.9 ml. of reagent blank. The samples were then read in the spectrophotometer at 635 mμ.

Kymograph recordings

The bottom shells of oysters were imbedded in cement (to add weight). Pieces of aluminum wire were hammered so that they were flat at both ends and bent into a loop. These were glued to the upper oyster shell with epoxy glue and thus provided a place to tie string. The other end of the string was tied to the stylus of the kymograph. A pen point filled with ink on the end of the stylus recorded the oyster movement on paper wrapped around a rotating drum. Drum speed was $12\frac{1}{2}$ cm. every hour. The oysters imbedded in cement were kept in C-1.

RESULTS AND DISCUSSION

Chlorophyll analysis

As figures 3 and 4 show chlorophyll is rapidly broken down to pheophytin in the oyster. This makes accurate analysis of chlorophyll in oyster tissues impossible, and this phase of the studies has been abandoned. However, it may be of interest to note that there was mostly chlorophyll a rather than pheophytin in the stomach of P-1 oysters. The chlorophyll a also appeared to be high in the feces sample. These could imply poor utilization of the food available in P-1 by the oysters.

Identification of algae

These studies were also set aside because of the difficulty of obtaining volumes (which are necessary to determine concentration of algae), the fact that few algae other than diatoms seemed to survive intact and in identifiable form, and uncertainty as to the correct identification of the algae. It may be of interest that besides the diatoms there were also present Peridinium fragments, planktonic crustacean fragments, rotifers, oyster gametes, and in one fecal sample, annelid larvae.

Wet weights and dry weights

On the average, the percent dry weight of C-1 oysters was higher than the percent dry weight of P-1 oysters, though there was overlap (see Table 5). The overlap may imply that there is some adjustment to P-1 water by the oysters. Generally, though, the P-1 oysters have less solid material and are in poorer condition than the C-1 oysters.

Glycogen analysis

Attempts to determine the glycogen content of oyster tissue have been unsuccessful, partially because of learning experience, and partly because the reagent was old.

Kymograph recordings

Kymograph studies of the oysters in the ponds are complicated by wind interference. However, an

attempt will be made in spring to study the oysters (when they resume feeding). Studies in the laboratory at present indicate that an oyster in C-1 water (water that had been brought into the lab and gradually warmed up) began feeding at a lower temperature than it did in P-1 water. Also it took the oyster longer to open in P-1 water, and it did not open as widely. The P-1 water was 0.6° warmer than the C-1 water.

CONCLUSION

Chlorophyll studies on the oyster, Crassostrea virginica, indicate that they may not utilize food as well in P-1 water as in C-1 water because chlorophyll was not broken down as readily in P-1 oysters. The percent dry weights indicate that the oysters of P-1 were generally in poorer condition than the C-1 oysters. Kymograph recordings seemed to indicate some physiological shock when a feeding C-1 oyster was transferred to P-1 water (when oysters were first added to the ponds, mortality was higher in P-1). However, over time there is some adjustment, as can be seen by the overlap in percent dry weights. Thus, the studies have indicated that some factor inhibits growth in P-1. The initial mortality is higher and the condition poorer (as indicated by the percent dry weight and glycogen content) in P-1 than in C-1. Feeding and utilization of food is also poorer (chlorophyllanalysis and kymograph studies).

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TABLE 1.

CHLOROPHYLL AND CAROTENOID CONTENT OF C-1 OYSTERS IN mg./kg.

sample	chl a	chl b	chl c	carotenoids
feces	138	18.1	61.0	65.6
pseudofeces	90.8	1.23	40.2	76.0
stomach	3.15	0.250	1.07	1.77
digestive gland	129	20.0	104	142
crystalline style	1.32	1.42	6.08	3.04
water	0.031	0.001	0.017	0.016

TABLE 2.

CHLOROPHYLL AND CAROTENOID CONTENT OF P-1 OYSTERS IN mg./kg.

sample	chl a	chl b	chl c	carotenoids
pseudofeces	18.8	0.789	7.03	9.87
stomach	0.459	0.343	0.121	0.303
digestive gland	324	6.56	128	215
water	0.237	0	0.074	0.114

TABLE 3.

CHLOROPHYLL A AND PHEOPHYTIN CONTENT OF C-1 OYSTERS IN mg./kg.

sample	chl a	pheophytin
feces	320	0
pseudofeces	66.7	35.7
stomach	1.87	1.91
digestive gland	12.3	197
crystalline style	0.530	1.52
water	0.029	0.002

TABLE 4.

CHLOROPHYLL A AND PHEOPHYTIN CONTENT OF P-1 OYSTERS IN mg./kg.

sample	chl a	pheophytin
pseudofeces	14.9	8.90
stomach	0.32	2.71
digestive gland	23.8	499
water	0.198	0.059

TABLE 5.

WET WEIGHTS AND DRY WEIGHTS OF C-1 AND P-1 OYSTERS

date	sample*	wet weight	dry weight	% dry weight	average % dry weight
9-29-70	C_1	5.6	1.4	25.0	C ave=23.7
9-29-70	C_2	4.9	1.1	22.4	
9-29-70	P_1	11.1	1.9	17.1	P ave=19.7
9-29-70	P_2	4.8	1.0	20.8	
9-29-70	P_3	10.4	2.2	21.2	
11-6-70	C_1	4.33	0.87	20.1	C ave=21.5
11-6-70	C_2	9.52	2.17	22.8	
11-6-70	P_1	7.57	1.67	22.1	P ave=21.3
11-6-70	P_2	5.80	1.19	20.5	
11-29-70	C_1	7.20	1.66	23.1	C ave=23.4
11-29-70	C_2	8.33	1.97	23.6	
11-29-70	P_1	6.68	1.30	19.5	P ave=19.5
11-29-70	P_2	7.41	1.44	19.4	
12-22-70	C_1	3.41	0.79	23.2	C ave=22.6
12-22-70	C_2	6.58	1.45	22.0	
12-22-70	P_1	7.64	1.71	22.4	P ave=21.9
12-22-70	P_2	3.45	0.74	21.4	
1-3-70	C_1	9.20	1.95	21.2	C ave=22.6
1-3-70	C_2	9.88	2.36	23.9	
1-3-70	P_1	5.27	1.03	19.5	P ave=19.2
1-3-70	P_2	3.37	0.56	16.6	
1-3-70	P_3	8.20	1.77	21.6	
1-28-71	C_1	7.19	1.52	21.1	C ave=21.9
1-28-71	C_2	8.00	1.81	22.6	
1-28-71	P_1	3.46	0.48	13.9	P ave=15.6
1-28-71	P_2	7.35	1.27	17.3	

* C_1, C_2, etc. are samples from pond C-1 and are <u>not</u> symbols for pond C-1, pond C-2, etc.

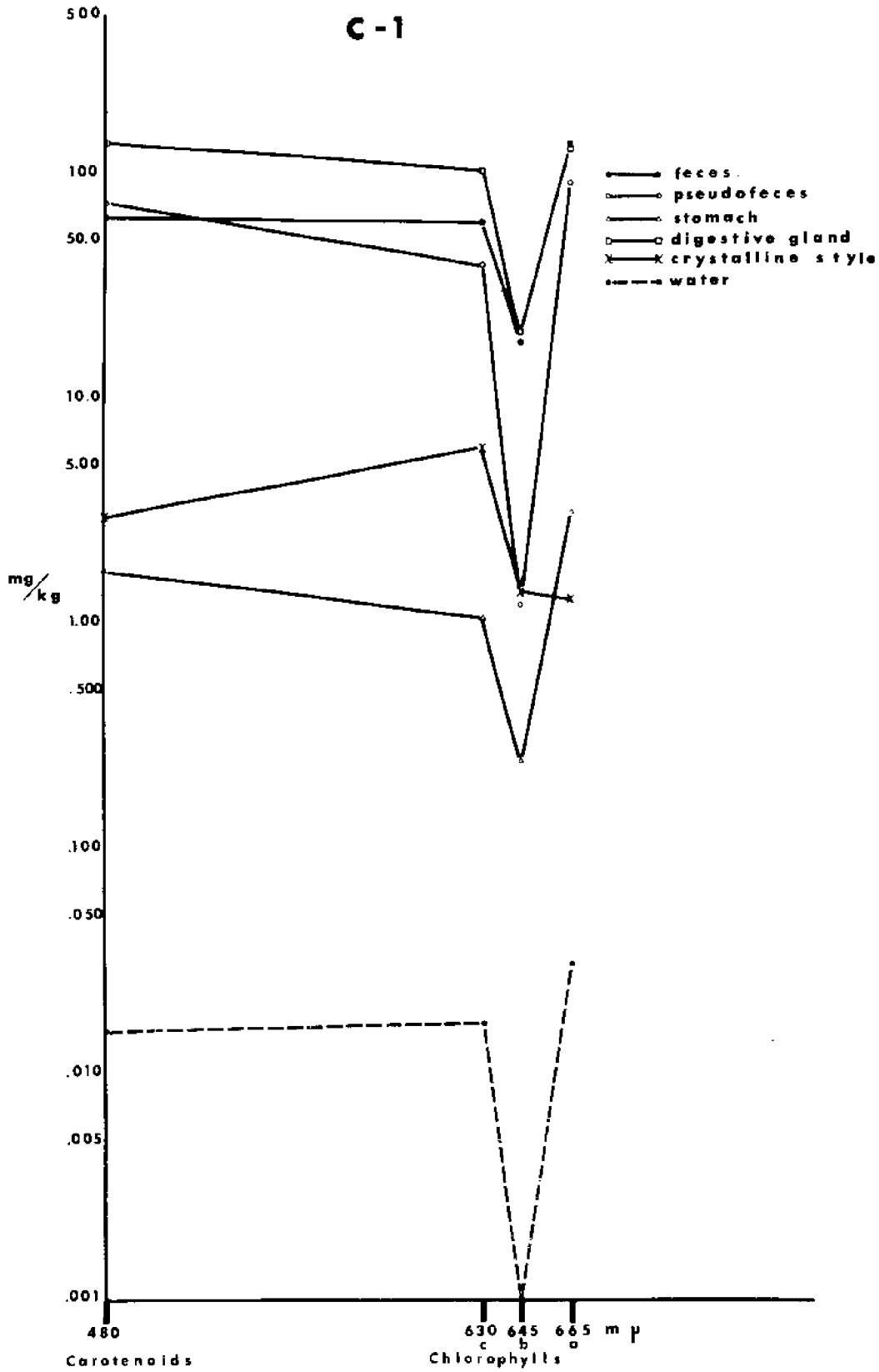

Fig. 1. Chlorophyll and carotenoid content of C-1 oysters in mg./kg.

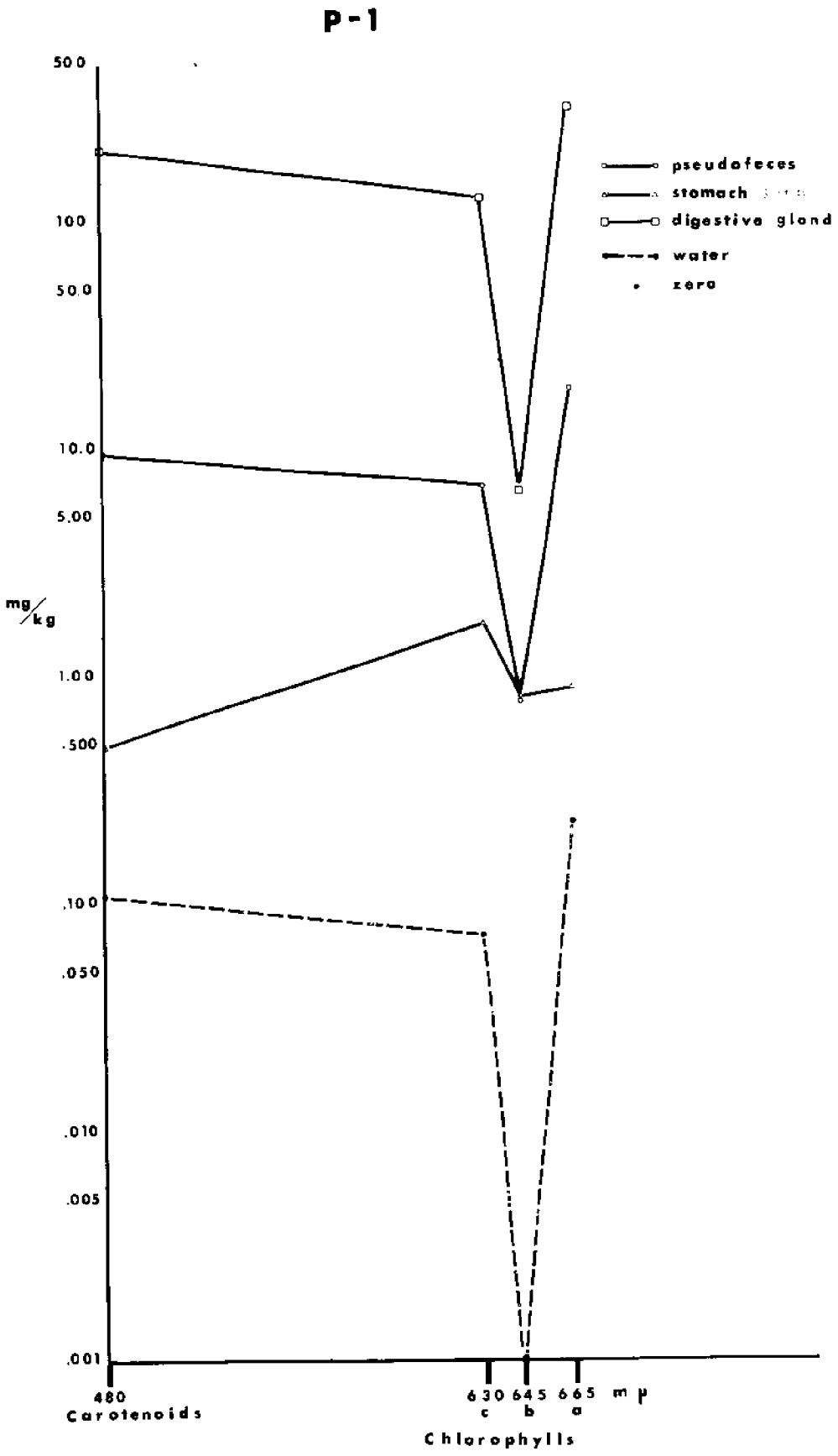

Fig. 2. Chlorophyll and carotenoid content of P-1 oysters in mg./kg.

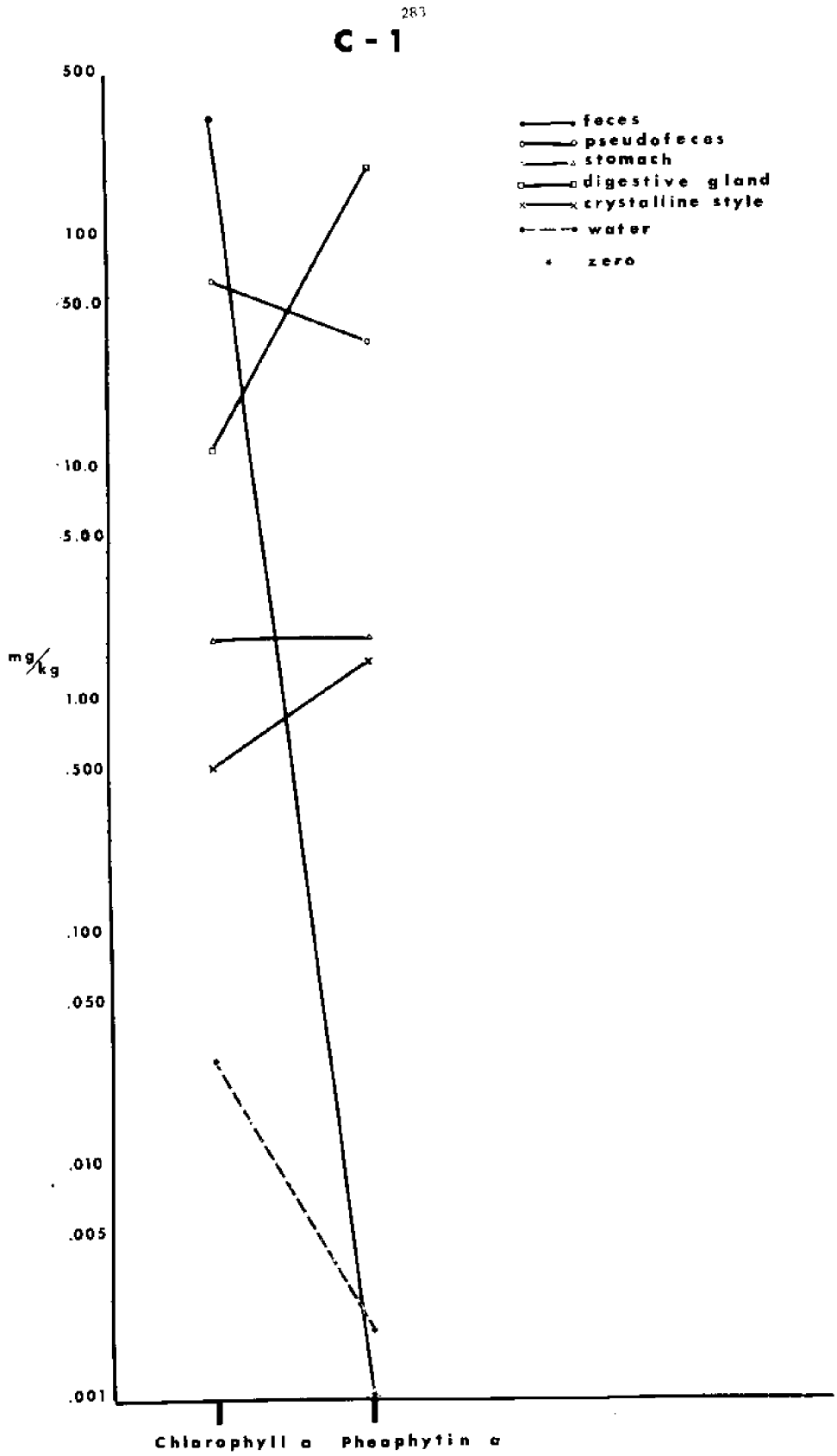

Fig. 3. Chlorophyll a and pheophytin content of C-1 oysters in mg./kg.

