Self-Organization of Ecosystems in Marine Ponds Receiving Treated Sewage

By Howard Odum

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SELF-ORGANIZATION OF ESTUARINE ECOSYSTEMS IN MARINE PONDS RECEIVING TREATED SEWAGE

Data from experimental pond studies at
Morehead City, North Carolina, 1968-72*

Howard T. Odum+

With data contributed by:

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With contributions from the Center for Wetlands, University of Florida, Gainesville.

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ABSTRACT

These are the results of an experimental study of the marine ecosystems developing in six shallow ponds - three waste ponds receiving sea water mixed with sewage treatment effluent from the municipal plant of Morehead City, N.C., and three control ponds receiving sea water mixed with city tap water. After multiple seeding of many species, acclimation and self organization of ecological succession was studied 1968-1972. The ponds were microcosms with much of the essence of estuarine eutrophication.

Production and respiration (1800 g O2/m2/yr) were much higher in the waste ponds than in the control ponds (790 g O2/m2/yr). Growths of marsh grass, Spartina alterniflora; blue crabs, Callinectes sapidus; grass shrimps, Palaemonetes pugio and vulgaris; a "soup" of copepods, Oithona; and capitellid bottom worms were abundant. Diurnal extremes of oxygen and pH in the waste ponds reduced the variety of larger animals in summer. Dominant fishes were air-breathing, lower food chain consumers - Fundulus heteroclitus, Cyprinodon variegatus, and some mullet.

The eutrophic ponds had a winter dominance of a nanoplankton xanthophyte alga, Monodus guttula, with exceptional quantities of chlorophyll a, 300-500 mg/m3. During three successive springs the Monodus bloom was precipitously replaced in early May by a more diverse flora and blue-green algal mat in the shallows.

The control ponds had more normal pH and a mixed and more oligotrophic plankton flora. By the third year, two of the ponds contained extensive beds of Ruppia maritima and one sustained a turbid plankton system with a prominent oyster reef, Rangia clams and snapping shrimp. Carnivores were flounders and penaeid shrimp. Low winter temperatures killed some larger fishes that were unable to migrate to deeper waters.

These extremes simplified and channeled food chains serving the ideals of aquaculture, in the case of the eutrophic ponds a good annual yield of bait fishes of about 23 g fresh weight/m2. Yield of carnivorous fishes and blue crabs was 7-12 g fresh weight/m2.

The waste ponds supported waterfowl and could be managed to stimulate food fish species by allowing the small pond fish to swim into less fertile waters containing the larger fish.

Comparisons of the ponds with nearby Calico Creek estuary and its marsh both receiving treated sewage effluent showed many similarities: greater rates of growth of marsh grass, high aquatic productivity, many aquatic birds, and lowered species variety among water-breathing animals.

Stimulating self organization of adapted ecosystems with multiple seeding developed an adapted ecosystem rapidly, one possibly suitable as an interface between the wastes of human economic development and the public waters.

PREFACE

From 1967 to 1971 at Morehead City, North Carolina, an experimental study of self organization of estuarine systems was made, comparing three marine ponds receiving treated sewage with three control ponds receiving ground water. Sponsored by Sea Grant, the project had many roles:

1. It was one of the first conscious tests of the self-organizing principle for developing domestic ecosystems to interface between the wastes of the economy and public waters.

2. The basic question of self organization in estuarine ecosystems was studied by monitoring which available organisms through species interaction, through reward loop feedbacks, and selection, were retained as important members of the emerging complex ecosystem. By multiple seeding of species, larvae, etc. genetic limitations were minimized the way they are in estuaries that mix well with the sea. The colonization, organization, increase in metabolism, and diversity were studied for a three-year period.

3. The project was a test of the utility of "ecological engineering". The human-guided, self-organizational process for generating new ecosystems is called "ecological engineering" when its purpose is generating ecosystems symbiotic with the human economy.

4. The project was organized as a systems ecology study with models developed before, during, and after data were obtained so as to measure items believed important and iteratively develop concurrent understanding of the whole and the parts. Although not formally sponsored by the International Biological Program, the project extended to estuaries the team systems approach to understanding complex ecosystems. After trial-and-error, a simulation model based on those parts and relationships believed important and evaluated separately was used to generate the main events in the three-year period of self organization.

5. A primary objective was presenting a total estuarine ecosystem in one place. This ideal, advocated by the early founders of Ecological Monographs, recognized the ultimate value of presenting details on ecosystems whether they were immediately related to a question or not. This viewpoint has long held that whole systems should be represented in their entirety with many purposes, questions, and levels of structure and function presented together. This ideal seeks to encompass a whole system in one place, to represent complexity, to unify holistic understanding, to provide basis for explanatory models, to make concepts of behavior of parts consistent with trends in behavior of the system through simulation, to gain predictability of whole ecosystems, and to find better ways of thinking of nature than one part at a time.

6. Controlled experiments on an ecosystem scale are rarely possible. But this project provided a field scale, replicated experimental test of the characteristics of estuarine ecosystems developing with and without treated sewage wastes. The project was a test of larger microcosms as a technique for study of ecosystem essence. The ponds provided a bounded, discrete system with replications.

7. The study was unusual among estuarine studies in having so many kinds of measurements made in a small controlled area simultaneously. Considerable understanding emerges from having so much collateral background information with which to consider each observed phenomenon. Just knowing what is happening among animals often helps explain plants, etc.

8. With emphasis on student classwork, theses, and dissertations, the project generated common interests within the new Marine Science Graduate Curriculum at the University of North Carolina, and initially most of the faculty and students participated. Work on shared pond ecosystems helped teach interrelationships and the unity of environmental processes.

9. The organization of project workers that emerged through self organization of human interactions may provide some insights on means for research project management that retains individual creativity while achieving needed focus. In giving instructions to new people joining the work, it was suggested that half their time go to meet promised project objectives and half into following interesting leads and ideas that they might develop.

10. In recent years, understanding of ecosystems has increased with many new quantitative theories about organization, homeostasis, system structure and function. However, there are few estuarine ecosystems where simultaneous measurements of so many components at the same time have been made available so that ecosystem level theories can be tested and models validated.

11. Since individual initiatives by participants were encouraged, some of the studies were done in great detail, whereas others were short efforts made later to fill in blank areas in understanding of the ecosystem.

12. Other papers may be stimulated by these data. Many, including former project participants, have been prevented from using the results of this project because the various data were not generally available. Some of the results have important general implications. Other details of less interest internationally are important to those doing detailed studies in North Carolina estuaries.

Parts of the data were in four progress reports.* Some of the studies were published in separate papers. However, for various reasons, most of the data have not been published heretofore. Part of the delay in publication was the continuing difficulty within the field of ecology to agree on ways to publish whole system studies. Most scientist are trained to expect publications to have single purpose, focus on few questions, and consider only one level of phenomena at a time. Even after the International Biological Program, no standard way emerged for publishing total ecosystem studies without dividing results into topical papers. Most journals oppose whole system documentation on grounds of complexity and of length.

To facilitate additional summary papers, to make results available to everyone, and to complete our obligation to society for the public funds spent, this data report is provided in a concise form without much interpretation or comparisons with other work. The format of this data report with minimum discussion represents a compromise approach to get all data in one place as promised in the original proposal. Integration of these data with the general literature is left to shorter papers and reviews that may follow in the scientific literature.

ACKNOWLEDGEMENT

With H.T. Odum and A.C. Chestnut as initial principal investigators, 1967-1970, the project, "Optimum Ecological Designs for Estuarine Ecosystems in North Carolina," was supported by the Sea Grant Program of the National Science Foundation with NSF Grant GH-18 and transferred as Grant GH-03 when Sea Grant was moved to National Oceanographic and Atmospheric Administration. Principal investigators for 1970-1971 were E.J. Kuenzler and A.C. Chestnut and for 1971-1972, C.M. Weiss.

Experimental ponds were constructed with funds from North Carolina Board of Science and Technology with grants No. 180 and 232. C.C. King prepared pond maps and legal description for a lease from Morehead City. Construction contract was with Howard Construction Co, Newport, N.C. J. Lamb arranged pumping systems.

Work was administratively organized in phases given in Table 1. Persons making various contributions are acknowledged in Table 2.

E.J. Kuenzler, A.B. Williams and J. Day provided suggestions and criticisms of this data report.

H. T. Odum
January, 1985

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* Progress reports: edited by Odum and Chestnut (1968, 1970); Kuenzler and Chestnut (1971a, 1971b); Kuenzler, Chestnut and Weiss (1973).

Table 1. Administrative organization of project work and personnel involved.

Phase	Principal Worker	Faculty Responsible
Pond Operation and Management	W. Laughinghouse W. Smith, P. Parks, R. Klemm	A.F. Chestnut and H.T. Odum
Total Metabolism, Diversity	Martha Smith,* S. Masarachia, M. May, C. Hall	H.T. Odum
Phosphorus System	H. McKellar L.C. Davidson M. Adams	E.J. Kuenzler
Carbon System	J. Day*	C. Weiss
Nitrogen System, Bottom Fauna	P. Hebert,* M. Raps	C. Weiss
Macroscopic Algae	C. Rhyne*	M. Hommersand
Bloom Factors	D. Talbert	M. Hommersand
Plankton Algae	S. Wyman, P. Cambell	E.J. Kuenzler
Metabolism of Organic Substrates	J. Marsh	E.J. Kuenzler
Fungi	B. Rao	W. Koch
Bacterial Populations	A. Rabin, A. Harvell, M. May, T.L. Herbert	R. Mah, J. Staley
Zooplankton; Larvae	A. McCrary, R. Dowd	C. Jenner
Crustacea, Fishes	M. Beeston,* R. Field, E. Walton, R. Hyle	A.B. Williams, F. Schwartz
Shellfish	W. Smith, W. Laughinghouse B. Muse	A.F. Chestnut
Bottom Production, Food Values	D. Oakley, R. Dillon D. Leeper	W. J. Woods
Coordination Chemistry, Halogens	F. Davis*	D. Johnson
Microzoa	J. Hall, A. Powell	R. Riedl
Marsh Vegetation	D. Marshall	H.T. Odum

Table 1. (cont.)

Phase	Principal Worker	Faculty Responsible
Marsh Insects and Crabs	R.L. Knight, A. Camp E. Danya	E.A. McMahan
Marsh Snails, Littorina	T. Hunter*	A. Stiven
Sediment and Foraminifera	A. LeFurgey	St. Jean
Fouling and Borers, Birds	R. Dowd, M. Canoy E. Lindgren, C.J. Spears	A. Williams
Fishes of Ponds and Calico Creek	R. Hyle*	F.J. Schwartz
Energy System Analysis	A. LeFurgey, W. Smith	H.T. Odum

* Indicates the work which constitute a graduate thesis

Table 2. Contributions of Participants*

Adams, M.--phosphorus exchange (Adams, 1969).

Beeston, M.--crustacea, fishes (Beeston, 1970, 1971a, 1971b).

Camp, A.--marsh insects (Camp, Knight, and McMahan, 1971; McMahan, Knight, and Camp, 1971, 1972).

Campbell, P.--Phytoplankton populations and taxonomy (Campbell, 1971, 1973).

Canoy, M.--fouling blocks, DNA.

Chestnut, A.C.--pond organization.

Chestnut, A.F.--co-principal investigator, 1967-72; Institute Director, resident pond manager, oysters, glycogen (Chestnut, 1970; Muse and Chestnut, 1973).

Davidson, L.C.--phosphorus measurements (Kuenzler and Davidson, 1962).

Davis, F. E.--calcium and chlorinity, pH (Davis, 1971a, 1971b).

Day, J.--carbon metabolism, reader and critic of manuscript (Day 1970; Day Weiss and Odum, 1970; Day, 1971a, 1971b, 1983; Johnson and Day, 1968).

Dillon, R.--bottom metabolism (Dillon and Woods, 1970).

Dowds, R.E.--epifauna, wood borers (Dowds and Williams, 1971).

Farris, R.A.--macro-infauna (Farris and Williams, 1973).

Field, R.C.--fishes.

Fox, R.E.--fouling invertebrates (Fox and Williams, 1973).

Hall, C.--Computer program and calculations of metabolism from diurnal oxygen measurements (Hall, 1970; Odum, Hall, and Masarachia, 1970; Hall, C. and R. Moll, 1975).

Hall, J. R.--microzoa (Hall, 1971).

Harvell, A.--coliform bacteria (May and Harvell, 1970).

Hastings, M.--chemical analyses.

Hebert, P.--nitrogen measurements (Hebert, 1970).

Herbert, T.L.--bacteria, coliforms (Herbert, 1973, 1975).

Hommersand, M.--algae, experimental studies of Ulva and Monodus (Hommersand, 1968; Rhyne and Hommersand,1970; Hommersand and Talbert, 1971; Hommersand and Huang, 1973).

Huang, Y.--Monodus (Hommersand and Huang, 1973).

Hunter, T.--marsh snails (Hunter and Stiven, 1973; Stiven and Hunter, 1971, 1976).

Hyle, R.--zooplankton grazing, fiddler crab larvae, fishes (Hyle, 1970, 1971, 1973).

Ingram, R.--clay minerals.

Jenner, C.--zooplankton, larvae.

Johnson, J. D.--physical chemistry, pH, calcium (Johnson and Day, 1968).

Joyner, J.--oxygen measurements.

Knight R.L.--marsh insects and crabs (McMahan, Knight and Camp, 1971, 1972; Camp, Knight and McMahan, 1971).

Koch, W.--fungi (Rao and Koch, 1969).

Kuenzler, E.J.--principal investigator, 1970-72; phytoplankton, phosphorus, report co-author (Kuenzler and Davidson, 1968; Kuenzler, McKellar and Muse, 1970; Kuenzler, 1971; Laughinghouse and Kuenzler, 1971; Kuenzler and Chestnut, 1971; Kuenzler, Wyman and McKellar, 1971; Kuenzler, Chestnut, and Weiss, 1973)

Laughinghouse, W.--pond operator, oysters, regular measurements. (Laughinghouse, Smith, and Kuenzler, 1971).

Leeper, D.--plankton movements (Leeper, 1971a, 1971b; Leeper and Woods, 1973).

LeFurgey, A.--foraminifera, clay minerals, electron micrographs, and food webs (LeFurgey, 1971, 1972; LeFurgey and St. Jean, 1973, 1976).

Lindgren, E.--Limnoria.

Mah, R.--microbes.

Marino, B.--oxygen measurements.

Marsh, J. A,--metabolism of organics, bacteria (Marsh, 1970, 1971).

Marshall, D.E.--nutrient analyses, marsh grasses (Marshall, 1970).

Masarachia, S.--oxygen, pH, nitrogen, metabolism (Odum, Hall, and Masarachia, 1970; Masarachia, 1971).

May, M.S.--coliform bacteria, microorganism diversity (May, 1970, 1971, May and Harvell, 1970).

McCrary, A.--Zooplankton populations and taxonomy (McCrary and Jenner, 1968, 1970).

McKellar, H.--phosphorus metabolism (Kuenzler, McKellar, and Muse, 1970; Kuenzler, Wyman and McKellar, 1971; McKellar, 1971a, 1971b, 1971c).

McMahan, E.A.--marsh insects and crabs, diversity (McMahan, Knight, and Camp, 1971, 1972).

Miller, D.--crab burrows.

Muse, B.--oysters, glycogen (Kuenzler, McKellar and Muse, 1970; Muse, 1971, 1973; Muse and Chestnut, 1973).

Oakley, D.--nutrients.

Odum, H.T.--principal investigator, metabolism, models (Odum and Chestnut, 1968, 1970; Odum and Marshall, 1968; Odum, Hall, and Masarachia, 1970).

Outen, R.--invertebrates.

Owen, T.--autoanalyzer nutrient analyses.

Parks, P.--pond operator, regular measurements.

Patterson, C.--oxygen measurements.

Powell, A.--zooplankton (Powell and Williams, 1973).

Rabin, A.N.--bacteria (Rabin, 1970).

Rao, B.--Fungi (Rao and Koch, 1970).

Raps, M.E.--Nitrogen metabolism, fixation, ammonia diffusion (Raps, 1971, 1973a, 1973b).

Rhyne, C.--Macroscopic algae (Rhyne and Hommersand, 1971; Rhyne, 1973).

Rickards, W.L.--shrimp yields (Rickards and Williams, 1972, 1973; Rickards, 1974).

Riedl, R.--microzoa.

Schwartz, F.--fishes.

Smith, M.--free water oxygen, metabolism (Smith, 1971, 1972).

Smith, W.-- salinity, oxygen, birds, shellfish.

Spears, C. J.--birds (Spears and Williams, 1971, 1973).

St. Jean, J.--foraminifera (LeFurgey and St. Jean, 1973, 1976).

Staley, J.--bacteria.

Stelljes, H.--invertebrates.

Stiven, A.--marsh snails (Hunter and Stiven, 1973; Stiven and Hunter, 1971, 1976).

Talbert, D.--experimental studies of Monodus (Hommersand and Talbert, 1971).

Walton, E--mud crabs and snapping shrimp (Walton and Williams, 1971).

Weiss, C.--co-principal investigator, 1971-72, nitrogen, (Weiss and Wilkes, 1969).

Williams, A.B.--invertebrates, birds; editor and critic of reports; (Williams, 1968; Dowds and Williams, 1971; Farris and Williams, 1973; Fox and Williams, 1973; Powell and Williams, 1973; Rickards and Williams, 1972; Rickards and Williams, 1973; Spears and Williams, 1971, 1973; Walton and Williams, 1971.

Woods, W.--nutrients, nutrients in organisms, particulate fractions, radiotracer photosynthesis measurements (Woods, 1968; 1970; Dillon and Woods, 1970; Leeper and Woods, 1973).

Wyman, S.D.--phytoplankton (Kuenzler, Wyman, and McKellar,1971)

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*Including references to authored reports, theses, dissertations, and publications on the project.

INTRODUCTION

Life in estuaries is often observed changing in response to changing conditions. Utilizing the available species, self-organization processes seem to organize new ecosystems to maximize productivity and consumption of new resources. Understanding the self-organization process is a primary objective of basic ecology.

Learning to control the self-organization process is a promising means of environmental management. Intelligent, low energy control of self organization was termed ecological engineering (Odum et al., 1963; Odum, 1971). Conscious ecological engineering of new ecosystems to deal with waste waters was suggested (Odum, 1967).

The increasing eutrophication of the world's estuaries receiving wastes from economic expansion, provides opportunities to study self organization and develop techniques of ecological engineering. This is a report of a team project which studied self organization of ecosystems in experimental estuarine pond microcosms and the utility of ecological engineering for developing interface ecosystems for waste treatment and eutrophic aquaculture.

Experimental Ponds

Three ponds were constructed in 1968 in marshes adjacent to the Morehead City Sewage Plant on Calico Creek. The ponds were supplied with a mixture of estuarine water and treated sewage waste waters. Three control ponds were constructed adjacent to the Institute of Marine Sciences at Morehead City and supplied with a mixture of estuarine water and tap water. Figure 1a shows the locations of the ponds. Figure 1b shows the arrangements for water flow through mixing tanks so that replicate ponds received similar inputs.

Pond maps with details are given in Figure 2. Special substrates added for development of ecological subsystems are indicated including patches of marsh grass planted along margins, shell reef bar with live oysters on top, creosote-treated wood piling for attachment studies, pier pilings (treated with penta-chlorophenol), masonry plates for fouling studies, rows of concrete blocks and rubble as a micro-reef, and shallow sandy beach margins supporting benthic algae. Ponds were about one meter deep (mean depth 0.5 m). Maps with depth contours are given in Figure 3, and hypsographic curves used to estimate volumes are given in Figure 4. After basins were scooped out with a dragline, both sets were lined with about 41 cm of reddish clay to reduce permeability and give both sets of ponds the same substrate. The bottoms were then floored with about 30 cm of black marsh mud from the Calico Creek marsh site. Figure 5 is a cross section sketch of the ponds. Figure 6 contains views of the ponds just after construction. Later views after development of marsh grass and aquatic macrophytes in control ponds are shown in Figures 7 and 8.

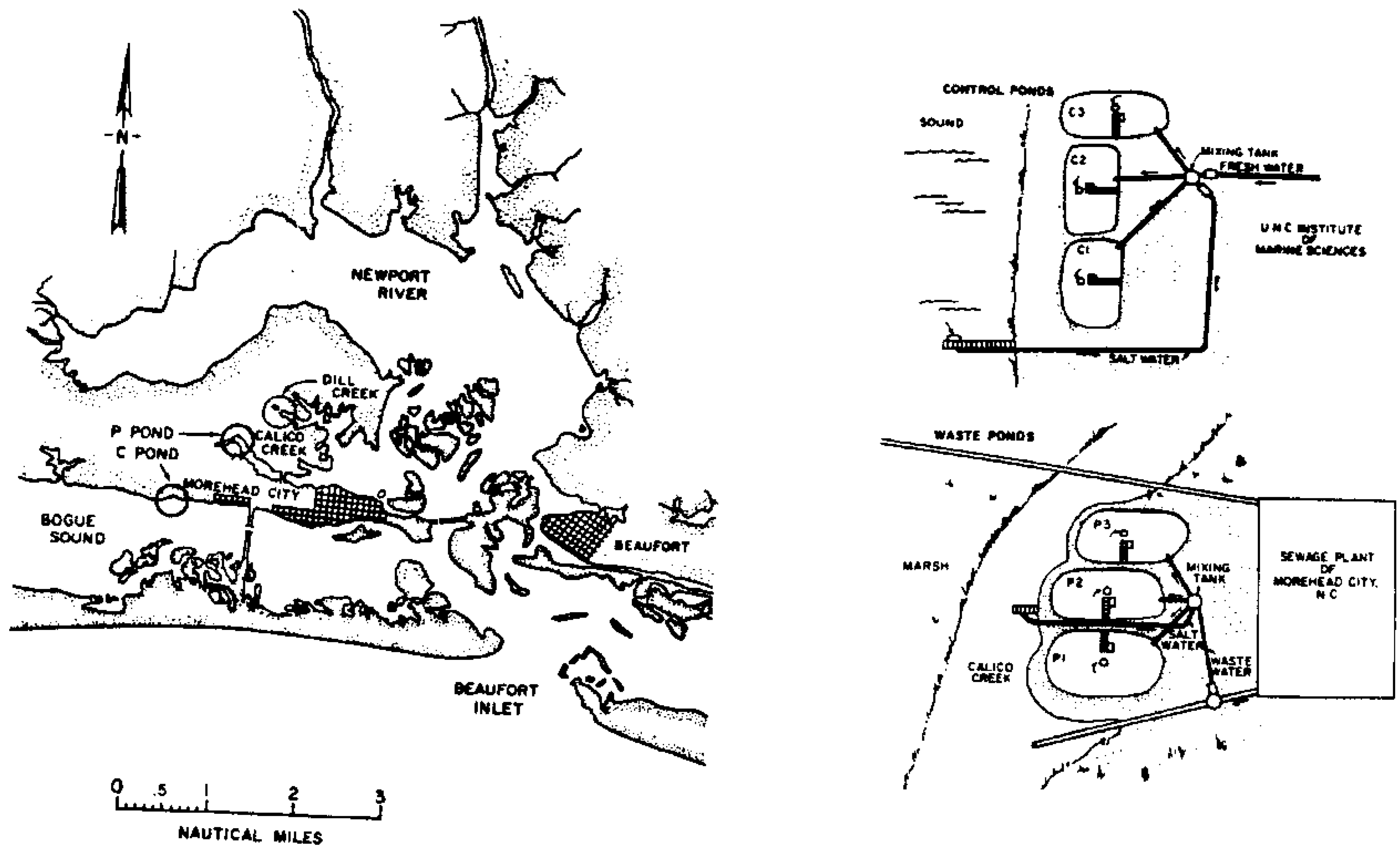


Figure 1. Experimental ponds. (a) Location in Morehead City, N.C. (b) arrangement of ponds and water flows.

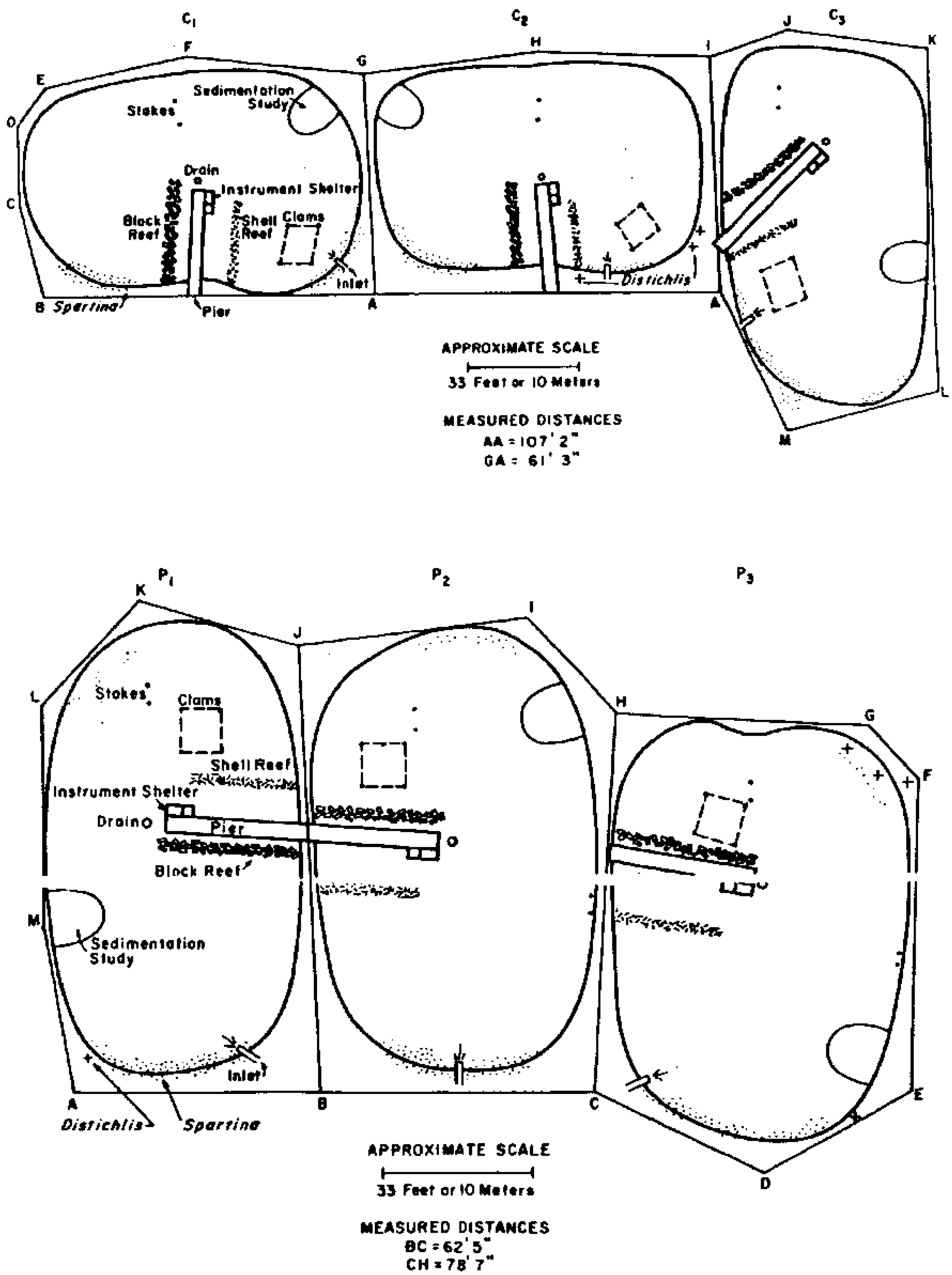


Figure 2. Maps of experimental ponds showing substrates. (a) control ponds (C ponds); (b) sewage waste ponds (P ponds).

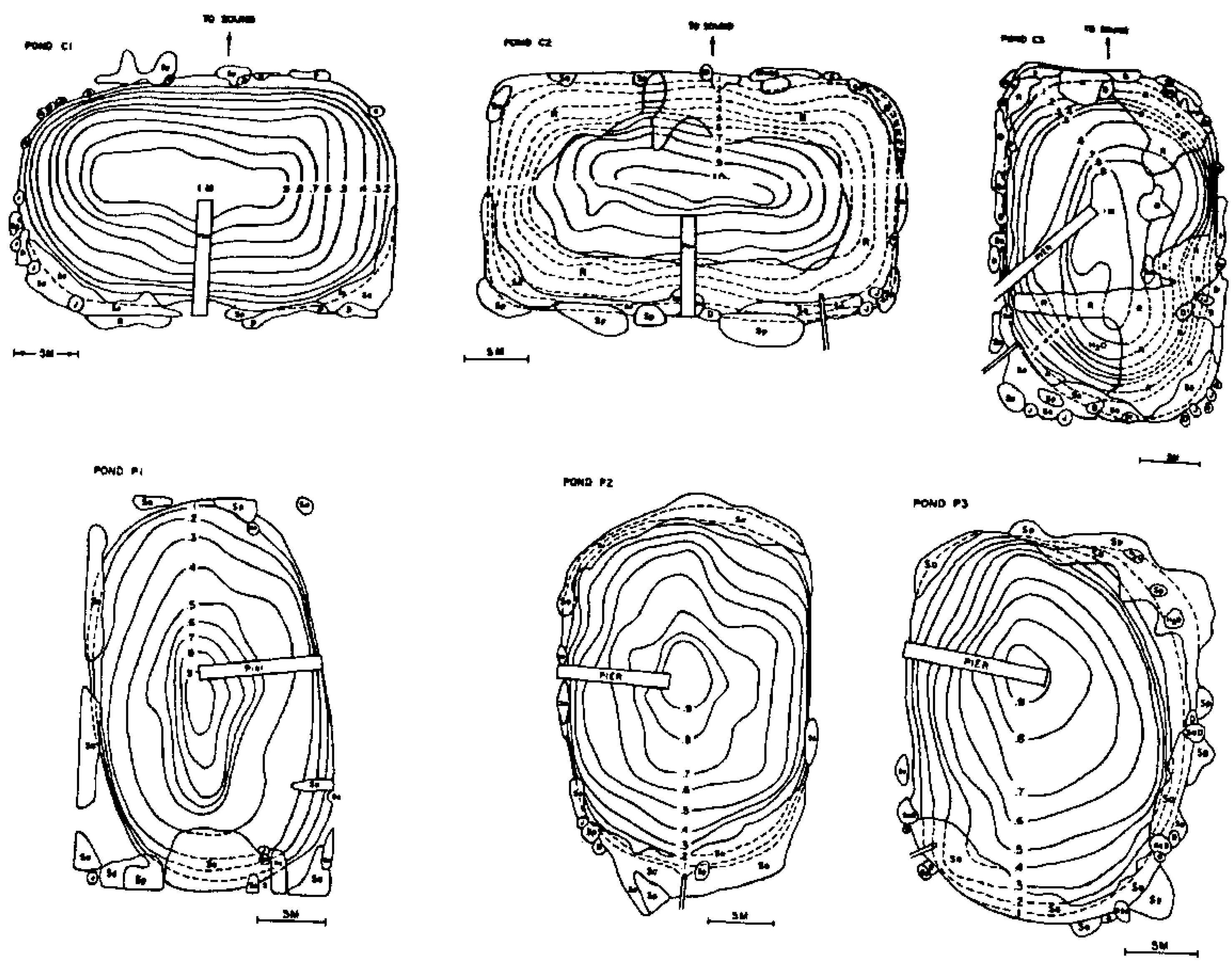


Figure 3. Depth contours in the ponds June 23, 1971.

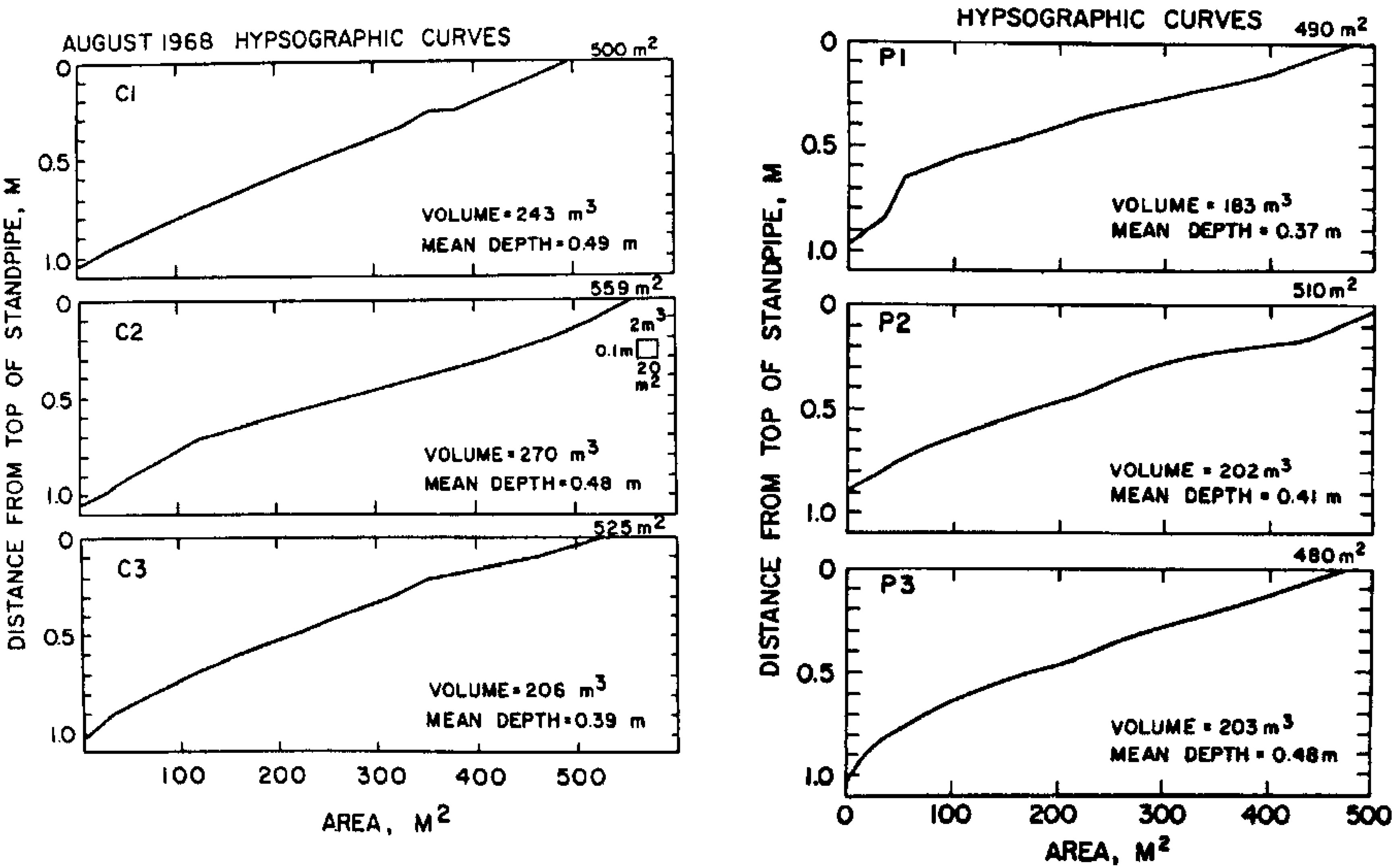


Figure 4. Hypsographic curves of the ponds.

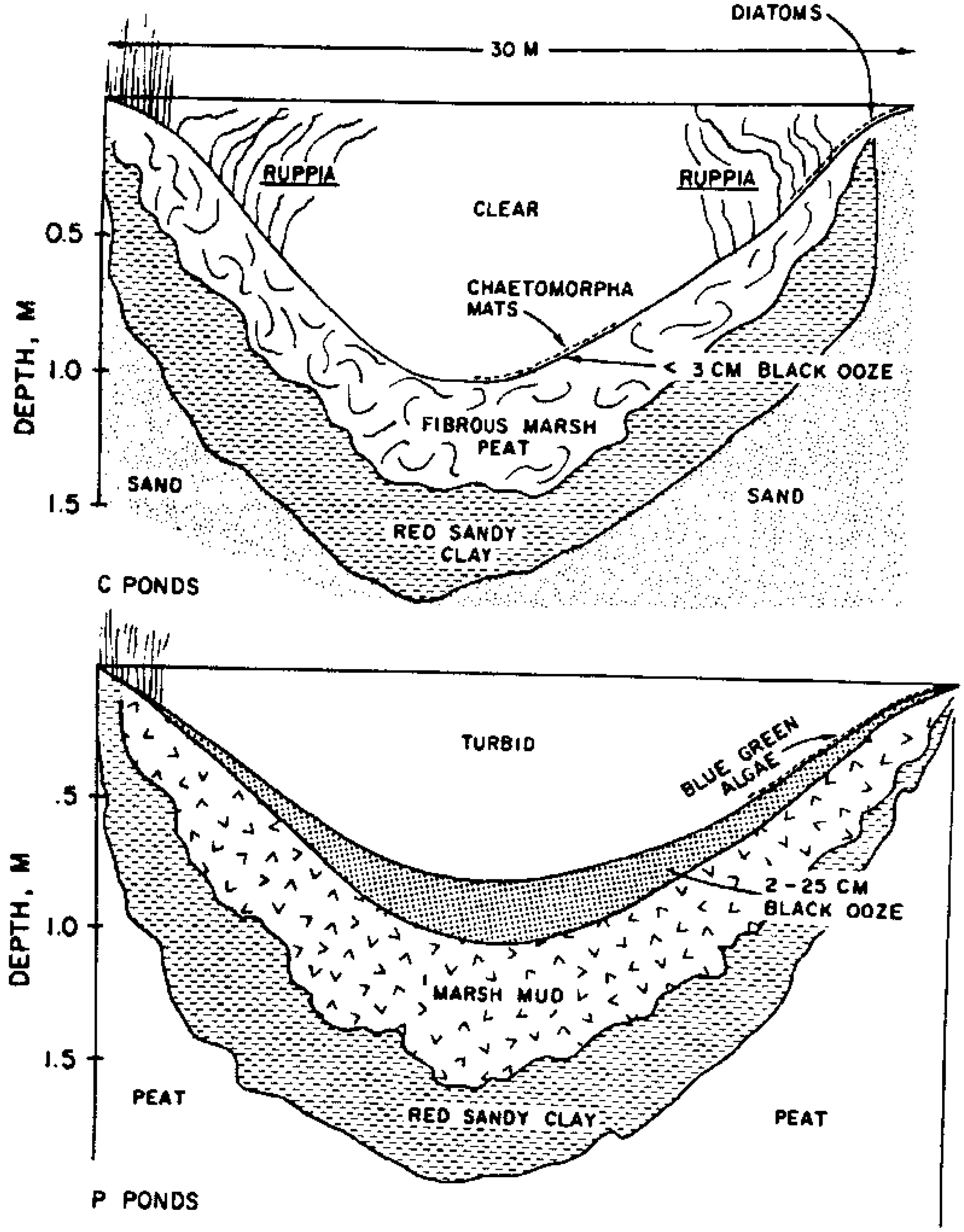
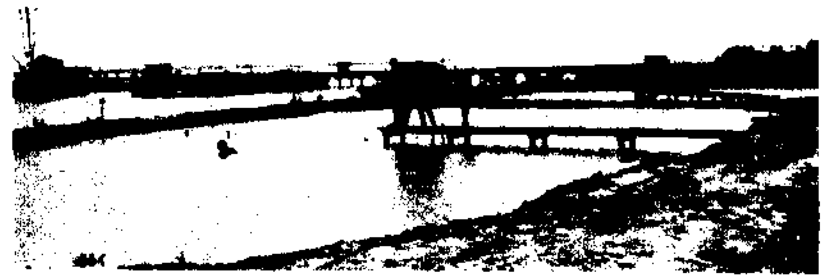


Figure 5. Cross sectional pattern of the ponds in July 1971.

a.



b.

c.

d.