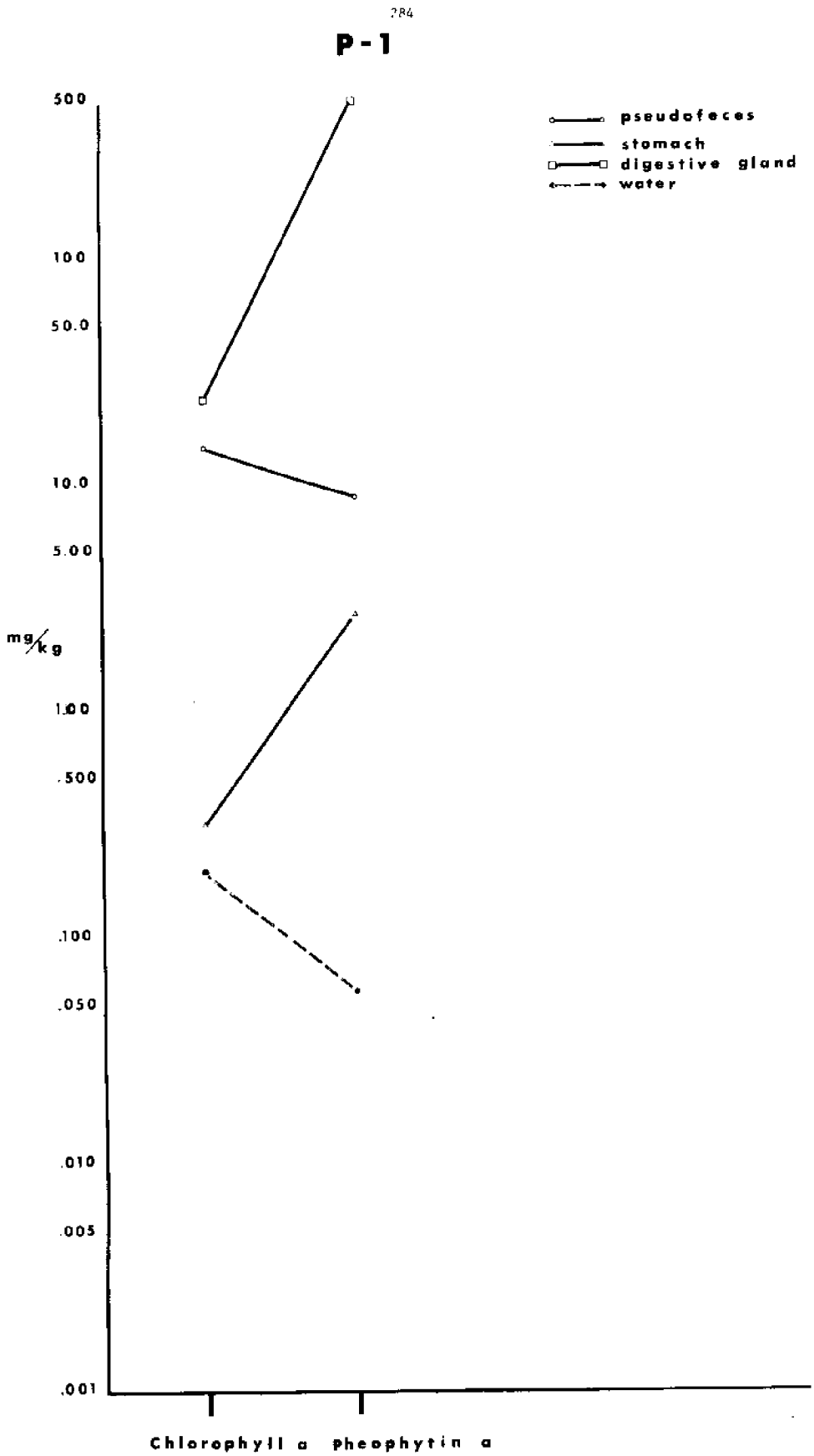

Fig. 4. Chlorophyll a and pheophytin content of P-1 oysters in mg./kg.

FISHES OF POND AND CREEK SYSTEMS

by R. A. Hyle
Curriculum in Marine Sciences
University of North Carolina

Introduction

Increasing awareness of the quality of the environment has brought the estuaries into national concern. Their importance as fin and shellfish production areas and wildlife sanctuaries is only too well known. Recently, for example, the Sport Fisheries Institute Bulletin (April, 1970) noted that in the state of Maine "the average annual crop of shellfish and bait worm harvest from each acre of estuary was worth $35,703 in the market place ...by comparison, the best market garden farms in Maine yield $2,000 of crops per acre per year". Further, it was emphasized that "general overall reduction of productivity of the estuaries by pollution or other factors, say 20, 40, 60, or 80 percent, etc., would cause a corresponding reduction in yield on the continental shelf".

A phase study of the fishes in the Sea Grant ponds and nearby creeks in the Newport Estuary was intiated in June, 1970. The Newport Estuary lies in the South Atlantic Estuarine Zone, and according to the National Estuary Study (U. S. Fish and Wildl. Serv., 1970) has been classified as a relatively unmodified estuary. Nevertheless, the Newport River Complex is receiving treated sewage wastes. The Calico Creek-Crab Point Bay area receives treated sewage wastes from the Morehead City Sewage Treatment Plant, and the Newport River proper receives treated sewage wastes from the Newport Sewage Treatment Plant.

The effects of sewage wastes on natural waters are well documented. An excellent review of literature on eutrophication appears in the National Academy of Science publication, Eutrophication (1969). Weiss and Wilkes (1969) present a discussion of estuarine ecosystems that receive sewage wastes. Some effects of water pollution on freshwater fishes are reviewed by Katz and others unpublished, 1970). Most effects of sewage wastes on estuaries have been labelled harmful, however, a new approach has been presented in the theme of this project which suggests the possible use of sewage wastes to foster productive "new" estuarine systems (Odum and Chestnut, 1970).

The main objective of this study is to compare the species composition, numbers and biomass of the fish populations of the control and waste ponds and of the creek systems and thereby assess the influence of eutrophication on the fish populations of the ponds and creek systems.

MATERIALS AND METHODS

Ponds

Sampling in the control and waste ponds was conducted on a quarterly basis (summer - June 8-17; fall - August 24-September 6; winter - December 10-13). A multiple mark-recapture study was employed for population estimates (Robson, 1960). Liquid nitrogen was used as a branding agent for marking, and a marking tool was designed and constructed by Mike Beeston to expedite the marking operation (Highell, 1969).

Undulations in the pond bottoms prevent an avenue of escape to fishes during seining; also "gear" and experiments in the ponds present obstructions making seining difficult. In view of this, sunken traps were utilized as a method of capture. The traps consisted of a lead net (1 1/2" stretch mesh), a set of battens of the same mesh and a funneled crib constructed from hardware cloth (1/8" mesh) and aluminum angle. The trap is shown in figure 1. All traps were unbaited and fished for 24 hours for 3 successive days in each set of ponds. The fishes were sorted to species and counted. Length and weights were taken of each sample. When large samples were caught, subsamples were taken for length and weight measurements. All fishes were returned to the ponds.

Portions of the waste and control ponds were seined during the sampling periods by the Crustacean Phase. Samples were examined for fish content. These data were also used in the pond species check lists.

Larval and juvenile fishes were separated from the seeding samples, which have been collected since the summer of 1967. All fishes were sorted (to species) and bottled in buffered

formalin.

Creeks

An effort has been made to extend this study into the nearby waters. After a preliminary survey of the Newport River Complex, Russell and Harlowe Creeks were chosen as control creeks. Calico, Russell and Harlowe Creeks (shown in figure 2) were sampled by trawling on a quarterly basis.

Due to gear difficulties, the summer sample was delayed and concurrent creek sampling was not possible with that of the ponds. Nevertheless, the creeks were sampled on July 8 and 9 with a small beam trawl. Trawling was done at flood tide. Each trawl set was for 10 minutes at a constant speed. All fishes were sorted to species, measured and weighed. The trawls were marked by shore points.

During the fall and winter samples, the creeks were sampled with a 20' otter trawl (1 1/2" stretch mesh with a 1/4" bag). The beam trawl was abandoned because it readily collected mud in the shallow creeks and made sampling inefficient and quite difficult. Again, trawling was done at flood tide and for 10 minutes at a constant speed. The trawl set was marked by shore points. All fishes were sorted to species, weighed and measured.

RESULTS

Ponds

A check list of fish species collected in the ponds during 1969 is presented in table 1. Fish species collected in the ponds during 1970 are shown in tables 2, 3 and 4. The check lists have been compiled from main and trap data.

Population and biomass estimates have not been calculated for the fishes of the control ponds; the mark-recapture data were not sufficient for population estimates since the catches were limited to one to several individuals.

Population and biomass estimates for two dominant species, Fundulus heteroclitus and Cyprinodon variegatus, in the waste ponds are given in tables 5 and 6. Cyprinodon minnows were trapped and marked during the winter sample, however, marks were not recaptured.

Mortalities occurred in the control ponds in September. On 9-17-70 12 Leiostomus xanthurus and one Paralichthyes

dentatus were discovered in C-3; one Paralichthyes dentatus was discovered also in C-2. Again on 9-17-70 mortalities occurred in C-3; 3 Leiostomus xanthurus were found. On C-19-70 one Paralichthyes dentatus was found in C-3, and one Leiostomus xanthurus in C-1. The dead fishes were unidentifiable but had begun to decompose; length and weight measurements were not taken.

The identification of the larval and juvenile fishes separated from the seining samples is incomplete and is being continued.

Creeks

Check lists of fish species collected in Calico, Harlowe and Russell Creeks are presented in tables 7 and 8. No fishes were captured by trawling during the winter sample in the creeks. Creek sample weights for the fall sample are compared in table 9.

DISCUSSION

It is assumed that the seeding has introduced the same species to both control and waste ponds, however, there appears to be a segregation of non-commercial and commercial species in the two series of ponds (Tables 2, 3 and 4). The mechanism is not clear, nevertheless the phenomenon is not new to highly eutrophic systems. Hasler (1947) in an early paper summerized the general pattern of change from coregonnies (whitefish) to coarse fish as eutrophication continued.

It is not possible to quantitatively compare the biomass of the two series of ponds, since small numbers of individuals were trapped in the control ponds; a mark-recapture study was not possible with just a few individuals. Nevertheless, the small numbers of fishes trapped, seined and observed in the control ponds appear to suggest low population numbers, compared to the high population numbers in the waste ponds.

High temperatures during September are probably responsible for the fish mortalities. Table 10 gives temperatures for both series of ponds on September 16 and 18.

Efforts in the creek systems have been of a pioneer nature. The beam trawl sampling of the summer led to the adoption of an otter trawl for later sampling. It is evident that quarterly sampling in the creeks is not sufficient to meet the objectives of the phase. Therefore, beginning in the spring of 1971 creek sampling will be conducted monthly, and trawl and trap stations may be employed. Temperature, salinity and oxygen will be measured at each station.

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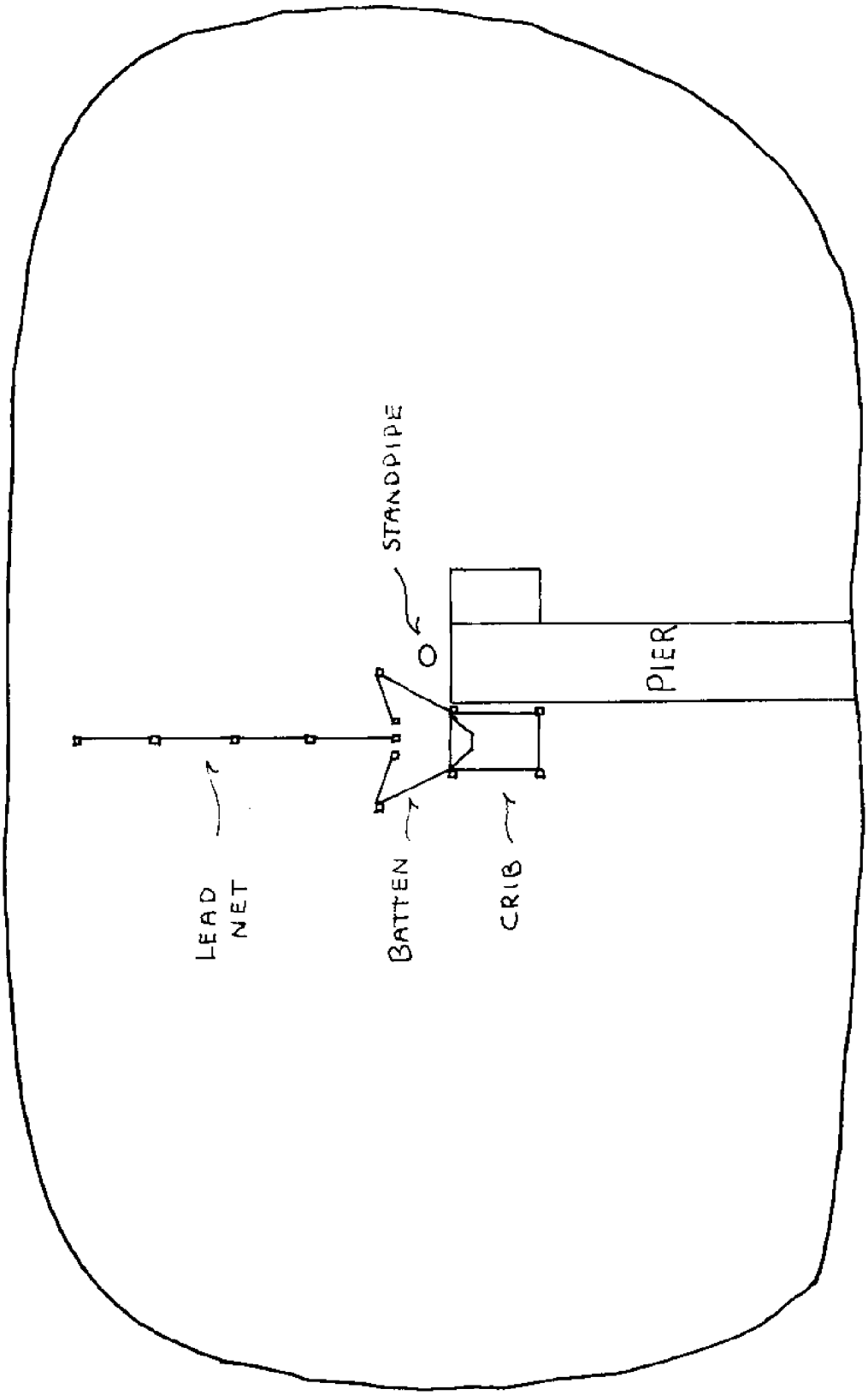

Figure 1. Diagram of trap arrangement in pond.

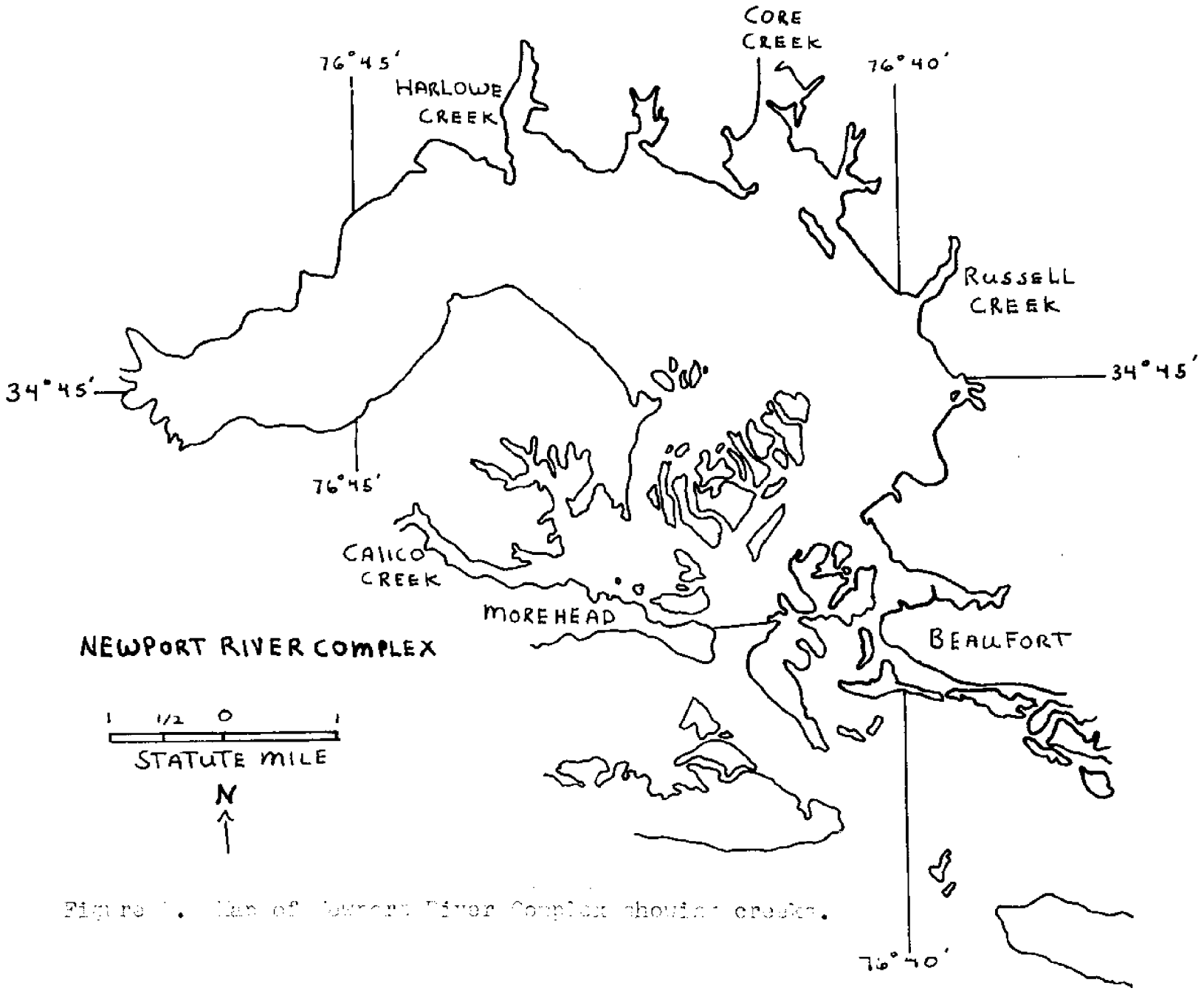

Figure 1. Map of Newport River Complex showing creeks.

Table 1 Fish Species Collected in Ponds.

Species	C-1	C-2	C-3	P-1	P-2	P-3
Brevoortia tyrannus		S,F	S			
Anchoa mitchilli	B	B	B,S			
Anguilla rostrata		B,S	S	B,S	S	B,S,F,W
Fundulus heteroclitus	B,F,W	S	B,S,F,W	B,S,F,W	B,S,F,W	B,S,F,W
Cyprinodon variegatus				B,S,F,W,	B,S,F,W	S
Gambusia holbrooki			B		B,S,F,W	B,F
Paralichthyes dentatus	B,S,F	S,F	B,F			
Membras vagrans	B		S			
Mugil cephalus			B			B,S,F,W
Lagodon rhomboides	B,S,F,W	B,S,F,W	B,S			
Leiostomus xanthurus	B,S,F	B,S,F	B,S,F			
Micropogon undulatus	B	B				
Gobiosoma bosci	B			B	B	

	Relatively Abundant	One to Several Individuals
Beeston 1969	B	B
Summer 1970	S	S
Fall 1970	F	F
Winter 1970-71	W	W

Table 2 Biomass Estimates of *Fundulus heteroclitus* and *Cyprinodon variegatus*. Weights are dry weights.

	Fundulus		Cyprinodon	
Pond	Estimated Population	Grams/M^2	Estimated Population	Grams/M^2
		Summer Sample		
P-1	790	3	290	0.8
P-2	670	4	60	0.2
P-3	2500	11		
		Fall Sample		
P-1	880	4	3000	6.0
P-2	510	4	180	0.5
P-3	3600	15		
		Winter Sample		
P-1	580	3		
P-2	990	6		
P-3	1200	5		

Table 3 Fish Species Collected in Creeks

Species	Calico	Russell	Harlowe
Anchoa mitchilli	F,S	F	F
Synodus foetens			F
Paralichtes rhomboides			S
Symphurus plagiusa			F,S
Mugil cephalus	S		
Orthopristes chrysopterus		F	F
Lagodon rhomboides	S	F,S	F
Eucinostomus gula	F	F,S	
Cynoscion regalis	F		F
Menticirrhus sp.			F
Leiostomus xanthurus	F,S	F,S	F,S
Chaetodipterus faber			F
Prionotus carolinus			F
Chasmodes bosquianus			F
Opsanus tau		F	F

	Relatively Abundant	One to Several Individuals
Summer 1970	S	S
Fall 1970-71	F	F

Table 4 Comparison of Creek Sample Weights* for Fall, 1970

Sample	Calico	Russell	Harlowe
8-26-70	550	380	430
9-4-70	1100	590	120

*Wet weight in grams.

Table 5 High Temperatures of Waste and Control Ponds

Pond	September 16	September 18
C-1	34.6°C	35.3°C
C-2	33.8°C	33.8°C
C-3	34.6°C	34.2°C
P-1	29.2°C	30.8°C
P-2	28.8°C	30.8°C
P-3	29.8°C	30.9°C

OBSERVATIONS ON BIRD ACTIVITY AROUND PONDS

by C. J. Spears and Austin B. Williams
Institute of Marine Sciences, University of North Carolina

Casual observation of bird activity by almost every investigator involved in the project indicated that birds were both a drain on and a contributor to the energy webs in a set of experimental estuarine ponds near the Institute of Marine Sciences on Bogue Sound and the Morehead City Sewage Treatment Plant north of town at the edge of a marsh bordering Calico Creek. No one was charged with responsibility for recording bird activity, but when it became evident that this should be done to help complete the accounting of energy flow, a person was sought who was experienced in observation of birds locally, knew the species well, and could devote fractional time to observation. One of the authors, C. J. S., teacher in the Carteret County Public School System, has done the field work and prepared this report in conjunction with A. B. W.

Recorded observations were initiated in early September 1970; therefore, at this writing little can be done but present summarizing notes. The influence of fall migration on occurrence can be seen clearly (Table 1) as the number and species began to drop during September.

Notes on feeding habits:

1) Spotted Sandpiper (Actitis macularia). Probably visiting area, feeding on insects.
2) Kingfisher, Eastern Belted (Megaceryle alcyon alcyon). Probably diving in all P ponds in effort to spear small fish.
3) Semipalmated Plover (Charadrius semipalmatus). Visiting insect feeder.
4) American Egret (Casmerodius albus egretta). Observed wading in P ponds spearing small fish. Mostly seen in P-1, occasionally in P-2. During middle of day these birds fly across creek to roost in pines.
5) Snowy Egret (Egretta thula thula). Seen mostly on banks of P-1 and P-2, also perched on stakes in ponds spearing fish. During midday these birds also roost in pines across creek.
6) Louisiana Heron (Hydranassa tricolor ruficollis). Perches on piers and pond banks. Not so abundant as snowy egrets.
7) Clapper Rail, Northern (Rallus longirostris crepitans). Feeding habits probably determined by tides, during high tides the species is found around P ponds. Rails follow ebbing tide out. (A rail was heard in Spartina growth near road in P-2 during last week in September, A. B. W.).
8) Green Heron, Eastern (Butorides virescens virescens). Seen spearing fish from piers, stakes and banks of P ponds.
9) More feeding observed in P-1 and P-2 than in P-3.
10) On basis of tracks, most activity in C ponds centers in C-1 and C-3.

TABLE 1

LIST OF BIRD SPECIES OBSERVED IN EXPERIMENTAL (P) AND CONTROL (C) PONDS DURING EARLY AUTUMN, 1970.

Date	Species	Number	Pond	Observation Time
9-9-70	Spotted Sandpiper	1	P-1	6:45 P.M.-7:30 P.M.
	Kingfisher	1	P-1	
	Semipalmated Plover	2	P-1 & 2	
9-10-70	None	-	P-Ponds	6:45 P.M.-7:45 P.M.
9-11-70	American Egret	4	P-1,2,3	5:00 P.M.-6:00 P.M.
9-13-70	Snowy Egret	10	P-2	6:45 A.M.-7:30 A.M.
	Louisiana Heron	1	P-2	
	American Egret	1	P-2	
	Clapper Rail	1	P-2	
	Least Sandpiper	2	P-2	
9-15-70	None	-	Control	4:30 P.M.-5:00 P.M.
9-22-70	None	-	P-Ponds	4:00 P.M.-4:30 P.M.
9-23-70	Sandpiper (?)	1	P-1	5:00 P.M.-5:45 P.M.
9-28-70	None	-	P-Ponds	3:00 P.M.-3:15 P.M.
10-5-70	None	-	P-Ponds	4:20 P.M.-4:45 P.M.
10-9-70	Green Heron	1	Control-3	4:10 P.M.-4:20 P.M.
10-10-70	American Egret	1	P-3	8:00 A.M.-8:30 A.M.
10-10-70	None	-	Control	8:45 A.M.-9:10 A.M.
10-11-70	None	-	P-Ponds	7:45 A.M.-8:15 A.M.
10-11-70	None	-	Control	8:20 A.M.-8:30 A.M.
10-27-70	Snowy Egret	2	P-1	10:10 A.M.-10:45 A.M.
10-28-70	None	-	P-Ponds	4:15 P.M.-4:30 P.M.
10-28-70	None	-	Control	4:00 P.M.-4:10 P.M.

Total number of visits to P-Ponds - 12
Total number of visits to C-Ponds - 5
Number of species seen in P-Ponds - 9
Number of species seen in C-Ponds - 1
Total hours of observation time at P-Ponds - 7 hrs. 15 mins.
Total hours of observation time at C-Ponds - 1 hr. 25 mins.
Most abundant species - Snowy Egret

CALCIUM

by Frederick E. Davis*
Department of Environmental Sciences and Engineering

INTRODUCTION

As part of the Water Quality phase of the Sea Grant Project a study of the calcium distribution in the ponds was undertaken. Since the study of the distribution of chemical species in natural waters has been greatly facilitated by the development of specific-ion electrodes, a secondary project to determine the feasibility of using the calcium specific-ion electrode under field conditions was concurrently investigated.

This report contains a brief summary of the electrode feasibility study and presents the preliminary data of the calcium distribution. Consideration of the possible chemical equilibria involved in the calcium distribution will be presented in a later report.

METHODS

Samples were collected in 200 ml polyethylene bottles from a depth of one foot near the outflow standpipe of each pond. Bogue Sound, Calico Creek, sewage and tap water samples were collected at the mixing tanks. The samples were returned to the laboratory and allowed to equilibrate to room temperature. Electrode measurments were made within two or three hours of collection. Chlorinity and atomic absorption measurments were made within two or three days of collection.

The potentiometric measurments were made using a Corning model 104 Four Channel Analyzer in the millivolt mode. The calcium ion activity was measured with an Orion model 92-20 Calcium Ion Electrode. The reference was an Orion model 90-04 Reservoir Reference Electrode with a filling solution of 1.0 molar KCl saturated with $AgNO_3$.

The potential of the calcium specific-ion electrode is developed as shown in equation (1):

$$E = E_o + 29.58 \log \left[(Ca^{++}) + \sum k_i (I_i)^{\frac{2}{2}} \right] \qquad (1)$$

where E is the measured potential of the cell and E_o is the standard potential of the cell. The ( ) refer to ionic activity. The second term in the

* Work phase on Coordination Chemistry, with Dr. J. Donald Johnson, Jr.

log factor represents interference from other cations. This interference is a function of the activity of the interfering cation and the selectivity constant of the electrode for the interfering cation.

Figure 1 shows the response of the calcium electrode to calcium activity in solutions of various salinities. The zero salinity line are solutions of $CaCl_2$ in distilled water. The response in the range of calcium activities expected in the ponds (log Ca^{++} = -2 to -3) is linear with a slope close to 29.58 mv/decadecalcium activity as expected from equation (1). The vertical part of the response curves indicate that interfering cations are dominating the response at low values of calcium activity. However, these interfering cations seem to have little effect at the higher calcium activity levels. The displacement of the response curves in the 10 to 25 ppt salinity solutions by nearly 10 mv to the right of the zero salinity line indicates a difference in the E_o term of equation (1) for the response of the electrode in sea water-like solutions. However, there are only a few millivolts difference among the different salinity lines in the expected range of calcium activities. The differences in the E_o terms are caused, in part, by the differences in the junction potential of the reference electrode in solutions of differing ionic strength.

To correct for possible errors due to cation interference and junction potential differences, the standards for the electrode were made up in solutions with cation concentrations and salinities similar to those of the pond samples. The standards were made with reagent grade chloride salts of sodium, potassium, magnesium and calcium to prevent ion pairing of the calcium in the standards. Standards equivalent to pond water of 16, 18 and 20 ppt salinity were prepared. The calcium concentration ranged from 3 to 7 millimoles/l.

Since the calcium electrode responds to calcium activity the calcium standards were converted to activity standards by use of equation (2):

$$(Ca^{++}) = \gamma_{Ca}[Ca^{++}] \qquad (2)$$

where (Ca^{++}) is calcium activity, γ_{Ca} is the single ion activity coefficient for calcium and $[Ca^{++}]$ is calcium concentration.

Single ion activity coefficients for calcium were calculated from the mean activity coefficient for calcium chloride in sodium chloride solutions (Butler, 1968). The assumption was made that:

$$(\gamma)^3_{CaCl_2} = \gamma_{Ca}\,\gamma_{Cl}\,\gamma_{Cl} \qquad (3)$$

where γ_{CaCl_2} is the mean activity coefficient for calcium chloride and γ_{Cl} is the single ion activity coefficient for chloride ion. The pH convention fixes the single ion activity coefficient of chloride by equation (4) (Bates, 1968).

$$-\log \gamma_{Cl} = \frac{0.509\sqrt{I}}{1 + 1.5\sqrt{I}} \qquad (4)$$

I is the ionic strength of the solution. Now $_{Ca}$ can be calculated using equation (5).

$$Ca = \frac{(\gamma)^3_{CaCl_2}}{(\gamma)^2_{Cl}} \qquad (5)$$

Figure 2 shows a typical calibration curve for the calcium electrode. Prior to each set of samples a similar calibration curve was determined using the set of standards with the salinity closest to that of the samples. Calcium activity of the samples were determined from such curves and the calcium concentrations were calculated by the same method as described for the standards. The ionic strengths of the samples were calculated from their salinities by assuming that the principle of conserative ratios of major cations and anions in sea water holds for the ponds and that the sea water contributes most of the ionic strength of the pond water.

The total concentration of calcium in the samples was measured on a Perkin Elmer 303 Atomic Absorption Spectrophotometer. The wavelength used was 4227 A. The flame was air-acetylene. Samples were diluted with distilled water to bring the calcium concentration into the optimum working range of 1 to 10 ppm. The final diluted samples also contained 1% lanthanum and 5% HCl to suppress anionic chemical interference. Standards of 0,2,5,8,and 10 ppm calcium in 1% lanthanum and 5% HCl were prepared from reagent grade chemicals.

The chlorosity of the samples was determined by titration with $AgNO_3$ using K_2CrO_4 as the endpoint indicator. The $AgNO_3$ solution was standardized with I.A.P.O. Standard Sea Water. All samples and reagents were at room temperature. Chlorinity was calculated by equation (6):

$$Cl\,‰ = Cl\alpha \qquad (6)$$

where Cl is the chlorosity and α is the specific volume of the sample as calculated from Horne (1969). Salinities were calculated by equation (7).

$$S\,‰ = 1.8066\ Cl\,‰ \qquad (7)$$

RESULTS AND DISCUSSION

Table 1 is asummary of the calcium concentrations, both total and free, in the ponds and their source waters. The percentage of total calcium which is "complexed" (non-free) and the calcium chlorinity ratios have also been calculated. Since the exact fate of the non-free calcium has not been determined the term complex is used rather loosely in the following discussion.

The ratio of calcium to chlorinity in the source sea water (Bogue Sound and Calico Creek) is in good agreement with the average ratio of 0.02122 - 0.02126 for Atlantic Ocean water (Horne, 1969). However, the relativly high concentration of calcium in the tap water and sewage effluent have had an appreciable effect on the calcium to chlorinity ratios in the ponds.

The percentage of complexed calcium in Bogue Sound and Calico Creek water are in good agreement with 9 percent figure calculated by Garrels and Thompson (1962) for sea water and the 16 percent figure measured by Thompson and Ross (1968) in sea water. Garrels and Thompson's chemical model for sea water assumes that nearly all the complexed calcium is in an ion pair with the sulfate radical. There is nothing to indicate that this is not the case with the Bogue Sound and Calico Creek water also. Although the percentage of complexed calcium in the ponds for the 7/8/70 and 9/24/70 samples are in the same range as the above figures, the11/13/70 samples appear significantly higher. Furthermore it is questionable whether the calcium distribution in the ponds should even be compared to the distribution of calcium in sea water since the "fresh" water is making a considerable contribution to the total concentration.

The percentage of complexed calcium does not appear to be a function of either the total calcium concentration or the chlorinity of the ponds. Also there does not appear to be any significant difference between the percentage of complexed calcium in the P-ponds and the percentage in the C-ponds for any set of samples. However there is not enough data to say definatly that there is no difference in the two sets of ponds.

The most reasonable forms of the complexed calcium in the ponds would be either ion pair formation with sulfate or actual aqueous complexes with bicarbonate or mono-hydrogen phosphate radicals. The degree to which the complexing of calcium might be controled by these anions has not been calculated yet. Variations in the concentrations of phosphate and bicarbonate due to biological activity may explain the variations in the percentage of complexed calcium.

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TABLE 1

SUMMARY OF CALCIUM DATA
Ca_t - Total Calcium Ca_f - Free Calcium

Sample	Ca_t %	Ca_f %	Complexed(%)	Cl %	Ca_t/Cl %
			7/8/70		
P-1	0.200	0.175	12.5	8.09	2.50×10^{-2}
P-2	0.206	0.171	16.9	8.29	2.51
P-3	0.204	0.178	12.7	8.49	2.43
C-1	0.204	0.193	5.4	7.80	2.65
C-2	0.212	0.192	9.4	8.04	2.67
C-3	0.212	0.221	-4.2	8.21	2.61
			9/24/70		
P-1	0.264	0.226	14.4	10.52	2.51
P-2	0.262	0.236	9.9	10.52	2.90
P-3	0.254	0.215	15.3	10.52	2.41
Sewage	0.084				
C-1	0.228	0.181	20.6	7.75	2.94
C-2	0.226	0.179	20.8	8.05	2.81
C-3	0.212	0.167	21.2	8.05	2.64
Tap	0.084				
			11/13/70		
P-1	0.208	0.115	44.7	8.48	2.45
P-2	0.209	0.102	51.1	8.70	2.40
P-3	0.235	0.127	46.4	10.00	2.40
Sewage	0.148				
Creek	0.328	0.306	6.7	15.15	2.16
C-1	0.235	0.152	35.3	9.50	2.47
C-2	0.247	0.154	37.6	9.61	2.57
C-3	0.248	0.156	35.3	10.19	2.37
Tap	0.088				
Sound	0.372	0.337	9.4	17.61	2.13

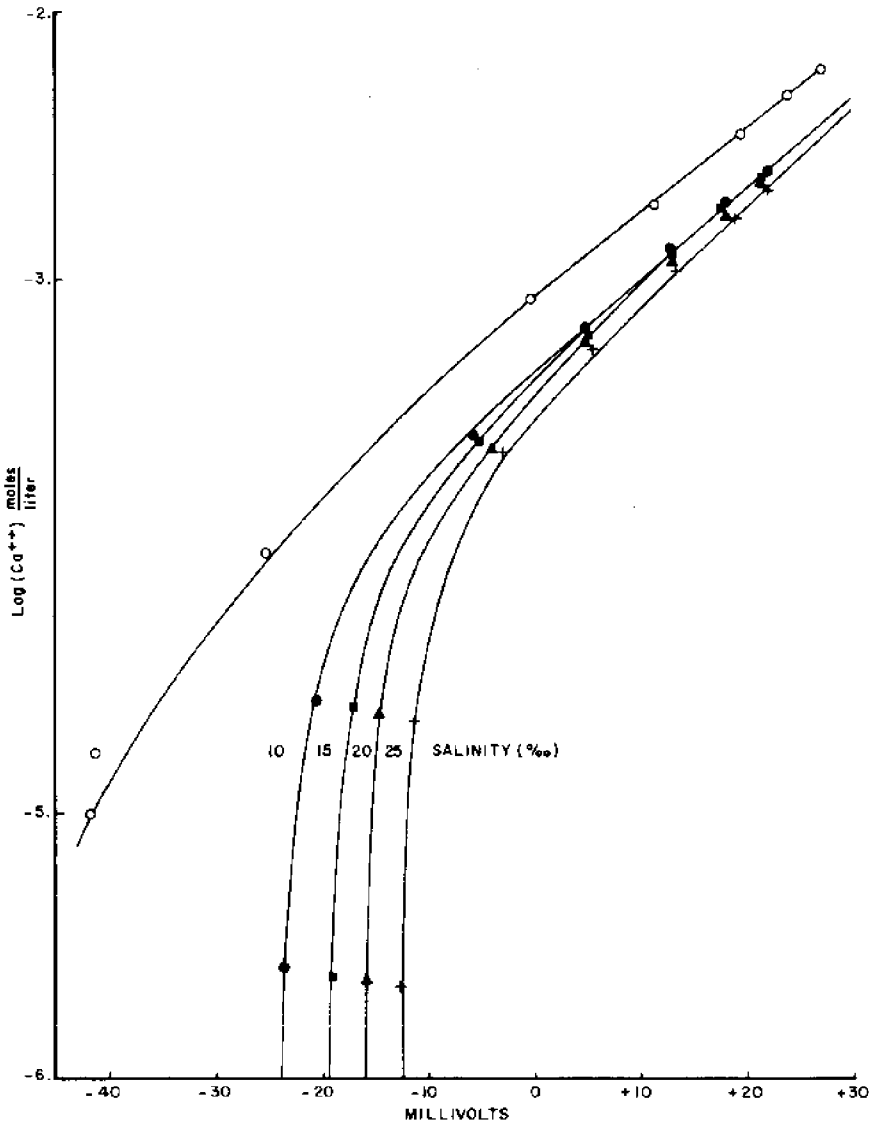

Figure 1. Response of the calcium specific-ion electrode in solutions of various salinities

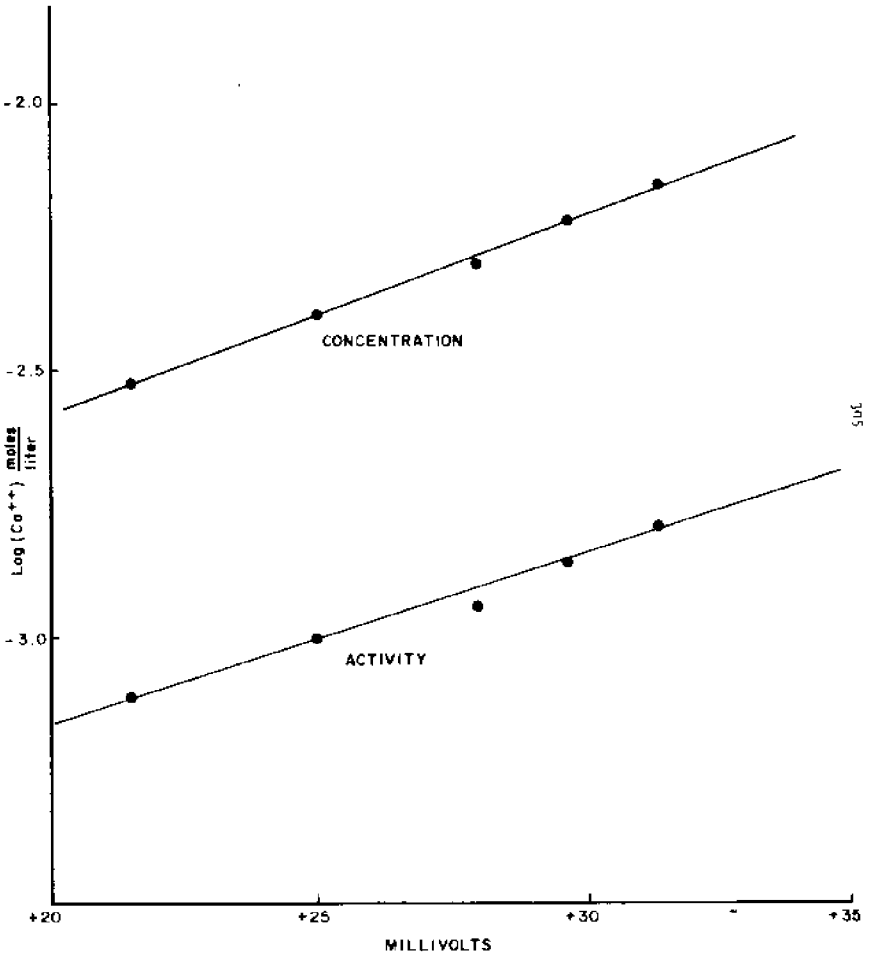

Figure 2. Calibration curve for the specific-ion electrode at 15 ppt salinity

A COMPARISON OF MICROARTHROPOD POPULATIONS IN SEWAGE-EXPOSED AND SEWAGE-FREE SPARTINA SALT MARSHES

E.A. McMahan, R.L. Knight, and A.R. Camp
Department of Zoology

INTRODUCTION

Salt marsh inhabitants include a great many kinds of insects. Biting dipterans such as mosquitoes and midges are readily noted, but the great variety and abundance of other insects in this marine-influenced environment have been documented only recently. Teal (1962) listed about 20 insect species, and a like number of arachnids, which he collected from a Georgia Spartina marsh or from its landward margin. Davis and Gray (1966) reported over 350 different kinds of insects from salt marshes near Beaufort, N.C., some species representing tremendous numbers of individuals. As detritus feeders, as sap- and leaf-feeders, and as parasites and predators on other marsh dwellers, insects are an integral part of the salt-marsh ecosystem.