Figure 6. Photographs of ponds at the time of filling in August 1968 (Canoy). (a) C-1 and C-2(foreground); (b) C-3, Bogue Sound behind; (c) P ponds facing west, P-1 in foreground; (d) P ponds facing east, P-3 in foreground.

a.

b.

Figure 7. Photographs of waste (P) ponds in August, 1972. (a) Pond P-3 with heavy Spartina along shore; (b) Pond P-2, sewage plant in background.

a.
b.
c.

Figure 8. Photographs of control (C) ponds in August, 1972. (a) Pond C-2 (foreground) showing Spartina patterns on shore and C-1 (background); (b) Pond C-2 with shore Spartina and Ruppia in water; (c) Pond C-2 with Ruppia and Pond C-1 in background.

Initial Filling, Acid Period

During construction in June 1968, black marsh mud was piled up at the control ponds and exposed to oxidation by air for several weeks. The smell of hydrogen sulfide was strong. After the mud was spread on the pond bottoms, estuarine water was introduced with a portable pump July 8. The waters were soon found to be acid, ph 3. The first of these ponds (C-1) received a mud slide and its standpipe was accidentally removed so that the acid water was immediately discharged. Ponds C-2 and C-3 remained acid until the continuous pumping system began October 31. Interesting phenomena during this period were studied. Differences observed later between C-1 and the other two control ponds may have resulted from these different treatments initially.

In the waste ponds, the marsh mud used for the floor was transferred without delay, and without much chance for oxidation, acid conditions did not develop. On Jan. 27,1969, 16 cm was added to the stand pipes of P ponds to make the two sets of ponds of comparable depth.

Water Flow and Salinity

Pumping of water into the ponds through mixing tanks began in October, 1968. The experimental plan was to mix estuarine waters with waste waters in the waste ponds and with tap water in the control ponds so as to maintain pond salinity in the mesohaline range, 15 to 25 ppt. The salinity of estuarine water available to the control ponds was steady so that inflows of estuarine water and tap water through the mixing tank maintained the desired salinity without much variation.

However, the estuarine water available to the waste ponds was from Calico Creek, a small estuary with wide range of salinity depending on rainfall. Consequently, in order to keep salinity in mesohaline range, pumping of treated sewage had to be varied. The hours of pumping into waste ponds were recorded; see Figure 9. Nutrient input was estimated by multiplying hours pumped times nutrient content of waste water and estuarine water. The estuarine water pumped into the waste ponds was fairly high in nutrients because the intake was only 60 m from the sewage plant outfall (Figure 1b).

Turnover time of the waterwas estimated from the flows divided by pond volumes and graphed in Figure 10.

The salinity resulting in both sets of ponds is given in Figure 11. The variation over a three-year period was not unlike that in many estuaries. Chronology of events affecting the ecosystems is included in Table 3.

A log book was kept of events and treatments involving the ponds. Important events include the following: Sewage plant failed for July 7-26,1969, and the intake pumps were turned off to avoid pumping raw sewage into the experimental ponds. Ponds were frozen,

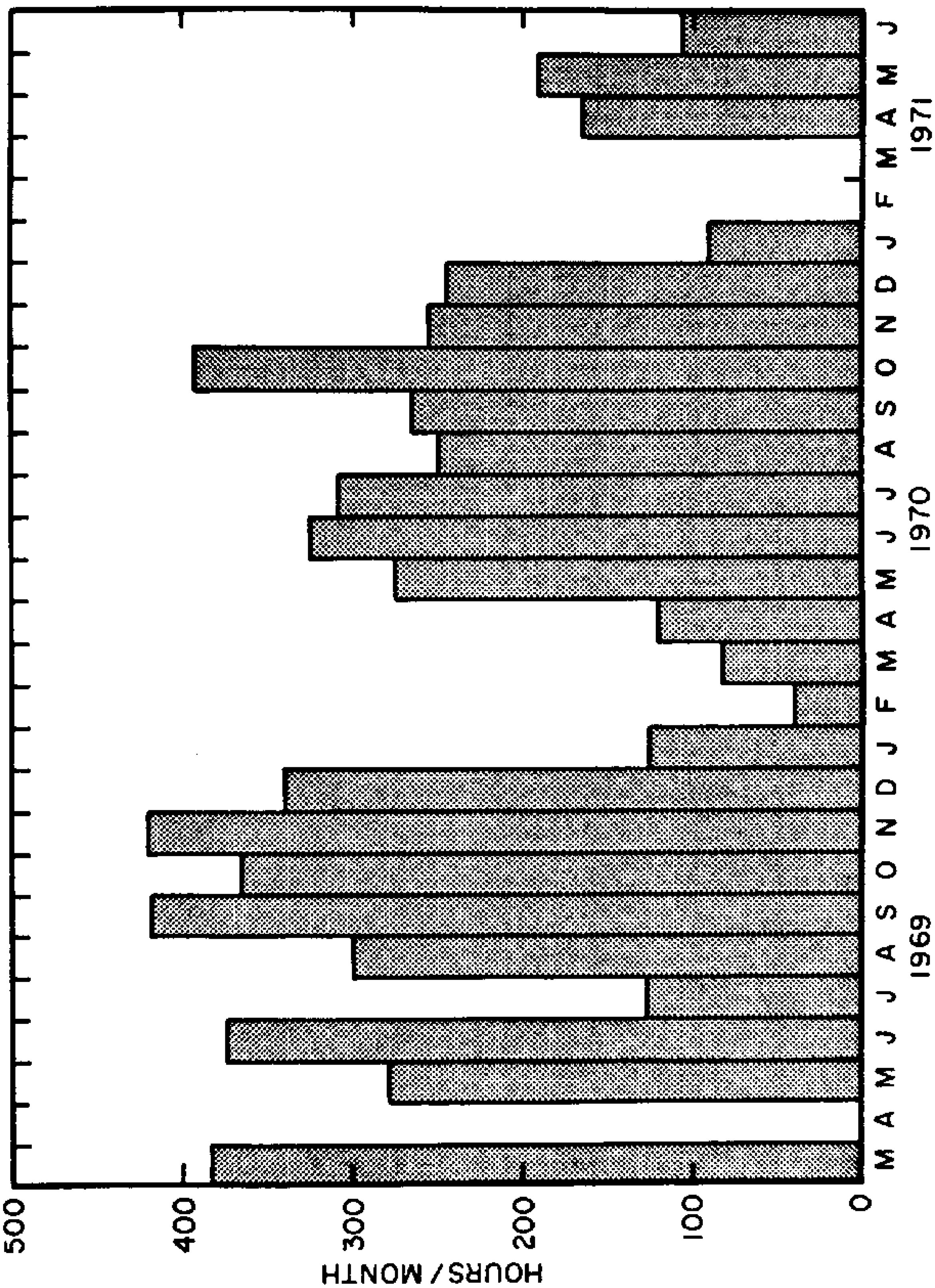


Figure 9. Hours per month of pumping wastewater into P ponds.

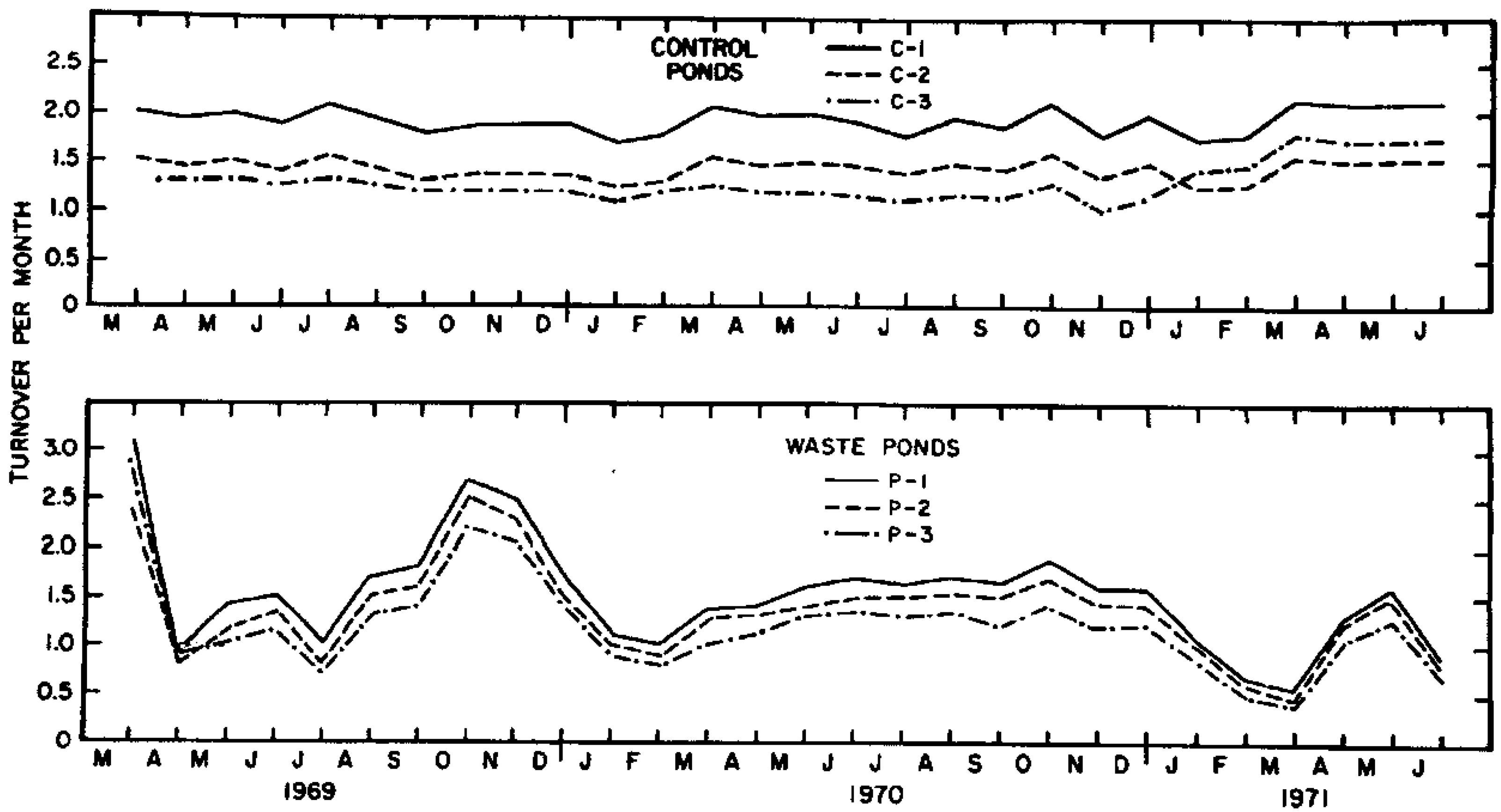


Figure 10. Record of turnover in ponds (inflow rate/pond volume).

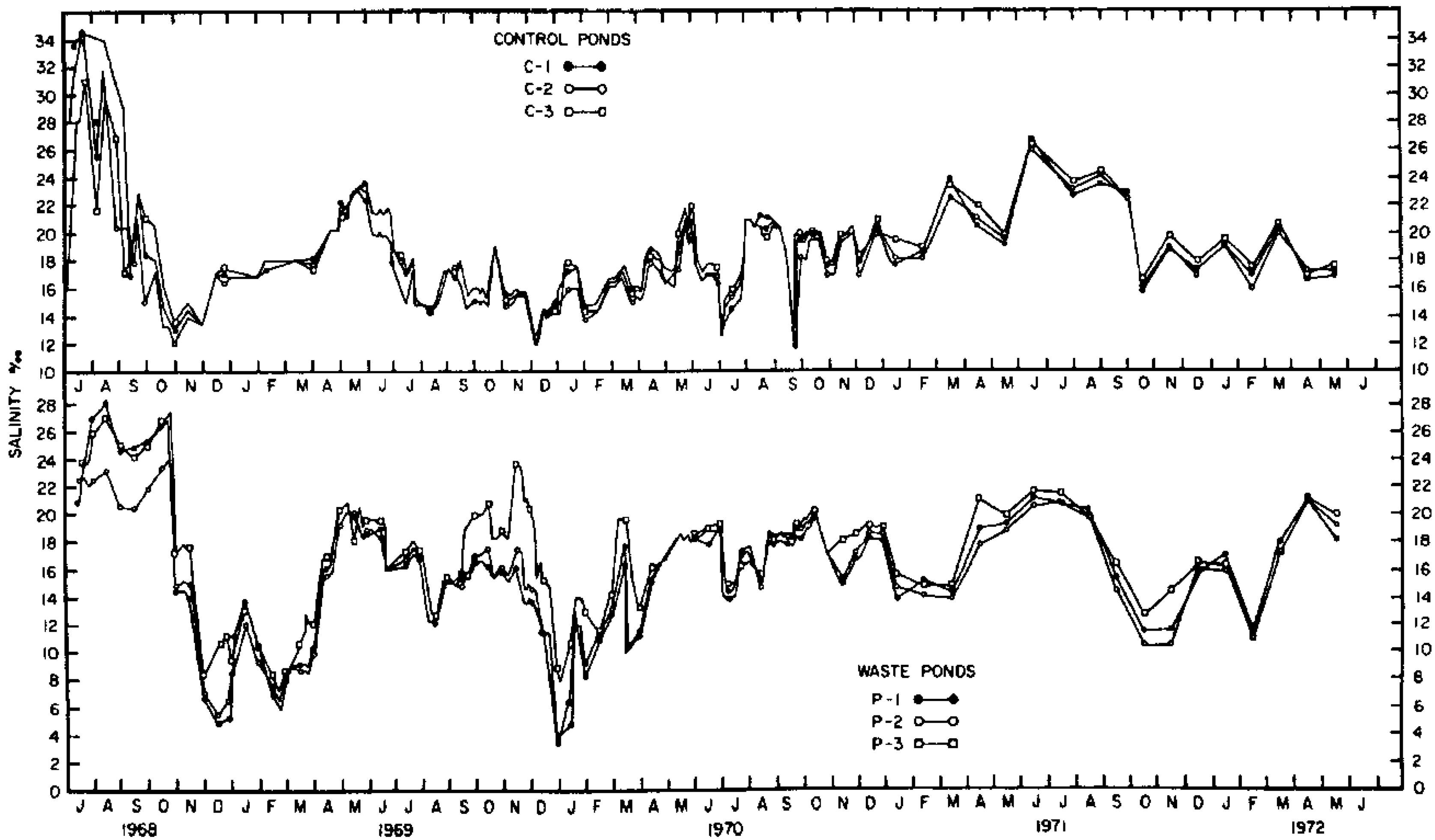


Figure 11. Record of salinity in ponds (W. Smith, 1968; Laughinghouse et al., 1971).

dead fish found and pumps out or turned off with hard freezes: Dec. 30, 1970, Jan. 13-26, 1970, Jan. 20-28, 1971, Feb 1-16, 1971. Pumps out for other reasons: Feb. 19-March 6, 1969, July 3-17, 1970, Aug. 19--Sept. 9, 1970. Pumps were out. Aug. 27-Sept. 7, 1971 and Sept 29-Oct. 4, 1971 with tropical storms.

After 1971 management changed and ponds were aerated and used to grow shrimp. Thus from Aug. 28, 1971 to March 26, 1972, only estuarine water without sewage was added to the waste ponds, but some waste water entered from the estuary, which was receiving sewage plant effluent. After 1972 salt water pumps were also stopped, and the waste ponds received only rain water and some salt water backflow when tides were exceptional.

Multiple Seeding of Species

The continuous self organization of estuarine ecosystems is facilitated by continuous seeding of species aided by tidal exchanges and migrations. In the ponds, some plankton species were found to enter through the pumps. Because Bogue Sound and Calico Creek are somewhat intermixed by tidal exchange in the course of a week, many of the same organisms were carried to the vicinity of the intake pipes of both sets of ponds. With exceptional storm tides, backflow introduced species through the standpipes, especially waste pond P-3.

To simulate nature and as an ecological engineering technique to minimize genetic limitations, multiple seeding of other species was carried out especially in spring of 1969 and 1970. Species were systematically introduced by adding estuarine waters, sediments, fouling communities, reef materials, plankton from plankton net tows, small fishes from seines, etc. Seeding efforts recorded in a log book are given in Table 3.

Some of the added species were measured for growth measurements, including Rangia clams, oysters, and shrimp. Similar quantities were added to each pond. During 1971-1972 seeding was primarily blue crabs, oysters, and shrimp.

Table 3. Pond Seeding

Date	Items and source
1968:	
July--Dec.	Backfilling through P pond stand pipes
July 8-17	Ponds filled with estuarine water
July 16,20; Sep. 10; Oct. 9	Macroplanktonic crustacea, Bogue Sound
July 16	Mats of Zostera and Ruppia planted
July 19	Grass shrimp, small fishes seined
July 26; Sep. 10	Zooplankton, net tows in Bogue Sound
Sep. 17	25 Rangia cuneata, Pamlico R.
Sep. 17	Polymesoda carolinensis, Pamlico R.
Oct. 31	Start of regular pumping into ponds
Oct. 31	Planting of Spartina and Distichlis
Nov. 8	Shells with Ulva, blue greens
Nov. 8	Pink shrimp, fishes from seining
Nov. 9,14	Oysters placed on shell reef
Dec. 3	21 Argopecten irradians, Boque Sound
1969:	
Jan. 9	100 Paleomonetes, Calico Creek
Jan. 14	Macrophytic algae
Jan. 25, March 3,4,10,31	Zooplankton tows, Bogue Sound
Apr. 1,2,4,14,15,16	Zooplankton tows, Calico Creek
Apr. 3,4,12,15;May 3;Jun 10	Spartina alterniflora, Calico Creek
May 20	Sections of bottom transplanted
July 18, Aug. 7	Clam-shell strings with oyster spat
Aug. 4	Baskets with year old oysters
Aug. 4	Shells with 6 weeks old oysters
Nov. 13	Storm backflooded P pond standpipes
1970:	
Mar. 13,14	Zooplankton net tows, Bogue Sound
Aug. 5	Juncus from Dill Creek
Sept. 29	C ponds seeded with lift pump
Oct. 1	P ponds seeded with lift pump
Oct. 14	Larvae seeded with lift pump*
Oct. 15	White, brown, pink shrimp, Bogue Sound
Oct. 15	Blue crabs
Oct. 15	P ponds seeded with lift pump
Oct. 28-Nov 2	Backflooding P-3 stand pipe
1971:	
Mar. 7	250 Mugil cephalus
Apr. 1	Menidia, Leisotomus, Lagodon

* Including: Bowmaniella dissimilis, Pseudodiaptomus coronatus, Hexapanopeus angustifrons, Alpheus heterochaelis, porcellanid zoeae, Mysidopsis bigelowi, Corophium, Sananiarids, Hexapanopeus augustifrons, Uca, brachyuran zoeae, Hippolyte pleuraranthe.

METHODS

Plan of Study

Using an administrative organization given in Table 1, main physical, chemical and biological features of the emerging ecosystem were monitored, including structures, functions, and species. Experiments were conducted to elucidate mechanisms and rates. Before Summer 1971, policy was against removing species or otherwise changing the ecosystem. Quarterly discussions of new data were held and an ecosystem model developed to summarize the facts and concepts of how the ecosystems were operating. Nutrient-rich waste ponds and control ponds were compared and the role of high nutrients studied. Features observed in ponds were compared with waste-receiving estuaries elsewhere. Most of the work considered phenomena at two levels, one involving components and the other the role of the process in affecting the larger ecosystem. Aquaculture possiblities were considered. In July 1971, after 3 years of self organization, the ponds were inventoried, including the seining out of all the fishes. Details of the many methods that were used follow:

Procedures

The following are the various chemical and biological procedures used to monitor the changes taking place in the ponds during self organization. Following project policy to minimize measurement effects on the ecosystems self organization, non-destructive measurements were used until the time a general inventory of larger plants and animals was made in all ponds in July 1971. The descriptions of methods given below are brief; more details may be found in the progress reports (Odum and Chestnut, 1970; Kuenzler and Chestnut, 1971, and Kuenzler, Chestnut, and Weiss, 1973).

Water Turnover

Flows reaching ponds were calibrated by catching water delivered per hour of pumping. Flows into control ponds were continuous. A strip chart recorded times of pumping into waste ponds. Turnover times were calculated as the ratio of the volumes of the ponds to the inflows.

Salinity

Salinity measurements were made with a Beckman RS-5 induction salinometer from Industrial Instruments or with a Goldberg refractometer of American Optical Co. (Behrens, 1965).

Solar Insolation

Light was recorded by an integrating photometer (Mark IV Sol-a-Meter) from DTI Division of Talley Industries, Inc. The ampere-hours accumulated were read daily and multiplied by factory calibration (177 gram calories per cm^2 per ampere-hour).

Evaporation

Evaporation was measured by measuring water loss from wide mouth jars placed within the ponds.

Temperature

Temperature was recorded with Model 80 Rustrak thermistor recorder, model 54 Yellow Springs Instrument Co. recorder, and a Rustrak Model 2133 temperature recorder. Temperatures were also measured with glass thermometer when Winkler Oxygen measurements were taken.

Dissolved Oxygen

Oxygen measurements were made with the Winkler method when diurnal studies were made on all six ponds. Continuous records on one pond at a time were made with a membrane electrode of a Yellow Springs Instrument Co. Model 54 Oxygen indicator receiving flowing water from a submersed pump.

A computer program in Pl/1 language by C. Hall (1970) was used to plot graphs of oxygen, percent saturation, temperature, and rate of oxygen change. Rate of exchange with atmosphere was found to be small and not affecting calculations of metabolic rate appreciably.

pH

pH was recorded with Fisher Accumet 210 pH meter operating with long leads to electrodes at end of the pier and Analytics Instrument Co. Model R-4 Meters were calibrated with pH 8 buffer weekly.

Carbon-dioxide Exchange

Diffusion of carbon dioxide across the air-water interface was measured by monitoring the air stream through a floating plastic dome with a Beckman model 215A infrared gas analyzer (Day,1971; Hall, Day and Odum,1971; Day,1983).

Carbonate Alkalinity

Carbonate alkalinity was determined by acid titration using a pH meter.

Total Inorganic Carbon

Total inorganic carbon was calculated from field pH determination and carbonate alkalinity using equations (Fair, Geyer, and Okun, 1966). See Day (1983).

Total Carbon

Total carbon was obtained with a Beckman total carbon analyzer model 915. A 40 ul sample injected into a CO2 free air stream was passed through a high temperature furnace and read as inorganic carbon dioxide by infrared analyzer. Where necessary samples were preserved with Mercuric chloride. Pond inflows were sampled over the time of pumping with a peristaltic pump.

Total Organic Carbon

Total organic carbon was calculated as the difference between total carbon and total inorganic carbon.

Particulate Carbon Outflow

Coarse particulate carbon fragments leaving the ponds through standpipe were collected with net, dried at 95 C and weighed.

Heterotrophic Uptake of Glucose

Using micropipette 10 to 200 ug/l of radioactive glucose was added to 10 ml duplicates of freshly collected water containing pond flora. A control pair received 0.3 ml of 2N sulfuric acid to drive out inorganic carbon. After flasks were incubated 1/4 to 1 hour they were sealed with cups containing filter paper suspended inside above the liquid surface. Then syringes were used through the cap to add 0.2 ml phenethylamine to absorb released carbon dioxide and 0.3 ml 2N sulfuric acid to drive carbon dioxide from solution. Filter papers with impregnated phenethylamine were placed in vials with scintillation fluid (4 g PPO and 50 mg dimethyl POPOP in purified toluene filled to 1 liter) and counted with liquid scintillation counter C 14 toluene as an internal standard. Rates of uptake and turnover times were analyzed graphically (Wright and Hobbie, 1966).

Uptake of glucose by sedimentary bacteria was measured after surface sediment ooze was suspended and diluted 100 to 1 in artificial sea water.

Nutrient Analyses

Various fractions of nitrogen and phosphorus were analyzed monthly and in special studies of diurnal change. See below. Later nitrogen analyses were analyzed with Technicon autoanalyzer procedures (FWPCA,1969).

Particulate Matter

Particulate matter was obtained from two liter samples of raw water passed through plankton centrifuge and through weighed and ashed fiberglass filters. Samples were dried at 85 C and ashed at 600 C.

Filtered Water

Water for chemical studies was filtered through Millipore filters, 0.45 u or 0.8 u.

Preservation of Water Samples

Where analyses were to be delayed, 4 mg mercuric chloride was used as a preservative and/or samples were frozen.

Phosphate Phosphorus

For monthly analyses filtered waters were analyzed with a method of Greenfield and Kalber (1954) modified by Woods (1965). In other measurements methods of Murphy and Riley (1962) were used.

Dissolved Organic Phosphorus

For monthly analyses filtered waters were digested with percholoric acid and determined as inorganic phosphate phosphorus (Hansen and Robinson, 1953). In other analyses filtered waters were autoclaved for 1 hour at 15 psi with 5 ml 5% K2S208 in 5% H2S04 (Menzel and Corwin (1965) and determined as inorganic phosphate.

Total Phosphorus

Raw waters were digested with perchloric acid and determined as inorganic phosphate (Hansen and Robinson).

Particulate Phosphate

Particulate matter collected on membrane filtered was autoclaved with persulfate and determined as inorganic phosphate.

Phosphate Flux with Radiotracer

100 ml samples were labeled with 0.05 ml radioactive ortho-phosphate 20-4 microcuries/m and incubated in the ponds. Six ml samples taken at 2,4,8,15,30, etc. minutes were filtered through 0.8 u membrane filters;, dried on a planchet and counted in a gas flow proporitonal detector. Duplicate samples were processed without filtering to determine solution radioactivity. Gas flow counting was compared and calibrated with scintillation counting. For details on methods see Kuenzler et al. (1970). Turnover times of dissolved organic phosphorus were calculated with method of Watt and Hayes (1963).

Nitrite Nitrogen

Filtered water was analyzed with method of Rider and Mellon (1946). In later autoanalyzer analyses, acidic sulfanilamide and N-1 naphthethylenediamine dihydrochloride was used to form azo dye and color read at 520 nm (FWPCA, 1969).

Nitrate Nitrogen

Filtered water was analyzed by the method of Mullin and Riley (1955). In later autoanalyzer analyses, samples were reduced with cadmium or hydrazine and processed for nitrite, subtracting out nitrite estimates (FWPCA,1969).

Ammonia Nitrogen

Filtered water was distilled and determination made colorimetrically (Riley, 1953). In later studies, procedure of Solorzano (1969) was used. In autoanalyzer analyses samples were reacted with alkaline phenol and sodium hypochlorite to form indophenol blue, intensified with sodium nitroprusside, and read colorimetrically at 630 nm (FWPCA, 1969).

Dissolved Organic Nitrogen

After micro-Kjeldahl digestion with selenium catalyst filtered waters were determined as ammonia. For autoanalyzer analyses filtered waters were processed as for total nitrogen (FWPCA, 1969).

Total Nitrogen

After micro-Kjeldahl digestion with selenium catalyst, raw waters were determined as ammonia. In autoanalyzer analyses samples were digested with acid at 360 C and processed as ammonia analyses (FWPCA, 1969)

Nitrogen Fixation

Nitrogen fixation was measured with acetylene reduction technique (Stewart et al., 1967) on 2 ml pond water or 2 ml of pond sediment, incubated for two hours. Reaction was stopped with Trichloroacetic acid and ethylene production measured with Varian Aerograph model 600D gas chromatograph, Sargent model 250T recorder, H-flame ionization detector and 2.7 m column packed with Porapak R, 50-80 mesh. Carrier gas was high purity nitrogen flowing a 25 cc/m.

Diffusion of Ammonia into Air

Diffusion of ammonia from pond was measured by measuring ammonia entering a floating plastic dome over 3, 12, and 24 hour periods. Ammonia entering the closed circulating air stream was collected in HCL and analyzed for ammonia with method of Solorzano (1969).

Secchi Disc

Depth of visibility of a 20 cm black and white disc was measured from the end of the pier at mid-day.

Photosynthesis and Respiration from Oxygen Measurements in Free Water

Diurnal sampling and analysis of oxygen in free water were converted to estimates of daily photosynthesis and respiration of the ecosystem (Odum and Hoskin, 1958; Odum and Wilson, 1962; and Copeland and Dorris, 1962). After diffusion exchange with air was found to be negligable, starting in April, 1970, triplicate oxygen measurements were taken in each pond at time of minimum at dawn and maximum around 4:30 p.m. (locations; shore, mid-pier, and end or pier). Metabolic rates were estimated from the difference between daily maximum and minimum. A study of vertical oxygen distribution was also made.

Photosynthesis and Respiration Measurements from Carbon Measurements in Free Water

Changes in total inorganic carbon during day and night were used to estimate daily ecosystem photosynthesis and respiration (Day 1971,1983). Corrections were made for carbon dioxide exchange.

Carbon dioxide Exchange Across the Air-water Surface

Carbon dioxide exchange was measured with a floating plastic dome (40.5 cm diameter) through which an air stream was passed and monitored with a Beckman 215A infrared carbon dioxide analyzer (Day et al., 1970; Hall et al., 1975; Day, 1971, 1983).

Photosynthesis and Respiration in Light and Dark Bottles

Changes in oxygen were measured in light and dark bottles suspended for 3 hours and 24 hours.

Photosynthesis and Respiration in Light and Dark Bell Jars

Change of oxygen in bottomless carbuoys was measured with a circulating pump apparatus that assured motion. Winkler analyses were made on samples drawn with hypodermic needles through rubber caps (Fromm, 1958).

Sedimentary Metabolism in Core Tubes

Relatively undisturbed bottom water and sediment were collected by pushing plastic coring tubes, (4.5 cm inside diameter) into the bottom of the pond. Metabolism of the sediment surface was estimated from changes in oxygen in the tube water above the sediment using oxygen electrodes.

Photosynthesis with Radiocarbon Methods

Uptake of radiocarbon labeled bicarbonate was measured in bottles containing pond water, bottom ooze, and glass slides with periphyton that had developed over a 5 week suspension in the ponds. After incubation for four hours at four depths bottle waters were filtered and counted in a gas flow counter.

Phytoplankton

Phytoplankton were monitored from samples 10 cm deep from ends of piers. Cells were concentrated in clinical centrifuge at full speed for 10 minutes. Cells were resuspended and examined live with a Unitron phase-contrast inverted microscope (Utermohl, 1931). Another 2-18 ml sample was counted at 400X after 2-10 hrs in a settling chamber with 1 drop iodine-potassium iodide solution (10 g

KI, 5g Na acetate, 70 ml distilled water).

After the first 1.5 years integrated samples were obtained combining pond water collected at 0.1,0.4, and 0.7 m and examined within 5 hours.

Plant Pigments

With good agreement procedures of Strickland and Parsons (1965) were used for chlrophylls and carotenoids, and procedures of Lorenzen (1967) for chlorophyll-a and pheo-pigments.

Chlorophyll Development in Flasks with Nutrient Omission

In 1968 nutrients (NO3, PO4, Si, Cd, Mo, Fe, Mn, Zn, Cu, vitamin B12, Thiamine, and Biotin) were added singly or omitted individually from flasks enriched with all the others and incubated at 120 foot candles at 25 C. Chlorophyll was determined after 1-3 days and compared to controls with no nutrients.

Bottom Plants

Ruppia was mapped in 1970 and in 1971 when as much as possible was pulled out and weighed wet. Photographs were made in summer 1972 of its regrowth.

Algal components of the bottom ooze were estimated by multiplying the loss on ignition at 500 C by the percent of organic detritus estimated in microscopic examination as due to algae. Large bottom mats of Chaetomorpha in the control ponds were removed by hand in 1971 and weighed wet.

Marsh Grass

Plots of Spartina alterniflora, Spartina patens, and Distichlis spicata were planted on shore of all ponds in spring 1969 and Juncus roemeriana in August. Areas of new growth were mapped each year, and some height and weight measurements made in 1970.

Zooplankton

Regular bimonthly samples of zooplankton were collected 1968-70 by towing a plankton net (30.48 cm diameter with mesh size 0.24 mm) by hand along the piers for a measured distance and calculating the volume of water filtered. All larger animals were counted; smaller animals were counted from aliquots. A #10 plankton net was used to estimate plankton passing in through intake pipe. Crab zooeae studied July 9,1968, were collected with a #2 plankton net. During the 1971 summer inventory, a Clarke-Bumpus sampler was used with #2 and #10 nets.

Small Bottom Animals

Triplicate cores of a 10 cm2 cross sectional area were taken monthly near ends of piers in Ponds C-2 and P-2. Meiofauna were extracted by sieving through .64 um mesh, flotation with magnesium sulfate, and centrifugation through sea water-glycerine interface (Teal,1960). Animals were stained with rose bengal. In the 1971 pond inventory the upper 2.5 cm of ooze was sieved with 250 u mesh, and number and diversity of animals determined. Biomass was estimated from animal volumes.

Foraminifera

Forams were sampled from the top 2 cm of pond ooze with a plastic coring tube 5 cm in diameter, preserved in isopropyl alcohol, and stained with rose bengal. After being washed over a 62 u sieve, samples were counted and biomass estimated from volumes (Murray,1968). Electron micrographs were made of representative individuals (LeFurgey, 1972).

Encrusting Animals

Two fouling plates (30 cm by 25 cm by 4 cm made of molluscan shells cast in concrete) were placed in each pond Sept. 17, 1968, and later examined for growth of barnacles, oysters, and bryozoa. Barnacles on surfaces of treated wood pier piles were also counted.

Wood Borers

Wood boring isopods, Limnoria, were introduced in 1970 and studied on creosote treated and untreated wood surfaces, concrete blocks, and paired plexiglass plates held together with brass bolts (Graham and Gay, 1945) and located 40cm, 80cm, and 120 cm from the bottom. Untreated pine stakes (4 x 4 x 50 cm) were submerged. The initially infected wood blocks used to introduce Limnoria and the pine stakes were cut off at intervals to examine for borers.

Concrete Block Micro-reefs

Reefs of concrete blocks were arranged in ponds Sept. 17, 1968, (Figure 2) and associated animals sampled later by working a seine (0.6 cm mesh) underneath and lifting out blocks and animals.

Afterwards the spot was seined with 0.8 cm mesh net. Crabs were marked by clipping anterolateral teeth and returned along with reconstituted reef. Selected crabs were dried to constant weight at 80 C. to estimate biomass from counts.

Shell Reef

Parallel to the piers (Figure 2) a long bar of scallop shells was arranged 30 cm high, 60 cm wide and 3-4 m long. Oyster shell carrying oyster spat and other organisms from Calico Creek was layered on top in November, 1968 and inventoried in 1971.

Submerged Screens Covered with Shells

Screens 49 by 59.5 cm with 15 mm metal mesh overlaid with 1 mm mesh plastic screen were fitted with handles for lifting, covered with shells, and submerged. Screens were abruptly lifted after week intervals to sample attracted animals particularly xanthid crabs and snapping shrimp.

Hydrophone

A hydrophone was used in August to verify locations of snapping shrimp and to listen for fishes.

Shellfish Growth

After intitial stocking of 225 notched Rangia clams (staked plot 3m by 3m, 0.4m deep; Oct. 17,1968) and oysters added on strings and in baskets, shellfish were measured and weighed and returned to ponds facilitating estimation of growth rates and mortality rates. Samples were ground and dried to constant weight at 85 C.

Glycogen in oysters was determined with diphenylamine and anthrone colorimetric methods after alkaline digestion of oyster meats in duplicate and triplicate (Durham,1959).

Blue Crabs

Blue crabs were estimated with mark and recapture methods (Robson and Regier, 1968). Crabs were caught with chicken wire crab pots, marked with Nesbit-Fiedler tags (Cronin,1949) or india ink, released and resampled after 3 weeks and in one day tests of methods. Biomass was estimated as the product of population estimate and mean weight of samples.

Grass Shrimp

Palaeomonetes were seined at night (3mm stretched mesh) weighed and most returned to ponds.

Penaeid Shrimp

Commerical shrimp species, Penaeus aztecus (brown shrimp) and P. duorarum (pink shrimp) were captured at night with seine adequate to trap animals at one end of each pond (1.5 m by 18 m; 1.3 cm stretched mesh). In 1972 measured Penaeus setiferus (white shrimp) were introduced and grown for 70 days with aeration to reduce mortality (Rickards and Williams, 1973).

Fishes

A mark-and-release study of fishes in 1969 used six unbaited, hardware cloth traps (cylinders, 75 cm by 40 cm with conical entrance 7.5 cm in diameter). All fish were caught when ponds were systematically seined out in July 1971. Species diversity was determined as species found per 1000 individuals collected.

Fiddler Crabs

Crab burrows (holes greater than 5 mm) were counted in a shore zone one meter wide. Uca larvae were surveyed in the plankton after the filling of the ponds in summer 1968.

Microarthropods in Marsh Grass

Micro-arthropods in marsh grass were sampled with sweep nets and vacuum suction; biomass and diversity were estimated.

Water Birds

A count of water birds per area of observation in ponds was made with two surveys. Also, those making regular dawn oxygen measurements recorded birds.

Clay Minerals in Bottom Sediment

Sediments were collected with a 5 cm diameter plastic tube and samples from water depths, 0.2m, 0.5 m and 1.0 m. After drying at 40 C, carbonates were removed with dilute HCl and heat, soluble salts were removed by washing with deionized water and centrifuging, and the clay size fraction was separated (Ingram,1970). In preparation for x-ray diffraction, clays were treated with magnesium acetate, magnesium acetate plus ethylene glycol, and postassium acetate plus heat. Powder was prepared by passing samples through 325 u sieve, placed in an aluminum and glass holder and x-rayed 4o 20 to 35o 20 with a Norelco-Phillips Diffractometer using copper K-alpha radiation. Indentification of lines was made with the ASTM index to inorganic compounds (Ingram,1970)

Kaolinite was identified by the 7A 001 reflection, constant through chemical treatments, disappearing on heat treating the K-treated sample to 500 C. Illite was identified by unchanged 001 reflection at 10A. The 060 reflection at 1.498A classifies it as dioctahedral, and equal intensities of 001 and 002 reflections indicate high aluminum content with little iron substitution (Grim,1968). A 14A hydroxy-interlayer mineral had basal reflection at 14A which changed after treatment with K plus heat, broadening at 50 C to 13-14A and shifting to 10A between 250 and 300 C.