Our study, carried out during the spring and summer of 1970, was an attempt to discern and evaluate possible effects of sewage effluent on sea marsh insects. Such effects may be direct or indirect. Exposure of a marsh to waste effluents may serve directly to enhance the food supply of detritus-feeders or lead directly to the poisoning of affected organisms. On the other hand, the effect may be indirect, as in stimulation of growth of the marsh grass which serves as food and/or shelter for marsh inhabitants.

Instead of trying to make a complete insect inventory of the study areas and to sample all populations equally, we concentrated on collecting comparable samples from Spartina alterniflora growing under the two conditions. We used standardized collecting procedures throughout, involving chiefly the use of a shop vacuum cleaner. The vacuuming technique sampled only microarthropods living primarily among the stems and leaves of the Spartina and, to some extent, those on the marsh floor. It usually missed the grasshoppers, dragonflies, large spiders, and other arthropods larger than a few mm in length and active enough to escape the suction. The collections consisted of representatives of 9 insect orders, plus spiders, mites, pseudoscorpions, and amphipods.

Three main groups of samples were collected from the Spartina using mainly the vacuuming technique. In this report they will be called 1) the Pond Collection, consisting of 3 sets of samples, 2) the Marsh Collection, consisting of 24 sets of samples, and 3) the Biomass Collection, consisting of 10 sets of samples. In addition, single samples were taken at two other (unpolluted) marshes.

MATERIALS AND METHODS

Collecting Sites

The Pond Collection was made from Spartina which, in the spring of 1969, had been transplanted by Marshall (1970) along the margins of the sewage-treated (P) and the control (C) ponds of the Sea Grant project (see Odum and Chestnut, 1970). By the spring of 1970 when the Pond Collection was made, these plants were well-rooted and, in the case of the P-Ponds, exceedingly luxuriant. This

pond Spartina grew partly in the water and so was exposed to pond and sewage influences, but not to tidal action.

The Marsh and Biomass Collections were made during the summer of 1970 in each of two natural marsh areas: an effluent-flooded marsh (P) adjacent to the Morehead City sewage treatment plant bordering Calico Creek, and a control marsh (C) bordering nearby Dill Creek. Both marshes were in the areas used by Marshall (1970) in his comparative study of Spartina marsh characteristics, but somewhat farther "downstream" in each case. Figure 1 shows the general location of the ponds and marshes.

The soft, gluey sediment in the P-marsh made walking in it, and hence collecting, a virtual impossibility. Therefore a walkway had to be constructed, consisting of boards that averaged 15" x 2.5" x 8'. They were laid end to end on the marsh surface and floated up and down with the tides. The width of the paths through the Spartina was the approximate width of the boards.

The walkway was laid out in a pattern providing a series of 36 collecting sites alongside the paths, each 5 m in length. Wooden stakes marked the limits of these sites (Fig. 2). The collecting areas in both the P- and the C-marsh were laid out in the pattern shown in Figure 2, but only the P-marsh pathways were paved with boards. In both marshes the long axis of the collecting area was parallel to and about a meter from the edge of an arm of the particular creek.

The height and lushness of the Spartina varied between collecting areas and also between collecting sites in the same area. These differences were quantified to some extent. Heights of 10 separate Spartina plants at each of the 36 sites in each marsh were measured. The 10 were selected by noting which plants coincided with marks that had been made at half-meter intervals along a cord stretched alongside the marsh grass bordering the walkways. The height range for the P-marsh plants was 48 - 143 cm and for the C-marsh plants, 29 - 105 cm. The respective average heights were 96.3 ± 10.8 cm and 70.1 ± 8.4 cm. This evidence of greater Spartina growth in the P-marsh corroborated observations by Marshall (1970) in the same areas.

Collecting Equipment

A Sears shop vacuum cleaner was the chief sampling device (Fig. 3A). It was a fiberboard cylinder 15" tall and 16" in diameter, with a 1 HP motor on the underside of the lid. Its 6-foot hose (3" diameter) was attached to a rigid, plastic, 4-foot extension tube with an expanded triangular opening. A 100-foot extension cord permitted the cylinder to be plugged into a Sears 1100-watt alternator for power (except for the Pond Collection, when it was plugged into electrical outlets at the ponds).

The foot half of a nylon stocking served as a collecting bag, hanging inside the cylinder and catching the microarthropods as they were sucked into the sampling tube.

For the Biomass Collection, a "biomass box" was used in conjunction with the vacuuming apparatus (Fig. 3B). It consisted of a plywood box measuring 1 square meter, with a height of 58 cm. It was built with 4 sides and a top, but without a bottom. In one side, a window 10" square was cut and outlined

by 3 slanted pieces of wood into which a "live" light trap ("Patio Pal") could be fitted tightly. The cylindrical light trap included a black light source for attracting insects, and a fan for drawing them inside where they fell into a net bag. The light trap, along with the vacuuming apparatus, was plugged into the alternator. A sweep net was used to capture insects that tried to escape when the lid was opened.

Sampling Procedures

Pond Collection

Samples for the Pond Collection were taken on March 27, April 25, and June 4. The pond Spartina nearest the electrical outlets (a stretch of marsh grass approximately 5 m long) was sampled at each of the 1-month intervals. The vacuuming procedure was that described for the Marsh Collection.

Marsh Collection

Each sample site in each marsh was chosen shortly before the day's collecting was begun by bouncing two dice off a wall. The one falling nearest the wall indicated by its upper face the "column" in the collecting area pattern (see Fig. 2), and the other's top face indicated the "row". If, for example, the dice fell in the order 3, 5, the collecting site designated was 27. After a particular site had been designated 3 times it was not used again; the dice were re-thrown.

Sampling for the Pond and the Marsh Collections consisted in thrusting the extension tube of the vacuuming cylinder toward the base of the Spartina, at a distance of about 1 meter from the walkway or Spartina margin, and moving it upward toward the tips, then thrusting it down to a point just adjacent to the previous point and again drawing it upward. Each sample was the result of 2 minutes of collecting.

One investigator (RLK) always carried out the extension tube sweeps in the Marsh experiment and tried to use the same procedure on all occasions. Usually he covered the 5 m sampling distance in about 30 seconds and returned more slowly over the same area during the next 90 seconds, ending where he began.

The collecting stocking, with its contents, was placed inside a killing jar containing ethyl acetate as soon as the 2-minute collecting period had ended. Separation of the microarthropods from the Spartina stems and other collected debris was carried out using dissecting microscopes and was usually started soon after reaching the laboratory. Great care was taken to find every tiny specimen. The samples were then preserved in 70% alcohol in labelled vials.

Biomass Collection

Exact sampling sites for the 10 sets of samples in the Biomass study were determined on each occasion by consensus of the experimenters who based their decisions on accessibility, lack of previous disturbance, and broad representation of variations in the Spartina. Figure 4 shows the sites chosen. In 3 sets out of the 10, the Biomass collecting in the two marshes was not done on the same day, but each collection was comparable to its partner in terms of

weather, time of day, temperature, and height of tide.

In making a sample, two workers held the biomass box between them at shoulder height. The top was firmly latched in place and a sheet of cardboard tightly covered the light trap opening. At the count of 3 both workers took several quick, predetermined steps to the chosen spot, and thrust the box vertically down until it was flush with the ground. The light trap was put into place in the window and the alternator started. The window cover was then removed, making the light trap the only means of escape from the box. The standard time for light trap operation alone was thirty minutes.

At the end of this time, the top was removed from the biomass box and vacuuming of the interior started. One person watched closely for grasshoppers and other quickly-moving insects that had not entered the light trap, and caught them with a net or by hand. Vacuuming was conducted in a thorough fashion for fifteen minutes. This included passing the collecting nozzle over most parts of the interior of the box and the entrance to the light trap, over the marsh floor enclosed, and over all parts of all the enclosed plants as far as that was possible within fifteen minutes. The light trap was allowed to continue running during this time. At the end of fifteen minutes the light trap catch and the vacuum catch were both placed in ethyl acetate killing jars and returned to the laboratory.

At the laboratory the standard procedure for preparing the insects for drying and weighing was careful separation of all of the small animals from the fine debris that had been captured with them.

The first three collections were preserved in alcohol before being weighed. This practice was replaced by dry sorting and immediate drying and weighing, to prevent extraction of lipids from the animals by the alcohol. The first three collections were dried along with their alcohol to make certain that no weight was lost. Drying was accomplished at an average temperature of 100° C. and was continued until no further weight loss was recorded. Collections were weighed to tenths of milligrams.

Light trap and vacuuming catches were weighed separately to give information on the relative usefulness of the two techniques. Collections #1 and #4 were sorted to order. In Collection #10, five extra minutes of vacuuming were given to test the effectiveness of the 15 minutes that had been decided on arbitrarily.

RESULTS

Pond Collection

The pond data were analyzed for species diversity, in insects only, using the procedures followed by McMahan and Sollins (1970) in their study of microarthropods in a Puerto Rican rain forest. The specimens were not precisely identified to species. Rather, each specimen was examined in turn, and the decision was made as to whether or not it belonged to a species different from those already examined. The species were identified as "Diptera 1", "Hymenoptera 5", etc. By this method, the 1775 insects collected on all 3 occasions from both the P-pond and the C-pond *Spartina* were separated into 142 different kinds.

Diversity indexes were determined 1) by dividing the number of species by the square root of the number of specimens (E.P. Odum, 1963), and 2) by noting the (projected) number of species per 1000 specimens. Table 1 shows the species diversity data for the 3 sampling days. Figure 5 gives the cumulative number of species for each collection, plotted against the cumulative number of individuals examined. The largest sample consisted of only 460 specimens, and only two points, the beginning and the end, were available in each case for plotting. The data, therefore, are too meager for more than speculative purposes, but for what they are worth the data have been extrapolated to 1000 specimens.

Both Table 1 and Figure 5 indicate that as Spring advanced insect diversity increased in both the P- and the C-pond Spartina, with diversity being greater in the latter in 2 of the 3 collections (Fig. 5).

Only 35 out of the total of 142 different insect species were found at both the collecting sites. The Venn diagram of Figure 6 shows that 47 species were collected only in the P-pond Spartina and 60 species were collected only in the C-pond Spartina. A large percentage of the species were represented by either 1 or 2 specimens (49% for P-ponds; 56% for C-ponds), but 6 of the 10 most common species at each site were the same. They consisted of 4 kinds of homopterans, 1 heteropteran, and 1 dipteran.

Figures 7 and 8 compare the numbers of specimens and species, respectively, for each of the insect orders represented in the P- and C-pond collections. They show that, in both cases, specimen density was greatest for Homoptera and Diptera, whereas species diversity was greatest for Hymenoptera and Diptera.

Marsh Collection

The P-marsh collection averaged 142 $\pm$ 79 microarthropods per sample, whereas the C-marsh collection averaged 139 $\pm$ 79. Figure 9 compares the catch for each marsh on each of the 24 collecting occasions in sequence. The two sets of samples varied together, indicating, perhaps, similarities both in collecting conditions and in population densities on each day. Explanations for the unusually high capture rate in collections #6 to #10 are not known. It did not appear to be correlated with temperature, tide cycle, or collection site.

Because of the relatively large number of specimens (6734) in the Marsh Collection and the tedious nature of the species diversity analysis, the latter has not yet been made on these data.

Relative specimen density by order was similar to that of the Pond Collection (compare Figs. 10 and 7), being greatest for Homoptera and Diptera. (Density data for spiders, mites, and amphipods are also shown in Figure 10). The heights of the bars indicate that for most insect orders the specimen density in the P-marsh did not differ greatly from that in the C-marsh. Statistical evaluation of the insect data by the t-test method did indeed show no significant differences. In the case of spiders and of amphipods variances were too great to permit application of the t-test, and the Mann-Whitney rank sum method of analysis was used (Campbell, 1967). It showed that a significantly (at the 99% confidence level) greater number of spiders and of amphipods were collected in the P-marsh than in the C-marsh.

Biomass Collection

Biomass data are in two parts, the light trap portion and the vacuuming portion, and the two were weighed separately. Table 2 gives these data for the two marshes. The average total biomass of insects and other microarthropods captured by the method described was 117.0 ± 68 mg/m^2 for the P-marsh and 140.6 ± 122 mg/m^2 for the C-marsh. Variances for these data were too great to permit use of the t-test method in comparing the two marshes, but analysis by the Mann-Whitney rank-sum method showed no significant differences between them. The vacuuming technique caught far more insects than the light trap in both marshes, but was especially effective in the C-marsh.

Table 2 also shows the results of 5 extra minutes of vacuuming that followed collection #10 at each marsh. The biomass in this extra collecting time equalled 13.5% of that of the regular collection for the P-marsh, and 24.6% for the C-marsh, indicating that the vacuuming period should have been longer then the one actually set.

Table 3 is a breakdown to Order (or to major group) of specimens in Biomass collections #1 and #4 for each marsh. It indicates the variety of specimens as well as the respective trapping effectiveness of light trap and vacuuming procedures. Approximately 10% of the captured specimens in these two collections were caught in the light trap, dipterans making up 74% of this catch. Like the Marsh Collection, the Biomass collections showed, in general, more spiders and amphipods in the P- than in the C-marsh.

Other Marshes

Two other marsh areas were sampled once each, using the 2-minute vacuuming method described. One was at Russell Creek, a tidal creek entering New Port River Estuary north of Beaufort, N.C.; the other was at Hoop Hole Creek, a small inlet on the sound side of Bogue Bank near Atlantic Beach. Both were rather extensive marsh areas (see Hunter's paper in this report for a description of the Hoop Hole Creek marsh).

The Hoop Hole Creek sample did not appear to differ greatly in size and makeup from the main Marsh Collection, but the sample from Russell Creek was outstanding for its domination by homopteran nymphs. Whereas the P- and C-marsh Collections averaged 45 and 37 homopterans (never exceeding 101) out of an average specimen count of about 140, the Russell Creek Collection had 2644 homopterans out of a total of 2701 specimens.

DISCUSSION

The salt marsh ecosystem has both above-water and aquatic components, all participating in a single system of mineral cycles and food chains. Cooper (1969) has summarized the literature on this ecosystem. The populations of microarthropods we studied were chiefly in the above-water group. Any effects of sewage effluent would therefore be expected to be mostly indirect, mediated through effects on growth of the Spartina on which they fed or in which they sheltered. These studies showed no obvious negative effects of the treated wastes.

In his study of Spartina characteristics in the Calico (P) and Dill (C) marshes, Marshall (1970) found significantly greater weights of the marsh grass per square meter in the Calico marsh than in comparable sites in the Dill marsh, and he recorded greater height and robustness there. Our own studies confirmed this difference in Spartina growth. Marshall felt that it probably reflected the increased nutrient supply to the P-marsh, and reported that only in nitrogen and phosphorus levels were the two marshes different in the parameters measured. In salinity, temperature, and pH they were very similar.

In spite of the difference in Spartina between the two marshes, there was no proven difference in insect density. A positive correlation might have been predicted for grazers such as grasshoppers or sap-suckers such as leafhoppers, if food supply were a limiting factor. That it is not limiting is indicated by work by Smalley (1958), Teal (1962) and others which showed that insect consumption accounted for only a tiny percentage of Spartina degradation in a salt marsh ecosystem. Smalley, in a study of the relationships between insects and marsh grass near Sapelo Island, Georgia, found that only 1% of the live Spartina was consumed by insects. The rest became detrital material or was washed out out of the area by the tides. Teal, who also worked in the Sapelo marshes, concluded that insects and other land-derived fauna of the salt marsh, although comprising nearly half the marsh fauna, are nevertheless "far less important in the energetics of the community than their aquatic counterparts." He listed only one grasshopper species (Orchelimum fidicinium) and one kind of leafhopper (Prokelisia marginata) as of importance in the marsh he studied.

Although insect densities were comparable in the two marshes of the present study, populations of spiders and amphipods were significantly denser in the P-marsh than in the C-marsh. Spiders are carnivores, catching their prey among the Spartina stems. Mechanisms through which sewage fertilization might affect spider populations would seem to require a relationship to herbivore prey density, yet there appeared to be no obvious increase in insect prey correlated with the increase in spiders. Such an increase in herbivores might be masked, however, by the predation pressure exerted by the resultant increase in spiders. While these studies show no correlation between augmented plant growth and herbivore increase the increase in carnivores (spiders) may have held the herbivore populations in check.

Amphipods are surface scavengers and detritus feeders. Explanations for their greater density in the P-marsh may be related directly to the increase in detrital food in the form of sewage wastes. As with the spiders, however, further studies of food chains, food preferences, and other aspects of the role of amphipods in the marsh ecosystem will be necessary before valid conclusions regarding their differential distribution in the two marshes can be drawn.

It may be interesting to note that there was little evidence of mosquitoes in either marsh during the study interval. Almost none were captured during sampling and none were observed. Other species may have been missed because of the restrictive nature of the collecting technique. For example, collembola, like amphipods, are detritus feeders on the marsh floor, yet they were rarely captured except in the Biomass study. In the latter, however, in which there was much more intensive vacuuming of the marsh floor, they appeared more often in the C- than in the P-marsh. Perhaps collembolans, in contrast to amphipods, are very sensitive to toxic effects of sewage effluent. Further studies, in which there is a special effort to collect collembolans, are needed.

The microarthropod standing crop in the Spartina marsh, according to our Biomass data, is relatively low. In his study of energy flow in the Georgia salt marsh, Teal (1962) calculated that 80.0 kcal/m^2 of insect tissue was produced per year. This figure included only those insects that fed on the live marsh grass, namely grasshoppers and leafhoppers. Our standing crop figure was 0.14 g/m^2 for the C-marsh, or about 0.7 kcal/m^2 (assuming about 5 kcal/gm). If we assume about 2 turnovers per year for the grasshoppers and about five for the leafhoppers and other microarthropods, we can estimate gross production for the C-marsh microarthropods as being about 3 times the standing crop or (on the basis of our data) 2.1 kcal/m^2/year. This is only 1/40 as great as Teal's yearly production figure (80.8 kcal/m^2), a discrepancy too large to be due entirely to the difference in climate in the two collecting areas (there is a longer growing season in Georgia).

Teal also described low species diversity in the Sapelo marsh and attributed the marsh ecosystem's stability to the broad diets of the few species present. In the marshes we have studied, however, species diversity appears to be considerably greater than previous reports have indicated. E.P. Odum (1963), for example, reported a diversity index (number of species/$\sqrt{\text{number of specimens}}$) of only 0.7 for a Georgia Spartina salt marsh, compared with one of 2.6 for old field vegetation. The comparable June figure for the P- and C-pond Spartina insects in the present study was about 3 (see Table 1). If species diversity is, after all, high in the Spartina marsh, stability of the ecosystem is not surprising. The vacuuming method of sampling might show greater diversity in the Georgia marsh.

The apparent differences between the Georgia and North Carolina marsh populations may be due in part to differences in tidal stress. The Georgia marshes have much larger tides (8-10 ft. spring tides versus about 3 ft. in Calico and Dill Creeks and no tides in the ponds). With less stress in the latter, higher species densities may have resulted and greater population stability.

Although populations of microarthropods in the Calico and Dill marshes appeared to be similar, collections from another marsh on Russell Creek gave a very different faunal composition, with homopterans greatly dominating. Some variations in components are to be expected. Davis and Gray (1966) pointed out from their studies that the various kinds of marsh insects occurred at different seasons and differed according to zonation. Seasonal or zonal variations, however, could not explain the tremendous dominance by leafhoppers in the Russell Creek marsh, and other factors were not studied.