Percentages of clay minerals present were estimated from the area under the x-ray peak multiplied by correction factors for layer structure (Freas, 1962).

Bacterial Counts with Fluorescent Microscope

Direct microscopic examination was made of specimens collected in sterile flasks 40 cm below the surface, and stored at 0 to 4 C. 0.5 ml sample was combined with 10 ml sterile diluting water and one ml acridine orange stain (100 mg acridine orange stain, 500 mg NaHCO3m 500 mg NaCl in 100 ml distilled water and filtered with 0.22 u membrane filter). Live cells to be counted were interpreted as fluorescing green in an epiflorescence microscope. Direct counts were also made by adding acridine orange stain to pond water which was then filtered on a black 0.45 u membrane filter, air- dried for a minute, mounted in immersion oil and coverslip and counted.

Low Nutrient Plate Counts of Bacteria

Similar samples were serially diluted with previously filtered sterile water from the ponds and plated out with habitat-simulating agar (1000 ml filtered water from each pond, 100 mg Difco yeast extract, 14 g agar and autoclaved) and incubated at 22 C. Colonies were counted at 21 days and diversity evaluated at 4 months. To improve growth another medium was used: 1000 mg yeast extract, 100 mg bacto-peptone,100 mg glucose, one liter of pooled, filtered pond water, and 10 ml vitamin extract (vitamin supplement formula in mg/liter: biotin, 2; folic acid, 2; thiamin HCl, 5; D Ca pantothenate, 5; B 12, 0.1; riboflavin, 5; nicotinic acid or nicotinamide, 5; pyridoxine HCl, 10; PABA, 5).

Coliform Bacteria

Standard methods for determination of sewage bacteria were used to plate out and verify coliform counts (U.S. Public Health Service).

Counts were also made of oysters collected from Calico Creek, where coliforms were high, transferred to experimental ponds for two weeks prior to coliform analysis and transferred to control ponds to determine the time for clearing coliforms.

Fungi

Water samples, sediments, and plant materials were collected and examined for fungi during summer 1969.

Part of the collections were baited by adding them to fungal substrates in petri dishes with 30 ml sterile water. Baits (sterile) included: hemp seed, boiled grass, microspathula of sweet gum pollen, human skin, snake exoskeleton, shrimp, human hair, onion cataphil, and cucumber seed.

Another part of the collections were plated (Vishniac, 1956) with antibiotic isolation medium (Fuller et al., 1964). The medium included the following autoclaved together: agar,12g; glucose, 1 g; gelatin hydrolysate (NBCo), 1 g; liver extract (NBCo, 1:20), 0.01 g; yeast extract, 0.1g; sea water, 1000 ml and after sterilization while hot, streptomycin sulfate USP (NB Co), 0.5 g; and Penicillin "G" USP Sodium (NB Co), 0.5 g. Another medium used was yeast extract-glucose-salt medium.

Where needed fungal slides were made using acid fuchsin or lactophenol with cotton blue.

Index of Species Diversity

Counts of diversity were made either as information in bits per individual (H) using the Shannon-Weaver formula or as cumulative species found in counting 1000 individuals. Where less than 1000 individuals were present or more than 1000 were counted, the index was derived from the observed line on semi-log plot at the intersection with the 1000 individual line.

RESULTS

The results of measuring parameters and processes during the ecosystem self organization are given in Figures and Tables that follow. Discussion is included here on the ecosystem components as considered separately.

Water Turnover, Salinity and Evaporation

Once pumping started, salinity (Figure 11) varied with that of waters being introduced, 13 to 26 ppt in control ponds and 5 to 25 in waste ponds. In the case of the waste ponds salinity was also affected by varying pumping rates (Figure 9). Resulting times of turnover of the ponds (Figure 10) were 2 to 3 weeks in control ponds, each one steady but consistently a little different, but varying from 1.6 to 8 weeks in waste ponds. Evaporation measurements (Figure 12) ranged 3 to 11 mm/day. Evaporation was high in summer when solar insolation and air mass saturation deficit was large. For these ponds averaging about 0.5m deep turnover due to evaporation ranges from 40% to 154% per week (Figure 4). When evaporation was high in summer most of the inflowing water evaporated.

Insolation

Insolation (Figures 13 and 14) had a twofold seasonal variation but the day to day range due to extended periods of heavy clouds was 10 fold. Some days in winter had less than 20 langleys.

Secchi Disk and Turbidity

Water clarity is indicated by Secchi disc measuremenmts in Figure 15. In the control ponds clarity was greater in winter and increased in summer with growth of phytoplankton populations. Pond C1 was most turbid with plant production by phytoplankton. By the third summer clarity was greatest in ponds C-2 and C-3 where bottom plants (Ruppia) predominated.

In the waste ponds where plankton were more concentrated than in control ponds, turbidities were highest especially in winter with the Monodus bloom. Waste ponds cleared in May each year with a crash of the Monodus and replacement with diverse summer phytoplankton.

Temperature

Temperatures (Figure 16) ranged from 0 C to 35 C with a 2 to 10 degree C diurnal range caused by the balance of insolation, back radiation, conduction and evaporation. Temperatures changed rapidly from day to day due to heating and cooling by the flows of different air masses changing with storm regimes. Freezing caused fish mortality several times, and once the ponds froze over (Jan. 9, 1970). Other temperature data are included with oxygen graphs and other data (see index).

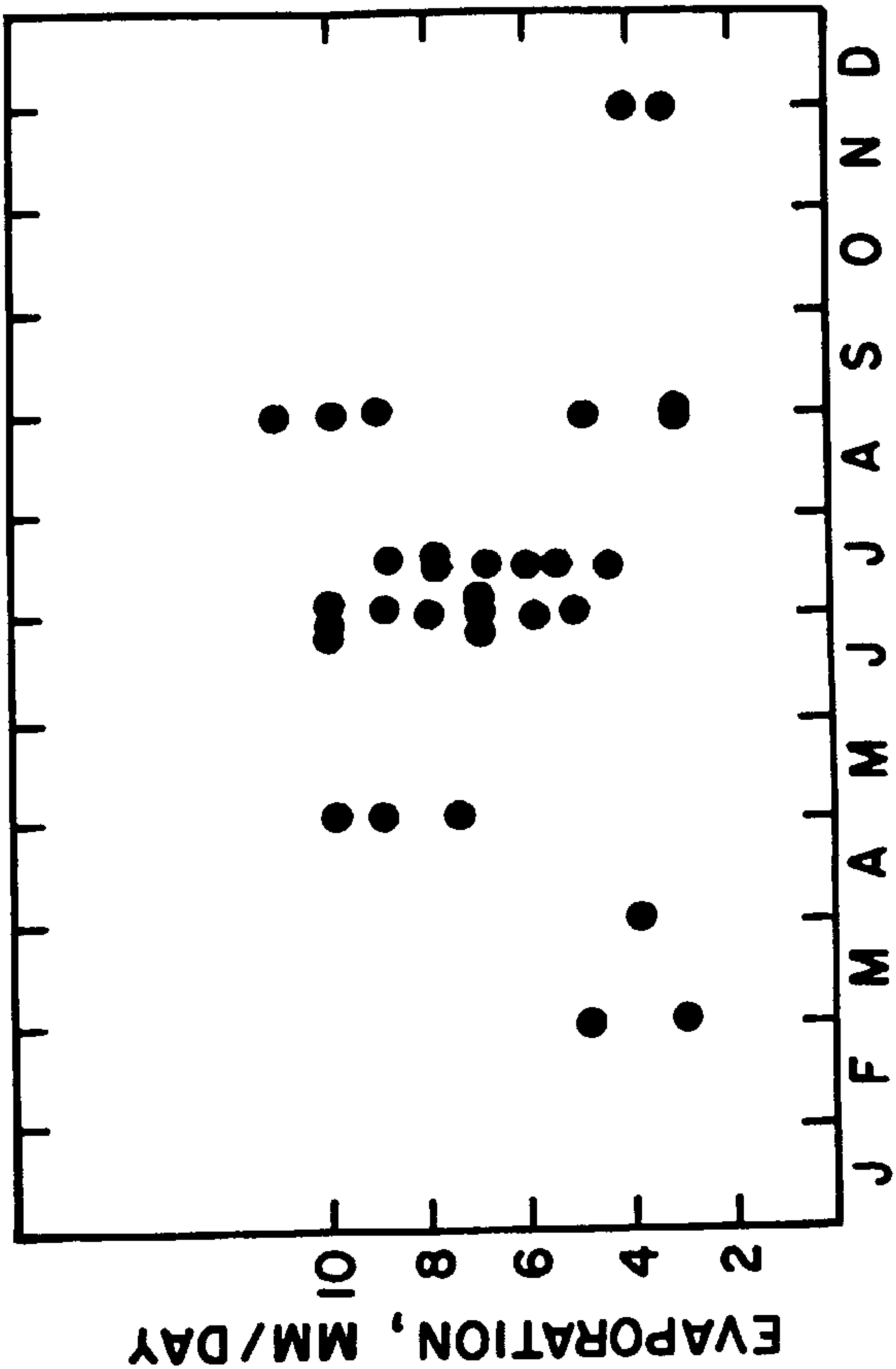


Figure 12. Evaporation rates during 1969-70.

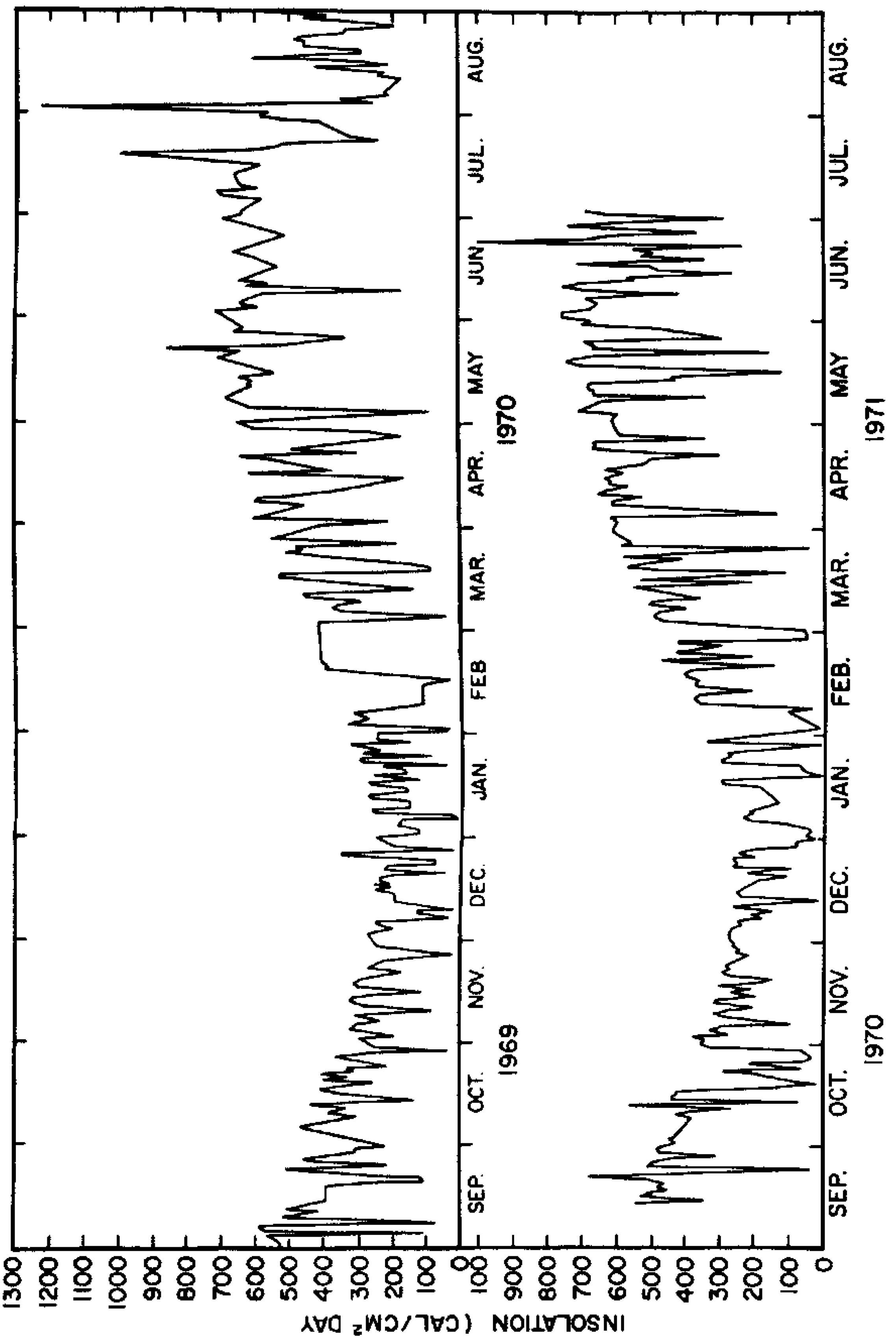


Figure 13. Daily values of solar insolation.

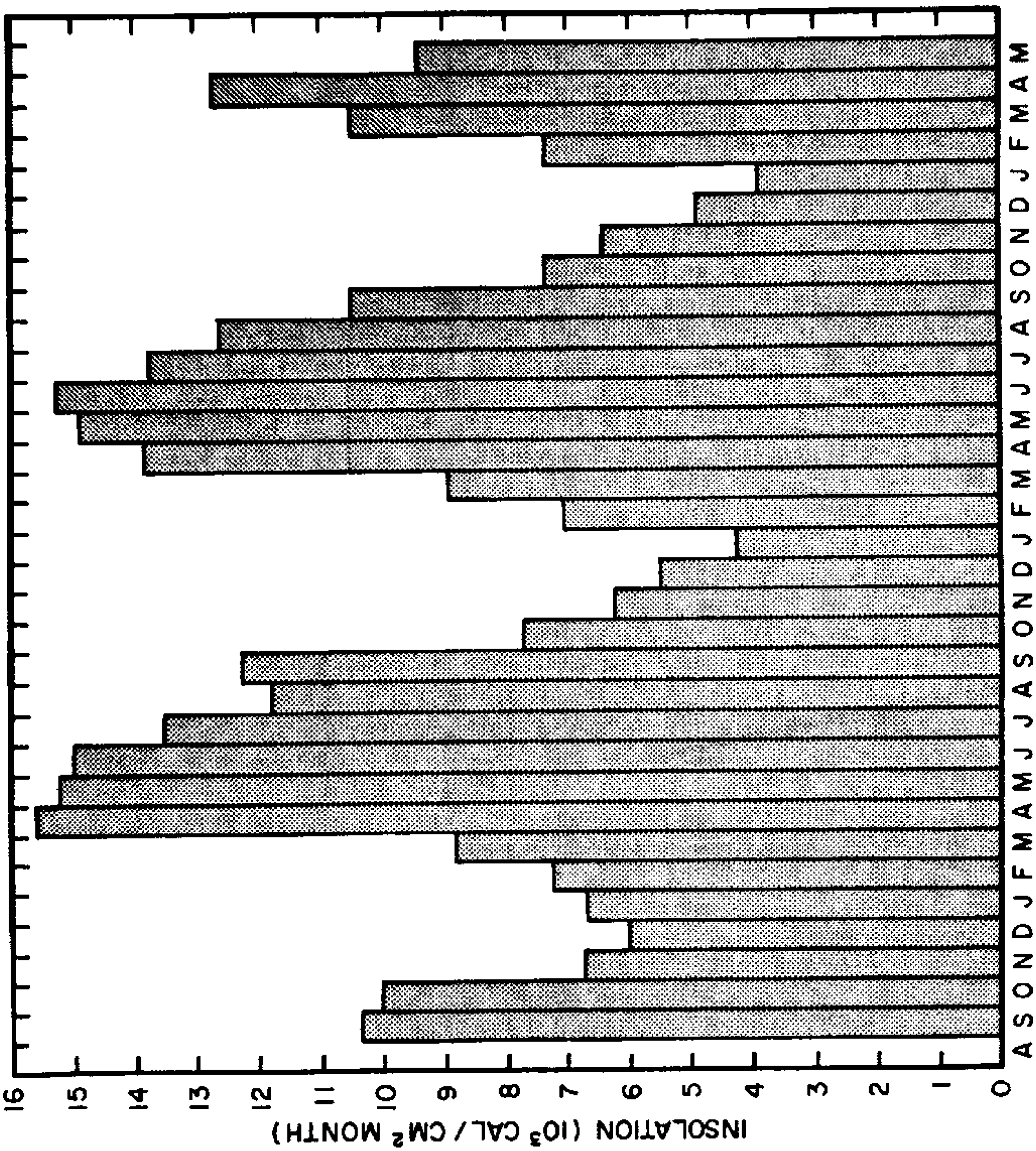


Figure 14. Monthly means of solar insolation.

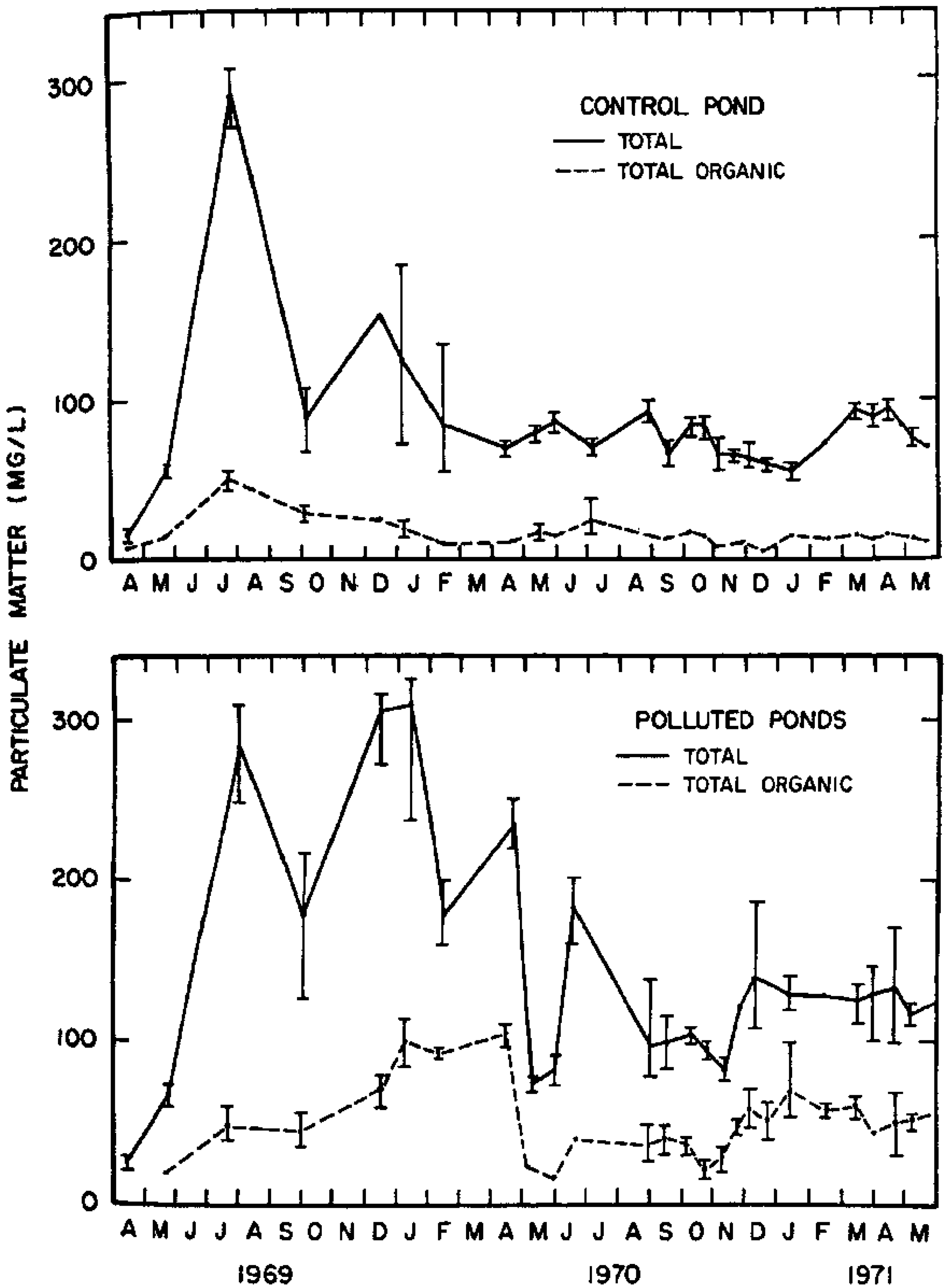


Figure 15a. Record of pond clarity. Particulate matter, 1969-1971

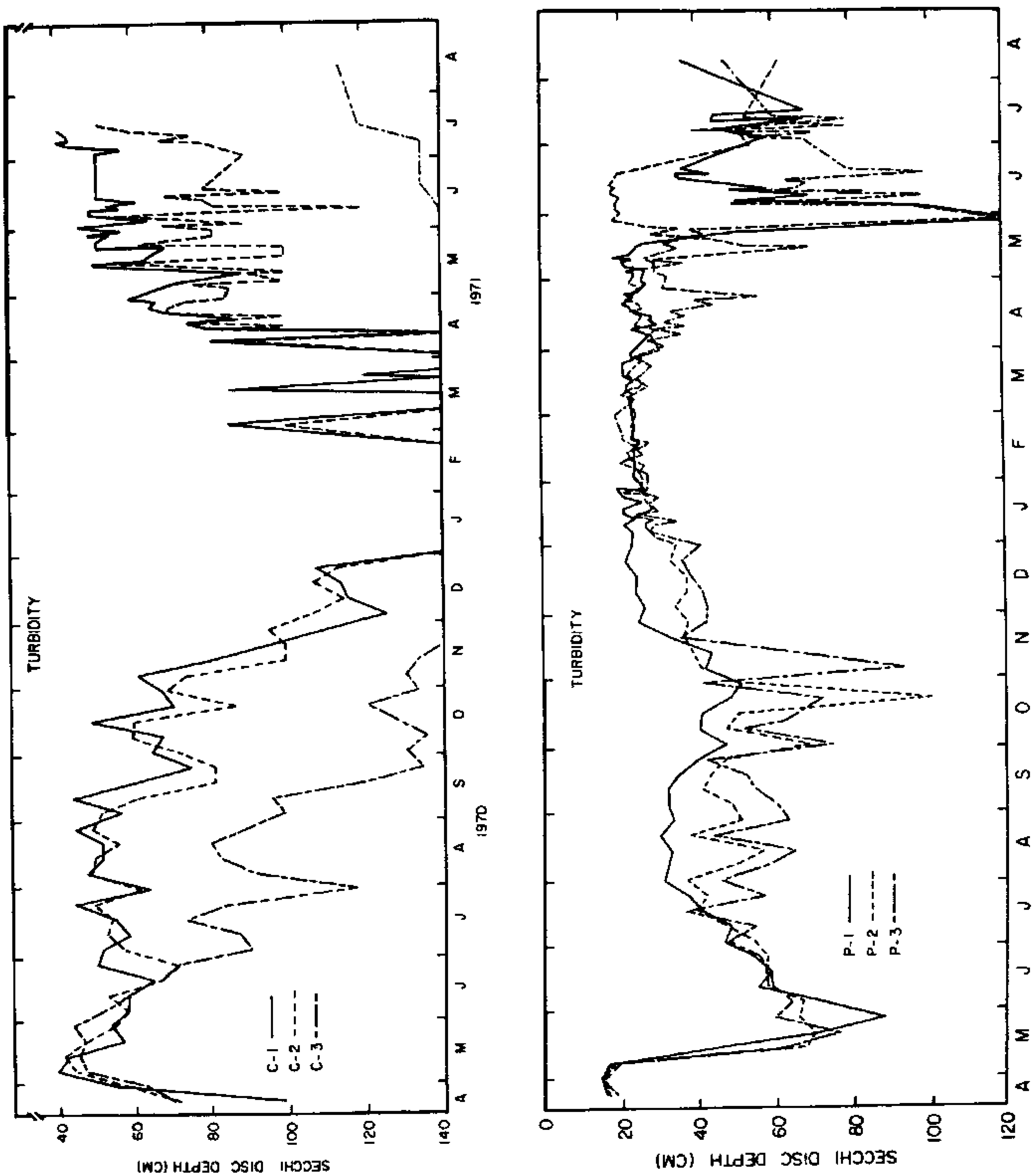


Figure 15b. Record of pond clarity. Secchi disk, 1970-1971

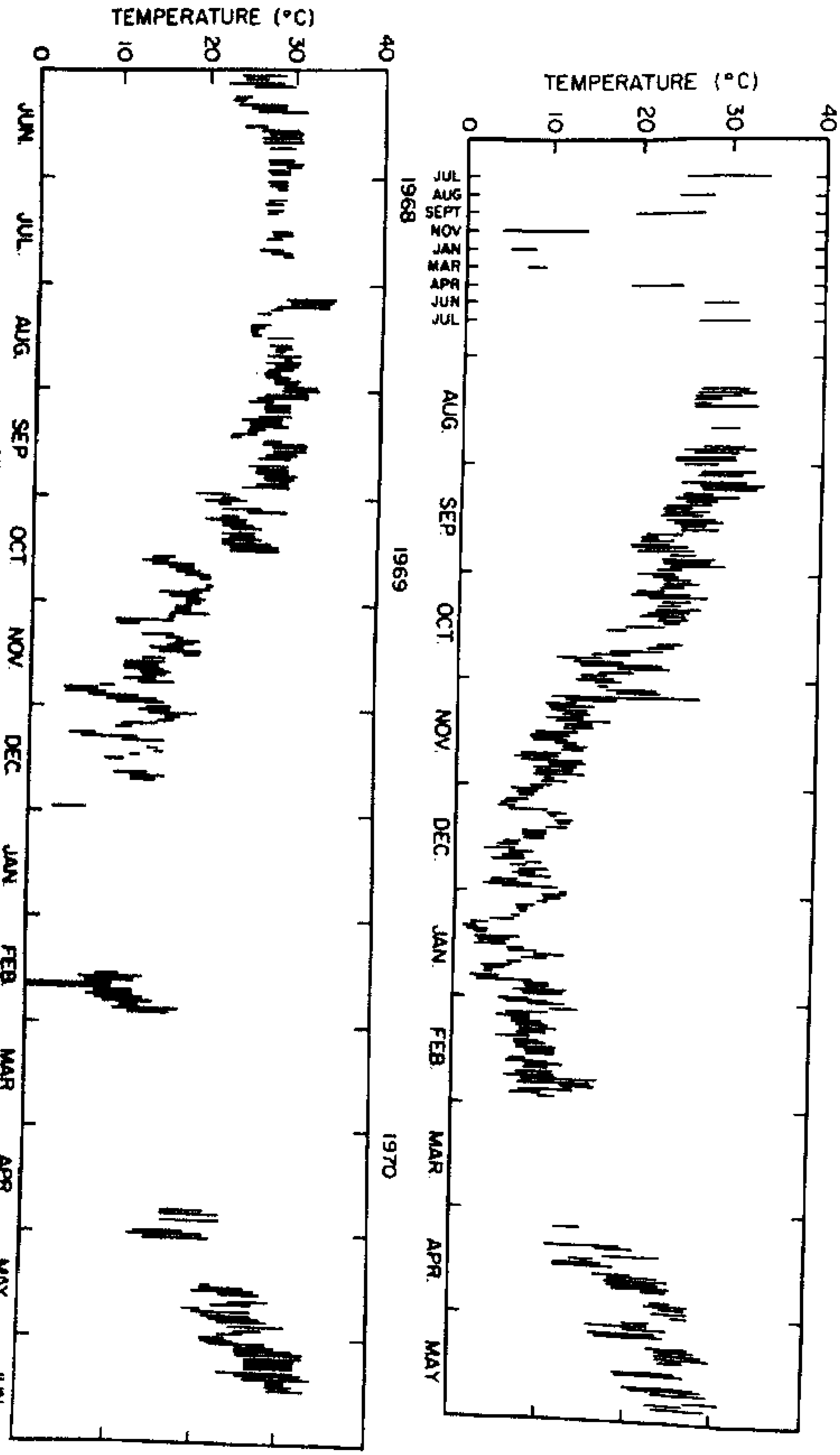


Figure 16. Daily ranges of temperature 1968-1971, combining data from all ponds.

Dissolved Oxygen and Ecosystem Oxygen Metabolism

Figure 17 contains diurnal records of freewater dissolved oxygen and rate of change of oxygen as routinely graphed to calculate ecosystem oxygen metabolism by the computer program. Figure 18 is a typical graph generated by the computer program used to make the calculations (Hall, 1970). Figure 19 is typical of the records during the period when only maxima and minima were measured. Ranges for a year are given in Figures 20 and 21. In Figure 22 and 23 oxygen data and resulting metabolism calculations for one of the waste ponds are compared with one of the control ponds for a month in each of the four seasons. Whereas the diurnal temperature ranges in waste and control ponds were similar, the oxygen ranges (Figure 22) were greater in nutrient-rich waste ponds especially in summer. Seventy two additional monthly graphs like those in Figures 22 were given for all ponds and months by Smith (1972). Table 4 has the means of these data. The level of oxygen was higher and supersaturated in winter due to a sustained bloom of Monodus. Waters were well mixed at dawn due to surface cooling, but during the day some oxygen gradient developed (Figure 24).

Ecosystem oxygen metabolism calculated from diurnal changes for 1968-1970 is given in Figure 25. These are presented as net daytime production (P net calculated by subtracting late afternoon values from dawn values) and night respiration (night R calculated by subtracting dawn values from previous late afternoon values). By plotting net production upward from zero and night respiration downward from zero, gross production (defined as P net plus night R) can be read as the combined length of the bars. Metabolism values for the set of oxygen data in Figure 22 are graphed similarly as Figures 23. Gross production in these graphs is the distance between the top and bottom point for each day. In general net production is proportional to night respiration. Waste ponds had higher photosynthesis and respiration rates than control ponds. Seventy two additional graphs for other months and ponds are available (Smith, 1972).

The means for these daily measurements for 1970-71 are given in Tables 4 and 5 including oxygen, temperature, salinity, and net photosynthesis (dawn - late afternoon) and night respiration (late afternoon - dawn). Monthly means are graphed in Figures 26. The much larger metabolism of waste ponds in all seasons is hidden somewhat by the different scales on the ordinate.

Gross production (sum of net P and night R) and insolation are given in Table 6 and their ratio calculated as an index of efficiency of gross photosynthesis. Efficiencies were several times higher in waste ponds.

Oxygen Metabolism in Bottles, Bell Jars, and Sediment Core Tubes

Oxygen metabolism of separate parts of the ponds was estimated by measuring oxygen change in containers. Data on oxygen change and metabolism in bottles containing pond water and its plankton are

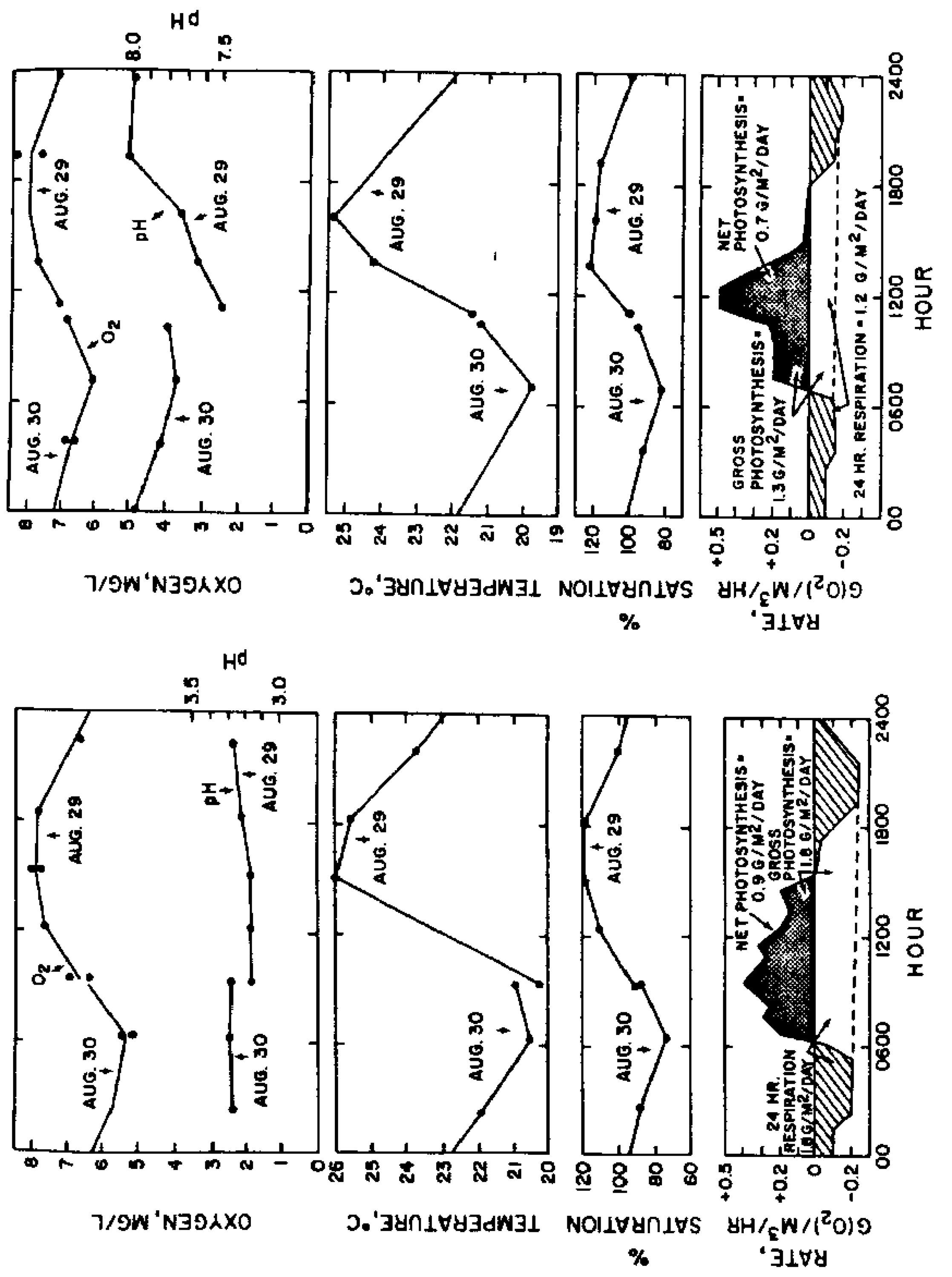


Figure 17. Dissolved oxygen and metabolic calculations for August 29-30. (a) Pond C-3 with pH 3.2-3.3; (b) Pond P-3 with pH 7.5-8.

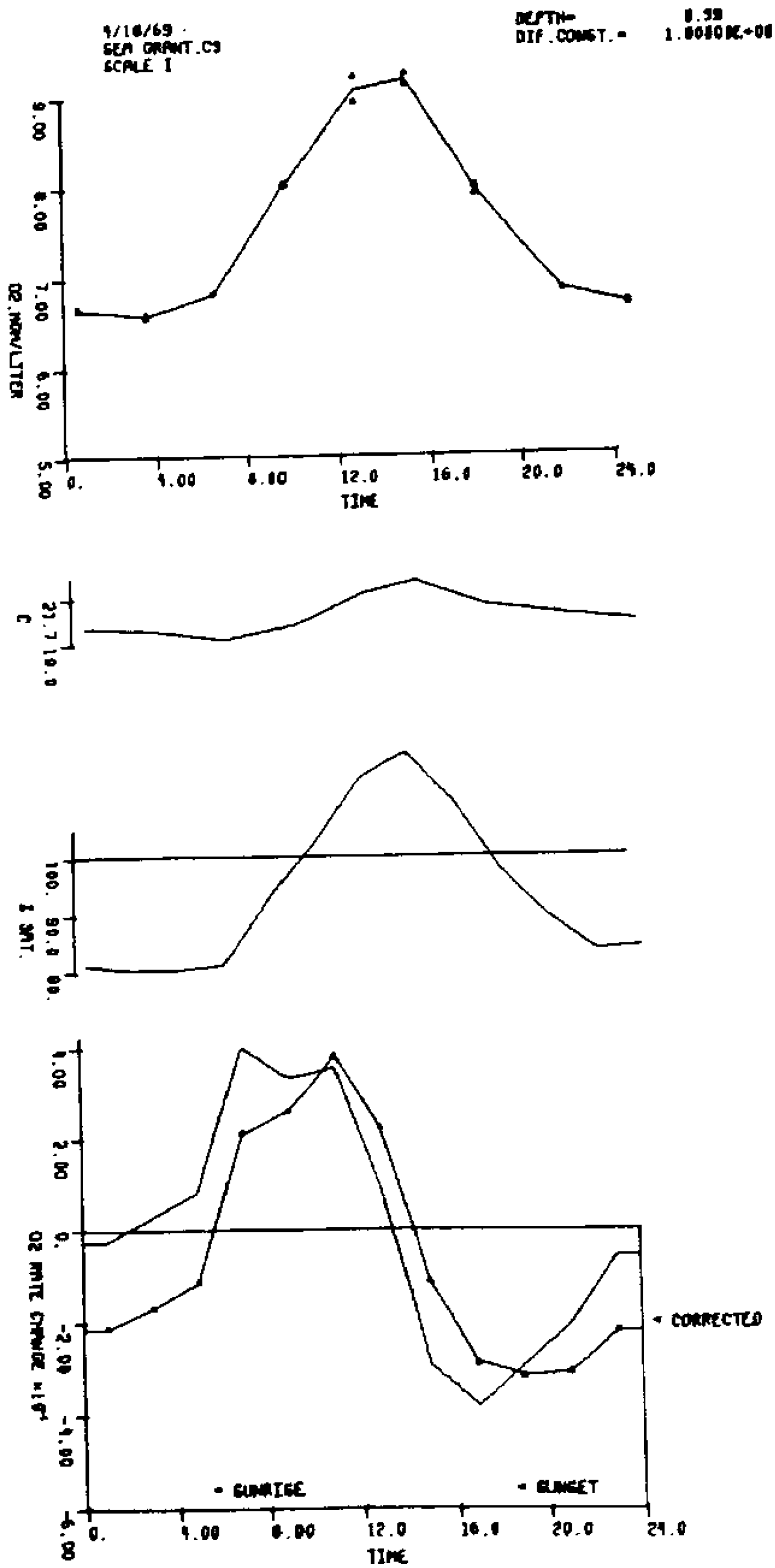


Figure 18. Example of diurnal oxygen records and calculations as graphed by the Primpro computer program (Hall, 1970).

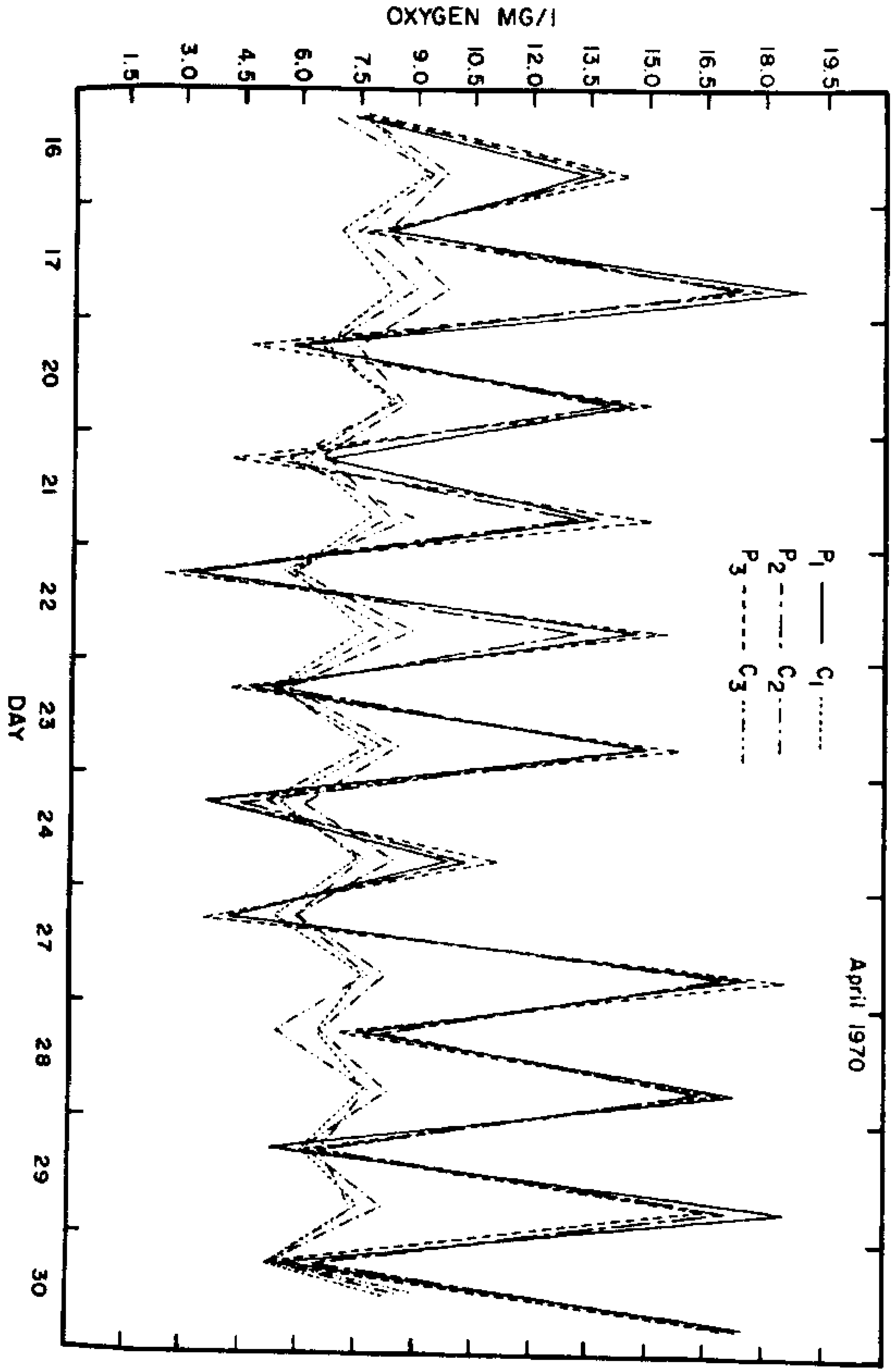


Figure 19. Daily record of early morning and late afternoon dissolved oxygen in all ponds in April 1970 (Smith, 1972).

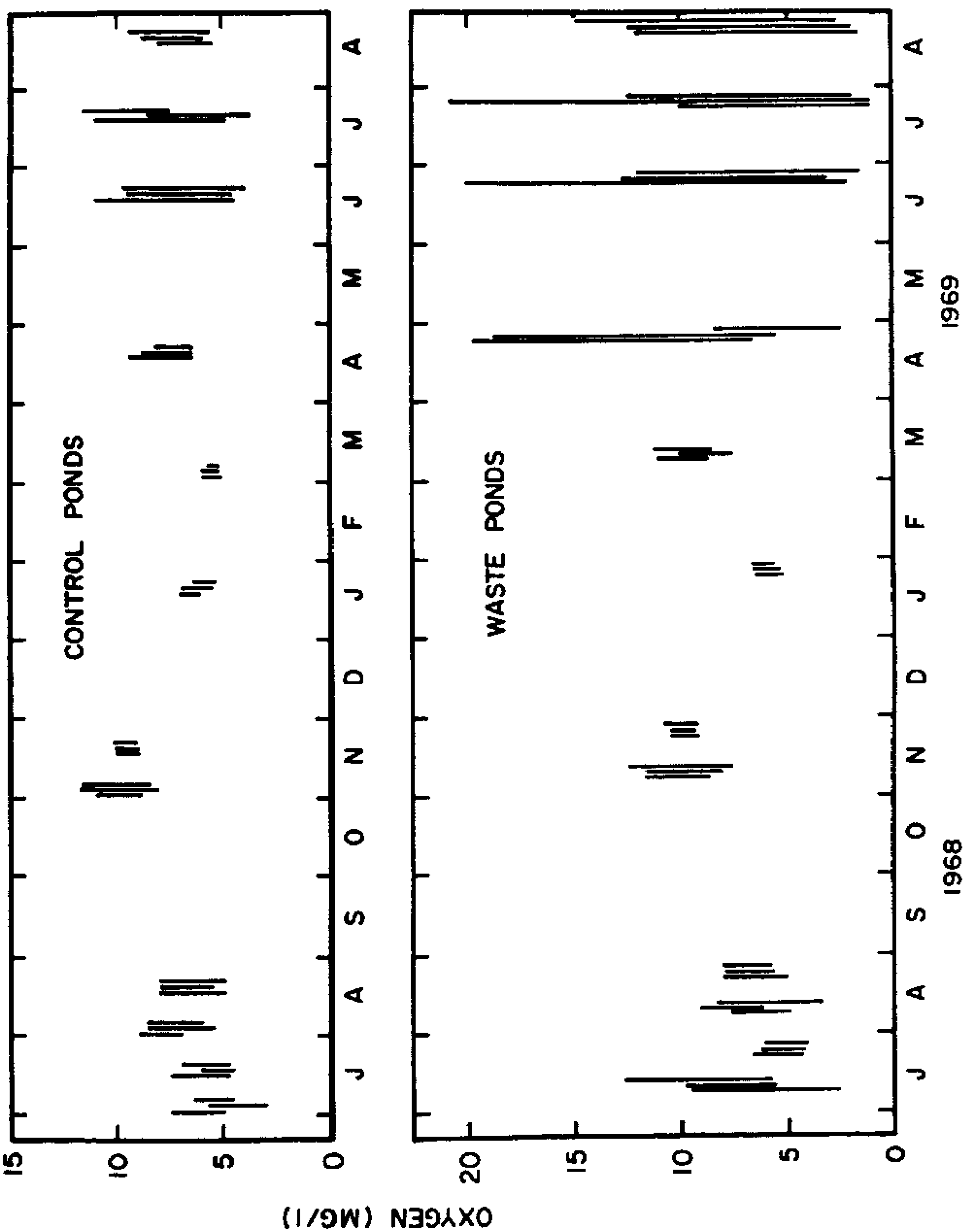


Figure 20. Daily ranges of oxygen 1968-1969, combining data from all ponds.

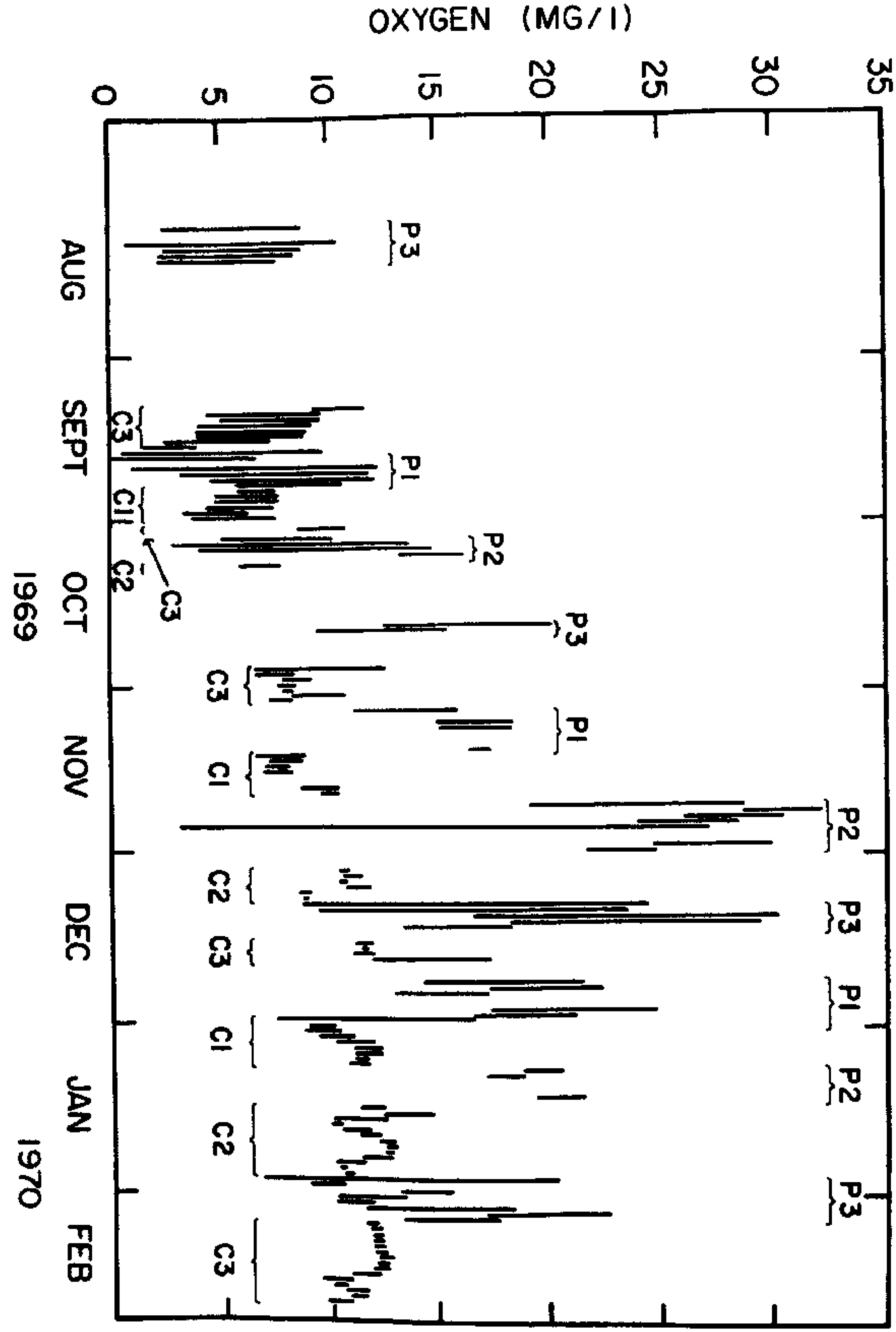


Figure 21. Daily ranges of oxygen 1969-70.

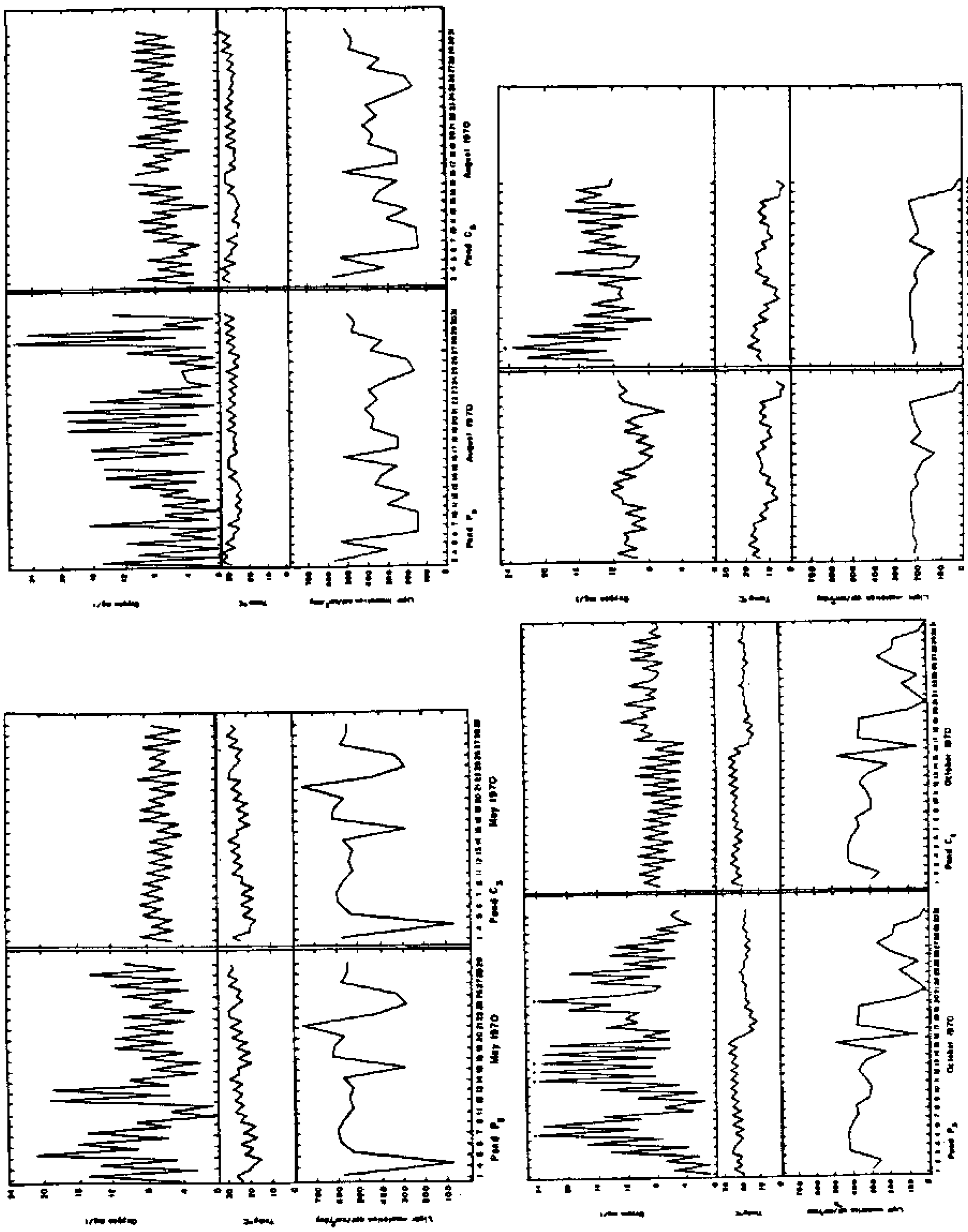


Figure 22. Daily record of early morning and late afternoon oxygen, temperature, and insolation for pond P-3 and pond C-3 in May, 1970 (Smith, 1972). (a) May, 1970; (b) August, 1970; (c) October, 1970; (d) December, 1970.

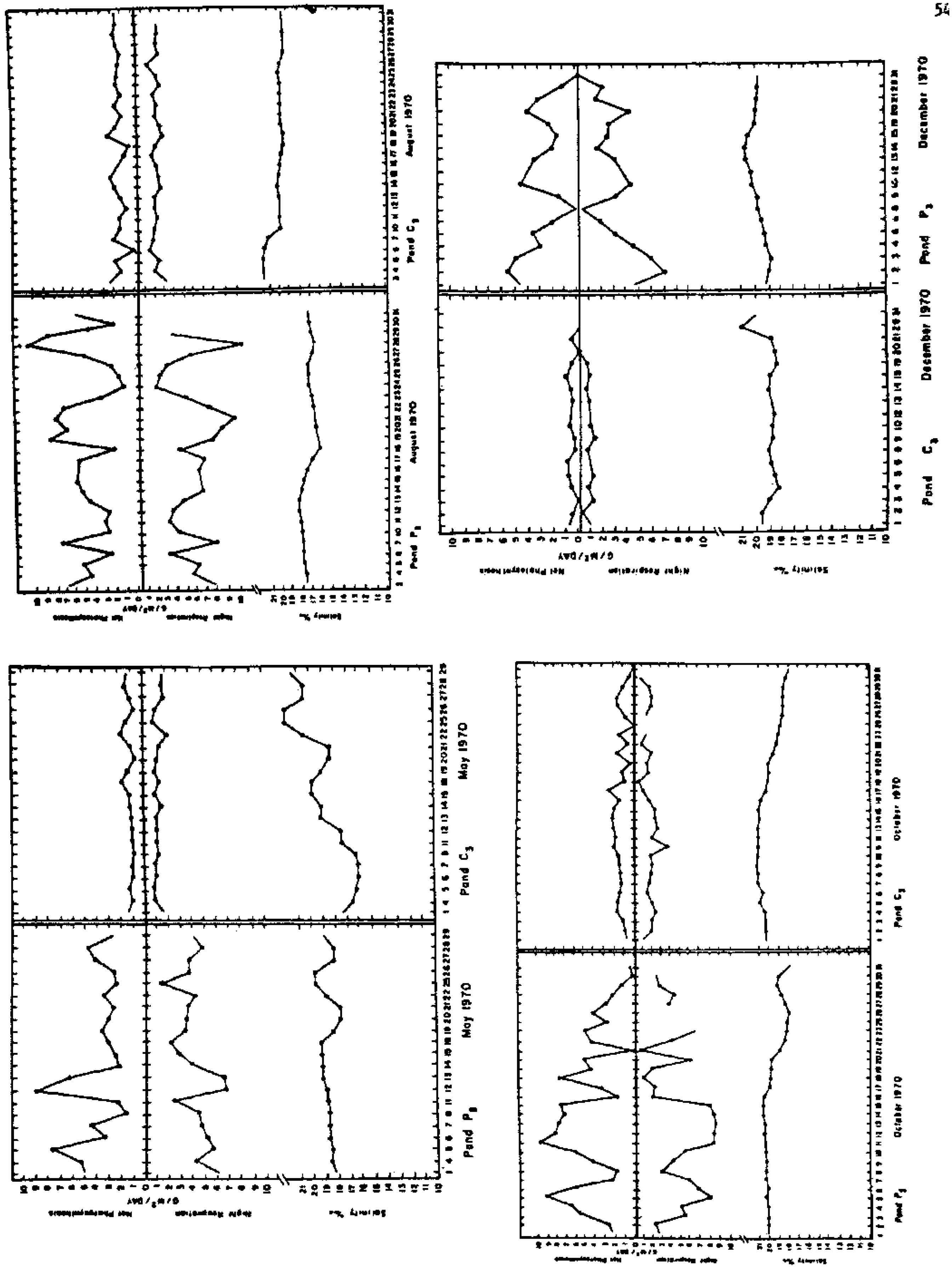


Figure 23. Oxygen metabolism and salinity for ponds P-3 and C-3 (Smith,1972) calculated from data in Figure 22 (Smith,1972). (a) May 1970; (b) August, 1970; (c) October, 1970; and (d) December, 1970.

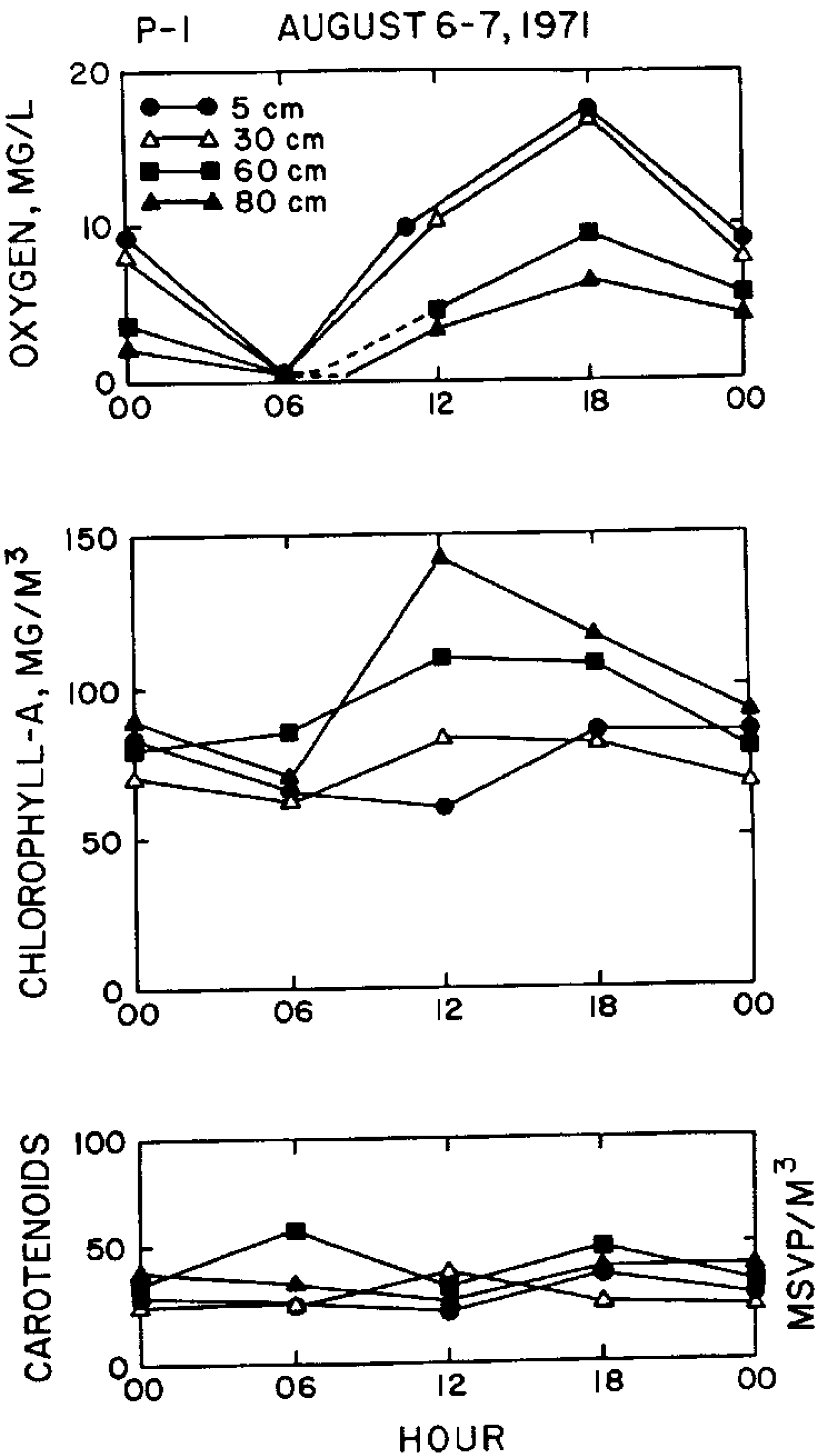


Figure 24a

Figure 24. Vertical distribution of oxygen and pigments at dawn and mid-day (Leeper and Woods, 1973). (a) diurnal graphs.

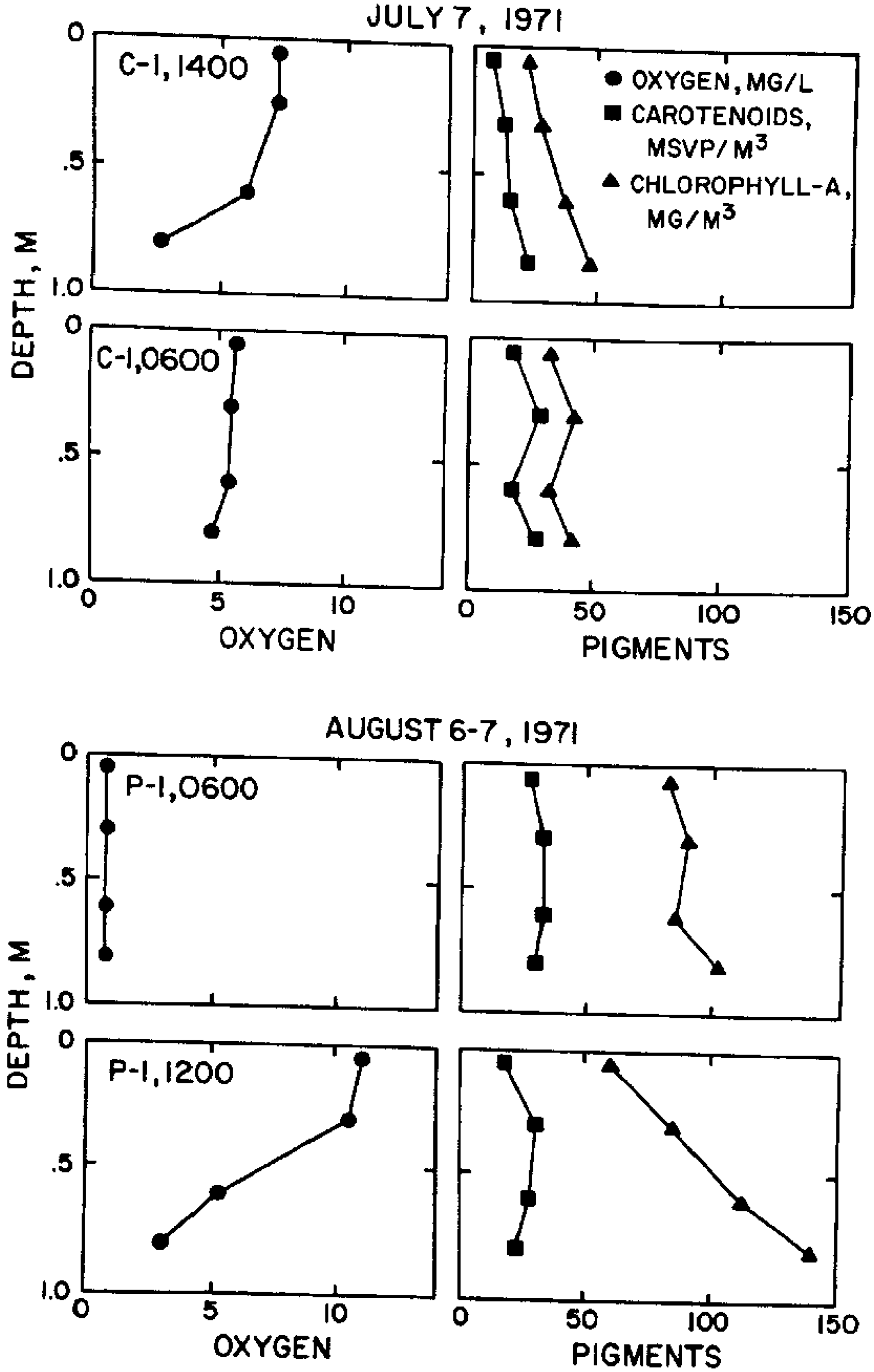


Figure 24b

(b) vertical.

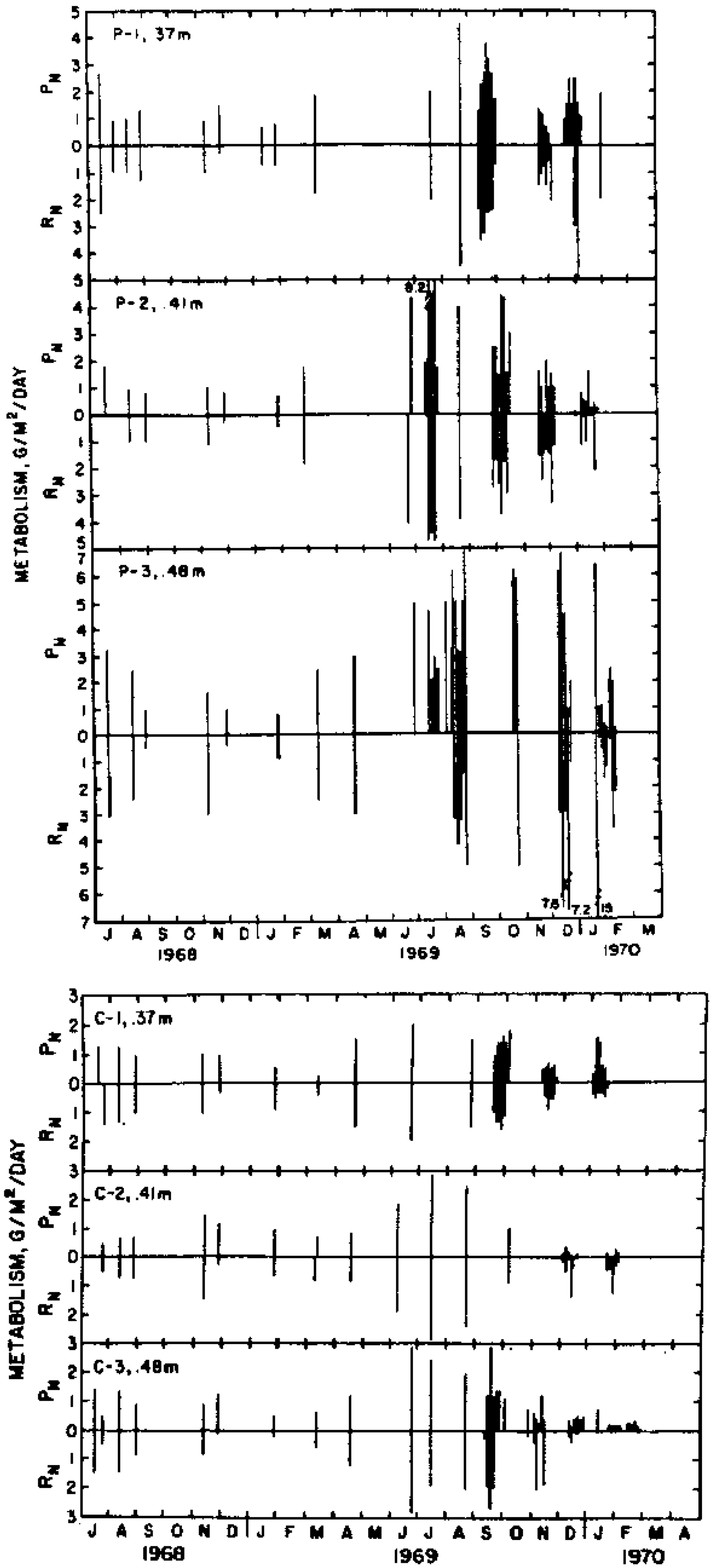


Figure 25. Daytime net photosynthesis and following night respiration calculated from diurnal oxygen data, 1968-1970. (a) waste ponds; (b) control ponds.

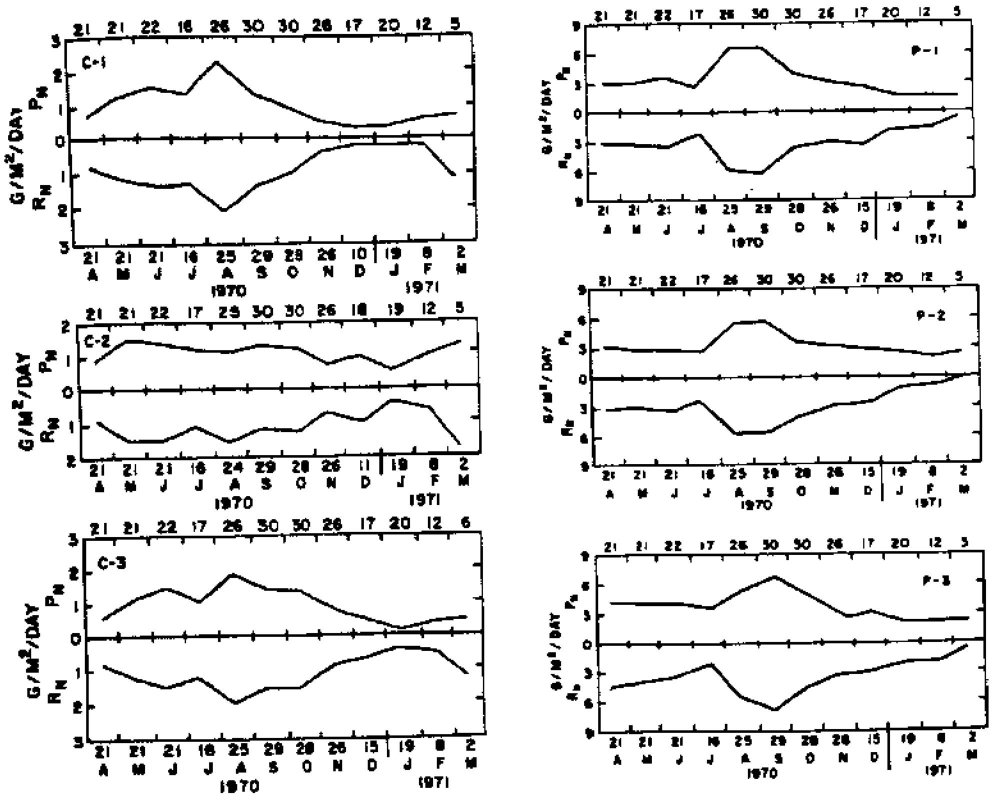


Figure 26. Monthly record of oxygen metabolism, 1970-1971 (Smith, 1972). (a) control ponds; (b) waste ponds. Note that ordinate scales are different.

Table 4. Comparison of seasonal mean data for waste ponds (P) and control ponds (C).

	Mean		Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.
a.m.	Oxygen mg/1	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
p.m.	Oxygen mg/1	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
o/oo	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
a.m.	Temperature °C	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°
p.m.	Temperature °C	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 5. Annual mean of daily metabolism, g/m^2/day.

	P net		R	
	1969-70*	1970-71+	1969-70*	1970-71≠
C-1	1.301	1.022	0.986	1.025
C-2	1.274	1.111	0.822	1.125
C-3	1.096	1.139	1.055	1.168
Mean	1.225	1.091	0.953	1.106
P-1	1.945	3.559	1.521	3.793
P-2	2.712	3.417	2.192	3.529
P-3	3.123	3.927	3.384	4.084
Mean	2.594	3.634	3.364	3.802

* Odum, Hall, and Masarachia (1970) 127 days

+ 246 days

≠ 226 days

Table 6. Efficiency of gross photosynthesis (Smith, 1972).

	Month	Mean Insolation Kcal/m^2/day	Mean Gross Production* Kcal/m^2/day		Efficiency +	
			C	P	C	P
1970	April	4059	6.44	27.8	0.16	0.69
	May	5018	10.40	26.8	0.21	0.53
	June	5016	12.04	27.6	0.24	0.55
	July	4375	9.40	19.7	0.21	0.45
	August	3848	14.88	44.9	0.39	1.17
	September	3985	10.80	49.4	0.27	1.24
	October	2664	10.04	33.4	0.38	1.26
	November	2223	5.88	25.2	0.27	1.13
	December	1812	4.80	23.2	0.27	1.28
1971	January	1450	2.80	14.7	0.19	1.01
	February	2567	3.92	12.0	0.15	0.47
	March	3643	4.84	11.4	0.13	0.31

* Mean oxygen production in g/m^2/day times 4 kcal/g.

+ % that mean oxygen production is of mean insolation.
If half of the insolation is used as in wavelengths available for photosynthesis, efficiencies are twice these.

given in Tables 7 and 8. Data on bell jars representing bottom metabolism are given in Table 9. Oxygen metabolism of water in sediment-containing core tubes are given in Table 10. Differences between waste ponds and control ponds were less in containers than in free water measurements. The sum of bottom metabolism measured in containers and bottle measurements (Table 11) was comparable to free water in control ponds but much smaller in waste ponds (Table 12). Containers may have inhibited the higher rates of metabolism.

pH and Ecosystem Carbon Metabolism

The alternation of daytime photosynthesis and nighttime respiration in the shallow ponds produced sharp rise and fall of pH (Figures 27 and 28) as well as oxygen as the daytime use of carbon dioxide by plants and nighttime release of carbon dioxide by ecosystem respiration shifted carbonate equilibria. Day (1971, 1983) studied diurnal pH variations, measurements of carbon fractions, and measurements of carbon dioxide exchange across the air water interface and used these to estimate carbon net photosynthesis and night respiration.

Diffusion exchange of carbon-dioxide measured in a floating plastic dome (Figures 28) indicated outward diffusion in the C ponds which were always supersaturated. C ponds received their freshwater component from tap water supplies drawn from artesian ground waters supersaturated relative to partial pressure of carbon dioxide in the air. However, outward diffusion was less during the day when photosynthesis raised pH. In waste ponds carbon dioxide diffusion was out during the night when respiration lowered pH and in during the day when photosynthesis raised pH. A diffusion coefficient (k) was calculated from the partial pressure of "free" carbon dioxide in the water (dissolved carbon dioxide in Figure 28). The "reaeration coefficient" varied during the day (Figures 28-29) and with weather regimes affecting the vertical circulation of the water column.

Typical graphs of diurnal patterns of carbon are given in Figure 30 including one in winter and one in summer. Changes of organic fractions in one day due to metabolism were a small percent of the stocks, and other factors such as variable stirring of ooze from the bottom may have affected samples as much. Suggestion of the daytime decline of inorganic carbon is observable as expected from plant photosynthesis and nighttime rise from respiration of plants, animals, microbes, etc.

Figure 31 is a typical graph of diurnal rate of change of inorganic carbon calculated from a diurnal graph of total inorganic carbon. After the carbon dioxide diffusion in or out of the air was subtracted, the residual graph indicated day net photosynthesis by the daytime fall and ecosystem respiration by the nighttime rise. Estimates of net daytime photosynthesis (P) and nighttime respiration (R) from these diurnal studies are given in Table 13.

An annual indication of pH trends is given in Figure 32, which also shows diurnal ranges. The pH moved up and down as the ratio of photosynthesis to respiration shifted with cloudiness over

Table 7. Oxygen bottle metabolism, Sept. 26-27, 1969* (Dillon and Woods).

Pond	Starting oxygen concentration	Dark bottles +	Net production in 24 hrs.		Gross production	P #/R
C-1	7.15	-2.22	Top	2.62	4.84	1.3
			bottom	3.02	-0.80≠	-1.7
			mean	0.20	2.02	0.8
P-1	7.35	-3.74	Top	15.38	19.12	4.5
			bottom	-3.95	-0.21 ≠	-1.4
			mean	5.71	9.45	2.4

* mean of two bottle each

+ Decrease due to respiration

≠ Negative gross production is possible if there is photorespiration or photo-oxidation

Ratio of daytime net production to night respiration (12 hrs.)

Table 8. Bottle metabolism, 3 hour measurements July 29, 1969 (Woods).