ACKNOWLEDGMENTS

Our work was greatly facilitated by the continuous cooperation and interest of the E. Rowley and the H. Sanders families who allowed us to work from their property adjoining the C-marsh. We also appreciate the contribution by the Atlantic Veneer Company, through Mr. Schnabel, of 50 large boards used in laying out the collecting areas in the P-marsh. For this aid we are deeply grateful.

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TABLE 1. COMPARISON OF INSECT DIVERSITY IN P- AND C-POND SPARTINA.

	P-Pond			C-Pond		
Sample	No. of Species	No. of Specimens	Index species/$\sqrt{\text{specimens}}$	No. of Species	No. of Specimens	Index species/$\sqrt{\text{specimens}}$
1 (Mar 29)	17	174	1.29	18	156	1.44
2 (Apr 25)	34	460	1.58	52	391	2.63
3 (June 4)	54	341	2.92	49	253	3.08

TABLE 2. INSECT BIOMASS FOR P- AND C-MARSHES ACCORDING TO COLLECTION AND TRAPPING TECHNIQUE.

Collection	Date	P-Marsh weights in milligrams			C-Marsh weights in milligrams		
		LT[a]	VAC[b]	Total	LT[a]	VAC[b]	Total
1	7-16-70 7-17-70	94.4	60.5	154.9	10.0	43.8	53.8
2	7-21-70 7-23-70	20.2	25.9	46.1	0.1	8.8	8.9
3	8-11-70 7-28-70	7.7	116.4	124.1	21.4	125.4	146.8
4	8-13-70	6.0	45.9	51.9	14.3	169.4	183.7
5	8-17-70	6.7	71.5	78.2	6.8	45.9	52.7
6	8-18-70	116.6	151.6	268.2	16.0	108.8	124.8
7	8-19-70	38.5	64.1	102.6	51.9	399.3	451.2
8	8-19-70	8.8	93.1	101.9	7.5	124.3	131.8
9	8-20-70	64.1	112.0	176.1	19.9	132.3	152.2
10	8-21-70	14.1	51.6	65.7	25.8	74.7	100.5
Total		377.1	792.6	1169.7	173.7	1232.7	1406.4
10 (extra 5 min)			8.9			24.7	

[a]Light trap
[b]Vacuuming

TABLE 3. BREAKDOWN OF TWO BIOMASS COLLECTIONS TO INSECT ORDER OR ARTHROPOD GROUP (NUMBER OF SPECIMENS).

	Collection #1				Collection #4			
	P-Marsh (July 16)		C-Marsh (July 17)		P-Marsh (Aug 13)		C-Marsh (Aug 13)	
	LT[a]	VAC[b]	LT[a]	VAC[b]	LT[a]	VAC[b]	LT[a]	VAC[b]
Orthoptera	5	1	0	4	0	0	0	0
Homoptera: adults					2	37	8	24
large nymphs	2	79	3	62	2	40	2	60
small nymphs					1	195	0	245
Heteroptera: adults					1	16	1	5
large nymphs	0	10	0	7	0	17	0	8
small nymphs					0	0	0	16
Diptera: large					1	1	5	0
medium	25	11	17	33	3	0	17	0
small					21	17	51	49
larvae					0	0	1	0
Hymenoptera	1	26	4	11	0	6	5	14
Thysanoptera	0	3	0	9	0	1	0	4
Collembola	0	0	1	0	0	4	0	105
Lepidoptera	0	0	0	0	0	0	0	1
Coleoptera	0	2	0	0	0	0	0	1

TABLE 3 (continued)

	Collection #1				Collection #4			
	P-Marsh (July 16)		C-Marsh (July 17)		P-Marsh (Aug 13)		C-Marsh (Aug 13)	
	LT[a]	VAC[b]	LT[a]	VAC[b]	LT[a]	VAC[b]	LT[a]	VAC[b]
Spiders	4	204	0	21	1	68	2	71
Mites	0	21	7	53	0	0	0	128
Amphipods	0	22	0	4	0	22	0	1
Ostracods	0	0	0	0	0	0	0	3
Pseudoscorpions	0	6	0	0	0	0	0	0
	37	348	32	204	32	424	92	735
Total #	385		236		456		827	

[a]Light trap
[b]Vacuuming

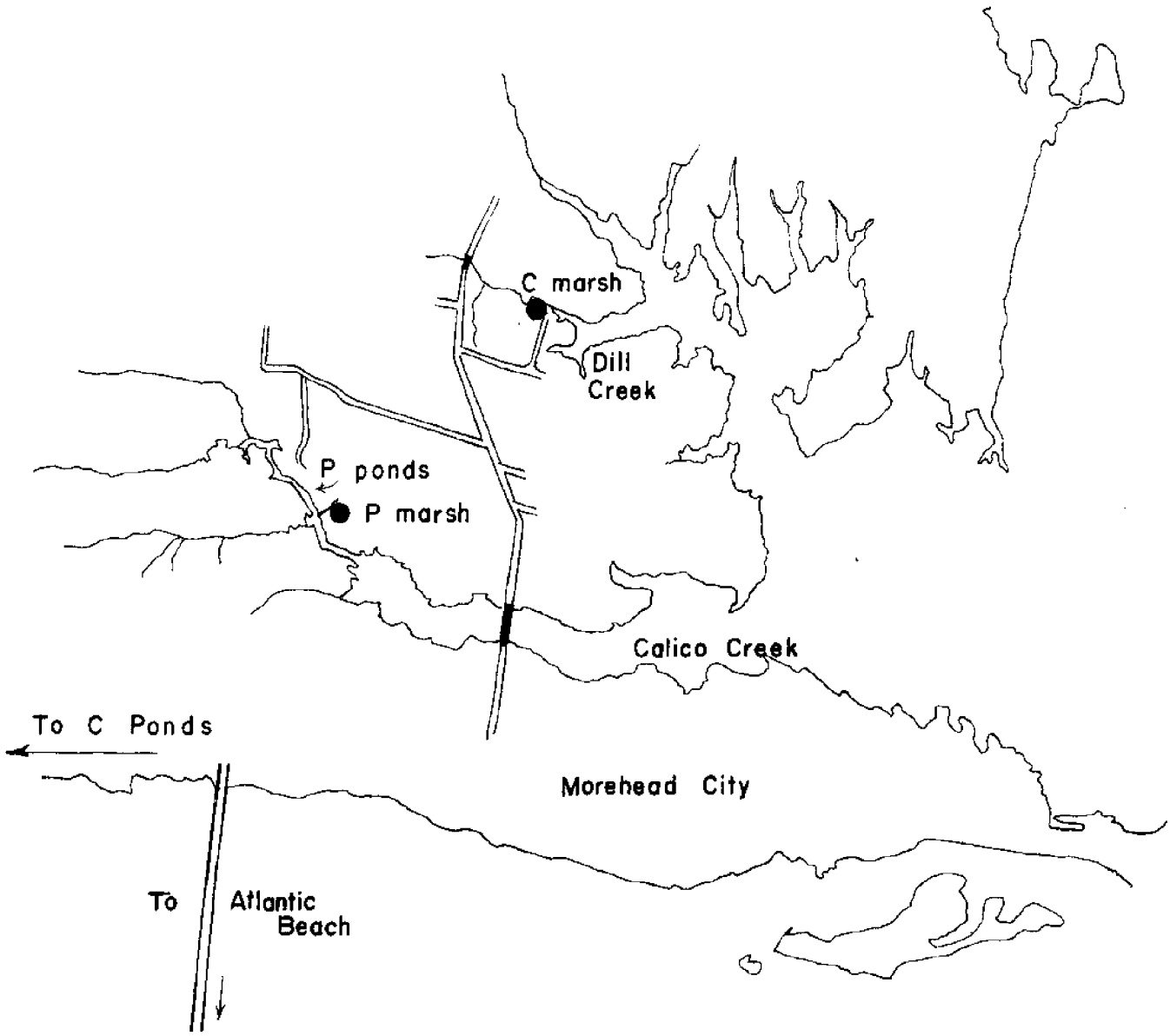

Fig. 1. Map of the Morehead City area showing the locations of the P- and C-ponds and the P- and C-marshes.

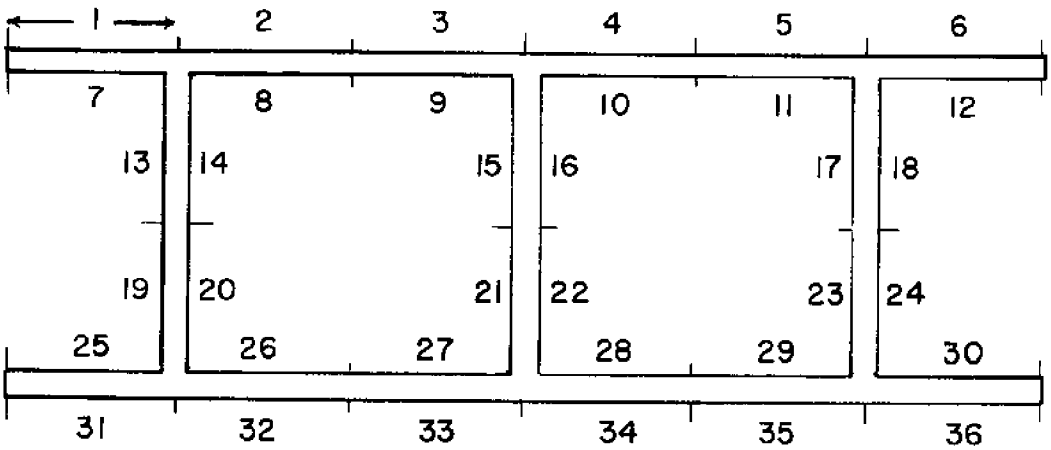

Fig. 2. Diagram of the 36 collecting sites for each marsh collecting area. The pathways in the P-marsh were paved with boards.

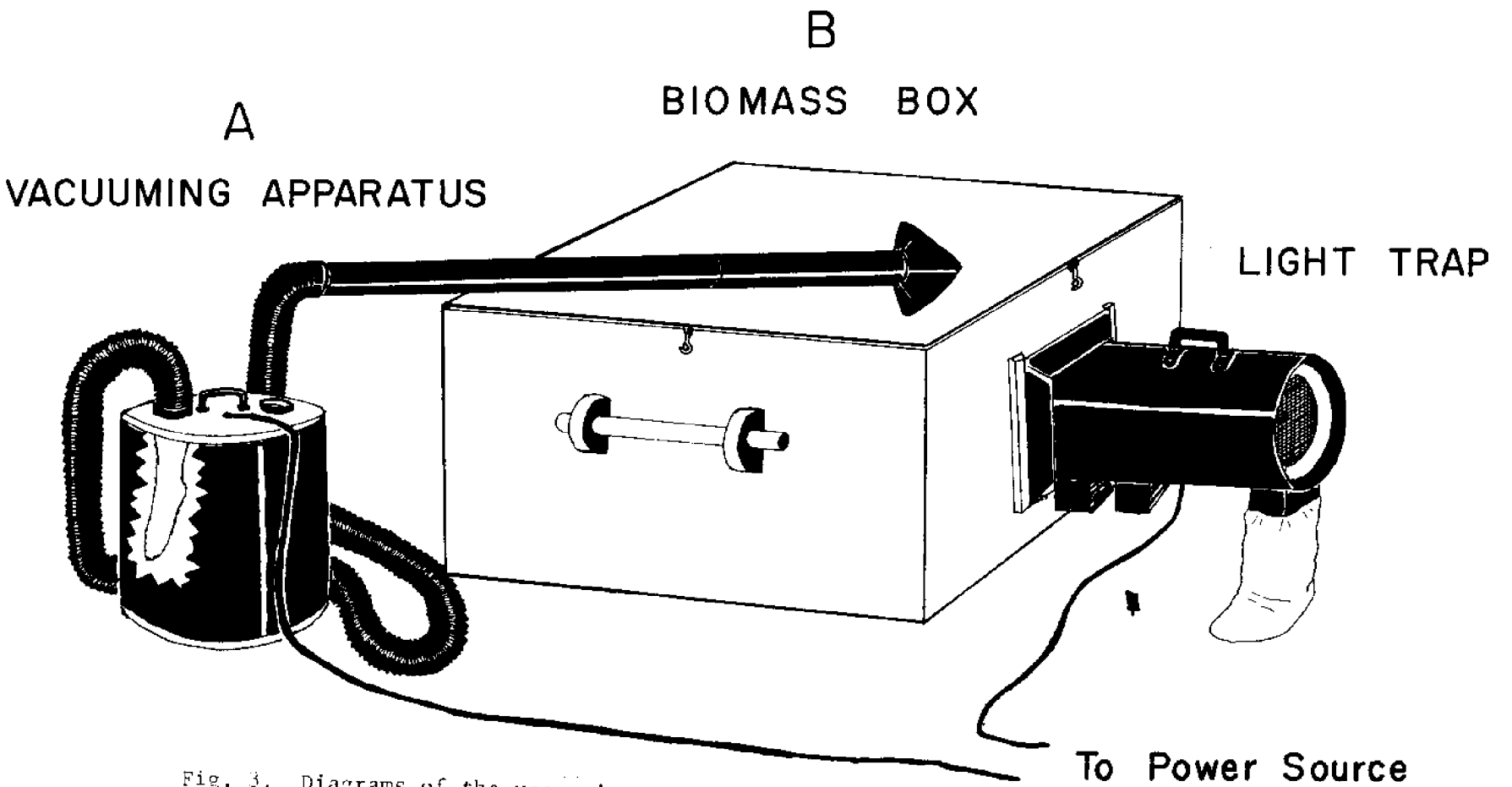

Fig. 3. Diagrams of the vacuuming apparatus and of the biomass box used in collecting specimens.

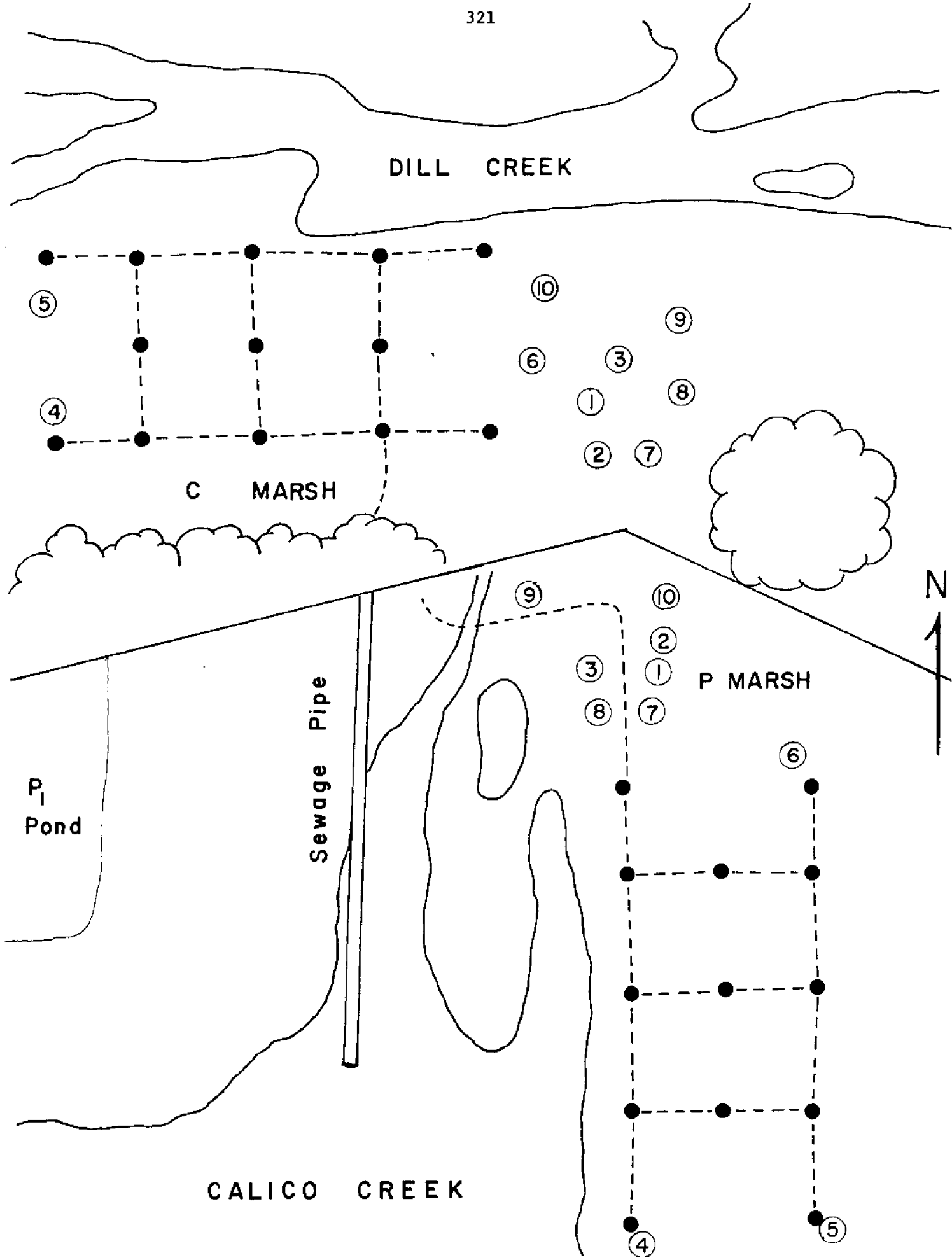

Fig. 4. Map of the two Marsh areas showing specific Biomass collecting sites.

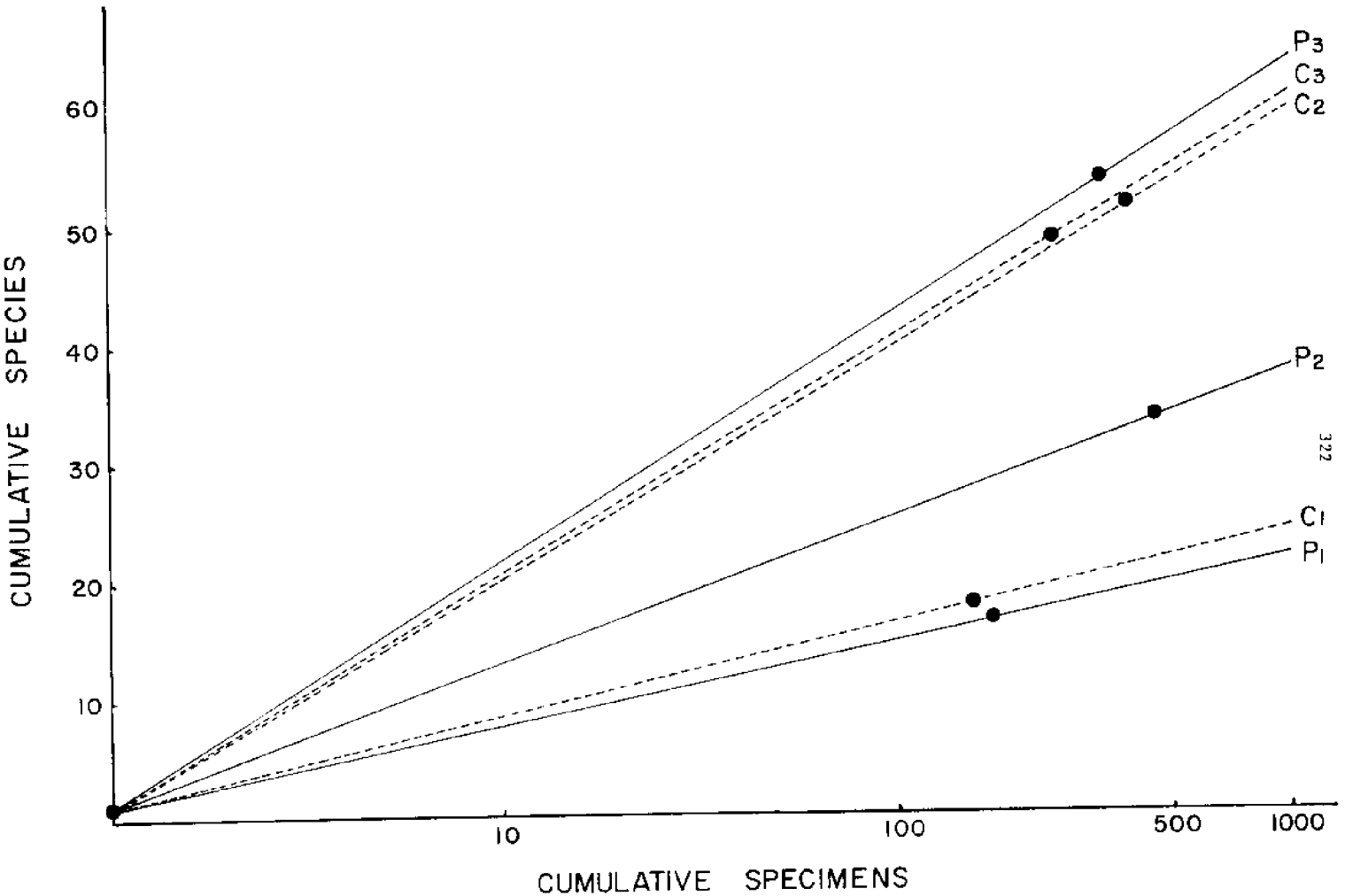

Fig. 5. Graph showing cumulative number of insect species plotted against cumulative number of specimens for each pond Spartina collection. Data are extrapolated to 1000 specimens in each case. P_1 represent data for the first collection (March 27) at the P-ponds; C_1 represents those at the C-ponds; etc.