Item	Pond		Oxygen change 3 hours Dark mg/l	Light mg/l	Light net production* (P_N) g/m^2/12 hrs.	Dark respiration+ (R_N) g/m^2/12 hrs.	P_N/R_N	Gross production ($P_N + R_N$) g/m^2/24 hrs.
Bottles	C-1		-0.39	2.15				
			-0.52	1.08				
			-0.60	0.81				
			-1.05	0.56				
			-2.56	0.59				
		mean	-0.81	1.04	2.04	1.59	1.28	3.63
	C-2		-0.62	0.89				
			-0.54	1.40				
			-0.63	0.75				
			-0.49	0.72				
		mean	- .57	.94	1.80	1.09	1.65	2.89
	C-3		-0.24	2.16				
			-0.67	1.89				
			-0.66	1.08				

Table 8 (cont.)

Item	Pond		Oxygen change 3 hours Dark mg/1	Light mg/1	Light net production* (P_N) g/m^2/12 hrs.	Dark respiration+ (R_N) g/m^2/12 hrs.	P_N/R_N	Gross production (P_N+R_N) g/m^2/24 hrs.
(cont.)	C-3		-0.59	0.19				
			-1.09	0.31				
		mean	-0.65	1.1	1.76	1.01	1.74	2.77
Bottles	P-1		-0.52	1.38				
			-0.30	1.94				
			-0.79	0.03				
			-0.17	0.09				
			-0.40	0.01				
			- .44	.69	0.65	1.02	0.64	1.67
	P-2		-0.85	0.79				
			-0.46	0.64				
			-0.70	1.22				
			-0.62	0.19				
			-2.37	0.84				
			-1.00	.74	1.21	1.64	0.74	2.85

Table 8 (cont.)

Item	Pond	Oxygen change 3 hours Dark mg/1	Light mg/1	Light net Production* (P_N) g/m^2/12 hrs.	Dark respiration+ (R_N) g/m^2/12 hrs.	P_N/R_N	Gross production (P_N+R_N) g/m^2/24 hrs.
P-3		-1.41	1.22				
		- .67	1.69				
		- .13	0.68				
		-1.72	0.31				
		-2.62	0.32				
	mean	1.31	0.84	1.61	2.52	0.64	4.13

* Net Production/ 12 hrs.
(mg/1/3 hrs.) ($\frac{12}{3}$ hrs./day) (mean depth≠)

+ Respiration (mg/1/3hrs.) ($\frac{12}{3}$ hrs./day) (mean depth≠)

≠ Depths C-1, 0.49m; C-2, 0.48m; C-3, 0.39m; P-1, 0.37m; P-2, 0.41m; P-3, 0.48 m

Table 9. Bottom metabolism measured with bell jars* (Dillon and Wood, 1970).

...em	Pond		Oxygen change 3 hrs. Dark mg/l	Oxygen change 3 hrs. Light mg/l	Oxygen change minus bottle values Dark mg/l	Oxygen change minus bottle values Light mg/l	Net Prod. (P_N) g/m^2/12 hrs.	Resp. (R_N) g/m^2/12hrs.	P_N/R_N	Gross Prod. (PN+R) g/m^2/24 hr.
...l jars	C-1		-2.36	2.33						
			-0.48	0.39						
			-0.80	1.87						
			-3.20	3.76						
			-5.36	5.93						
		mean	-2.44	2.86	1.63	1.82	1.59	1.42	1.12	6.90
	C-2		-2.70	1.59						
			-1.32	0.60						
			-0.27	1.65						
			-0.21	2.17						
		mean	-1.13	1.50	0.56	0.56	0.49	0.49	1.00	2.24
	C-3		-0.82	0.95						
			-1.71	2.02						
			-0.56	2.91						
			-0.14	0.24						
			-2.15	0.86						
		mean	1.08	1.40	0.43	0.30	0.26	0.38	0.69	1.40
	P-1		-1.43	0.36						
			-0.56	4.00						
			-0.99	1.10						
			0.87	1.16						
			-2.01	0.52						
		mean	-1.17	1.43	0.73	0.74	0.65	0.64	1.02	2.94
	P-2		-1.32	0.19						
			.00	1.70						
			.00	0.42						
			-3.49	4.30						
			-3.79	2.46						
		mean	-2.87	1.81	1.87	1.07	0.94	1.64	0.57	5.88

Table 9 (cont.)

Item	Pond		Oxygen change 3 hrs. Dark mg/1	Oxygen change 3 hrs. Light mg/1	Oxygen change minus bottle values Dark mg/1	Oxygen change minus bottle values Light mg/1	Net Prod. (P_N) $g/m^2/12$ hrs.	Resp. (R_N) $g/m^2/12$hrs.	P_N/R_N	Gross Prod. (PN+R) $g/m^2/24$ hr.
	P-3		-1.71	0.90						
			-0	0.02						
			-0.30	0.10						
			-2.90	1.62						
			-5.87	2.91						
		mean	-2.16	1.07	0.85	0.23	0.20	0.75	0.27	2.16

* Volume of water in bell jar made from cutting carbuoy: 16.0 liters; area, 731 cm^2. Calculation was made as follows:

$$\frac{(4)\ (\text{mg/1/3 hrs. in bell jar} - \text{mg/1/3 hrs. in bottles}^{+})\ (16.0\ \text{liters})}{(0.0731\ m^2)\ (1000\ \text{mg/g})} = g/m^2/12\ \text{hrs.}$$

\+ Table 10

Table 10. Oxygen metabolism in water in plastic tubes with sediment plug. (C. Lathrop, N. Meith, J. Murray, and J. Richey).

		Dissolved Oxygen (PPM)								
Time	Temp (C)	CN 1	CN2	u_{cn}	C 1	C 2	u_c	P 1	P 2	u_p
0130	21.5	9.0	9.0	9.0	8.0	8.0	8.0	8.0	8.5	8.2
0445	18.0	8.5	8.5	8.5	7.5	7.6	7.6	7.5	7.5	7.5
0745	18.0	8.1	8.2	8.2	7.0	7.0	7.0	6.7	6.5	6.6
1030	21.5	8.5	8.5	8.5	7.6	8.1	7.8	7.8	7.0	7.4
1300	27.0	9.1	9.2	9.2	8.5	9.9	10.9	10.9	8.7	9.8
1530	34.0	9.5	10.0	9.8	10.0	12.0	15.3	15.3	11.0	13.2
1745	30.5	8.5	9.0	8.8	9.0	11.5	15.0	15.0	13.5	14.3
2030	24.0	8.8	9.0	8.9	8.7	11.5	12.5	12.5	11.6	12.1

Time = time of day 9-27-69

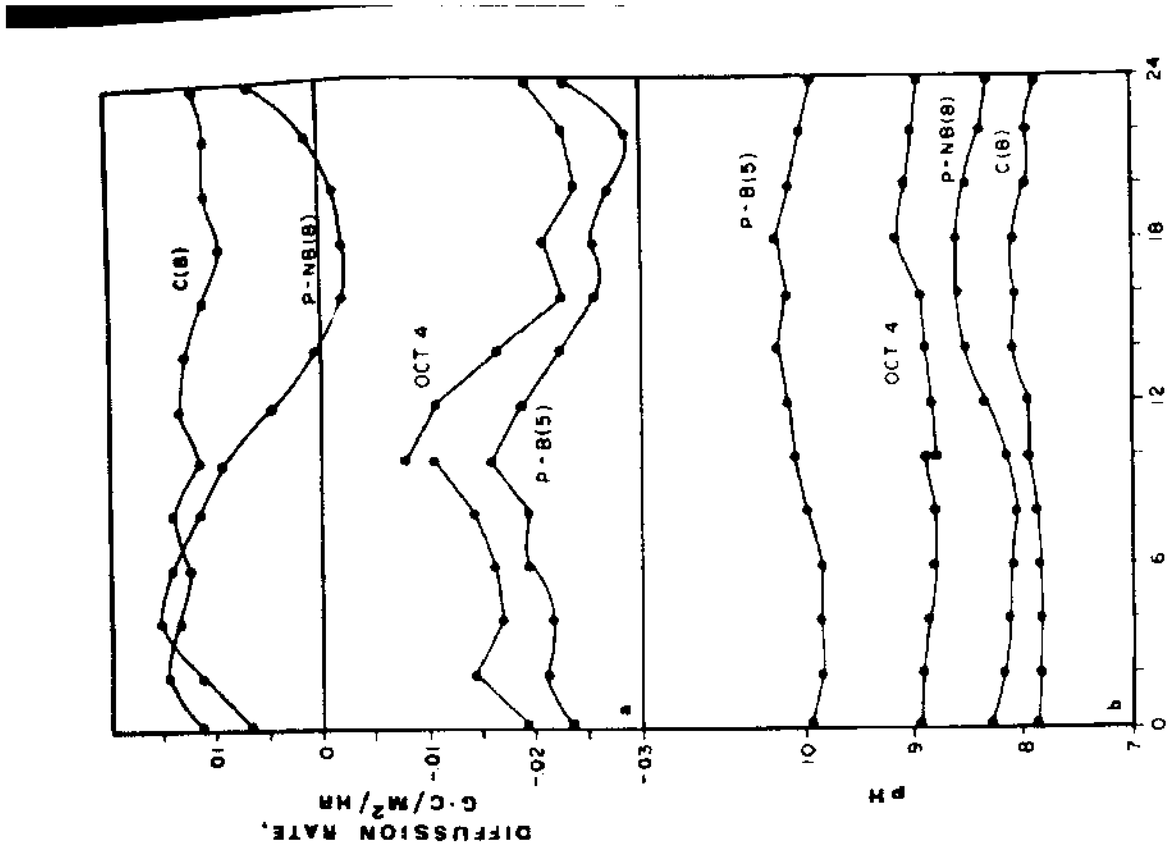
Temp = temp. of water in core tube

CN = control (No Sediment)

C = control pond samples

P = waste pond samples

u = average



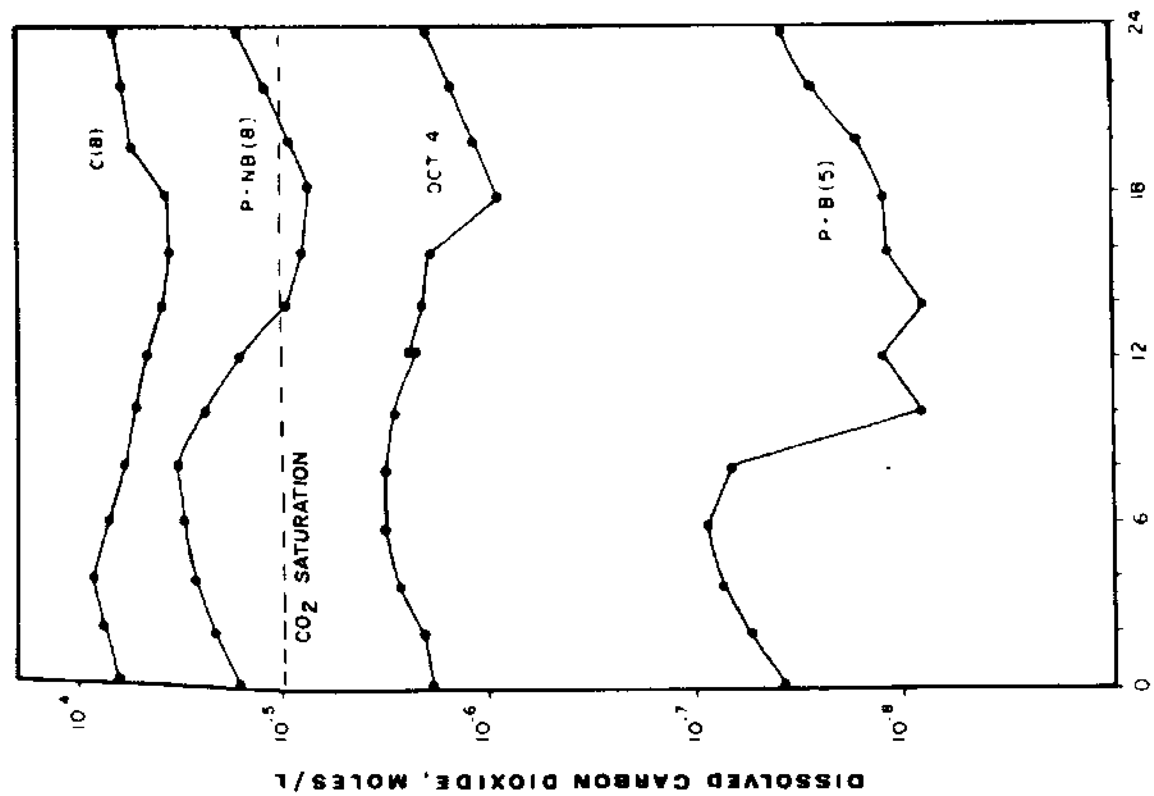


Figure 27. Diurnal patterns of dissolved carbon dioxide, pH, rate of diffusion, and metabolism in pond C-3 including curves for Oct. 4, 1970, and curves based on average pH curves P-NB(8) and P-NB(5); B bloom

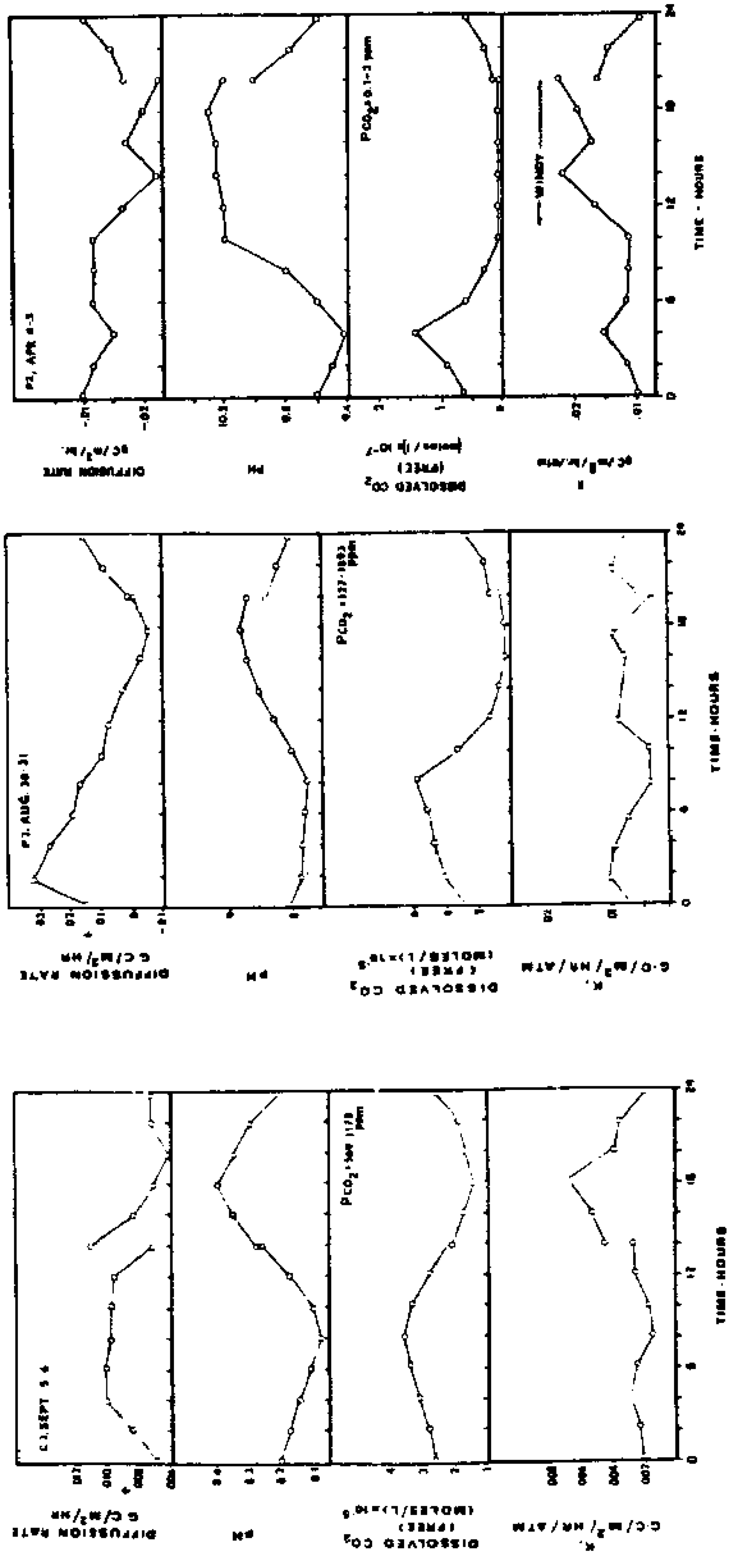


Figure 28. Diurnal variations in free carbon dioxide, pH, diffusion rate, and metabolism (Day, 1971). (a) pond C-3, Sept 5-6, 1970. (b) pond P-2, August 30-31, 1970; (c) pond P-3, April 4-5, 1970.

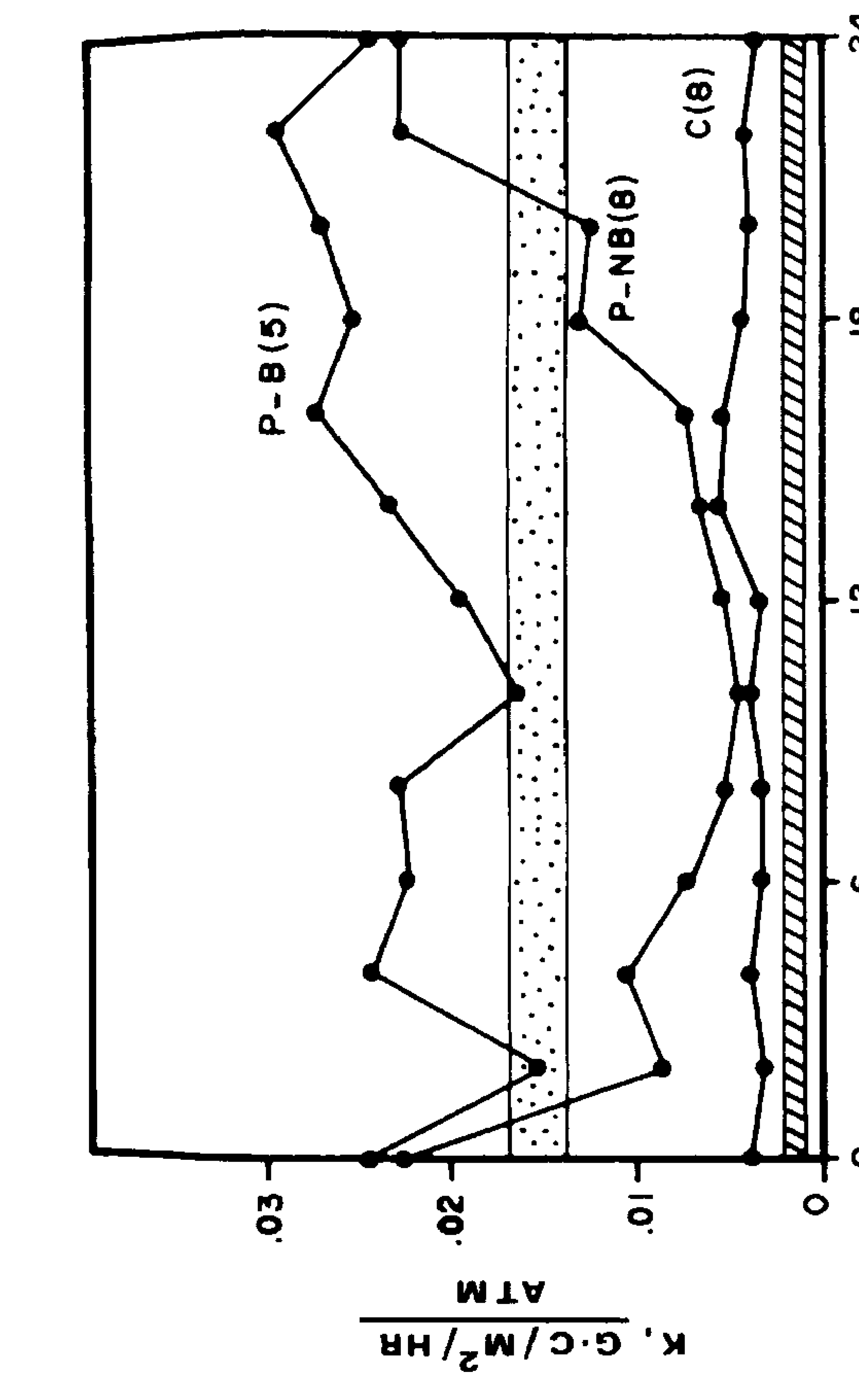


Figure 29. Mean diurnal variations of the diffusion coefficient for

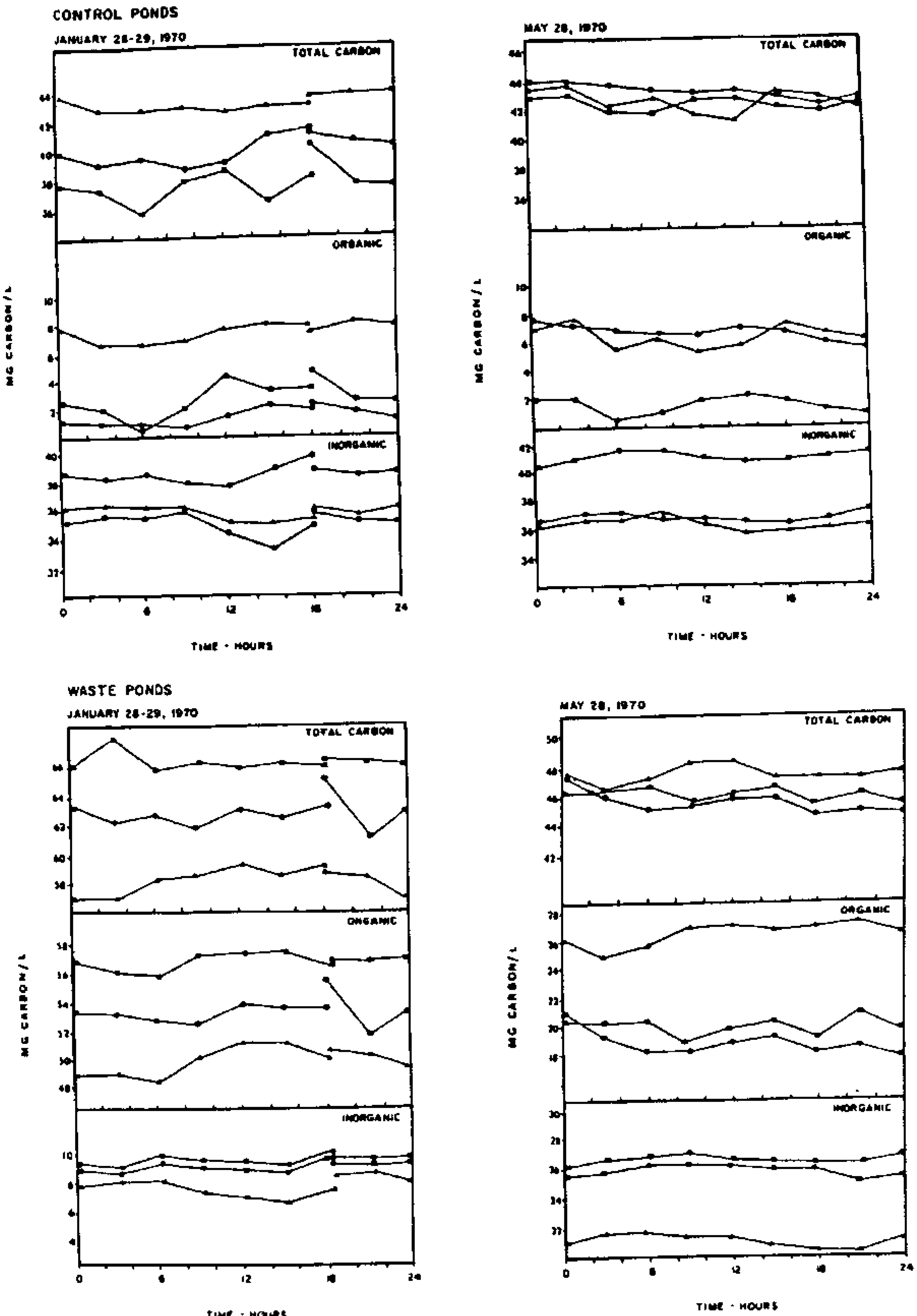


Figure 30. Diurnal variations in total carbon, organic carbon, and inorganic carbon in the ponds. Ponds C-1 and P-1, circles; ponds C-2 and P-2, squares; ponds C-3 and P-3, triangles (Day, 1971).

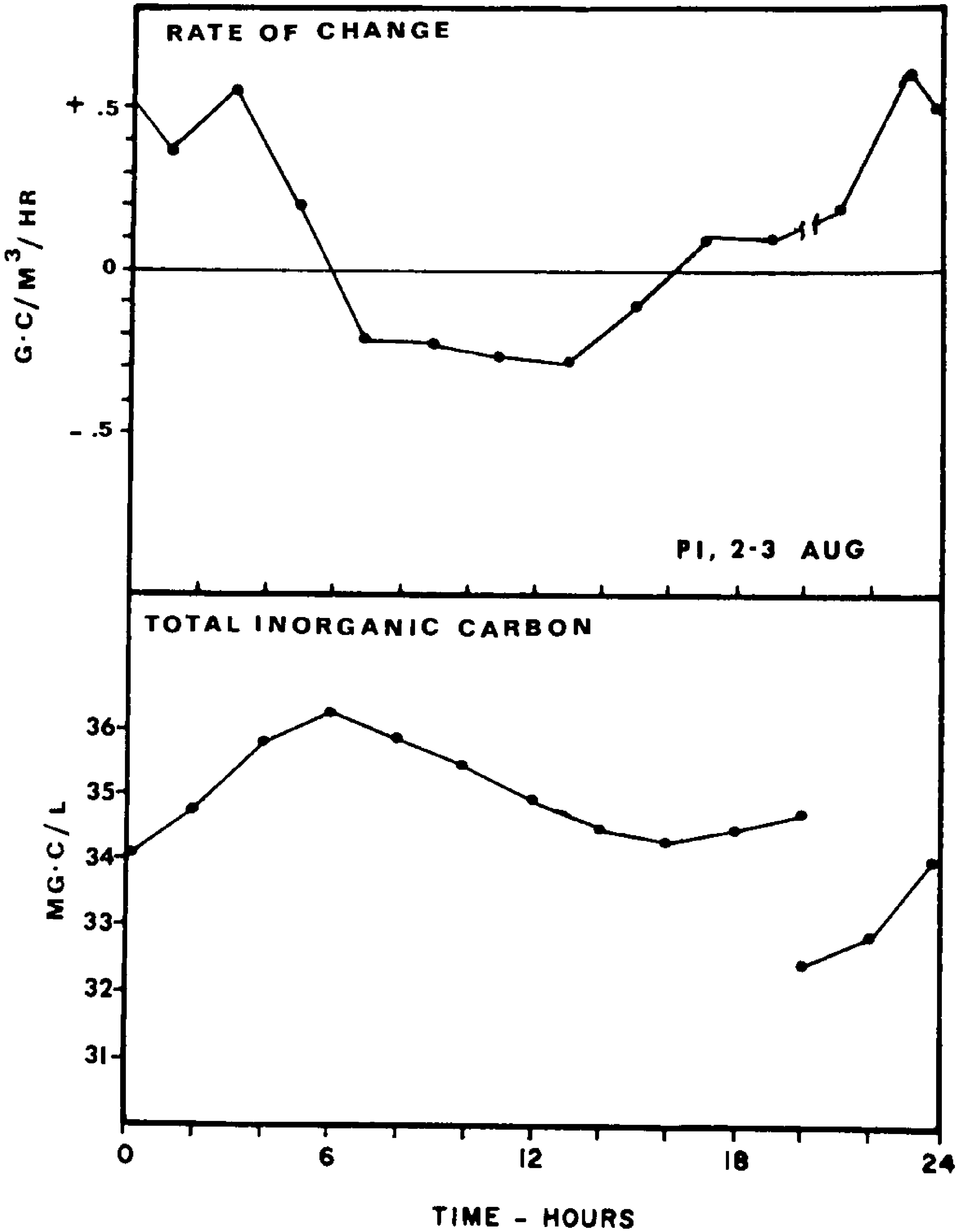


Figure 31. Record of pH in various ponds 1969-1971; a bar is the range for a single diurnal record in one pond.

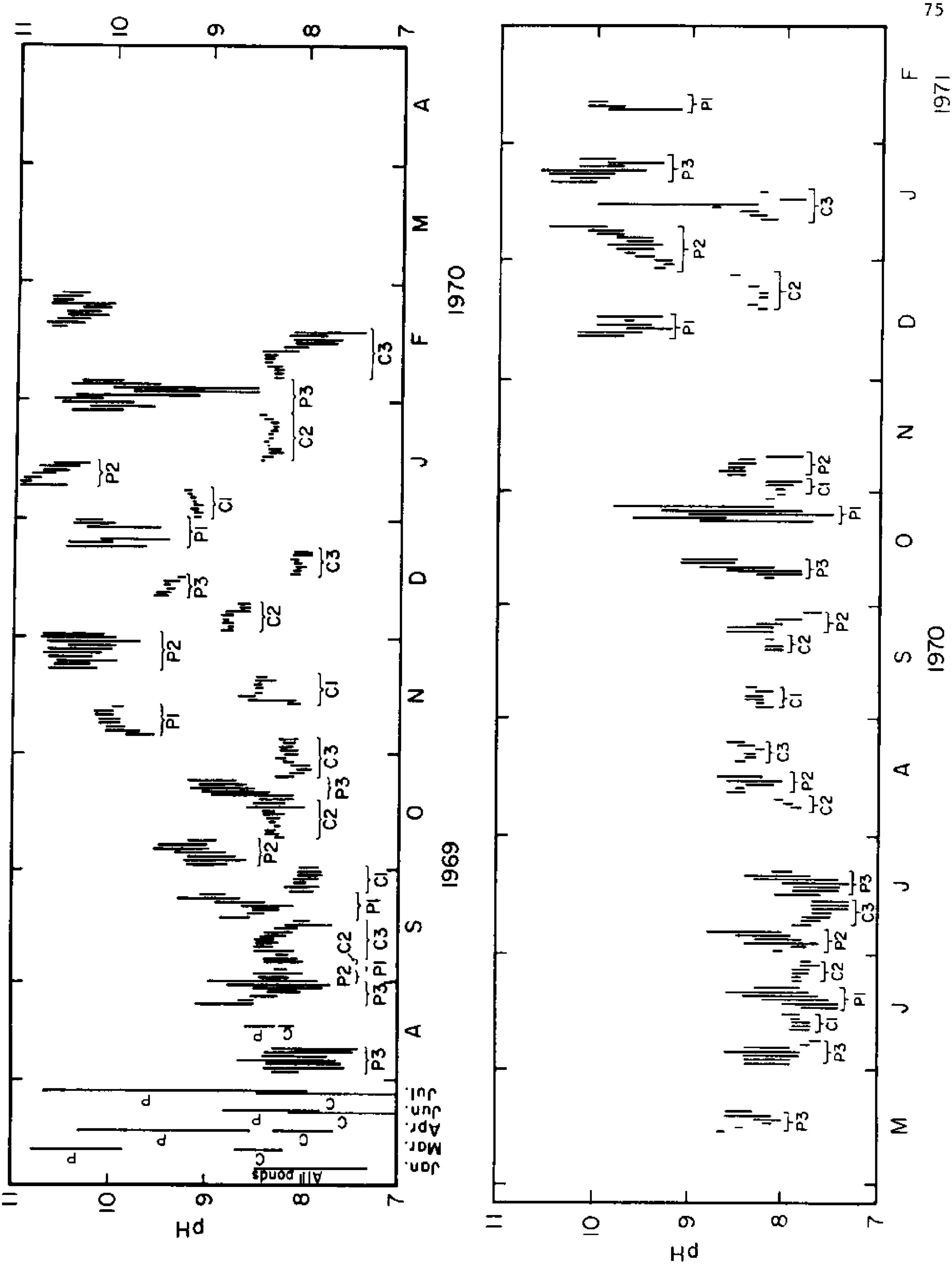


Figure 32. Diurnal variation in concentration and rate of change of total inorganic carbon in Pond P-1, August 2-3, 1970.

Table 11. Rates of nighttime respiration of bottom (Dillon and Wood, 1970).

Date	Pond	g O_2/m^2 /hr.	g C/m^2 /hr.
Nov. 8, 1969	P-1	.0088	.0033
	C-1	.0149	.0056
Jan. 30, 1970	P-2	.0024	.0009
	C-1	.0076	.0029
May 2, 1970	P-2	.0128	.0048
May 31, 1970	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 12. Components of oxygen metabolism, July 29, 1969.

$g/m^2/12$ hrs.	C-1	C-2	C-3	P-1	P-2	P-3
Net photosynthesis:						
Plankton, using bottles	2.04	1.80	1.76	0.65	1.02	0.64
Bottom, using bell jars	1.59	0.49	0.26	0.65	1.21	1.61
Sum	3.73	2.29	2.02	1.30	2.23	2.25
Free water mean	1.3	1.2	1.1	2.4	2.4	3.7
Respiration:						
Plankton using bottles	1.59	1.09	1.01	1.02	1.64	2.52
Bottom using bell jars	1.42	0.49	0.38	0.64	1.64	0.75
Sum	3.01	1.58	1.39	1.66	2.38	2.77
Free water mean	1.3	1.1	1.2	2.6	2.3	2.5

Table 13. Ecosystem carbon metabolism of the experimental ponds (Day, 1971).

Pond	Date 1970	non corrected		diffusion correction		corrected			molar O_2 equivalent	
		R	P	R	P	R	P	P/R	R	P
C-1	May 27	0.54	0.52	-0.13	-0.09	0.67	0.42	0.63	1.78	1.13
C-2	Aug. 5	0.34	0.58	-0.36	-0.14	0.70	0.43	0.62	1.87	1.15
C-3	Sep. 5	1.05	1.05	-0.12	-0.08	1.17	0.93	0.80	3.12	2.51
C-3	May 25	1.17	1.17	-0.20	-0.10	1.37	1.08	0.79	3.65	2.88
P-1	May 29	1.30	1.18	-0.02	-0.02	1.31	1.20	0.92	3.49	3.20
P-1	Aug. 2	1.41	0.74	-0.04	-0.08	1.45	0.66	0.46	3.87	1.76
P-1	Nov. 8	0.67	0.81	+0.44	+0.31	0.23	1.10	4.79	0.61	2.94
P-2	Oct. 4	1.11	1.19	+0.21	+0.21	0.91	1.40	1.54	2.42	3.73
P-2	May 30	0.74	0.44	+0.05	+0.03	0.69	0.47	0.68	1.84	1.25
P-3	Aug. 30	2.02	2.74	-0.23	-0.06	2.25	2.68	1.19	5.99	7.14
P-3	Aug. 29	1.39	1.82	-0.33	-0.09	1.73	1.73	1.00	4.60	4.62
P-3	Apr. 4	1.15	1.34	+0.14	+0.22	1.56	1.56	1.54	2.69	4.16

several day periods. In the waste ponds, starting in the fall each year, as temperature declined, a dense winter bloom of Monodus developed causing very high pH and oxygen level as photosynthesis exceeded respiration. Photosynthesis was less than in summer but the respiration decreased more. In control ponds the level of pH was more uniform. The high values in January 1971 were accompanied by a thick growth of benthic plants.

Day (1971, 1983) also developed annual records of carbon and its components. Total carbon in Figure 33, carbonate alkalinity in Figure 34, and total organic carbon, dissolved organic fraction, and microparticulate organic fraction in Figure 35. Carbonate alkalinity decreased and organic carbon increased during the winter net photosynthesis of the Monodus bloom. At the time of Monodus crash in May organic fraction was replaced by inorganic fraction as respiration increased. The rates of inflow and outflow of carbon are given in Figure 34. The sharp decline in inorganic input to control ponds in June 1970 was caused by a broken pump.

Radioactive carbon uptake rates by plankton in bottles, by benthos, and by periphyton on slides determined by Adams and Wood are given in Table 14a where higher rates were in waste ponds than control ponds.

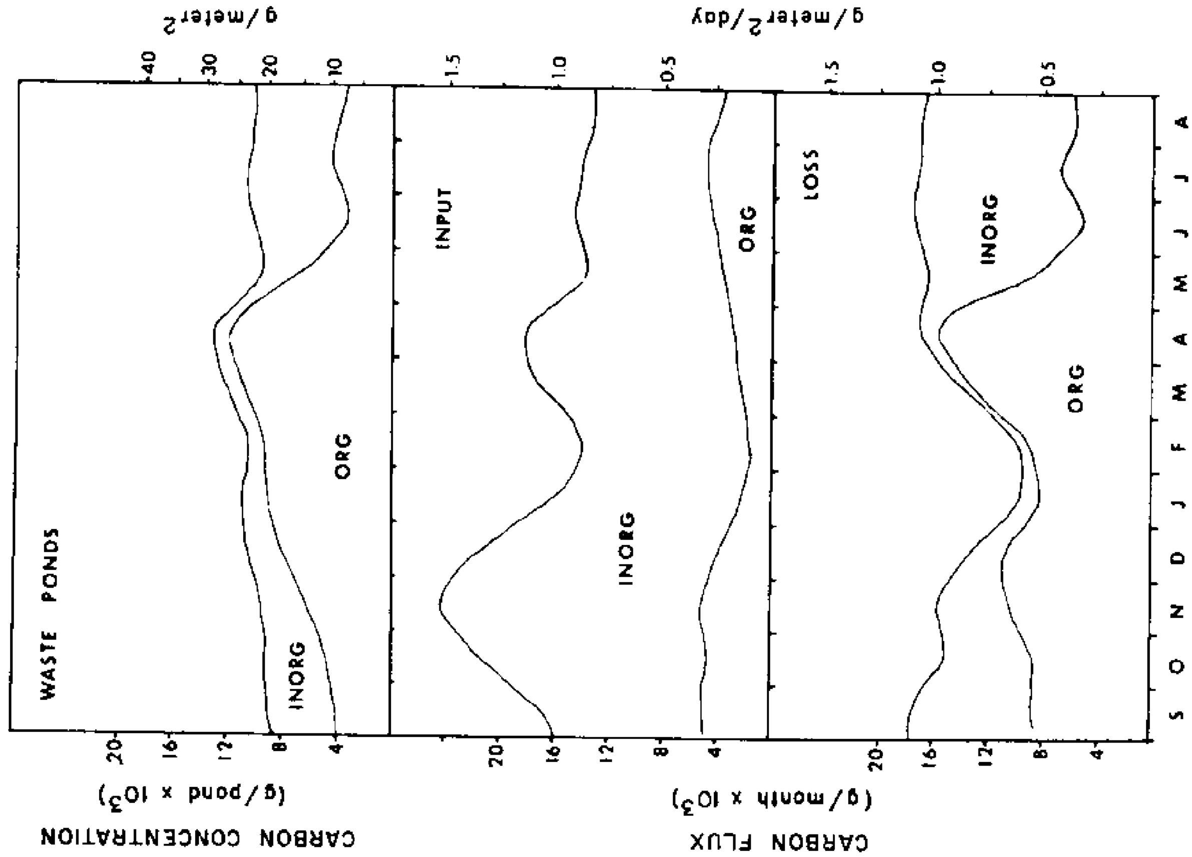
Since all ponds in their initial construction had been floored with organic rich mud from the Calico Creek marsh, there were, initially at least, contributions to ecosystem respiration from the initial organic stock. By 1971 bottom oozes were derived in part from three years of ecosystem processing. Loss on ignition estimates from 1969-1970 are given in Table 15 are highly variable, not indicating differences with time or between pond sets. Carbon content of the bottom oozes as estimated with two methods in 1971 is given in Table 16. Data are variable with higher values in waste ponds possibly comparable to the initial muds.

Simulation of Diurnal Carbon Metabolism

Day, Weiss and Odum (1971) simulated the patterns of diurnal carbon metabolism using a highly aggregated model given in Figure 36. The simulation curves, one of which is Figure 37, resembles observed graphs in Figures 27-28 and 30.

Phosphorus

As intended, high levels of phosphorus were pumped into the waste ponds compared to control ponds. Regular analyses made by W. Woods are given in Figure 38, and analyses of components by E.J. Kuenzler and H. McKellar in Figure 39. High but variable levels of dissolved phosphorus and particulate phosphorus were maintained in the waste ponds whereas very low levels of dissolved phosphorus (0.03 ug-atom/l) were maintained in the control ponds. From February - July 1970, there was 19 ug-atom/l inflowing into the waste ponds but only 3.2 ug-atom/l maintained in the pond waters, the rest presumably going to littoral and bottom uptake.



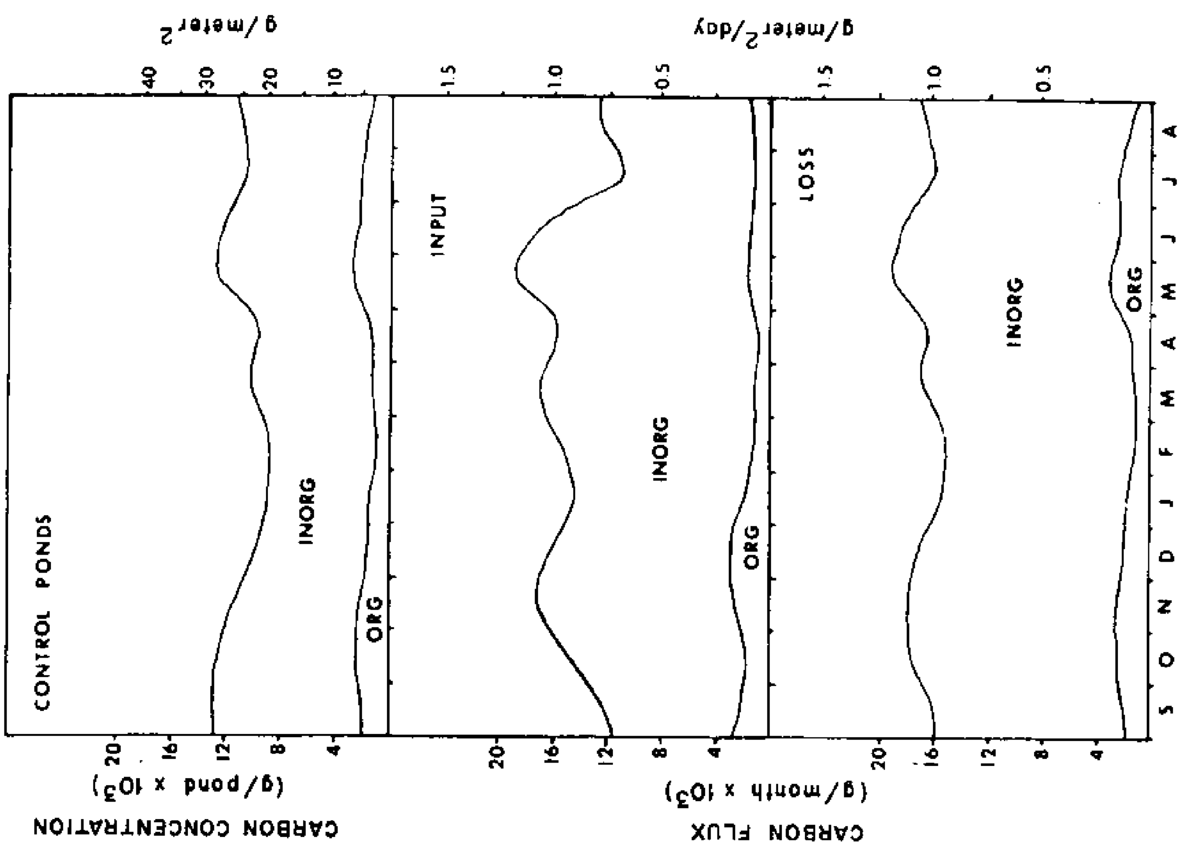


Figure 33. Annual carbon budget in 1970-1971 showing inflow, outflow and concentrations. (a) Control ponds; (b) waste ponds.

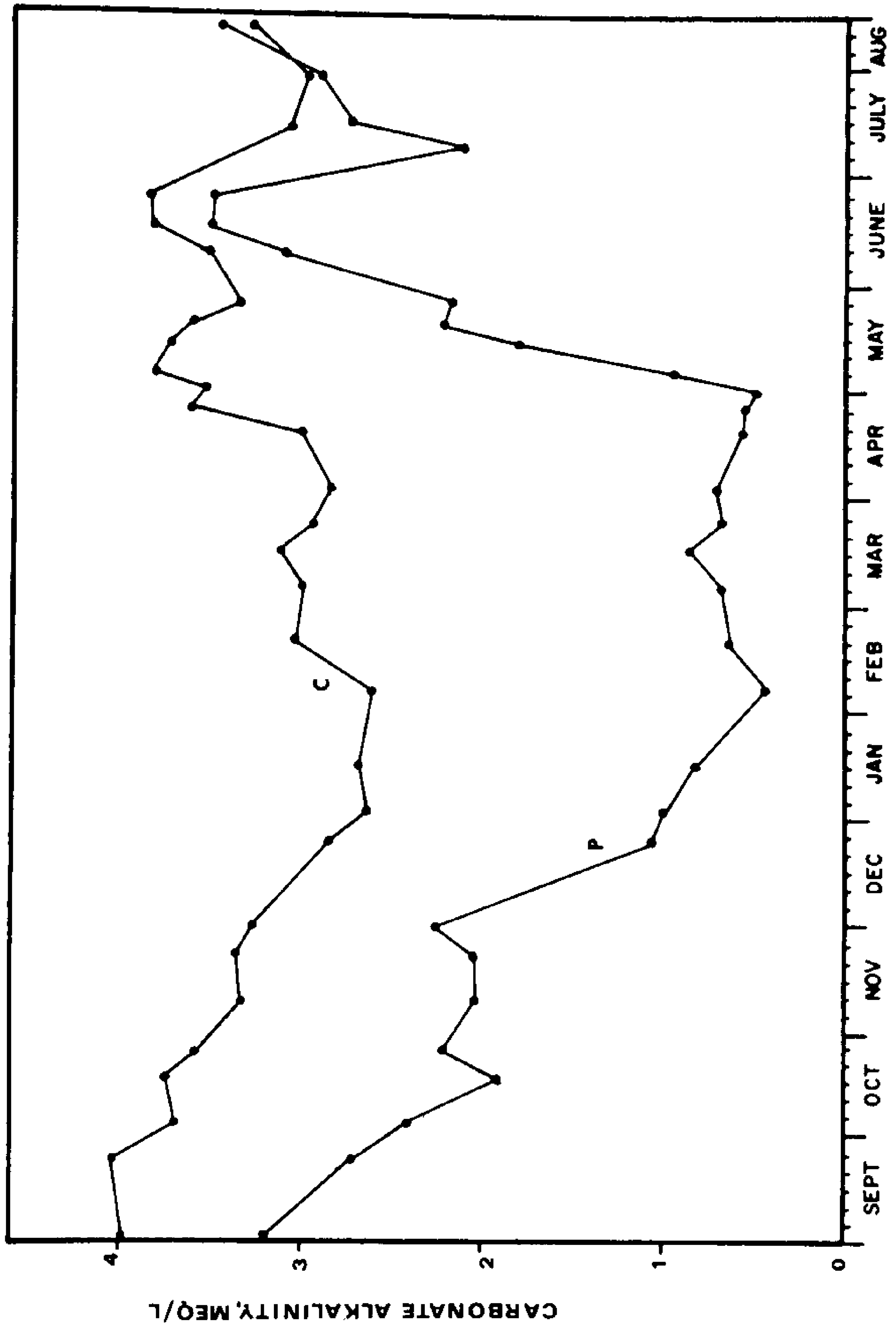


Figure 34. Annual record of carbonate alkalinity in control ponds (C) and waste ponds (P).

Table 14. Radioactive carbon measurements of productivity in containers in summer 1969 (by M. Adams and W. Woods). (a) Surfaces at various depths, g $C/m^2/hr$; (b) plankton bottles, g $C/m^3/hr$.

(a)

Depth in cm:	Benthos 15	25	35	50	Mean	Slides 15	50	Mean
Pond:								
C - 2	.17	.27	.16	.12	.18	.17	.09	.13
C - 3	.21	.17	.16	.16	.18	.17	.08	.13
P - 1	.43	.29	.18	.29	.34	.73	.71	.72
P - 2	.18	.22	.17	.07	.147	.111	.09	.10

(b)

Pond	Depth of Incubation, cm 15	30	50	Mean
C - 2	0.75	0.35	0.06	0.39
C - 3	0.71	0.42	0.07	0.40
P - 1	1.88	1.53	0.96	1.46
P - 2	2.22	1.40	0.58	1.40

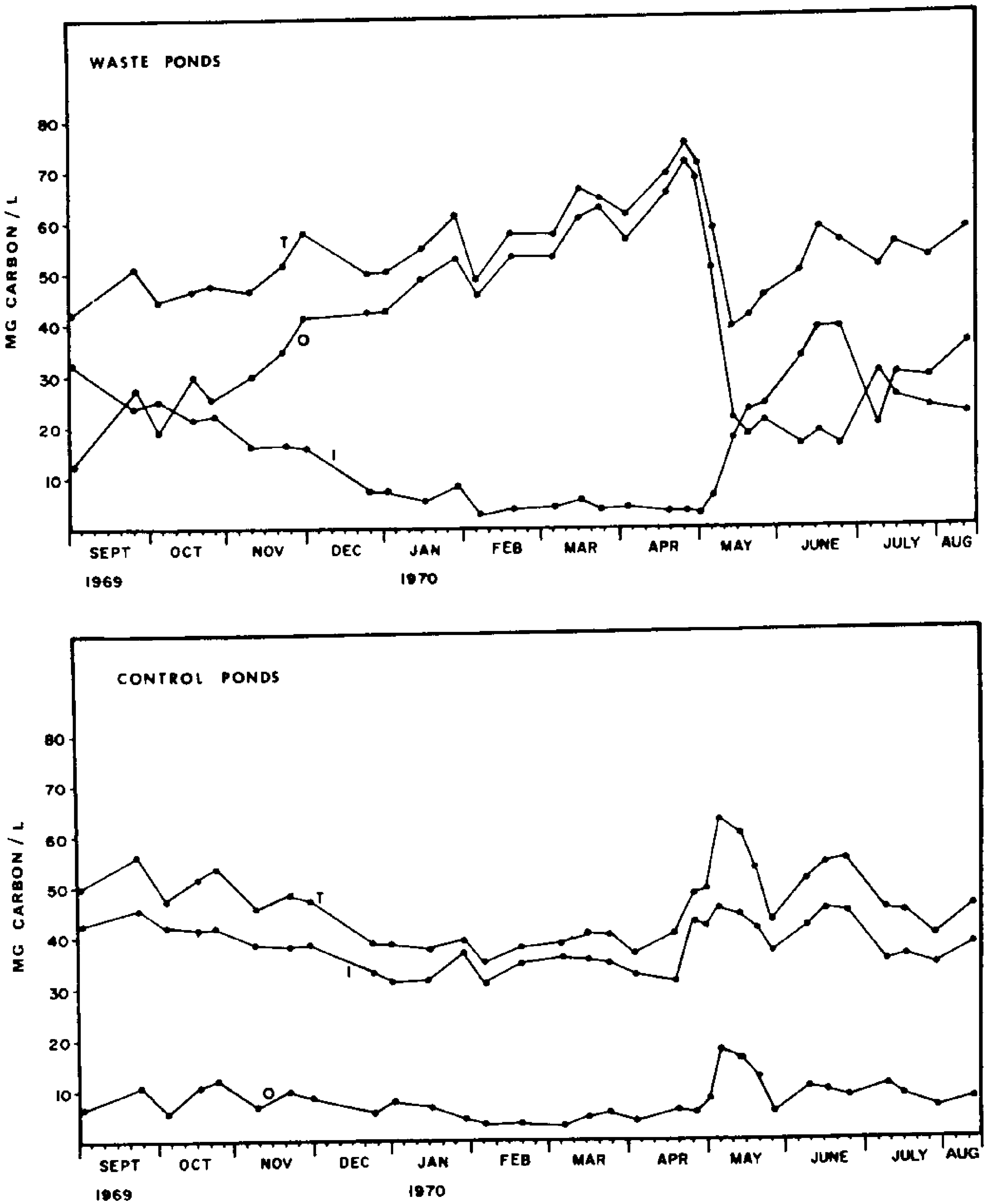


Figure 35. Annual record of dissolved and microparticulate carbon (less than 200 microns). Total organic carbon (T), organic carbon (O), and inorganic carbon (I).

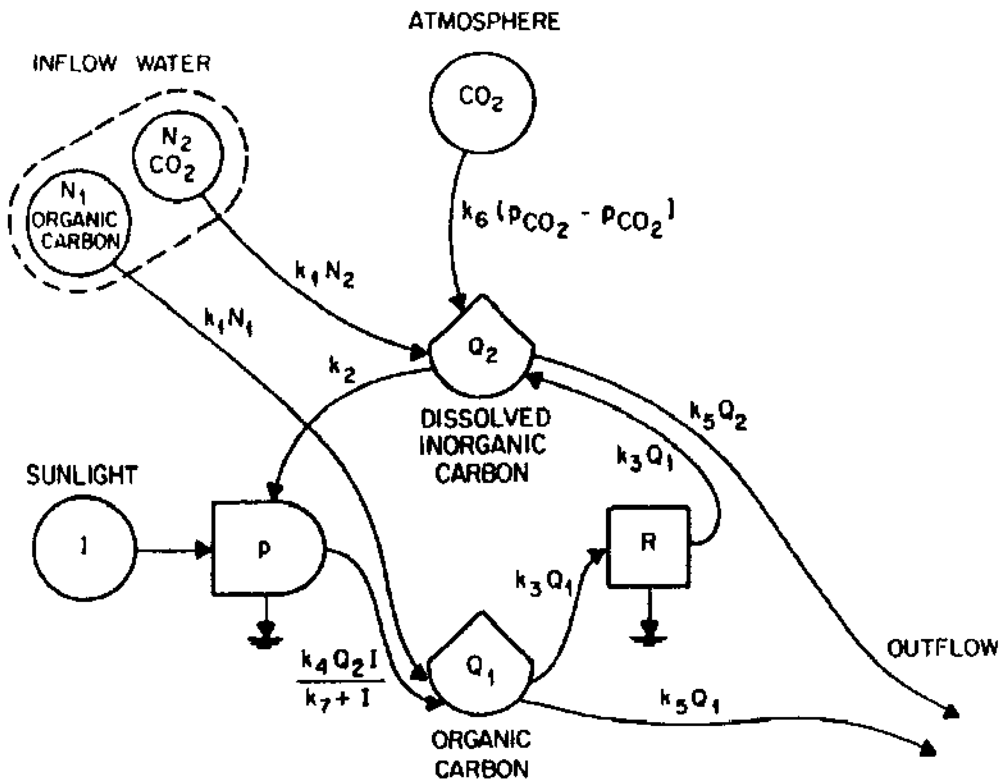
Table 15. Loss on ignition in upper one cm of sediments, from peripheral zones g/m^2.

Pond	Nov. 8, 1969	Jan. 30, 1970	Mar. 7, 1970	April 4, 1970	May 2, 1970	May 28, 1970
C-1	291	393	334	275	342	205
C-1	606	318	220	114	397	359
C-2	287	348	275	110	374	325
C-2	---	252	385	185	460	291
C-3	488	239	338	181	366	225
C-3	318	---	330	83	291	204
P-1	149	324	346	228	307	340
P-2	184	282	287	212	381	374
P-2	216	231	185	209	256	279
P-3	222	371	256	142	366	156
P-3	287	308	138	708	405	277

Table 16. Carbon determinations on bottom ooze collected from the center of the ponds, June 22-24, 1971.

Description	Chemical oxygen demand mg/g	Loss on ignition mg/g
Old Marsh Mud from ponds bottom (below recent ooze)*	68.8	103.7
	135.4	175.8
Control Ponds		
C-1	51.5	183.0
	13.7	25.9
	30.5	64.7
C-2	46.6	69.5
	10.7	21.3
	8.4	19.8
	71.5	123.5
	5.7	15.6
C-3	38.6	57.7
	80.0	98.8
	35.7	65.8
Mean		
Waste Ponds		
P-1	117.0	146.2
P-2	115.8	150.7
	91.5	120.6
	96.5	109.6
P-3	123.4	139.6
	96.7	89.4
	73.1	71.5
Mean		

*Three years after this mud was used to form a pond bottom.



$$Q_1 = \frac{k_4Q_2I}{k_7+I} - k_3Q_1 - k_5Q_1 + k_1N_1$$

$$Q_2 = k_1N_2 + k_6(p_{CO_2} - p_{CO_2}) + k_3Q_1 - k_5Q_2 - \frac{k_4Q_2I}{k_7+I}$$

Figure 36. Aggregated model of the main flows affesting diurnal changes in carbon; P, gross photosynthesis; R, ecosystem respiration, day and night (Day, Weiss and Odum, 1971; Day, 1971).

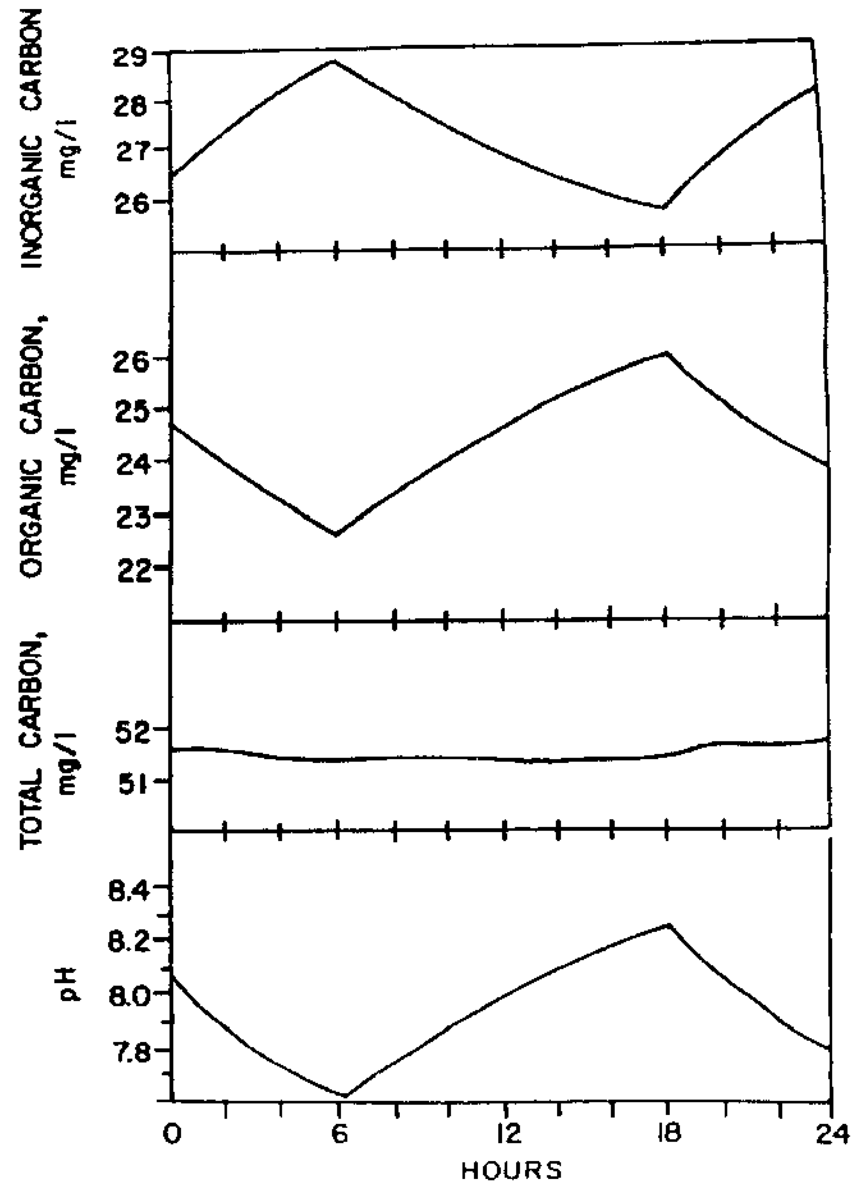


Figure 37. Diurnal variations in carbon fractions obtained from simulating carbon model in Figure 37 (Day, 1971).

McKellar and Kuenzler studied phosphorus dynamics by measuring rates of uptake and release of radioactive phosphorus by plankton (Figure 40) from which rates of turnover were calculated (Figure 39, Tables 17-18). The control ponds with lower levels of phosphorus kept the phosphorus bound in living particulate fraction, turning phosphorus over rapidly. The waste ponds with large pools of available phosphorus (25 ug-atom/l in summer) had lower phosphorus turnover rates. In winter with the development of the massive Monodus bloom, dissolved phosphorus fractions were reduced to 10 ug-atom/l. More phosphorus was being stored in cells in the particulate fraction with an increase in turnover rate.

McKellar (1971b) simulated diurnal phosphorus dynamics with an analog computer model, which is given as an energy language diagram with differential equations in Figure 41 and was calibrated with data in Table 19. Simulation results in Figure 42 compare reasonably with observed data in diurnal studies in Figure 43, suggesting that the overview represented by the pathways in Figure 41 is consistent with measurements.

Nitrogen

Waste ponds received much higher levels of nitrogen in the inflowing waters than received by the control ponds. Analyses of nitrogen inflow in Figure 45 and 46 were given by Raps(1971). Note the large decrease in all fractions in September 1971 when several pumps stopped.

Levels of nitrogen in various forms during the period of self-organization and study 1968-1971 were given by W. Woods (Figure 44) and for 1971 by C. Weiss and M. Raps (Raps, 1973) (Figures 45-46). Large pulses in particulate nitrogen and large dips in ammonium, nitrate, and nitrite tend to reflect periodic phytoplankton blooms. For most of the year, nitrate, nitrite and ammonium were below 5 ug-atom/l even in the waste ponds. Nitrogen was erratic in waste ponds and relatively stable in control ponds.

Nitrogen was also added by nitrogen-fixation (Figure 47a). Raps (1971) estimated average total nitrogen fixation potential to be about 3.4 mg N/m2/day in waste ponds and 2.0 mg nitrogen/m2/day in control ponds.

Some ammonia was lost by diffusion to the air when pH was high (Figure 47b). Raps (1971) estimated that diffusion out of waste ponds as ammonia nitrogen was 14 mg nitrogen/m2/day in summer and 3 mg nitrogen/m2/day in cooler months. Estimates for control ponds was less, 6 mg nitrogen/m2/day in summer and 1 mg nitrogen/m2/day in cooler months.

Nitrogen was outflowing through the standpipes at rates estimable from the product of concentrations in the ponds and rate of water overflow.

An eight-day diurnal study comparing morning and evening values of nitrogen fractions was carried out in July 1970 by Masarachia and

Table 17. Phosphorus concentrations, relative uptake rates of dissolved inorganic phosphorus (k), and turnover times during periods of high and low phytoplankton densities. Data for 1968-69 from Kuenzler (1971); for 1970-71 from McKellar (1971).

		P-CONCENTRATIONS µg at P l^{-1}			k	TURNOVER TIME hr.	
		DIP	PP	DOP	(% $min.^{-1}$)	DIP	PP
CONTROL PONDS							
High Density	1968-69	.05	1.4	.27	23	.073	2
	1970-71	.03	1.2	.23	23	.073	2.9
Low Density	1968-69	.04	.74	.33	1.3	1.3	24
	1970-71	.03	.30	.23	1.6	1.0	10
WASTE PONDS							
High Density	1968-69	9.2	60	2.9	.21	8	52
	1970-71	15	37	4.4	.13	13	32
Low Density	1968-69	54	3.6	7.9	.021	80	5.3
	1970-71	42	13	4.4	.04	42	13

Table 18. Rate of production of dissolved organic phosphorus in ponds (Kuenzler, 1971).

Date	Pond	Conditions		Chl - a ($mg\ m^{-3}$)	Phosphorus ($\mu gat\ l^{-1}$) DIP	DOP	PP	DOP produced (%/hr.) a	b	Turnover Time (hr.)
June 23 69	C-1	*In situ* light	27°C	32	0.20	0.84	1.60	19	15	.62
	C-2	" " "	"	24	0.08	0.02	1.08	33	18	(-)
	C-3	" " "	"	26	U	0.15	1.23	20	12	(-)
June 24 69	P-1	*In situ* light	27°C	130	47	-	5.2	55*	0.2*	-
	P-2	" " "	"	38	31	-	3.8	10*	0.1*	-
	P-3	" " "	"	32	31	-	3.8	25*	0.3*	-
Sept. 7 69	C-2	300 ft.-c.	27-28°C	10	0.13	0.17	0.62	25	19	(-)
	P-2	" "	"	21	43	26	5.3	15	1.3	1.3
Dec. 18 69	C-1	Dark	7°C	.43	.14	.31	.44	62	2.6	(-)
	C-2	"	"	2.7	.05	.23	.52	55	2.9	(-)
	C-3	"	"	15.3	.05	.16	.98	2.4	2.2	2.8
	C-2	300 ft.-c.	7°C	2.7	.05	.23	.52	32	1.5	(-)
	C-2	Poisoned	7°C	2.7	.05	.23	.52	0	0	∞
Feb. 27 70	C-1	Dark	9°C	1.1	.09	.09	.19	10	0.5	1.6
	C-2	"	"	.74	.04	.19	.14	19	1.1	1.1

Table 18 (cont.)

Date	Pond	Conditions		Chl - a ($mg\ m^{-3}$)	Phosphorus ($\mu gat\ l^{-1}$) DIP	DOP	PP	DOP produced (%/hr.) a	b	Turnover Time (hr.)
Feb. 27 70 (cont.)	P-2	Dark	9°C	410	6.6	1.2	50	20	2.1	(-)
	C-1	*In situ* light	9°C	1.1	.09	.09	.19	0	0	∞
	C-2	" " "	"	.74	.04	.19	.14	10	0.7	5.9
	P-2	" " "	"	410	6.6	1.2	50	0	0	∞
July 31 70	C-2	Dark	27°C	12	-	-	-	3.9	1.3	-
	P-2	"	"	180	36	3.7	16	0	0	∞
	C-2	600 ft.-c.	27°C	12	-	-	-	3.4	1.1	-
	P-2	"	"	180	36	3.7	16	0.5	0.01	21
	C-2	3,000 ft.-c.	27°C	12	-	-	-	4.1	1.4	-
	P-2	"	"	180	36	3.7	16	0	0	∞
	C-2	Poisoned	27°C	12	-	-	-	0	0	-
	P-2	"	"	180	36	3.7	16	0	0	∞

* calculated from DOP measurements after 12 hour incubation.

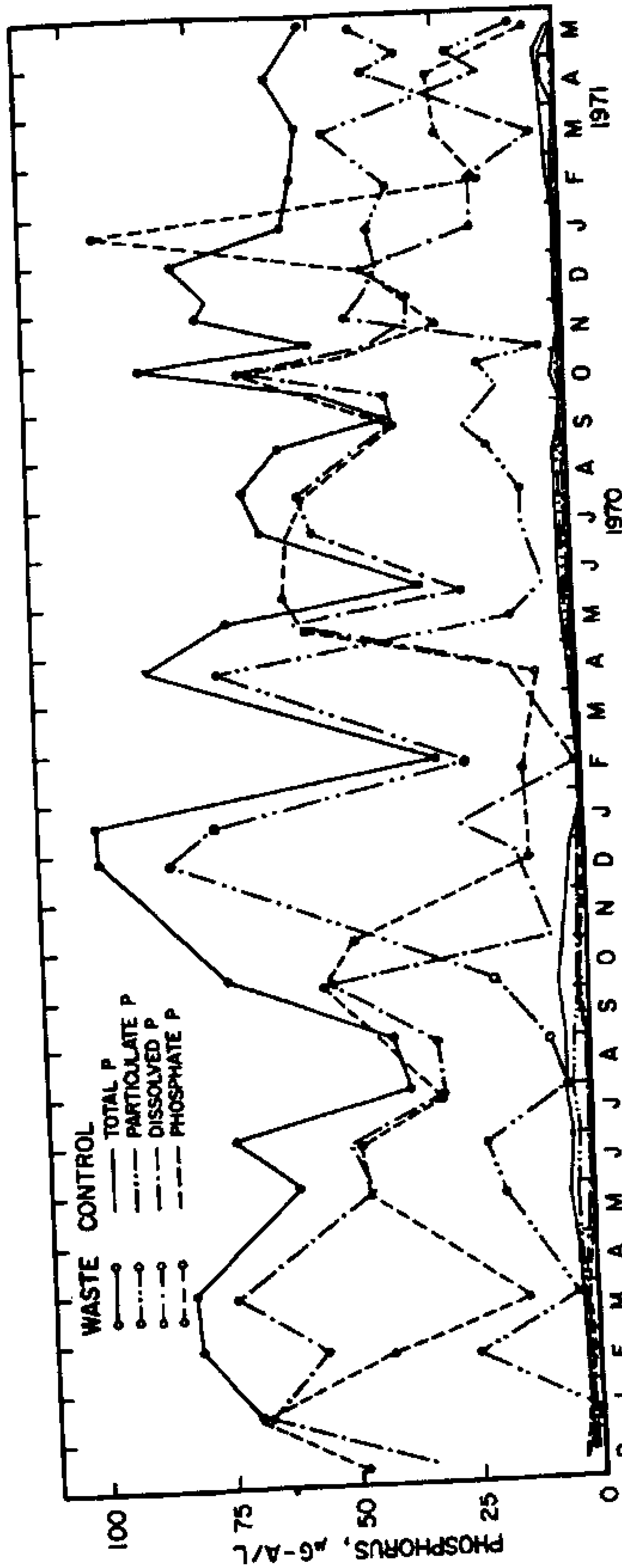


Figure 38. Annual record of phosphorus content in control ponds and waste ponds 1968-1971 (W. Woods).

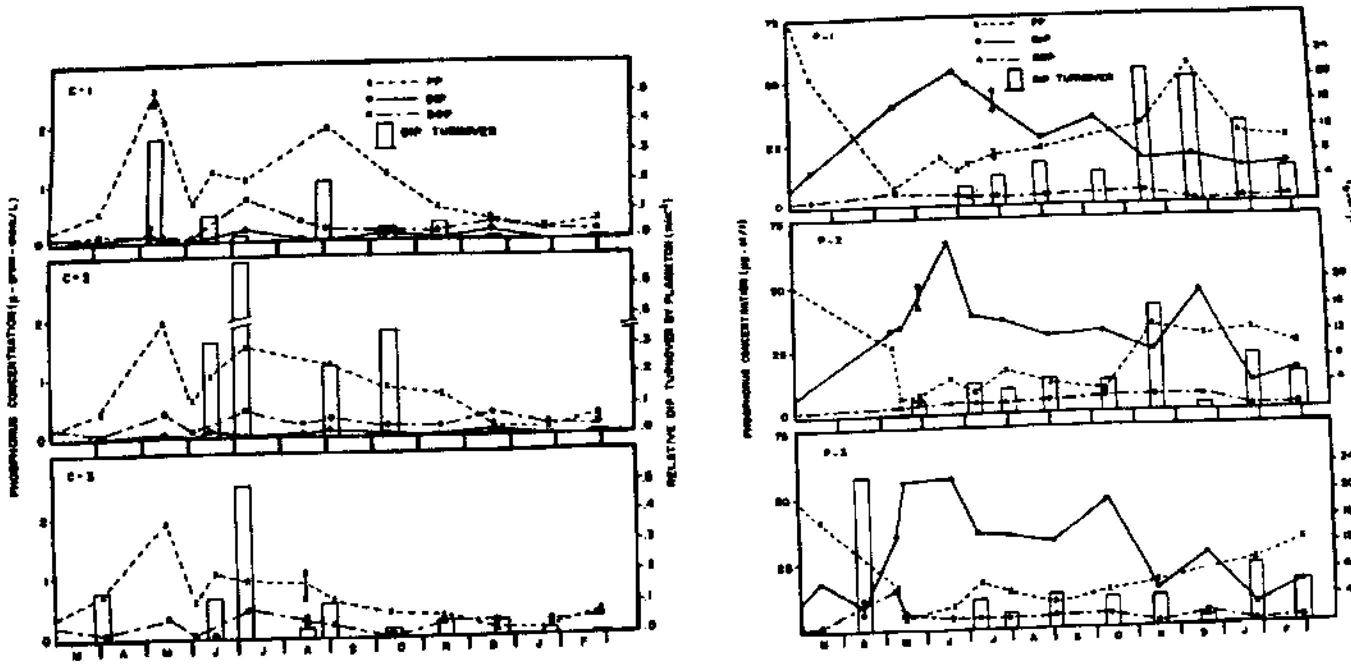


Figure 39. Seasonal variations in phosphorus fractions and uptake rates of dissolved organic phosphorus (DIP turnover) 1970-71. (a) Control ponds; (b) waste ponds.

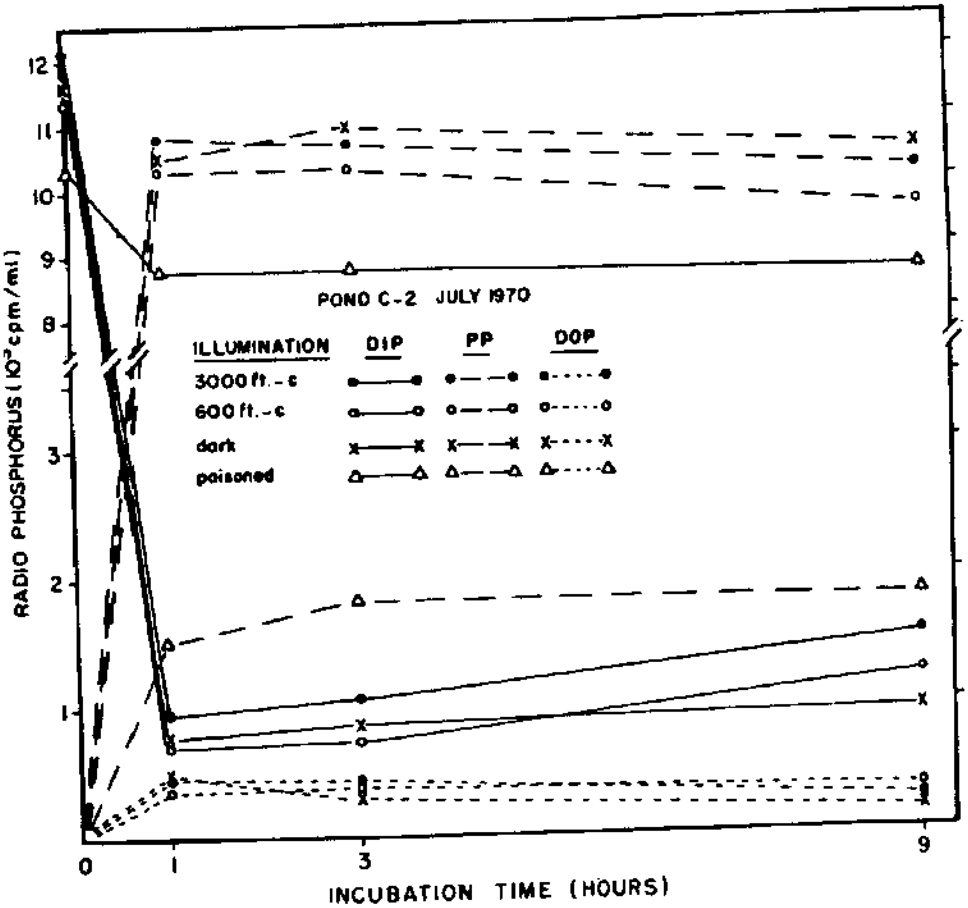


Figure 40. Uptake of radioactive phosphate by plankton at different light intensities and its return to water as radioactive organic phosphorus (Kuenzler and McKellar).