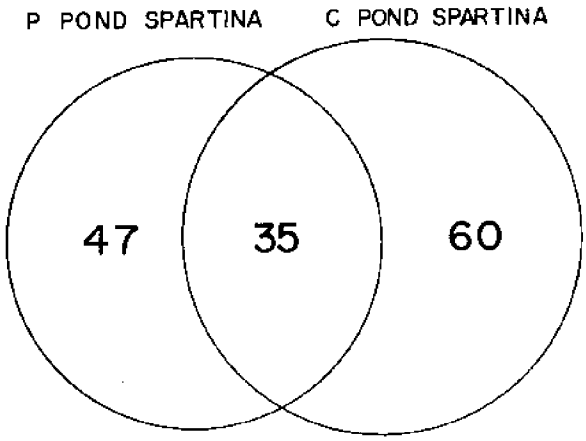

Fig. 6. A Venn diagram indicating degree of overlap of insect species collected from the P-pond Spartina with those collected from the C-pond Spartina.

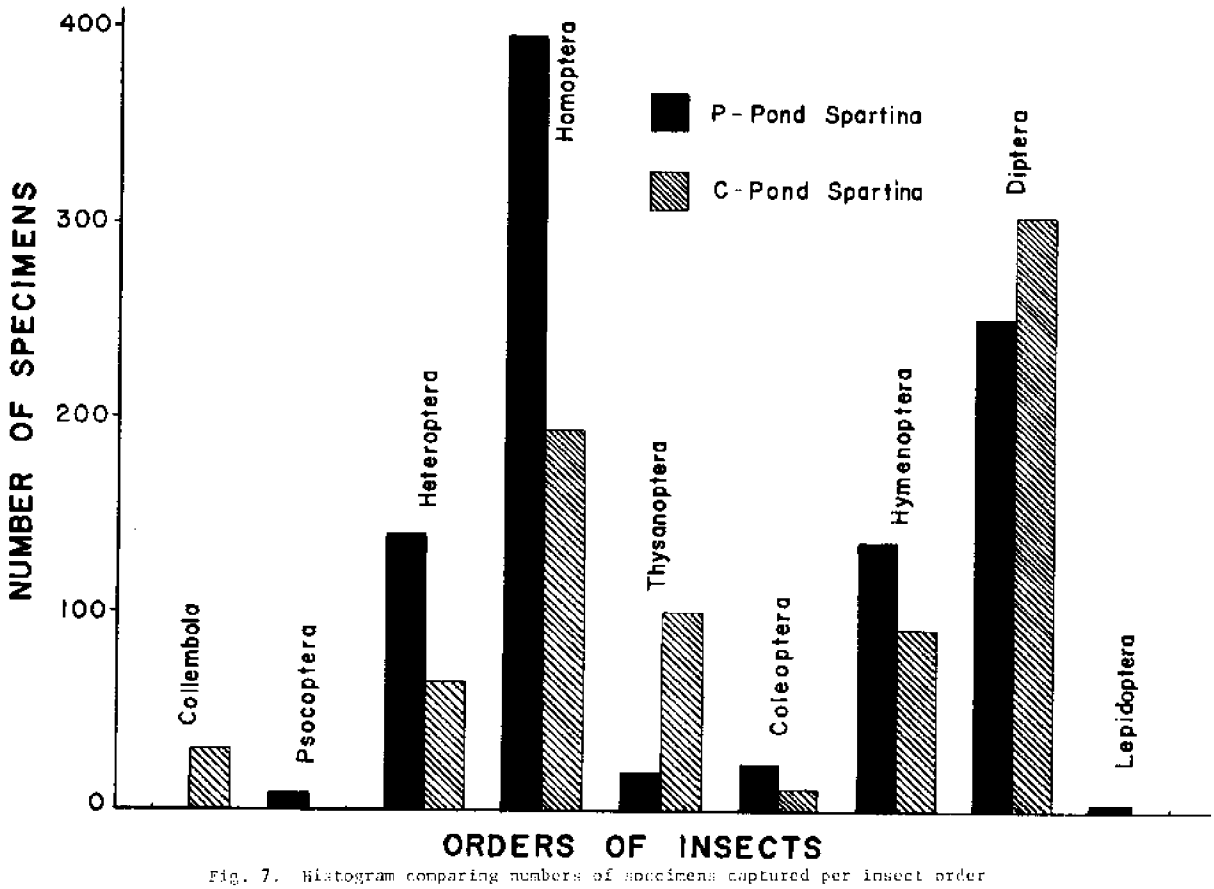

Fig. 7. Histogram comparing numbers of specimens captured per insect order for P- and C-pond Spartina.

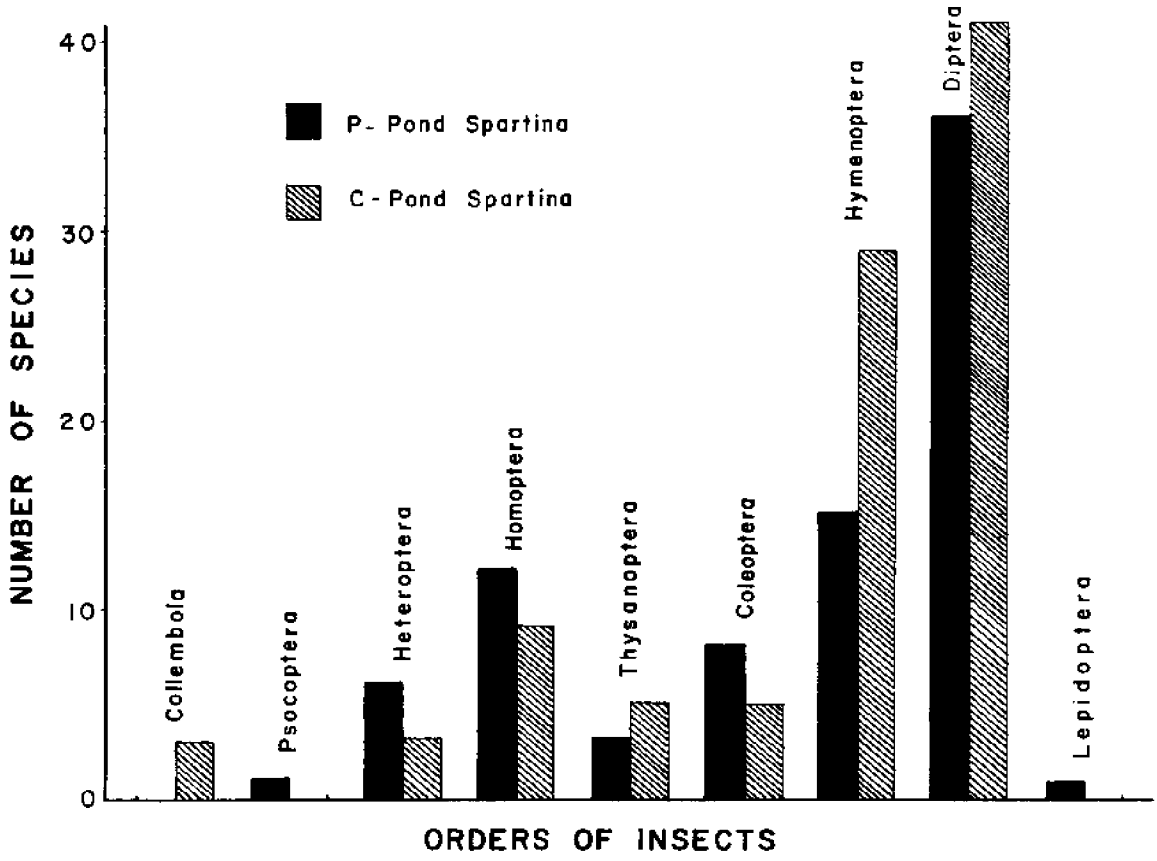

Fig. 8. Comparison of numbers of species encountered per insect order for P- and C-pond Spartina.

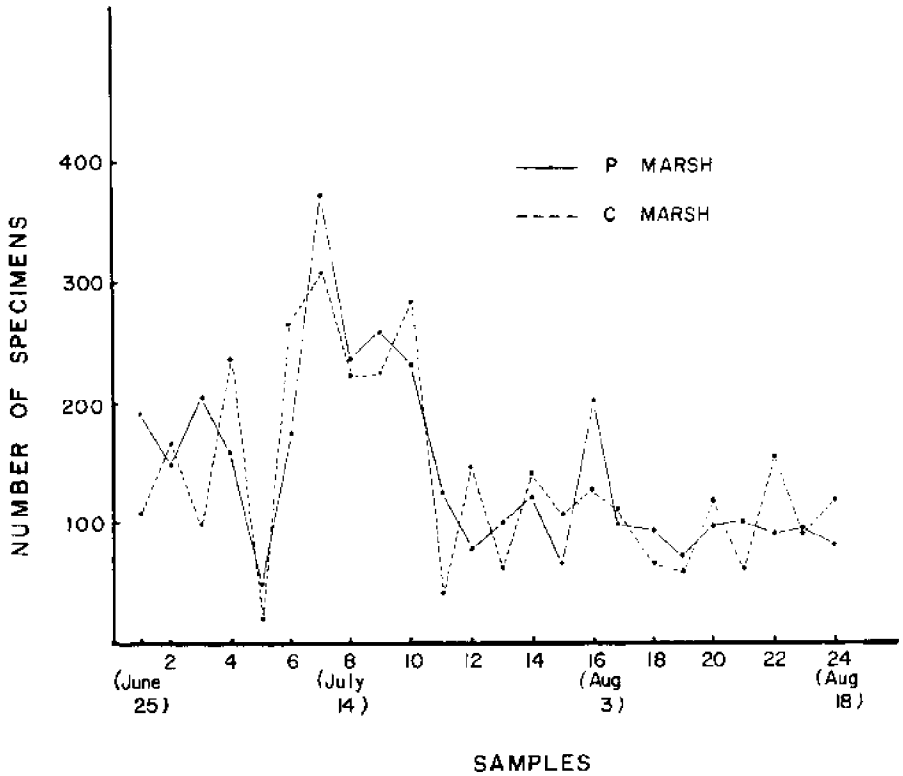

Fig. 9. Comparison of numbers of specimens caught at P- and at C-marsh for each of the 24 collecting dates.

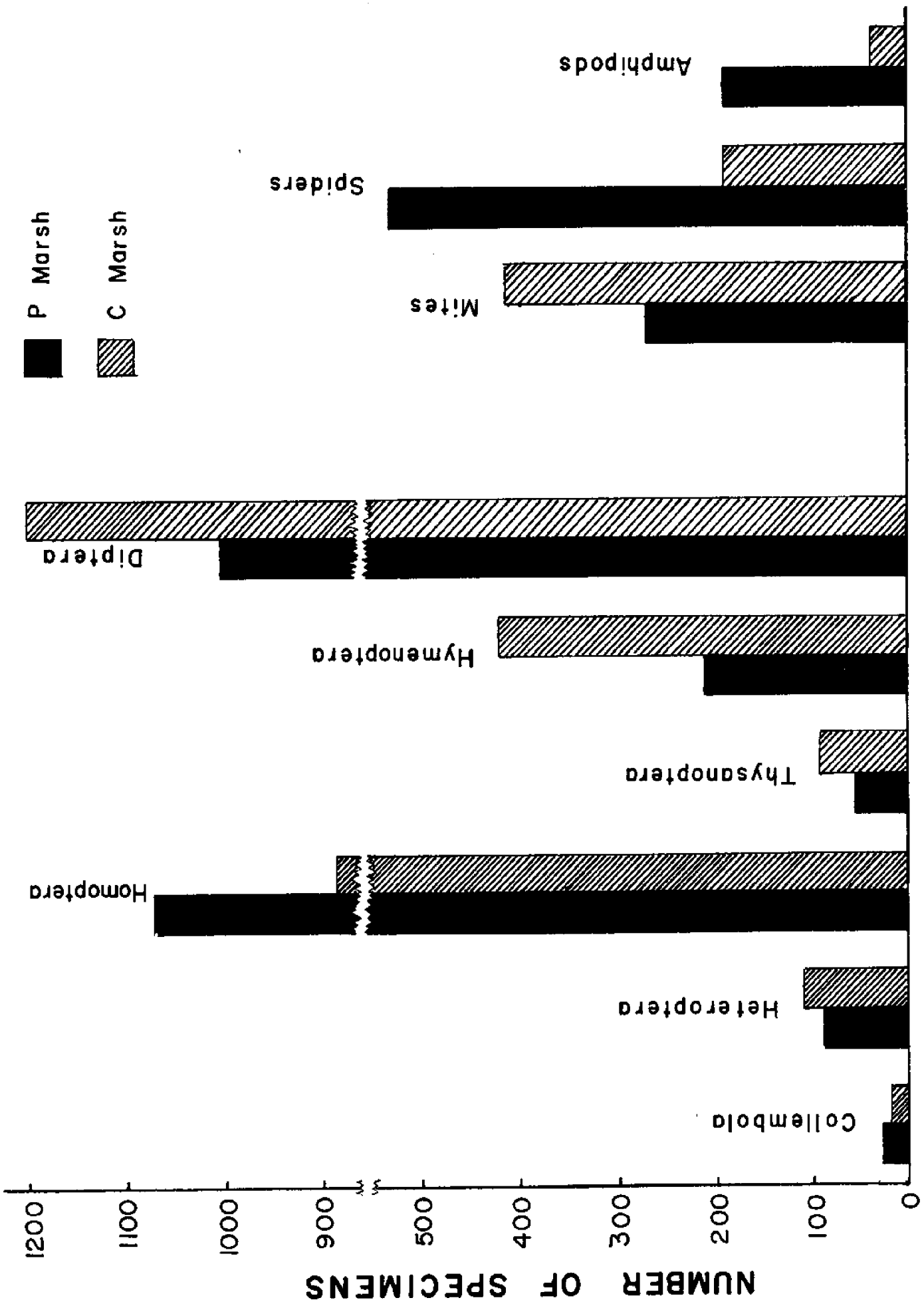

Fig. 10. Comparison of numbers of specimens collected at P- and C-marshes

A PRELIMINARY COMPARISON OF FIDDLER CRAB POPULATIONS IN A SALT MARSH RECEIVING TREATED WASTES AND IN A CONTROL MARSH

A.R. Camp, R.L. Knight and E.A. McMahan
Department of Zoology

Three species of fiddler crabs are found in the salt marshes along the east coast of the United States. The largest is Uca minax, which is usually found in brackish marshes with a substratum of mud. Uca pugnax also prefers a vegetated muddy substratum but waters of higher salinity. Uca pugilator frequents the sandier substrate of tidal creek banks below marsh vegetation. The three species overlap in their habitat distributions, however, and populations of U. pugilator are especially likely to be affected adversely by competition with the other two (Teal, 1958). All three species are found in abundance in marshes around Morehead City, N.C., in whose vicinity effects of treated sewage on marsh and pond ecosystems are being studied (Odum and Chestnut, 1970).

As detritus feeders, fiddlers take mud and sand into the buccal cavity, sort it with their mouthparts, and retain the bacteria, decaying marsh grass, and other organic materials contained in it. Because of these feeding habits, effects of sewage wastes on fiddler populations might be expected to be direct.

In the summer of 1970 a preliminary comparison of populations of fiddler crabs living in the sewage-exposed marsh on Calico Creek (P-marsh) and in a control marsh on Dill Creek (C-marsh) was carried out. The study areas were very near those of the Microarthropod study (See the paper by McMahan, Knight, and Camp in this report), and are shown in Figs. 1 and 4 of that paper. Although the mud flat areas near the P-ponds (Odum and Chestnut, 1970) appeared to be suitable for U. pugilator, none were definitely identified in the present study. Only U. minax and U. pugnax were noted, reflecting perhaps their exclusion of U. pugilator through competition.

Three brief comparative studies were made using different sampling procedures: 1) Fiddler densities around the P-ponds and around the C-ponds were compared by counting burrow holes within comparable zones. In addition, an attempt was made to study burrow changes through photographing particular areas of two mud flats on sequential days. 2) Pitfall traps were used in the P-marsh and in the C-marsh to sample the respective populations. 3) A square meter of each marsh, to a depth of 8 inches, was excavated and sieved to ensure the capture of all crabs contained in it.

METHODS AND RESULTS

Burrow Study

Burrow counts were made on July 13 and 14. The study areas consisted of a zone one meter wide surrounding each of the 3 P-ponds and each of the 3

C- ponds, and adjoining the water's edge. Since each pond's perimeter was about 90 m, the zones were marked off in intervals of 5 m, making about 18 separate sections in which burrows were counted. Every hole of 5 mm or greater within these sections was assumed to be a fiddler burrow. The study zones were above the tidal level and were never under water. They therefore received no tidal action or sewage effluent. The P-ponds adjoined large mud flat areas (on Calico Creek) which were teeming with fiddlers. The C-ponds, on the other hand, were located on Bogue Sound near a sandy beach. The C-ponds had been seeded in 1968 with U. pugnax and U. pugilator, and the burrows on their perimeters in 1970 were presumably dug by these crabs or their offspring. Thick stands of Spartina alterniflora, S. patens, and Distichlis spicata along the northern margins of all the ponds made detection of burrows that might have existed in these vegetated regions difficult.

Table 1 shows that there were almost twice as many Uca burrows around the P-ponds as around the C-ponds. This seems attributable mostly to the presence near the P-ponds of a large natural population of fiddlers which could serve as a constant source of immigrants, while the C-pond population had been seeded there and was isolated from a natural population. Substrate and perhaps dominant species may also have been different.

The photographic study was inconclusive but indicated a rather high degree of burrow permanence, at least over a 4-day period.

Pitfall Study

Six pitfall collections were made at the P-marsh and six at the C-marsh between August 11 and August 18. Trapping occurred within a square enclosure made by forcing four sheets of plywood (each 1 m x 58 cm) into the marsh floor. Two stakes attached to each sheet and projecting about 20 cm below the bottom margin permitted the sheet to be firmly anchored. The four sheets were separated at each corner by a gap of about 1 cm, covered by plastic screening. The latter permitted water to enter and leave the enclosure, but retained crabs and other organisms that had been trapped within. In the center of the pen a tin can (15.5 cm in diameter and 17.5 cm deep) was sunk to lip level in the substrate to form a pitfall trap. Collections from the trap were usually made after it had been left undisturbed for about 24 hours. The water was strained from the can through a nylon stocking and the retained organisms were transferred to the laboratory for examination. The can was replaced for another 24 hour trapping interval.