Figure 40(a) Pond C-2, July 1970

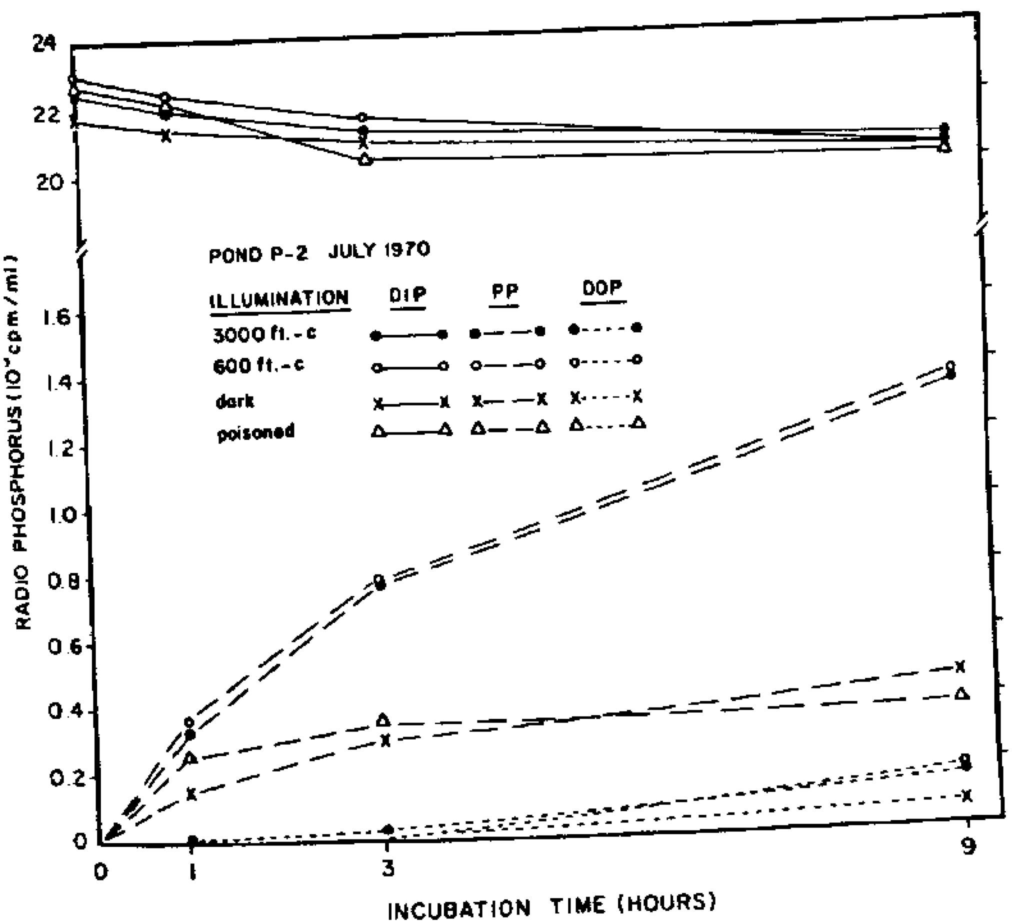


Figure 40(b) pond P-2, July, 1970

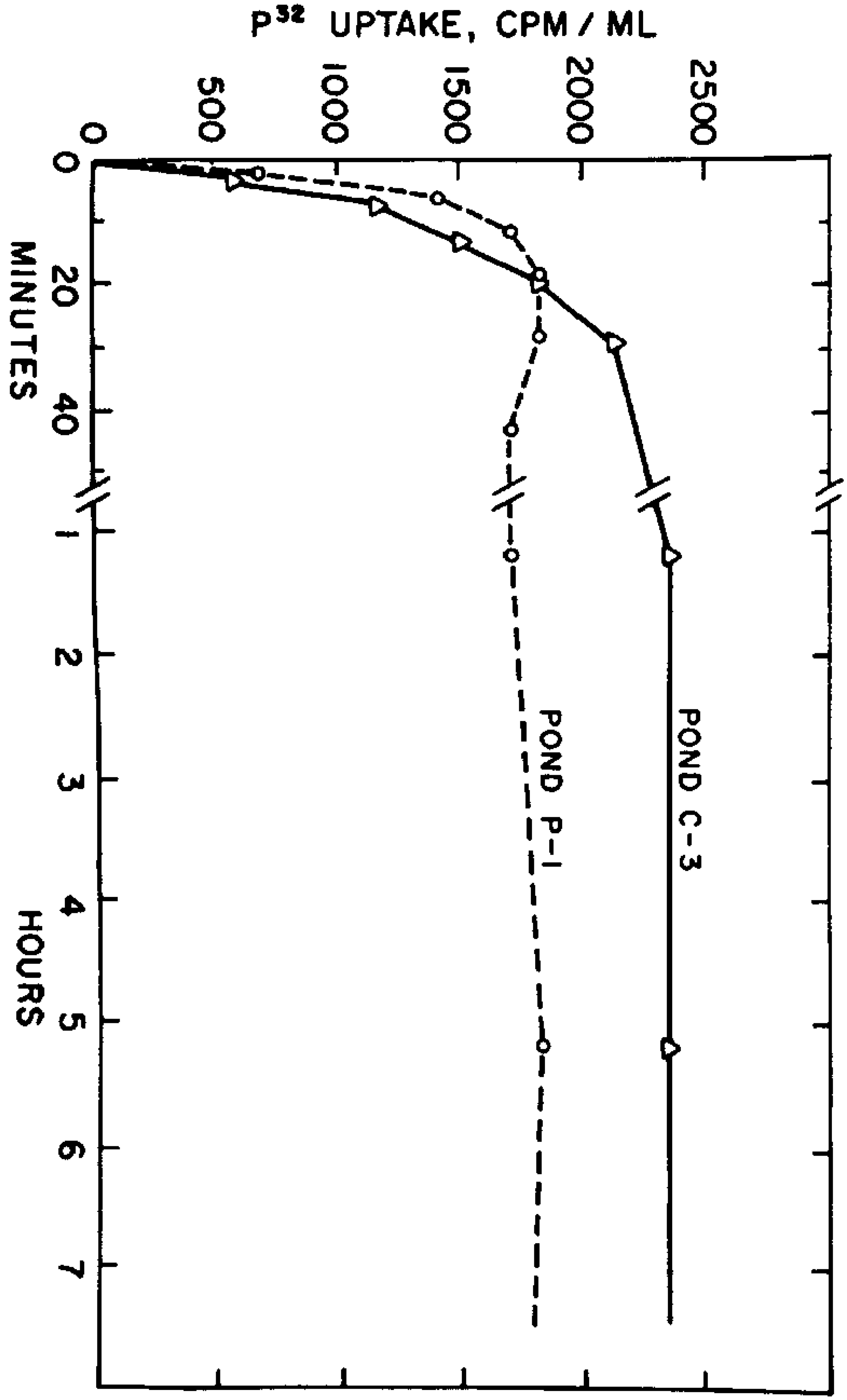


Figure 40(c) August 1968 when pond C-3 was acid

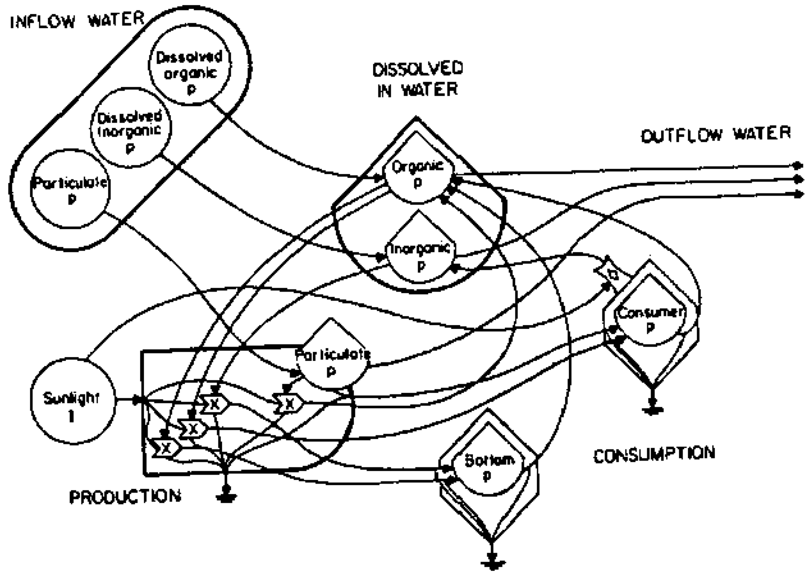


Figure 41. Simulation model of main flows affecting phosphorus (P) over day-night periods, Modified from McKellar, 1971b. Values of coefficients are in Table 19. Equations:

dQ/dt = J2 + k51*Q5 + k41*Q4 - k12*Q1 - k14*Q1 - k16*Q1

dQ2/dt = J1 + k12*Q1 + k32*Q3 - k23*Q2*I - k25*Q2 - k26*Q2

dQ3/dt = J3 + k23*Q2*I + k43*Q4 - k5*Q5 - k32*Q3 - k35*Q3*I - k34*Q3*I - k36*Q3

dQ4/dt = k14*Q1*I + k34*Q4 - k41*Q4 -k41*Q4

dQ5/dt = k25*Q2 + k35*Q3*I - k51*Q5 - k53*Q5

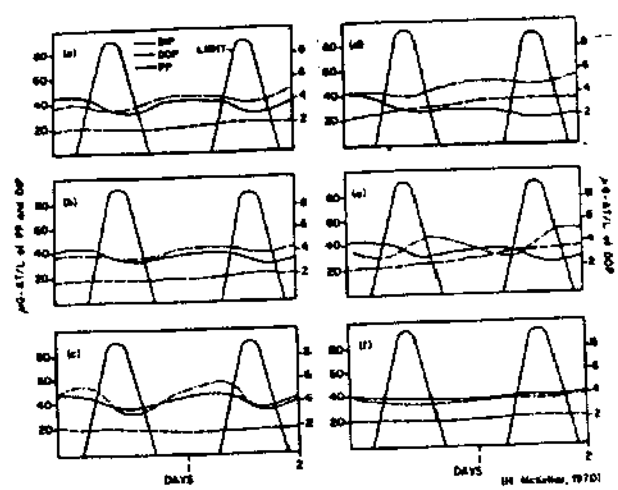


Figure 42. Simulation of diurnal phosphorus variation with model in Figure 41 (McKellar, 1971b); DIP, dissolved inorganic phosphorus; DOP, dissolved organic phosphorus; PP, particulate phosphorus. (a) model reproduction of observed fluctuations; (b) model response to nulled inflow and outflow rates; (c) model response to nulled planktonic exchanges; (d) model response to 3-fold increase in DIP uptake rate by the plankton; (e) model response to nulled consumer exchanges; (f) model response to nulled bottom exchanges.

Table 19. Phosphorus exchange rates, transfer coefficients, and observed concentrations in pond P-1, July 28-29, 1970 used to calibrate the simulation model in Figure 42 (McKellar, 1971).

Compartment	Exchange	Rate (J) µg-at/l·hr	Transfer Coefficient (K) hr^{-1}
DIP	Planktonic uptake	.27	.0067
	Bottom uptake	1.20	.0592
	Bottom release	.83	.0922
	Consumer release (day)	.24	.0261
	(night)	.14	.0152
	Inflow	.08**	.08
	Outflow	.10**	.0025
DOP	Plankton uptake	.14	.0350
	Plankton release	.13*	.0124
	Bottom uptake	.04	.0200
	Bottom release	.08	.0089
	Consumer uptake	.19	.0950
	Consumer release	.14	.0152
	Inflow	.01**	.01
	Outflow	.01**	.0025
PP	Consumer grazing	.23	.0110
	Inflow	.05**	.05
	Outflow	.05**	.0024

COMPARTMENT CONCENTRATIONS	µg-at/l·hr
DIP	43.60
PP	21.00
DOP	4.00
Consumers	9.20
Bottom	9.00

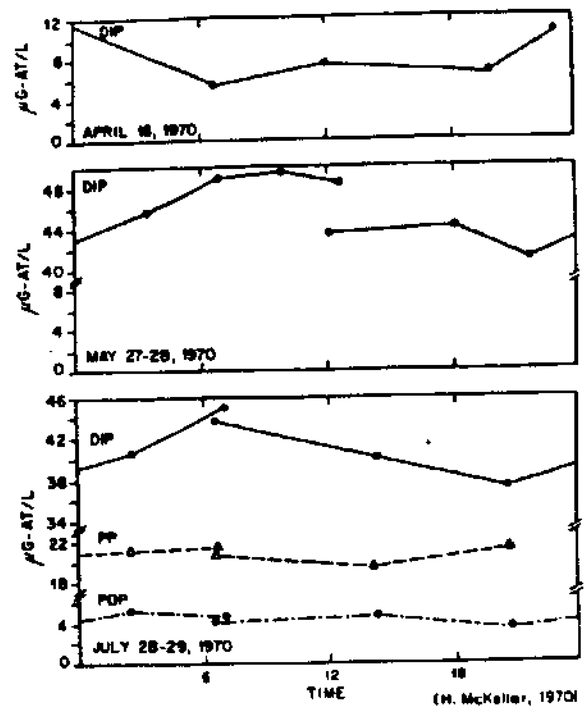


Figure 43. Diurnal records of phosphorus (McKellar, 1971b).

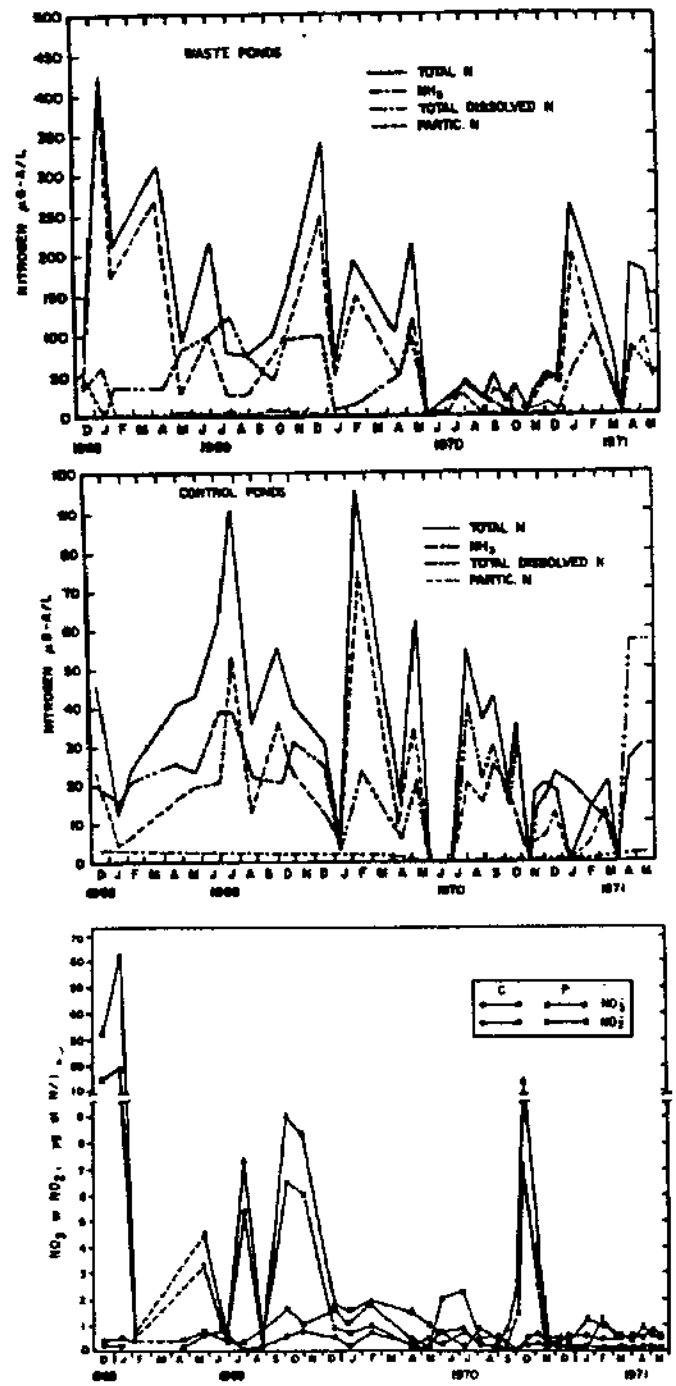


Figure 44. Record of nitrogen 1968-1971 (W. Woods). (a) Total nitrogen and fractions in waste ponds; (b) total nitrogen and fractions in control ponds; (c) nitrate and nitrite in ponds.

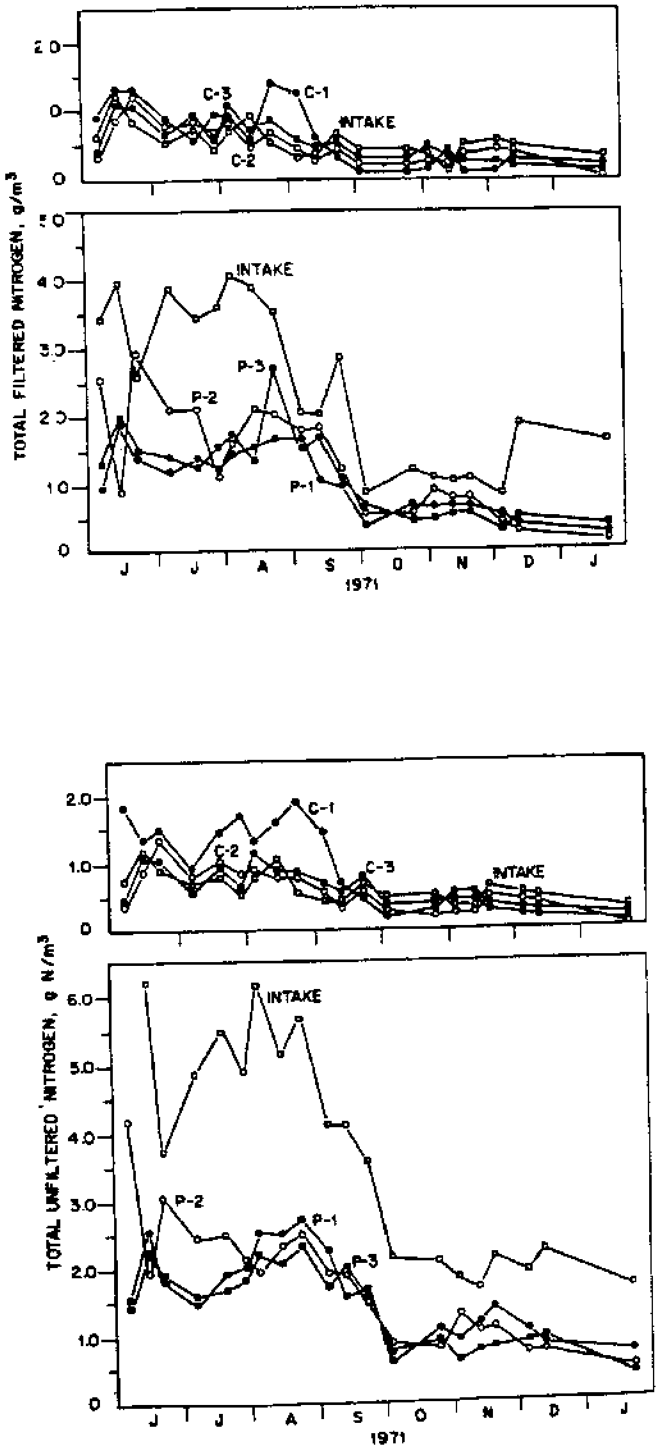


Figure 45. Total nitrogen in 1971 (Raps, 1973).

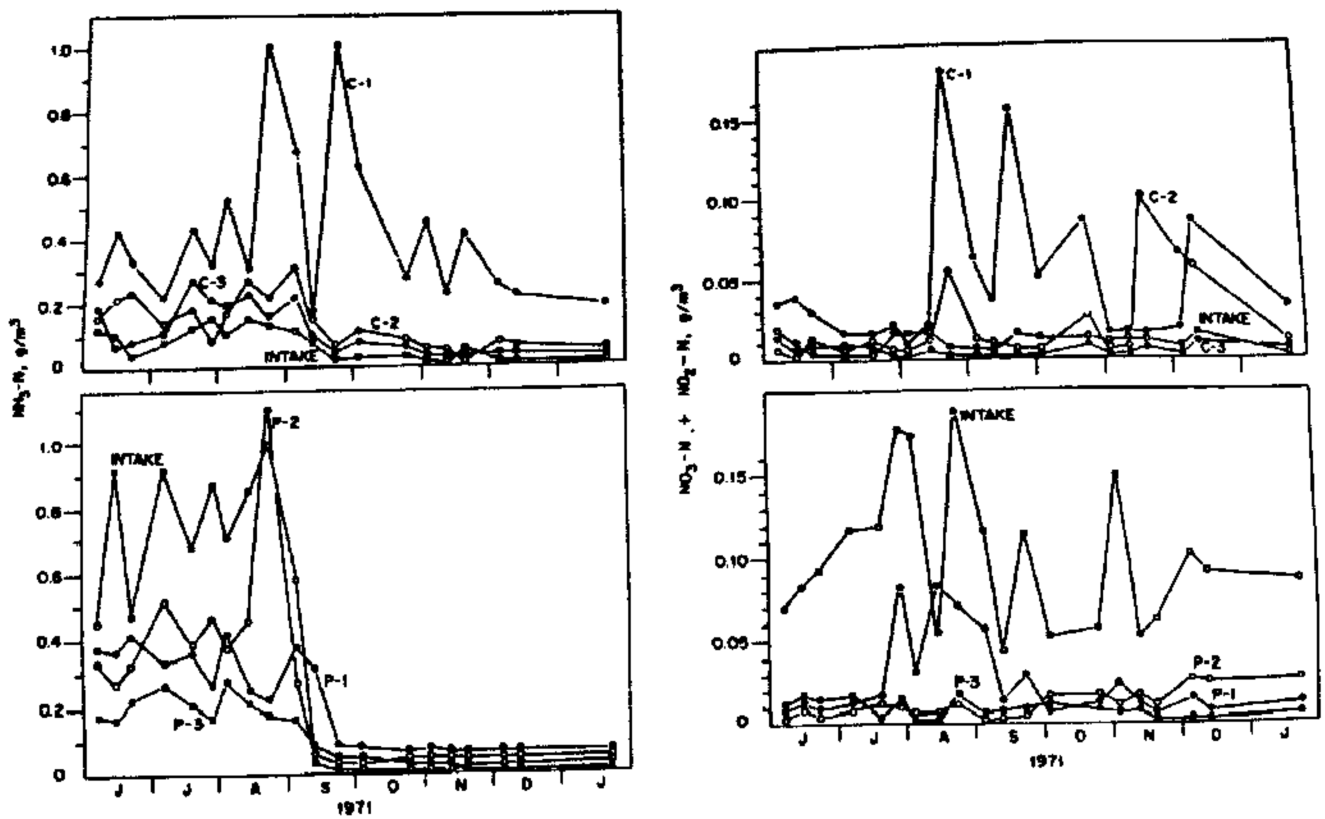


Figure 46. Nitrogen components in 1971 (Raps, 1973). (a) nitrate and nitrite; (b) ammonia.

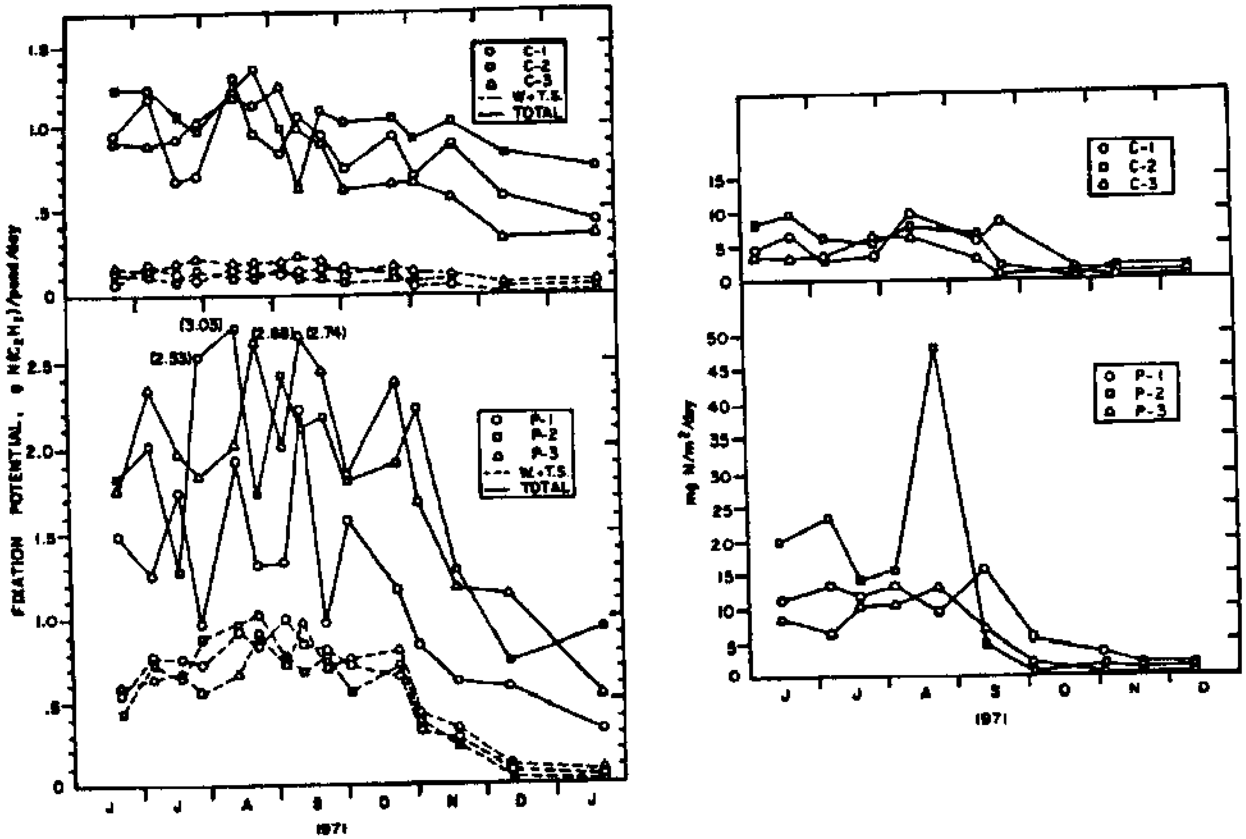


Figure 47. Gaseous exchange of nitrogen with air in 1971 (Raps, 1973). (a) Nitrogen fixation potential; (b) rate of outward diffusion of ammonia.

Weiss (Figure 48). The contributions of daytime photosynthesis and nighttime respiration show up as zig-zag increase of organic nitrogen fractions during the day and decrease at night whereas ammonia was generated at night.

Masarachia (1971) provided preliminary nitrogen budget calculations for August 1970 (Table 20). Dissolved organic nitrogen, nitrite, and ammonia concentrations were respectively, one-half, one-eighth, and one-third of the inflowing water measured in the mixing tank, whereas particulate oxygen increased from inflow to ponds as nitrogen was incorporated in biota. Control ponds were discharging about as much as gained with 15% or less going to growth of animals, benthic plants and sediments. Even so, there were substantial growths of shellfish in pond C-1 and Ruppia beds in ponds C-2 and C-3. In the waste ponds with three times as much inflowing nitrogen half was discharged the other half going into macro-growths and sediments. There was ten times as much nitrogen metabolism as in the control ponds even though the oxygen production was only two to three times larger.

Plants, Phytoplankton, and Chlorophyll

The plant producers of the ponds were the phytoplankton, benthic micro-algae, heavy beds of Ruppia that developed in ponds C-2 and C-3 in the second and third years (Figure 8), and the Spartina alterniflora marsh grass that spread around the shore by the second and third year.

The phytoplankton that developed in the ponds were monitored by E.J. Kuenzler and P.H. Campbell (Campbell, 1971, 1973). The dominants are shown in Figures 49-51 and records of these populations are given in Figures 52-53. The phytoplankton that dominated were usually different in waste ponds from control ponds, although some species such as Nitzschia closterium bloomed in both.

The waste ponds were rich in nutrients compared to control ponds and many species had more than a thousand cells per milliliter. In the waste ponds a heavy winter bloom with large biomass predominantly Monodus guttula, developed during each winter in which the sewage pumping regime was maintained. In contrast, a high diversity, lower biomass phytoplankton prevailed in the warmer seasons with a sudden switch in regimes in the first week of May in 1969, 1970 and 1971. The most important species were Chaetoceros muelleri, Prorocentrum Minimum, Nitzschia closterium, Oocystis parva, Hemiselmis virescens, Oocystis parva and Chroomonas amphioxeia. In the waste ponds in 1970, the yearly average was 2 million cells per milliliter. Because of the Monodus, phytoplankton biomass in winter (Table 21) was 7.6 g/m2, 20 times that in summer or that in the control ponds.

After pumping started, the most important species in control ponds were Monallantus stichococcoides, Nannochloris atomus, Cyclotella caspia, Nitschia proxima, Monochrysis lutheri, and Hemiselmis virescens. In control ponds in 1970 the yearly average was 30,000 cells per milliliter with blooms ten times that. Biomass

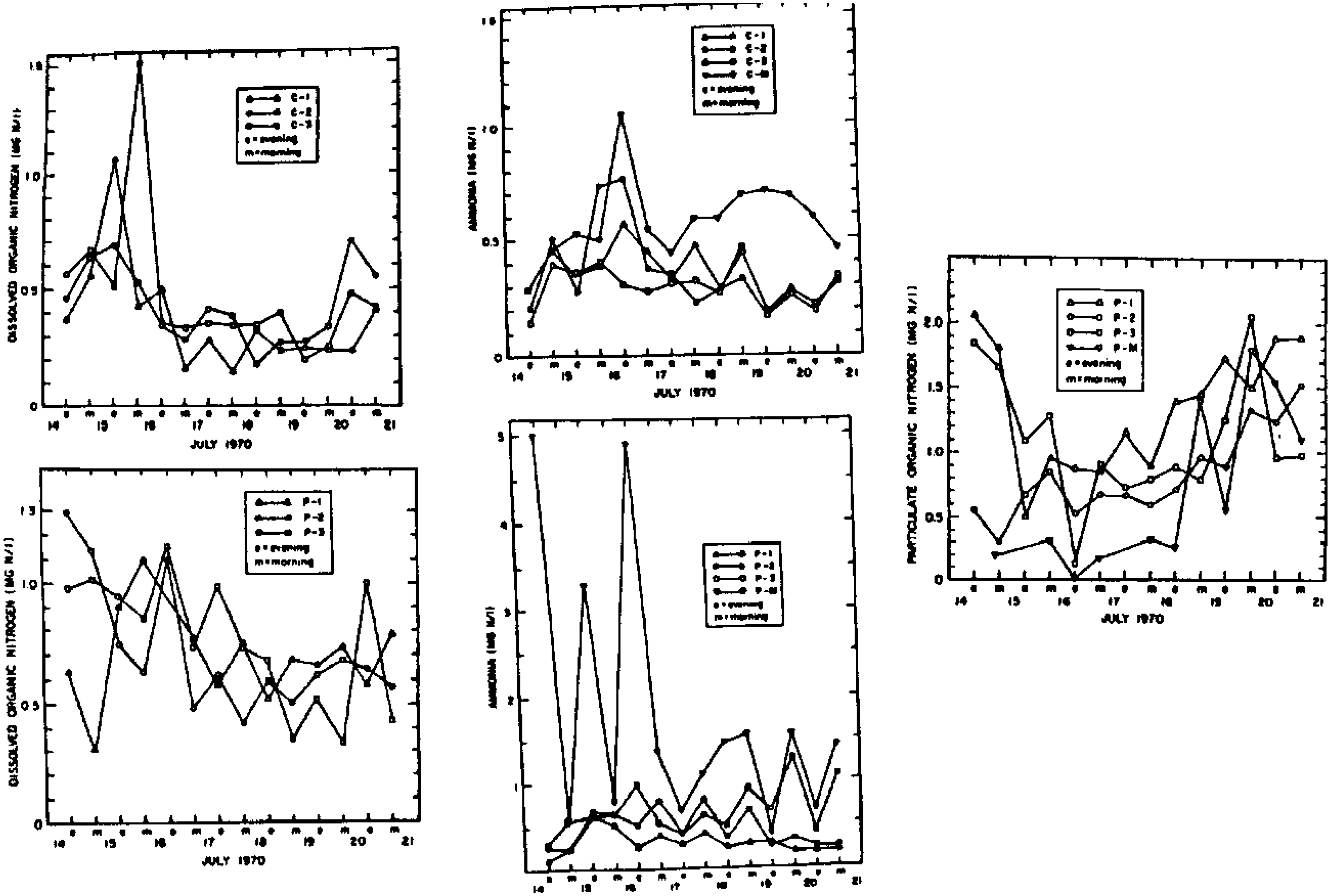


Figure 48. Eight day study of diurnal nitrogen changes (Masarachia, 1971). (a) Dissolved organic nitrogen; (b) ammonia; (c) particulate organic nitrogen.

Table 20. Nitrogen budgets in ponds in August, 1970 (modified from Masarachia, 1971), g nitrogen/day.

Pond	Inflow (I)	Outflow(O)	Loss to animals (I-O) and sediments
P-1	46	27	19
P-2	46	23	23
P-3	40	25	15
C-1	15	13	2
C-2	11	10	1
C-3	10	8	2

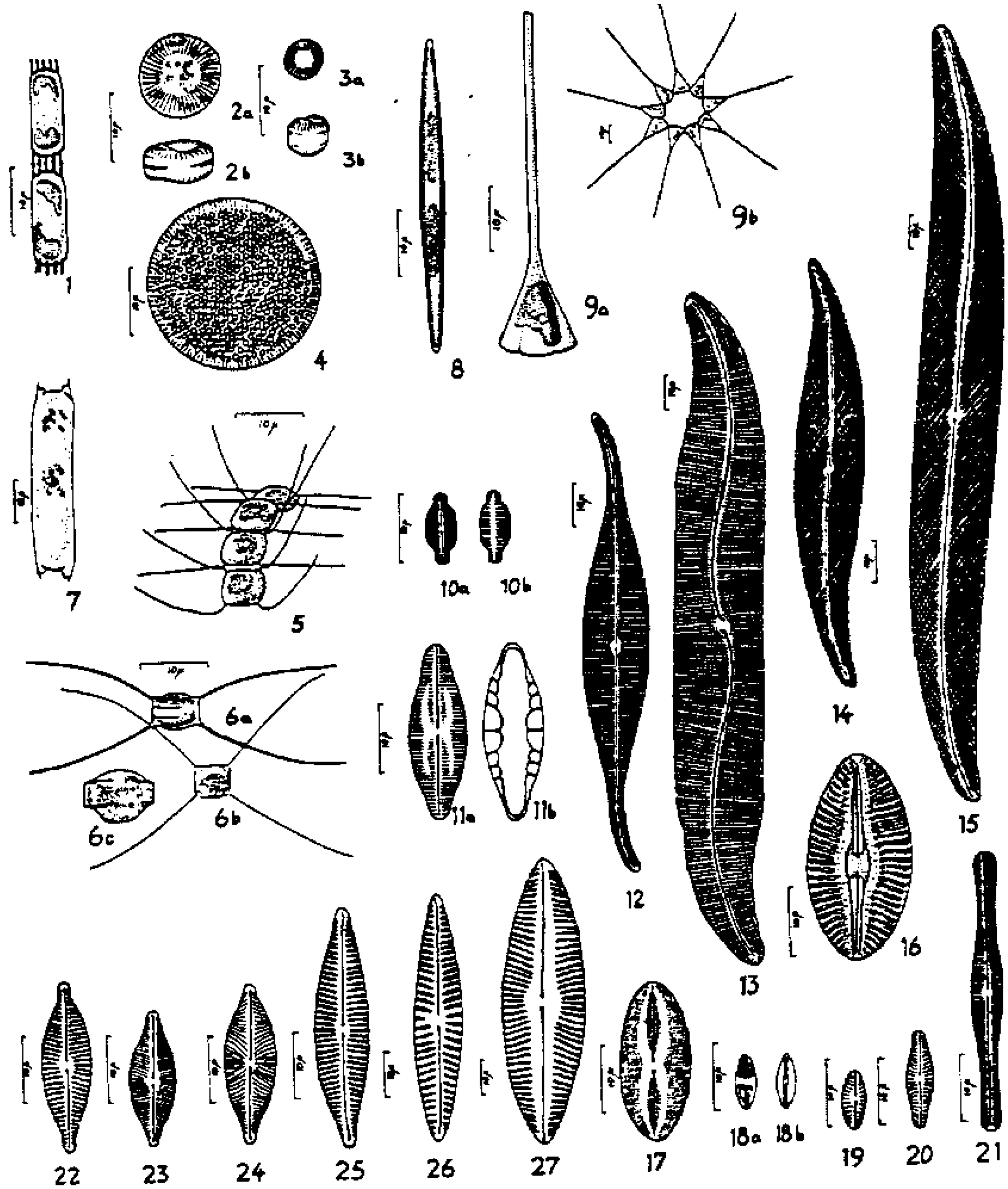


Figure 49. Diagram of principal phytoplankton dominants (Campbell, 1971): 1. Skeletonema costatum (Grev.) Cl.; 2. Cyclotella striata var. ambigua Grun.; 3. Cyclotella caspia Grun.; 4. Coscinodiscus sublineatus Grun.; 5. Chaetoceros debilis Cl.; 6. Chaetoceros muellieri Lemm., 6b thinly silicified cell from October, 6c resting spore.; 7. Cerataulina bergoni Per.; 8. Synedra tabulata (Ag.) Kutz.; 9. Asterionella japonica Cl., 9b colony.; 10 Acnanthes orientalis Hust.; 11. Mastogloia pumila (Grun.) Cl.; (Ehr.) Rabh.; 12 Gyrosigma fasciola (Ehr;.) Griff. & Henfr.; 13 Gyrosigma balticum (Ehr.) Rabh.; 14. Pleurosigma salinarum Grun.; 15. Navicula pygmaea Kutz.; 18. Navicula arvensis Hust.; 19. Navicula cf. muralis f. agrestis (Hust.) Lund.; 20 Navicula cf. friska Carter; 21. Navicula rogallii Hust.; 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.