More fiddlers and other organisms were captured in the C-marsh pitfall trap than in the P-marsh trap. Table 2 shows the results. At first, other obvious organisms in addition to crabs were counted, but this was discontinued after the third collection. It is interesting to note that ostracods and copepods were captured in the C-marsh trap, but not in that of the P-marsh.

This sampling method was not a very good one, because the can trap remained full of water and organisms could come and go at will. Furthermore, the can sometimes pushed up out of the mud, so that its lip was not at substrate level. During collection 3 at the P-marsh it floated out and upended, eliminating that particular collection.

Excavation Study

On August 19 and 20 a square meter of marsh in the P-marsh and in the C-marsh, respectively, was enclosed within the plywood sheets described under "Pitfall Study." Two investigators (ARC and RLK) positioned them as quickly as possible in an attempt to prevent the herding away of crabs in the area. The rather thick vegetation in both marshes permitted them to be fairly successful at this by impeding the movement of the crabs. All crabs seen within the enclosure were captured, after which the enclosed Spartina was pulled up by the roots and rinsed vigorously in a water-filled container to dislodge any attached crabs. The substrate and root masses remaining within the enclosure were then dug out to a depth of about 8 inches and transferred to large plastic containers. These were taken to the laboratory and their contents strained through a series of graduated sieves to catch all tiny crabs.

The C-marsh sample of excavated marsh contained nearly 3 times as many crabs as the P-marsh, as shown in Table 3. In the P-marsh collection, fiddlers large enough to be identified with any certainty appeared to be divided almost equally between U. minax and U. pugnax, whereas in the C-marsh collection, they appeared to be entirely U. pugnax. Another apparent difference lay in the somewhat greater number of crab species (5) taken in the C-marsh as compared with the P-marsh (3).

DISCUSSION

This study of fiddlers in a sewage-exposed and in a control marsh was too brief to do more than indicate possible areas for continued investigation.

Both the pitfall trap and the excavation methods of sampling the two populations showed greater numbers of fiddlers in the Control than in the waste-flooded marsh. This might indicate a toxic effect of the effluent. The greater concentration of U. minax in the P- than in the C-marsh might indicate, furthermore, that the effluent tipped the scales in favor of this species, in its competition with U. pugnax. On the other hand, factors other than the wastes may have been more important in determining these apparent results. The muddy substrate in the P-marsh was deeper and the Spartina more lush than in the C-marsh. The C-marsh was also narrower in its extent than was the P-marsh study area. Laboratory tests of waste tolerances in fiddlers, in conjunction with further field studies, seem indicated.

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Table 1. Comparison of Densities of Uca Burrows Around C-Pond and P-Pond Margins (in 5 m sections)

C-Ponds			P-Ponds		
1	2	3	1	2	3
15	21	14	27	20	29
1	39	2	7	23	21
1	3	7	20	23	0
15	12	18	43	28	0
13	7	5	44	26	3
4	17	0	52	64	29
23	12	1	66	29	21
3	1	10	44	42	36
14	21	3	48	26	24
3	2	12	29	39	4
4	0	11	43	24	23
4	14	15	21	1	23
5	2	4	29	0	18
19	15	9	22	21	10
28	9	12*	23	41	49
42*	23*	21*	8*	0*	0*
28*	14*	40*	0*	0*	0*
32*	21*	10*	1*		0*
		0			
254	233	194	527	407	290
	681			1224	

* Dense vegetation in these sections made burrow detection almost impossible.

Table 2. Comparison of Pitfall Sampling in C-Marsh and in P-Marsh

Collection Date	Uca	Callinectes	Sesarma	Minnows	Ostracods	Nematodes	Annelids	Mites	Shrimp	Insects
C-Marsh										
1. Aug. 11	2	0	0	2	0	0	0	0	1	0
2. Aug. 12	2	0	0	0	10	4	1	1	0	1
3. Aug. 13	14	1	3	1	7	7	2	0	0	0
4. Aug. 14	0	0	0	3						
5. Aug. 17	13	1	3	1						
6. Aug. 18	0	0	0	1						
Total	31	2	6	8						
P-Marsh										
1. Aug. 11	1	0	0	3	0	0	0	0	0	0
2. Aug. 12	0	0	0	1	0	0	0	1	0	0
3. Aug. 13*										
4. Aug. 14	2	0	0	1						
5. Aug. 17	1	0	0	1						
6. Aug. 18	3	0	1	4						
Total	7	0	1	10						

* Pitfall trap washed out.

Table 3. Comparison of Excavation Sampling of Crabs in C-Marsh and P-Marsh

Crab	Number of Specimens	
	C-Marsh	P-Marsh
Uca pugnax	111	20
Uca minax	0	21
Uca? (undetermined very small)	34	14
Callinectes sp.	1	1
Sesarma sp.	8	0
Eurytium sp.	1	0
Total	155	56

NUTRIENT INPUTS AND THE RESPONSE OF SALT MARSH GASTROPOD POPULATIONS: A PRELIMINARY REPORT

Alan E. Stiven and J. T. Hunter

Department of Zoology, University of North Carolina, Chapel Hill

INTRODUCTION

The relationship of herbivore production to resource availability, and the question of population regulatory processes in macrodecomposer herbivore-detrital systems are central ecological questions today (Hairston et al., 1960). In addition, in coastal salt marsh and estuarine areas of the U.S., the effect of increased nutrient inputs from city effluents on the growth, production, and survival of the important decomposer and herbivore groups is of major importance.

One system in which these questions can be studied is the salt marsh ecosystem. Several prior studies in the past 10-15 years have provided much background information. Teal (1959) placed the components of the salt marsh ecosystem into the context of an energy flow diagram and Odum & Smalley (1959) further advanced the understanding of the system's metabolism with a comparison of a herbivorous and a detrital feeding invertebrates. The principle detrital feeder in Odum & Smalley's study was the common marsh snail, Littorina irrorata. Teal's study had indicated that the molluscs play an important role in the transfer of energy into secondary production. Odum & Smalley quantified this by examining the ingestion processes of the L. irrorata population and the assimilation efficiency of detritus ingestion. These herbivorous "macrodecomposers" have been shown to play an extremely important role in estuary enrichment (Odum, 1961), which form the base for much of the fishery industry of the east coast.

In the salt marshes in and around the Morehead City-Beaufort area, Littorina irrorata is one of the major detrital feeders with populations ranging from 100 to 300 snails/m^2. Their principle food source is the detritus of the marsh bed, formed mainly by the decomposition of the predominant marsh grass, Spartina alterniflora. They also utilize the epiphytic biota of the Spartina stem. As the detritus floor in all areas is considerably thick, including areas of both high and low relative snail populations, one might suspect at first that the snail populations are not resource limited, as suggested by Hairston et al. (1960) in their general treatment of resource limited population

components of ecological systems.

The main focus of this project, therefore, is an examination of the ability of the major detrital feeders of the estuary to respond to the increased nutrient inputs into the system from the addition of effluents. Their ability to handle increased nutrients should be reflected in changed population characteristics. This question and the coupled hypothesis of a resource limited detrital consumer will be examined first in the gastropod Littorina irrorata.

METHODS

Preliminary sampling was carried out in the summer of 1970 in six study marshes on Bogue Sound, with Calico Creek representing the high nutrient ("polluted") marsh, and demographic characteristics of the gastropod as well as features of the Spartina recorded. Experimental cages were installed in four of the marshes (Calico, Hoope Hole, and Dill Creek) to initiate a population-resource manipulative phase of the study.

During 1971-72 the actual levels of major nutrients and the amount and composition of total organic detritus in each of the study marshes will be established through chemical analyses of the organic detritus and sediments, living Spartina and Littorina. Energetic transformations will be determined by standard calorimetry respiration and input feeding experiments. Total epiphytic biota levels will be analyzed for each marsh.

Population demographic characteristics will continue to be followed for each Littorina population. These characters will be related through regression procedures to nutrient resource levels and primary biomass and production levels.

Tests of resource limited Littorina hypothesis have involved the establishment of caged populations in the three most divergent (with respect to nutrient inputs) marshes. Resource availability has been varied by varying gastropod population size per cage. Littorina responses involve measurements of individual and population growth of marked snails and mortality rates.

PRELIMINARY RESULTS

Preliminary results indicate that the Spartina and Juncus (marsh grasses) in the salt marsh at Calico Creek are larger in diameter. There is no significant difference between comparable areas of unpolluted and polluted marsh in height or in density (stems/m^2), but there is a significant difference in the dry weight/m^2 .

Littorina population characteristics based on preliminary sampling are given below in Table 1.

Table 1. Population characteristics of Littorina irrorata in six marshes near Morehead City, N.C.

Marsh	Crude Density (No./m^2)	Mean Crude Dry Wt (g/ind)	Mean Length (cm)	g/m^2
Calico	154	.19	2.40	29.7
Hoope Hole	110	.10	2.09	11.1
Oceana	~ 260	--	2.0	--
Broad	~ 100	--	1.95	--
Dill Creek	~ 125	--	1.75	--
Gabs	~ 100	--	2.00	--

Based upon 50 snails sampled randomly from both Calico and Hoope Hole Creeks, regression of dry body weight Y to cube root of X (length x width x opercule opening) were found to be:

Calico Y= 0.247X - 0.271
Hoope Hole Y= 0.342X - 0.381

Such regression (with appropriate confidence intervals to be computed later) will serve as future predictive equations for marsh snail biomass estimates.

These early observations on the first two marshes above indicate not only that the size of the population is larger in Calico but the size of the individuals is considerably larger than that of the populations of other marshes. On the average, the Calico Creek population is about 40% larger (154 vs. 110) and the average shell-less dry body weight is about 90% larger (0.1928 g/ind vs. 0.1010 g/ind). These combine to give an almost three fold difference in dry body weight/m^2 between the marshes (29.69 g/m^2 vs. 11.11 g/m^2). The question of such a large difference in dry body weight and population size may be due to a greater longevity and a positive growth rate throughout their lifespan. Future research will deal more usable food per unit of detritus in relation to ingested food and comparative population and individual growth patterns.

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LABORATORY STUDIES CONCERNING THE GROWTH AND REPRODUCTION IN THE SEAWEED, ULVA CURVATA

Charles F. Rhyne and Max H. Hommersand

Department of Botany

University of North Carolina at Chapel Hill

INTRODUCTION

The research reported here has been carried out on Ulva curvata (Kutzing) De Toni, which together with species of the closely related genus Enteromorpha comprise the dominant vegetation of the oyster beds in Calico Creek. Calico Creek, which is a eutrophicated estuarine tidal creek located adjacent to Morehead City, N.C., drains both residential and limited agricultural areas and receives raw and treated sewage through the town's treatment plant. In our annual report last year we reported on field and laboratory studies which showed that U. curvata appears to have a heteromorphic life cycle in which the gametophytes are minute filamentous plants that develop early in the winter. These are succeeded by sporophytes which develop into large thalli in mid-winter and become reproductive in February, March and April. The thalloid form of this species reproduces synchronously on a semilunar rhythm. The released zoospores encyst and survive the summer as encysted cells or minute filamentous plantlets on the oyster shells.

The Calico Creek material appeared to be a favorable organism for laboratory studies. After achieving satisfactory culture conditions in the laboratory with whole plants and discs cut from thalli, studies were designed to follow the influence of certain environmental parameters on growth and reproduction. In particular, photoperiod, light intensity, temperature, and nitrogen levels (as NH_4-N), have been analyzed in the laboratory in the hope of elucidating possible causative factors affecting growth and reproduction. It appears that light intensity or mean daily illuminance interact with nitrogen levels to regulate vegetative and reproductive conditions in the cell.

MATERIALS AND METHODS

Studies on growth and reproduction were conducted in a walk-in room in which temperature and photoperiod was controlled independently for each experimental set-up. Plant material used in these experiments were taken at two different times. Material used in experiments shown in figures 1-7 were collected in April, 1970, while material used in the data of figure 8 was collected in December, 1970. Plants collected in April were maintained in low light, low temperature conditions.

Discs, 0.5 to 1.80 cm^2 in size, were cut from Ulva thalli with a cork boring tube. Discs were regularly taken from a particular area of the thallus to normalize for variations in growth potential of different parts of the thallus. Discs were measured individually at the beginning of each experiment and generally there was never more than 4 to 5% variation in size. The area of each disc was measured on a grid having 100 squares/cm^2.

Reproduction was followed under the microscope by observing cell cleavage leading to swarmer formation. Growth measurements and observations on reproduction were made daily for each disc. In the early experiments the culture medium was unfiltered Calico Creek water supplemented with 1 ml/1 von Stosch enrichment medium and 0, 0.26, 0.78, 1.82 and 13.0 mg N/1 as ammonia nitrogen. Unsupplemented Calico Creek water had 0.06 to 0.09 mg N/1 as ammonia-nitrogen. Later, experiments were carried out with millipore filtered water (0.45u pore size) without von Stosch medium. Von Stosch medium is primarily NO_3 and PO_4 salts, Fe, Mn and EDTA with biotin, thiamin and B_{12} vitamins.

Illumination: Most of the experiments were conducted with the use of 40 watt cool white fluorescent bulbs allowing approximately 200 to 900 ft-c. The temperature-light intensity gradient experiment used high intensity "Power Groove" fluorescent bulbs allowing up to 1200 ft-c. Photoperiods were controlled by time clocks.

An experimental design with crossed gradients of light intensity and temperature was used for obtaining growth and reproduction data plotted in the graphs. The design for this system is described in the 1969-1970 annual report. Temperature gradients were 5° to $25^{\circ}C$ with light intensity gradients from 50 to 1200 ft-c.

A second design was utilized in growth and reproduction studies which consisted of 200 and 500 ml culture flasks containing filtered and unfiltered Calico Creek water which was changed every other day. A continuous supply of filtered air was bubbled through the culture medium of some of the flasks. The discs used were 0.50 and 1.80 cm^2 in size at the beginning of each experiment.

Ammonia determinations were carried out by the method of Solorzano, 1969.

RESULTS

Observations on the effects of photoperiod, temperature, light intensity and NH_4-N levels upon growth and reproduction can be observed in figures 1-5. Growth was measured at the end of a seven-day period while reproduction was observed daily. In the areas of the graphs where no growth is indicated reproduction occurred before the 7th day. Figure 1 shows the effects of the photoperiods 8,$\overline{16}$

and 18,$\overline{6}$ hours, which lie outside the range plants are subjected to in their natural habitat. In the field at this latitude the photoperiod ranges from ca. 9 hr, 45 min daylight in December to 14 hr, 30 min daylight in June. Figures 2 and 3 show results with 10 and 12 hr photoperiods using Calico Creek-von Stosch medium. Figures 4 and 5 indicate results when photoperiods of 10 and 12 hr are used with NH_4Cl levels of 1.0 mg/l (260 ug NH_4-N) and 3.0 mg/l (790 ug NH_4-N). The light intensity was extended to 1200 ft-c in the experiments plotted in figures 3 and 5.

It can be seen in figure 1 that very little growth ensues with photoperiods of 8,$\overline{16}$ and 18,$\overline{6}$ hours, as both reproduction and some cell death occurred where no growth is indicated. Figures 2 and 3 show that growth tended to be limited in a 10,$\overline{14}$ hr photoperiod, with reproduction occurring in the 15° to 25°C range. With 12,$\overline{12}$ hr photoperiods (figure 3) growth improved but again reproduction took place at temperatures in the 20° to 25°C range. Higher growth rates were obtained with a lower incidence of reproduction when 1.0 and 3.0 mg/l NH_4Cl is added (figs 4,5). In figure 4 a tendency toward higher growth rates is seen under conditions of high light and temperatures.

Figures 6 and 7 show the growth and reproduction potential for material collected five months before the experiment. Discs, 1.80 cm^2, were placed in 200 ml culture flasks and subjected to variables of nitrogen levels, light intensity, and bubbling rates of air. Unfiltered Calico Creek water was changed every other day and temperature was set at 18°C. Growth was plotted as percent increases in area per day beginning with the day of inoculation. It can be seen in figure 6 that growth doubles in approximately 3.5 to 5 days at 200 ft-c. Of the seven discs used only that in Calico Creek water was observed to reproduce before the termination of the experiment. Figure 7 shows the same material subjected to 800 ft-c illumination. All discs reproduced in 1 to 3 days.

Figure 8 shows an experiment conducted with material collected in December, 1970, with discs cut to 0.50 cm^2 and maintained in 500 ml flasks. The water was millipore filtered, changed every other day, and maintained at a constant temperature of 14°C. In the experiment growth and reproduction was observed in relation to low and high light intensity and NH_4-N levels. Growth is plotted on semi-log graph paper as a function of daily measurements in disc size. Experiments were done in triplicate with Calico Creek water containing 60 ug/l NH_4-N and Calico Creek water containing 260 ug/l NH_4-N. Only the highest and lowest growth rates of each set of three were plotted. The data show the following: (1) growth is a log rhythmic function prior to reproduction; (2) low and high NH_4-N levels give nearly the same growth rate in low intensity light; (3) a higher growth rate is obtained at higher light intensities in the high NH_4-N medium; (4) reproduction takes place in 3 to 4 days after transferring the Ulva material to high light conditions with all discs grown in the low NH_4-N medium.

DISCUSSION

Field studies conducted earlier indicated that thallus growth was rapid during the months from January to March, with semi-lunar reproductive periodicities associated with high spring tides.

Growth and reproduction experiments using Ulva curvata discs indicate that a rhythm is not present in the laboratory, at least not a semi-lunar reproductive rhythm. However, these studies have implicated certain environmental parameters as having a coordinating function in the regulation of growth and reproduction.

Data shown in figures 1-5 appear to indicate control of growth and reproduction by temperature, light intensity, photoperiod and NH_4-N levels. Growth appears to be prolonged when NH_4Cl is introduced into the culture medium as seen in figures 4 and 5. Whereas, figures 2 and 3 show the greater occurrence of reproduction when low NH_4-N (Calico Creek water only) levels are used.

Figures 6 and 7 point to a relationship between light intensity and NH_4-N concentrations. It appears that concentrations of 7.0 and 50.0 mg/l NH_4Cl may well be inhibiting growth to a certain degree. Figure 6 shows that discs grown in Calico Creek water with additions of NH_4Cl allowed vegetative growth with reproduction occurring only in the medium containing a minimum of NH_4-N. When the remaining 6 discs were transferred to high light (fig 7), reproduction proceeded to occur in all discs. It would appear that higher light intensities favor a faster uptake of NH_4Cl, whereupon the NH_4-N level is reduced to zero sooner than at lower light intensities.

Figure 8 shows growth and reproduction in relation to low and high light intensities and NH_4-N levels. Here we see growth differences when low and high NH_4-N grown discs are switched from low to high light conditions. Reproduction is observed in the low NH_4-N material while the high NH_4-N grown discs continued their vegetative growth but at higher rates. With both low and high NH_4-N in the medium discs grew approximately the same rates with low intensity light. Growth averaged 36%/day for the discs grown in low NH_4-N, while discs grown in high NH_4-N increased in area an average of 51%/day when subjected to high light intensities.

It was seen that reproduction did not occur in the high NH_4-N until 6 days after transfer from low to high intensity light when discs, 0.5 cm^2, were grown in 500 ml flasks (fig 8). In contrast, discs, 1.80 cm^2, grown in 200 ml culture flasks reproduced in 1-3 days when treated according to the same regimen. This larger ratio of culture medium to disc size allows the NH_4-N level to remain at a relatively higher level promoting vegetative growth over reproduction.

These observations are rather interesting from the standpoint that the depletion of certain nutrients has long been known to induce

sexuality in micro-organisms. The depletion of nitrogen, particularly NH_4^+, was found to be the controlling factor along with light in regulating gametogenesis in Chlamydomonas by Sager and Granick (1954). The control of reproduction by NH_4^+ has also been discussed by Kandeler (1969) working with Lemna gibba, an aquatic higher plant.

Warburg experiments were conducted in the laboratory in which O_2 evolution rates of discs were measured as a function of low and high NH_4-N levels. It was observed that O_2 evolution rates are identical under these conditions. These findings, however, do not rule out the importance of photosynthesis in relation to growth and reproduction in different nitrogen concentrations.

Light intensity or daily mean illuminance and nitrogen appear to be vital interacting factors controlling the potential for either vegetative growth or reproduction. Light and nutrient cycling in nature may act through daily and tidal rhythms to produce the observed semilunar reproductive periodicities.

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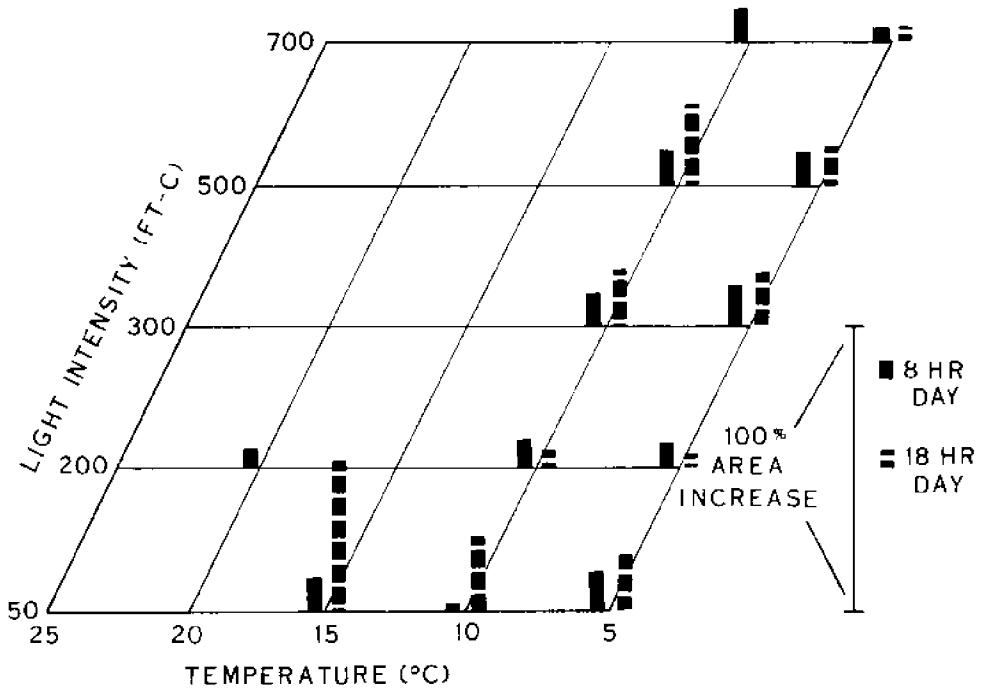

Figure 1. Light Intensity-Temperature effects upon Growth and Reproduction in Five curvata discs. Photoperiod: 8,10 and 18,7 hours. Medium: Calico Creek water-von Stosch enrichment (1.0 ml/l)

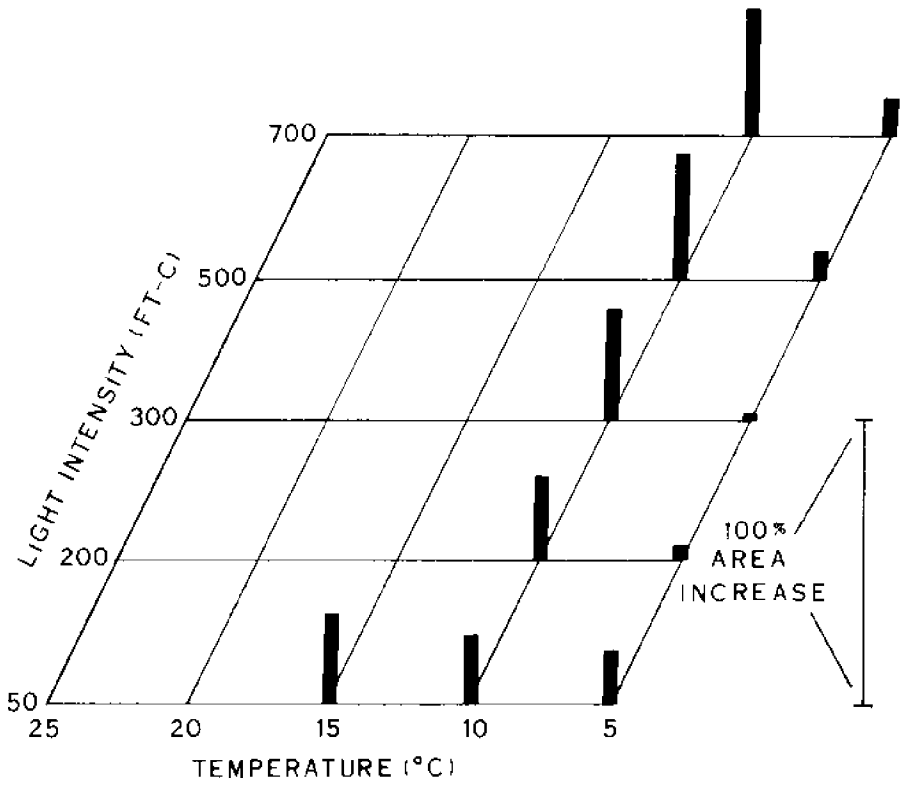

Figure 2. Light Intensity-Temperature effects upon Growth and Reproduction in Five curvata discs. Photoperiod: 10,14 hours. Medium: Calico Creek water-von Stosch enrichment (1.0 ml/l)

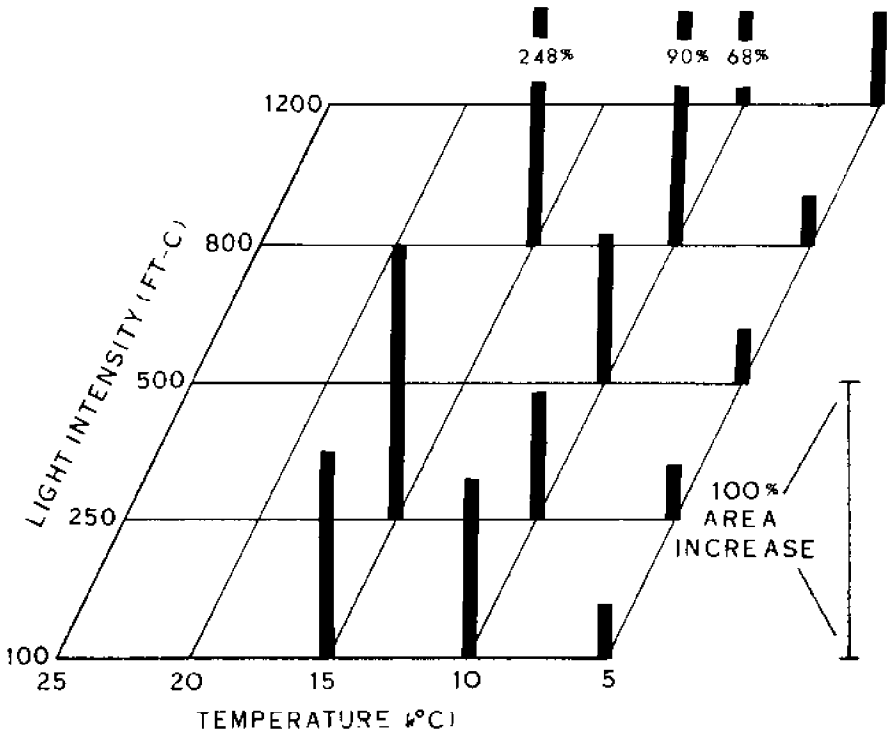

Figure 3. Light Intensity-Temperature effects upon Growth and Reproduction in Ulva curvata discs. Photoperiod: 12,12 hours. Medium: Calico Creek water-von Stosch enrichment (1.0 ml/l)

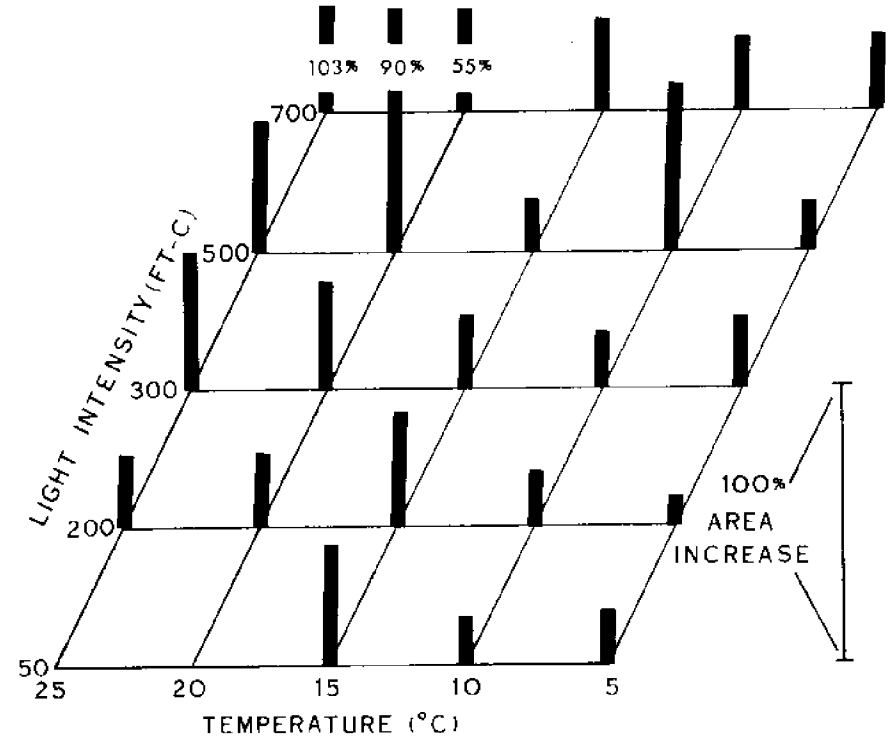

Figure 4. Light Intensity-Temperature effects upon Growth and Reproduction in Ulva curvata discs. Photoperiod: 10,14 hours. Medium: Calico Creek water-von Stosch enrichment (1.0 ml/l) with 1.0 mg/l NH_4Cl

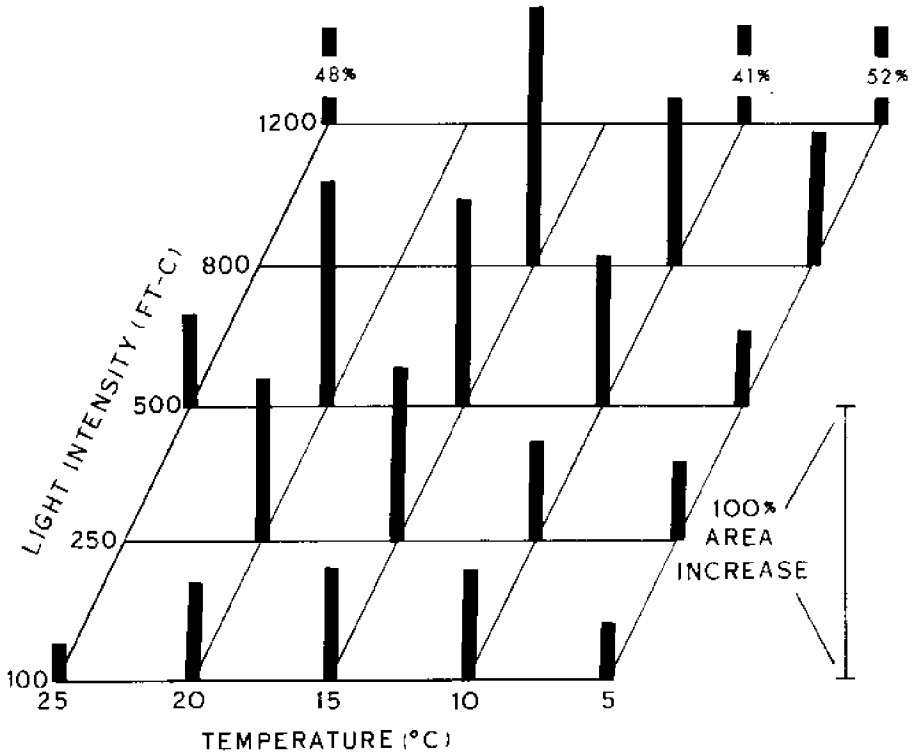

Figure 5. Light Intensity-Temperature effects upon Growth and Reproduction in Ulva curvata discs. Photoperiod: 12,12 hours. Medium: Calico Creek water-von Stosch enrichment (1.0 ml/l) with 3.0 mg/l NH_4Cl.

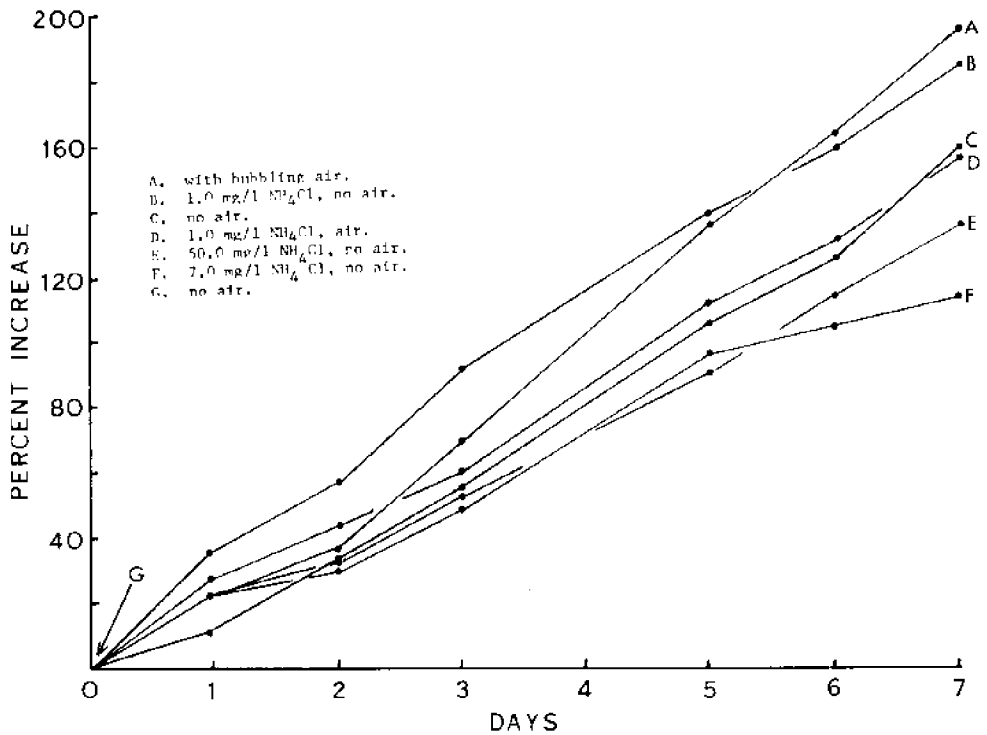

Figure 6. Growth rates (% area increase from day 0) and Reproductive Potential in Ulva curvata discs. Temperature: 18°C. Photoperiod: 12,12 hours. Medium: A-F, Calico Creek water-von Stosch enrichment (1.0 ml/l). G, Calico Creek water. Light Intensity: 250 ft-c.

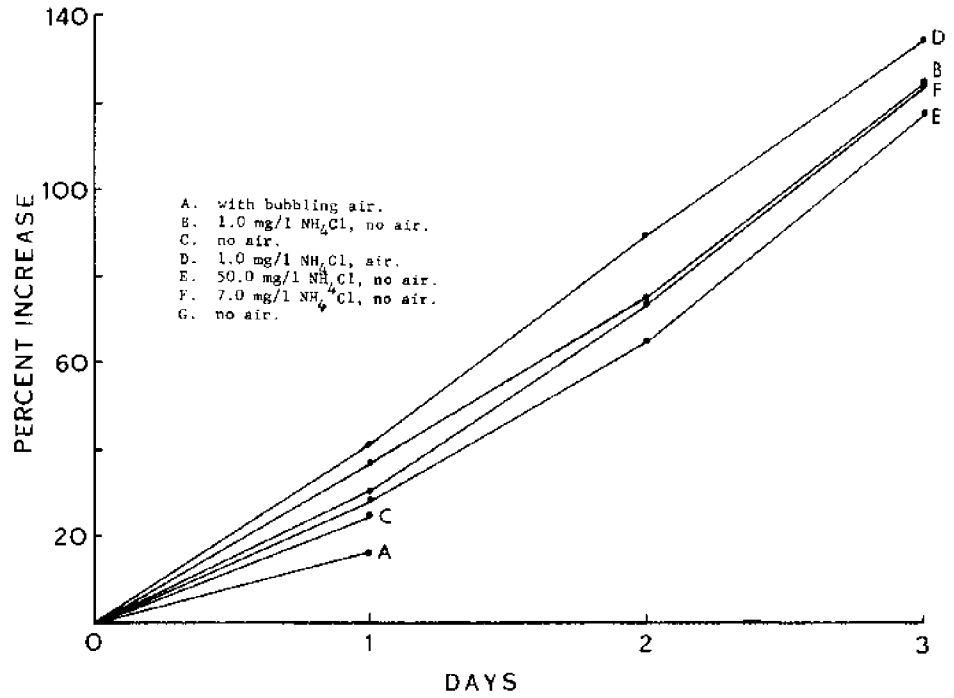

Figure 7. Growth rates (% area increase from day 0) and Reproductive Potential in Ulva curvata discs. Temperature: 18°C. Photoperiod: 12,12 hours. Medium: A-F, Calico Creek water-von Stosch enrichment (1.0 ml/l). G, Calico Creek water, Light Intensity: 800 ft-c.

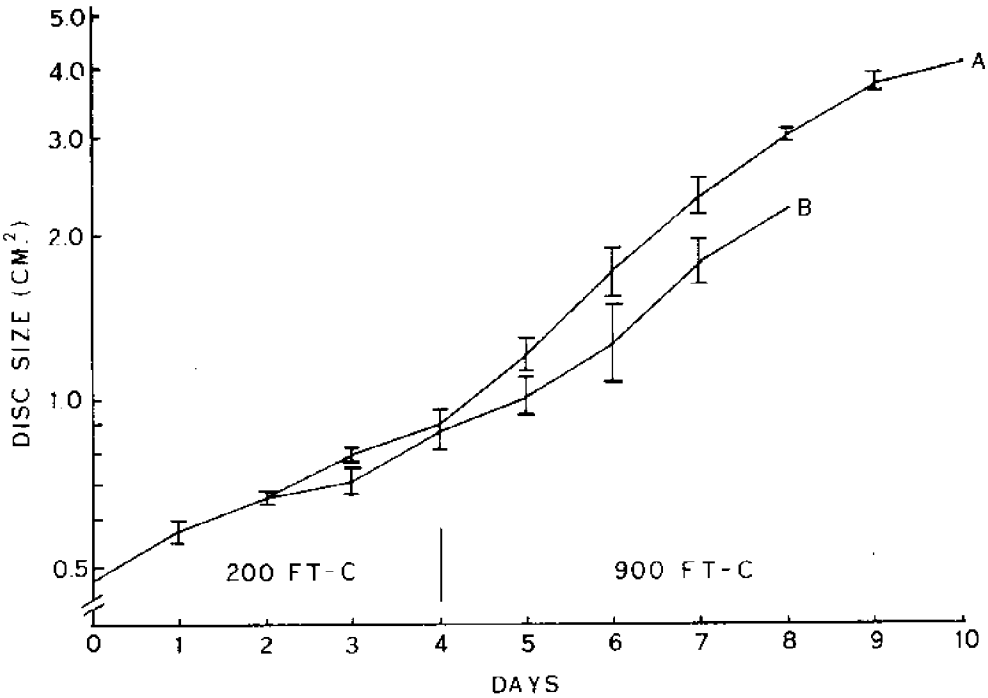

Figure 8: Growth rates (disc size) and Reproductive Potential in Ulva curvata discs.
A. Calico Creek water with 1.0 mg/l NH_4Cl.
B. Calico Creek water.