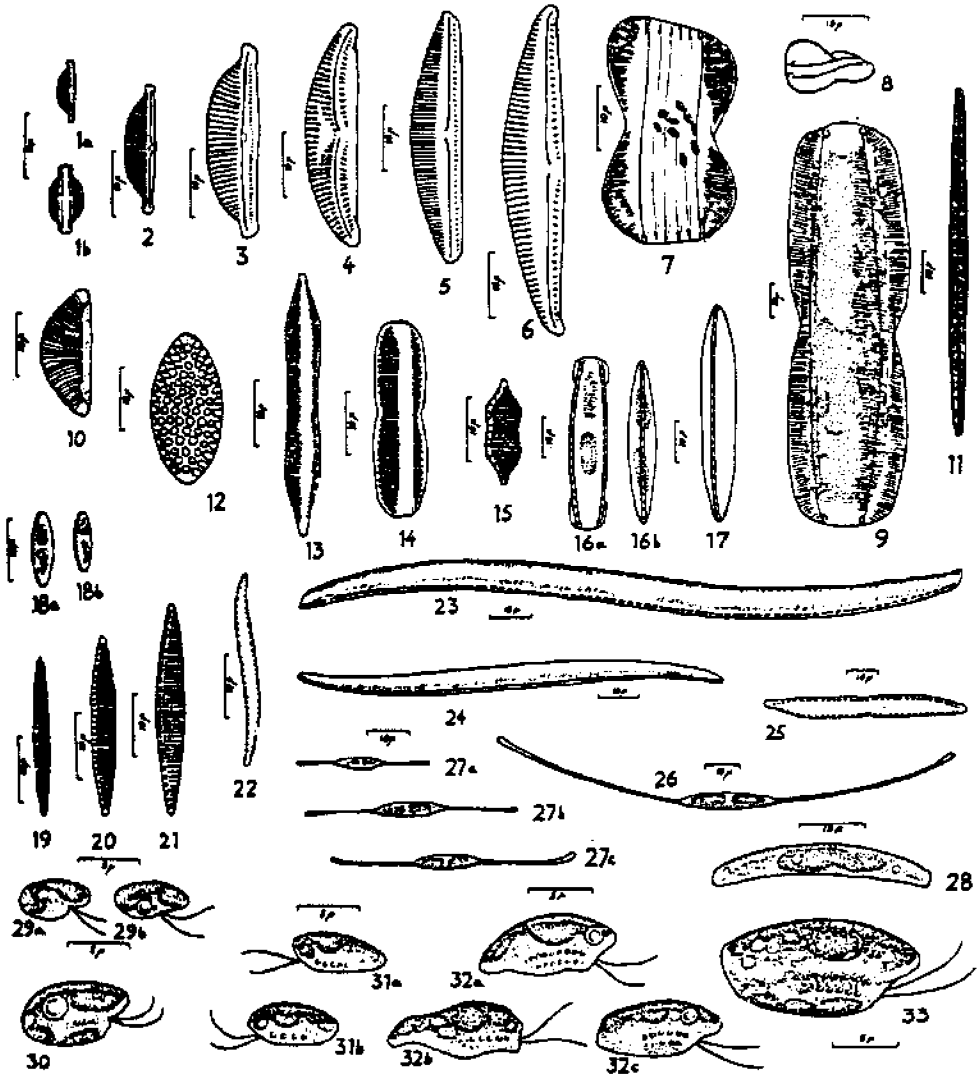


Figure 50. Diagram of principal phytoplankton dominants (Campbell, 1971). 1. Amphora cf. delicatissima Krasske; 2. Amphora cf. tumida Hust.; 3. Amphora granulata Greg.; 4. Amphora ovalis var affinis Grun.; 5. Amphora angusta Greg.; 6. Amphora angusta var. ventricosa Greg.; 7. Amphiprora paludosa var. duplex Donk.; 8. Amphiprora paludosa vr. hyalina Eulenst.; 9. Tropidoneis lepidoptera Greg.; 10. Rhopalodia musculus var. producta Grun.; 11 Bacillaria paradoxa ;Gmelin; 12 Nitschia compressa (Ball.) Boyer; 13. Nitzschia apiculata (Greg.) Grun.; 14. Nitzschia hybridaeformis Hust.; 15. Nitzschia apiculata (Greg.) Grun.; 14 Nitzschia hybridaeformis Hust.; 15. Nitzschia panduriformis var. minor Grun.; 16. Nitzschia spathulata Breb.; 17. Nitzschia cf. angularis Sm.; 18. Nitzschia cf. communis var. hyalina Lund; 19. Nitzschia proxima Hust.; 20. Nitzschia frustulum (Kutz.) Grun.; 21. Nitzschia grossestriata Hust.; 22. Nitzschia cf. serpenticula Cholnoky; 23. Nitzschia sigma (Kutz.) Sm.; 24. Nitzschia sigma var. rigidula Grun.; 25. Nitzschia obtusa var. scalpelliformis Grun.; 26. Nitzschia longissima (Breb.) Ralfs.; 27 Nitzschia closterium W. Sm.; 28. Genus ? species. ?; 29. Hemiselmis virescens Droop; 30. Chroomonas diplococca Butcher; 31. Chroomonas minuta var. apyrenoidosa (Hulburt); 32. Chroomonas amphioxela (Conr. & Kuff.) Butcher; 33. Cryptomonas pseudobaltica Butcher.

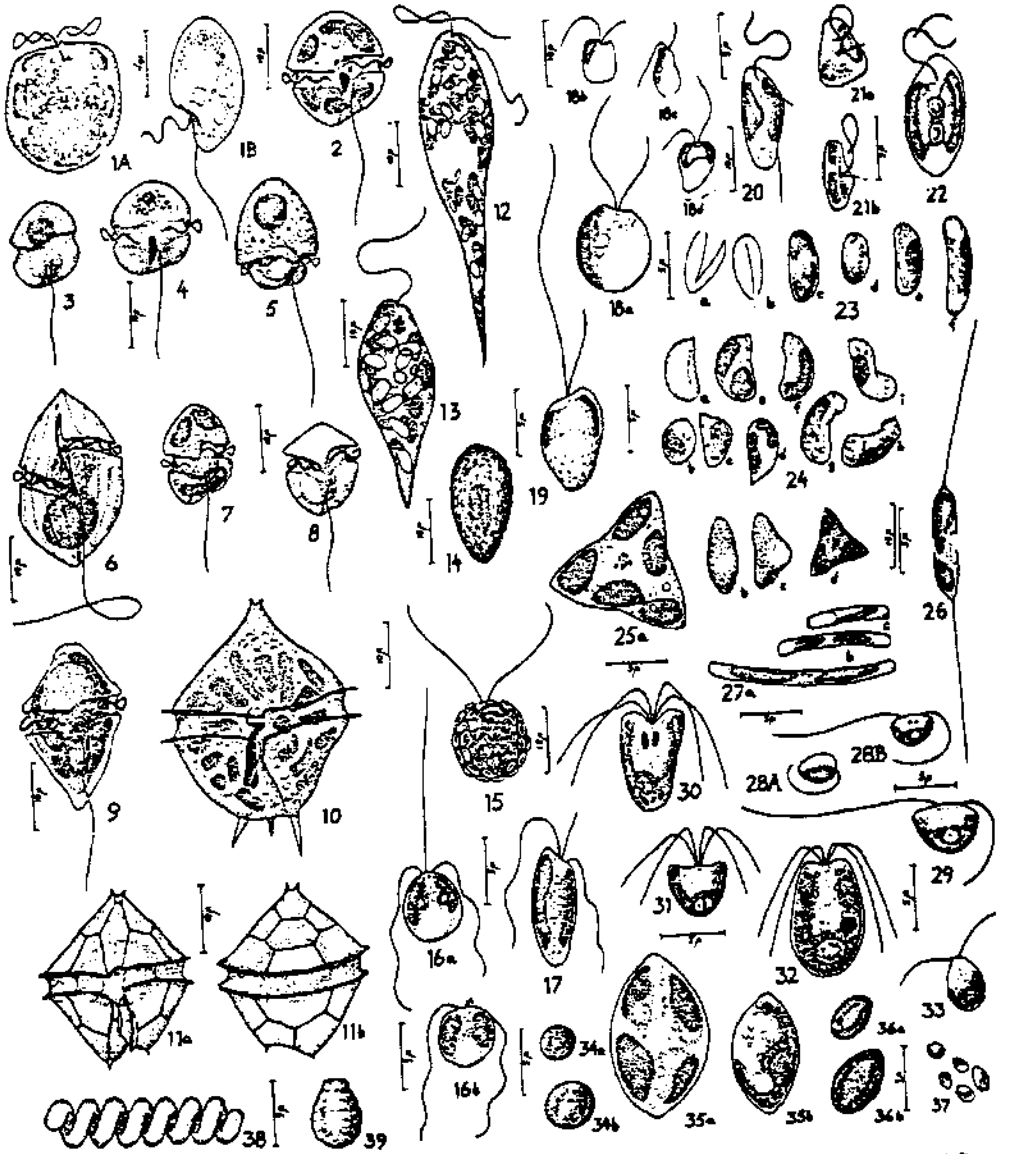


Figure 51. Diagram of principal phytoplankton dominants (Campbell, 1971): 1A. Prorocentrum minimum (Pav.) Schiller; 1B. Oxyrrhis marina Duj.; 2. Gymnodinium danicans Campbell; 3. Gymnodinium sp.; 4. Gymnodinium roseostigma Campbell; 5. Katodinium asymmetricum (Massart) Fott; 6. Gyrodinium dominans Hulburt; 7. Gyrodinium estuariale Hulburt; 8. Gyrodinium metum Hulburt; 9. Heterocapsa triquetra (Ehr.) Stein; 10. Peridinium aciculiferum Lemm.; 11. Peridinium achromaticum Lev.; 12. Eutreptia cf. lanowii Steuer; 13. Euglena aff. proxima Dangeard; 14. Trachelomonas ? obovata Stobes; 15. Hymenomonas carterae (Braar. & Fag.) Braarud; 16. Chrysochromulina sp. 16a: haptonema extended.; 17. Prymnesium parvum Carter; 18. Ochromonas ? minuscula Conrad; 19. Ochromonas ? vallesiaca Skuja; 20. Pavlova gyrans Butcher; 21 Monochrysis lutheri Droop; 22. Nephrochloris salina Carter; 23. Monallantus stichococcoides Pascher, 23a, b: cast off walls.; 24. Monodus aff. guttula Pascher, 24a: cast off wall. 24d-f: from autumn, 24 g-i: from winter.; 25. Goniochloris pulschra Pascher 25b-d: cell wall surfaces.; 26. Centritractus aff. belonophorus Lemm.; 27. Genus ? species ? ; 28a. Pedinomonas minor Korsch.; 28B. Nephroselmis gilva Parke & Rayns; 29. Heteromastix pyriformis (Carter) Manton; 30. Pyramimonas plurioculata Buther; 31. Pyramimonas micron Conr. & Kuff.; 32. Tetraselmis maculata (Kylin) Butcher; 33. Chlamydomonas sp.; 34. Nannochloris atomis Butcher; 35. Oocystis parva West & West; 36. Genus ? species ?; 37. Genus ? species?; 38. Spirulina subsalsa Oersted; 39. Calycomonas ovalis Wulff.

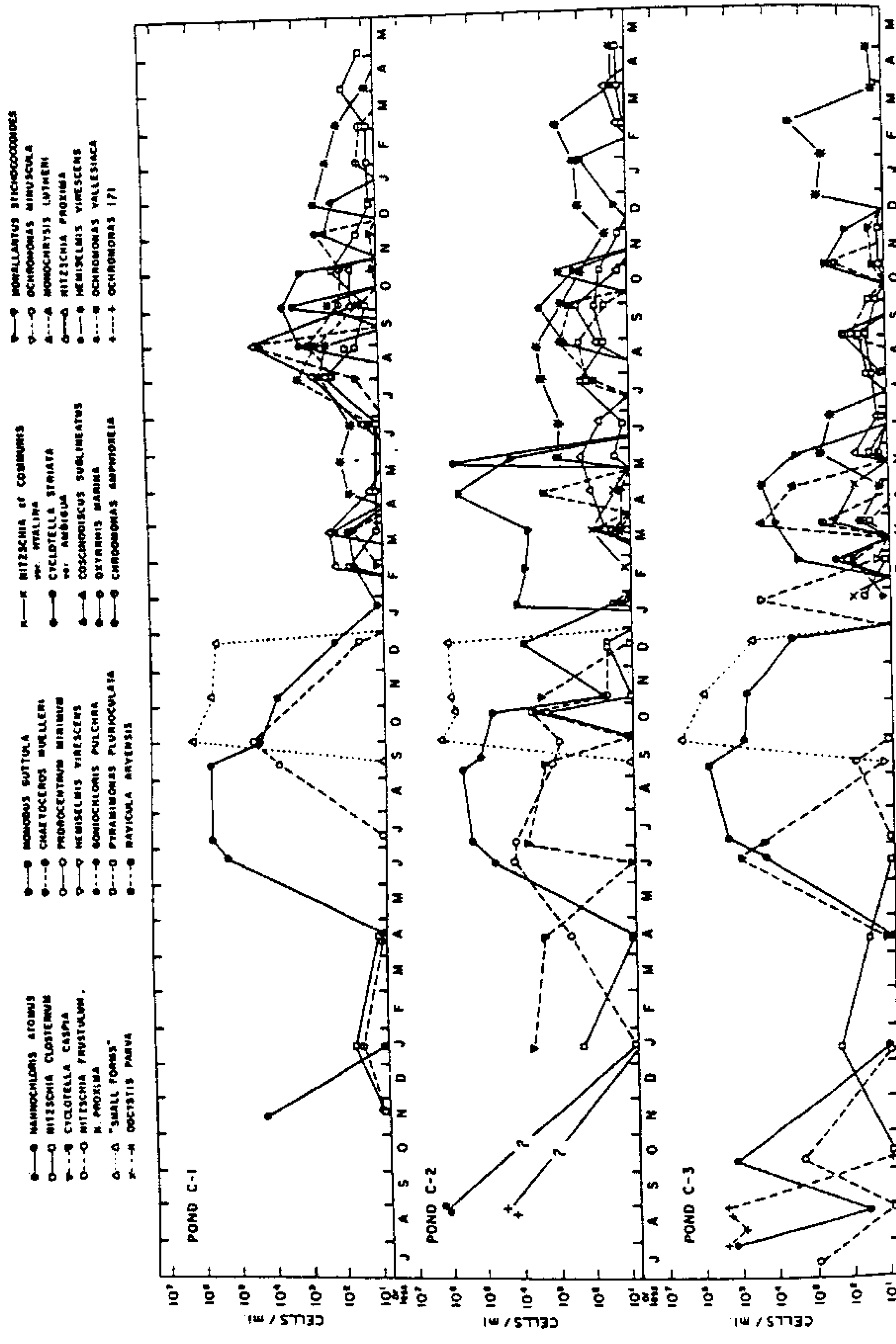


Figure 52. Seasonal distribution of dominant phytoplankton species in the control ponds 1968-1971 (Campbell, 1973).

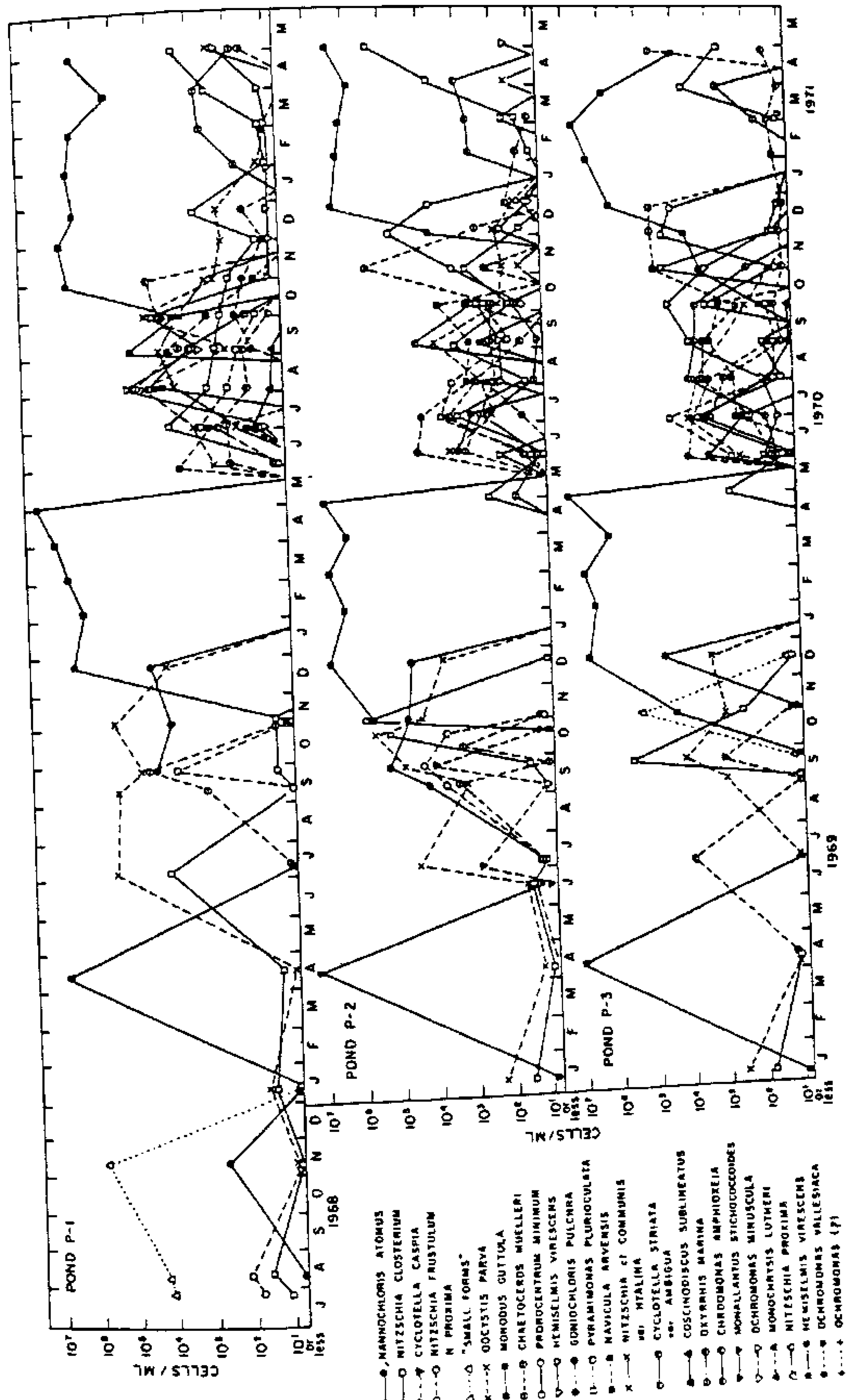


Figure 53. Seasonal distribution of dominant phytoplankton species in the waste ponds 1968-71 (Campbell, 1973).

(Table 21) was about 0.032 g/m2 winter and summer. The dominant species changed some as the ponds matured. Massive growths of Ruppia maritima developed in 1970 and 1971. Monallantus, Nannochloris, and Cyclotella predominating in 1969 were partially replaced by Hemiselmis virescens. Part of the time, different species dominated blooms in each control pond such as winter bloom of Ochromonas miniscula and spring bloom of Monochrysis lutheri only in pond C-3.

Data on seasonal Chlorophyll in the plankton were given by W. Woods, E.J. Kuenzler, and H. McKellar (Figures 54 and 55). Before pumping started chlorophyll was higher in control ponds (21-87 mg/m3) than in waste ponds (5-18 mg/m3). In this period Kuenzler and Davidson (1968) performed enrichment experiments with phosphorus, nitrogen, silicon, trace minerals, and vitamins (Figure 56) and found only nitrogen stimulated chlorophyll development in waters from control pond C-1 (normal pH) and waste pond P-1.

After pumping started, summer chlorophyll concentrations in control ponds were higher (20-40 mg/m3) than winter ($<$ 4 mg/m3). However, in the waste ponds the reverse was true with extremely high chlorophyll (300-1030 mg/m3) in the continuous winter bloom of Monodus. Photosynthetic production, however, did not accompany the high winter chlorophyll, but ocurred in summer (Figure 26) when light intensities were high. In summer chlorophyll was less but still highly eutrophic (10-300 mg/m3).

Vertical distributions of chlorophyll (Figure 24) are typical of data obtained by Leeper and Woods (1973), showing some daytime stratification and increase in chlorophyll.

Ruppia beds developed in 1970 in control ponds C-2 and C-3 with their leaves floating as a dense surface mat (Figure 8), displacing much of the phytoplankton and benthic algal production. The area of surface plant beds in 1971 is drawn on the maps in Figure 3. Attempts to pull out and weigh all the Ruppia in June 21, 1971 were only partly successful, yielding 425 kg wet weight from pond C-2 and 165 kg from pond C-3, which are respectively 760 and 314 g/m2. By midsummer, 1972, the beds had grown back and were more extensive. Although seeded several times with Ruppia, Pond C-1 maintained a high turbidity (Figure 15), and phytoplankton prevailed over macrophyte benthos.

Nor did aquatic higher plants develop in the waste ponds where phytoplankton turbidities were extreme and nighttime oxygen near zero. Inventory in July 1971 removed 42.6 kg wet weight of large mats of bottom algae, Chaetomorpha linum, about 10 g/m2 dry weight. In sunny shallows of the waste ponds, microscopic algae, including blue-greens and small diatoms, were abundant in bottom ooze. These were estimated by C.F. Rhyne from organic matter determinations (Tables 14 and 15) and microscopic examinations of the fraction photosynthetically active (Table 22).

Marsh grasses grew rapidly from patches planted in 1968-69 to form heavy beds around the shore by 1971 and 1972, even though there

Table 21. Approximate biomass of living phytoplankton in the ponds during 1970,* g dry weight/m (Kuenzler, 1971).

	Winter (Jan. - Mar.)	Summer (Jul. - Sept.)
Control Ponds:		
C-1	0.007	0.083
C-2	0.020	0.011
C-3	0.065	0.005
	0.031	0.033
Waste Ponds:		
P-1	10.4	0.77
P-2	5.6	0.12
P-3	6.8	0.15
	7.6	0.34

* $\text{Biomass} = \dfrac{[\sum(\text{cell count})\ (\text{cell vol.})]\ (0.2\ \text{g dry wt./cm}^3)}{\text{mean depth of ponds}}$

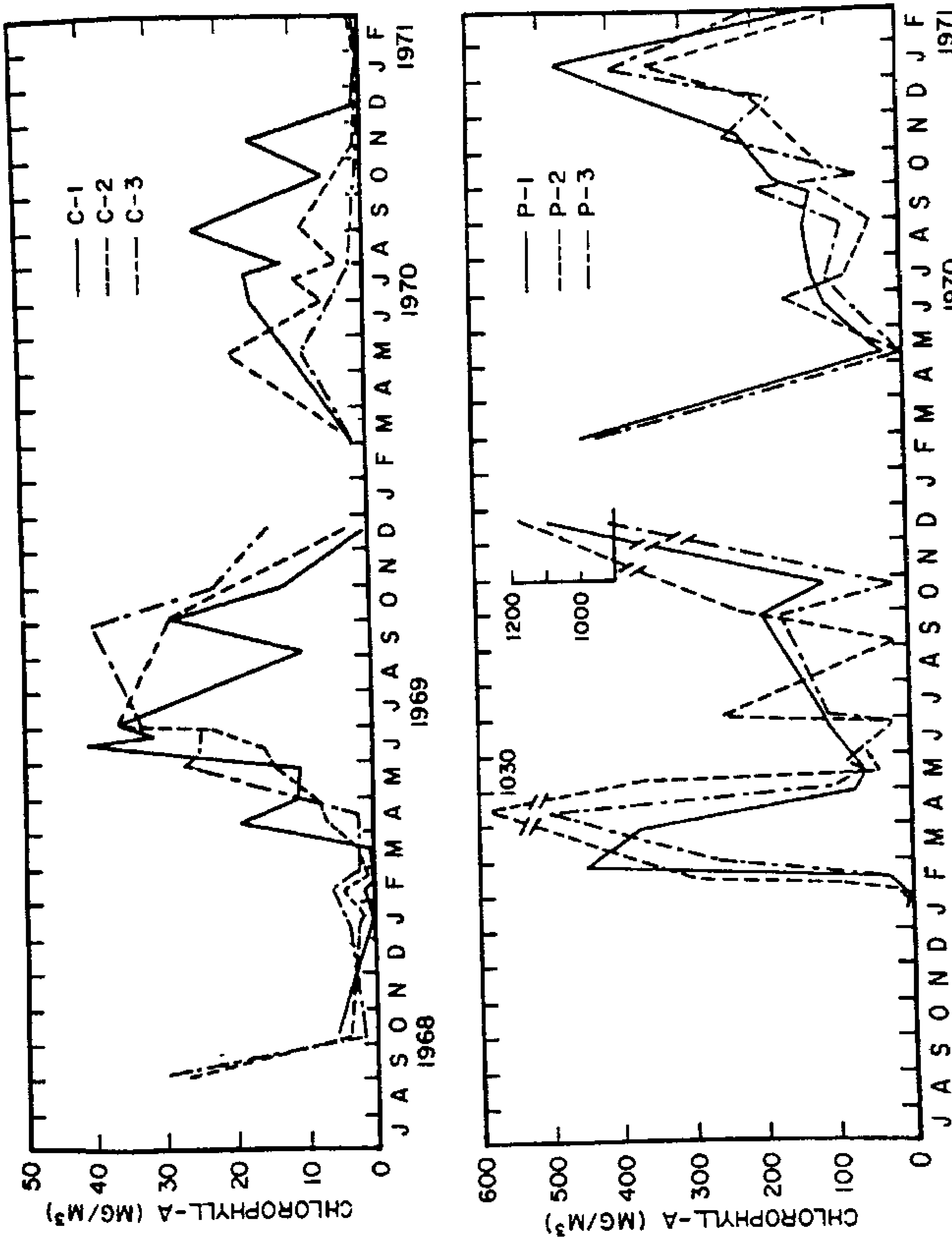


Figure 54. Plankton chlorophyll concentrations in ponds (W.Woods).

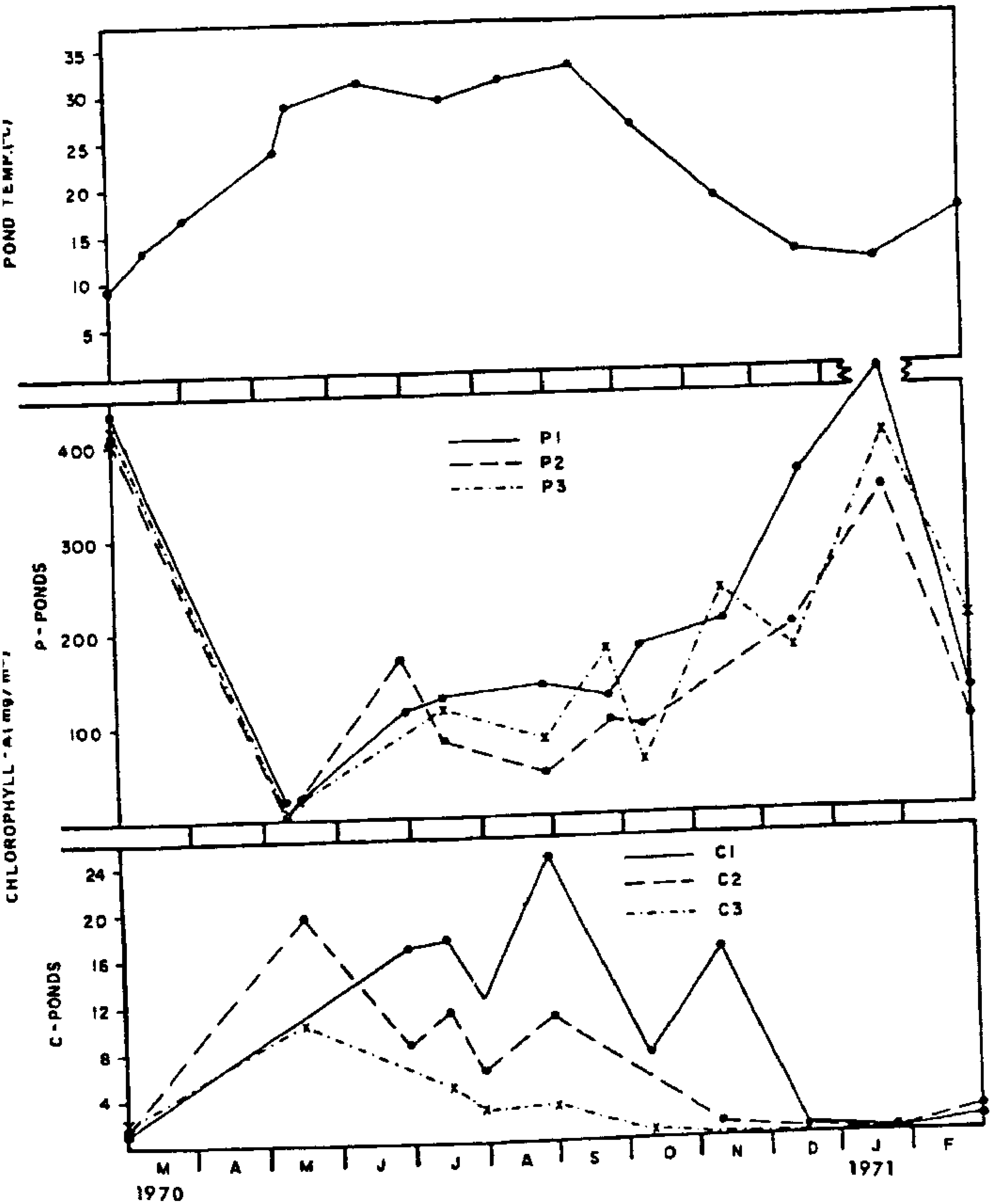


Figure 55. Seasonal trends in pond temperature and chlorophyll concentration (McKellar, 1971a).

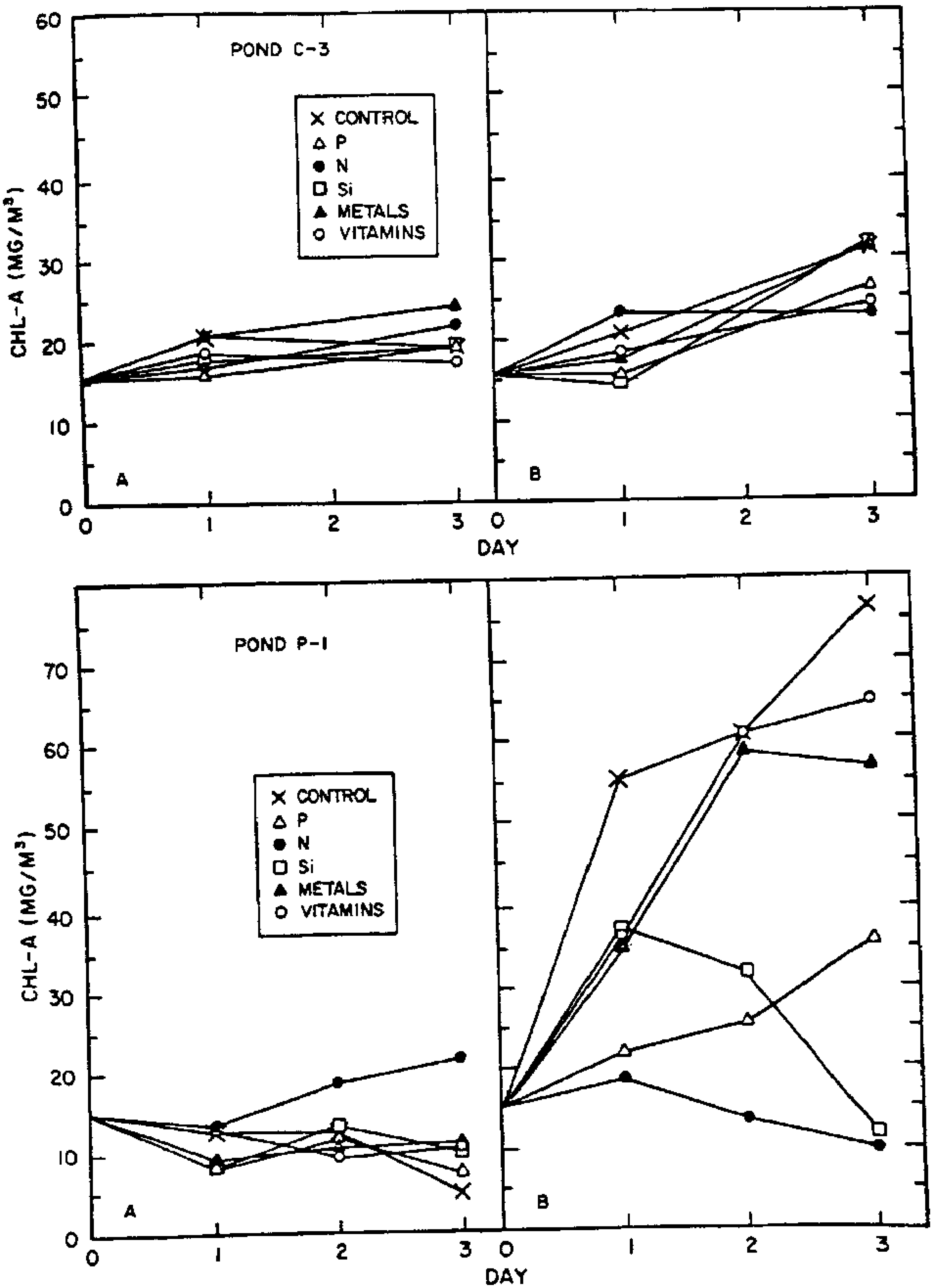


Figure 56. Effect of enrichment on development of Chlorophyll in pond waters prior to start of pumping (Kuenzler and Davidson, 1968) A-items indicated were added one at a time; B-all items were added except the one indicated.

was no tidal mixing or water-level variation. Characteristics of the Spartina beds in the first year are given in Table 23. Plant beds in summer 1971 were mapped (Figure 3). Compare photoviews of Spartina in Figure 57a after one year with growth in the same place after four years (Figure 57b). Spartina alterniflora in the waste ponds was taller (1.3m), flowering profusely, phenologically ahead of Spartina in the sewage-fertilized, tidal marsh nearby. Spread of Spartina was facilitated by the tunnelling of fiddler crabs ahead of the grass through which runners were emerging. Dead grass of a preceding year decayed in situ, rather than being removed by high tides. The heavy beds of marsh were obviously a major part of the biogenic cycle of production and consumption of the ponds. Flowering in August 1972, Spartina patens formed patches 2 m in diameter landward (uphill) from the S. alterniflora just as in the tidal marsh.

Growth of Spartina alterniflora in the control ponds (Figure 8) also spread around the shore invading bare areas, but growth was less, height less, and the rate of invasion less than in the waste ponds. Some Distichlis developed in advance of the Spartina with outgrowing linear runners. Juncus patches in control ponds remained alive after four years without much expansion. In the waste ponds, the Juncus was mostly overgrown, one small patch flowering in August 1972.

Adaptive Physiology of Monodus

Hommersand and Talbert (1971) examined Monodus with an electron microscope and conducted physiological studies on conditions that favor growth of Monodus guttula. Growth rates for varying temperatures, salinities, pH, and growth media are given in Figure 58. Many of the conditions stimulating laboratory growth (high pH, wide range of salinity tolerance, cool temperature at low light intensity) were those found in the waste ponds in winter, helping to account for the dominance of the species. The neutral buoyancy found in Monodus due to fat content favors existence in pond waters with little circulation. In the laboratory, as in the field in early May, rising temperature caused excess of cell respiration over photosynthesis, loss of oil, and sedimenting of populations.

Table 22. Estimates of algal biomass in bottom ooze (C.F. Rhyne).

Date	Ponds	Total Organic Matter	Estimated Photosynthetic Percentage	Estimated Photosynthetic Biomass
July 12, 1969	P-1	74.93 g/m^2	50%	37.47 g/m^2
	P-2	137.59	50	68.80
	P-3	110.58	50	55.30
August 9, 1969	C-1	70.40	10	7.00
	C-2	159.50	10	16.00
	P-1	141.50	33	46.70 } 100 g dry/m^2
	P-2	276.90	33	91.38 } 100 g dry/m^2
	P-3	497.60**	33	164.20 } 100 g dry/m^2
Sept. 20, 1969	C-1	135.20	25	33.80
	C-2	130.20	25	32.55
	C-3	126.80	25	31.70
	P-1	134.30	25	33.60
	P-2	99.90	25	25.00
	P-3	149.80	25	37.50
Oct. 26, 1969	C-1	77.80	25	19.45
	C-2	87.58	25	21.89
	C-3	102.40	25	25.60
	P-1	97.30	25	24.32
	P-2	61.47**	25	15.37
	P-3	73.80	25	18.45

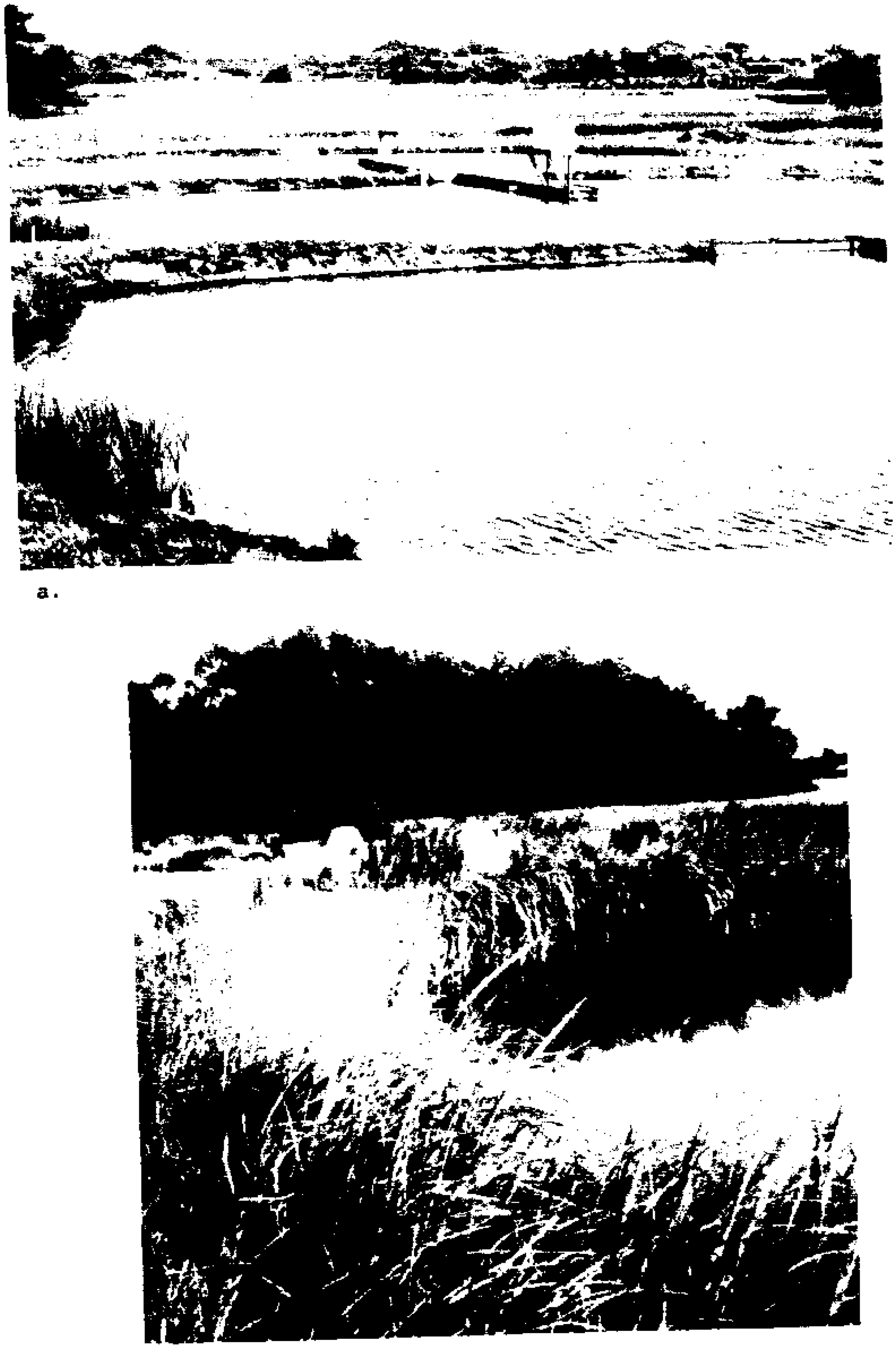
* - single sample
** - two samples

Table 23. Characteristics of growths of <u>Spartina alterniflora</u> in the ponds in the first year of growth after transplanting (D.E. Marshall).

Parameter	C-1 dry	C-1 wet	C-2 dry	C-2 wet	C-3 dry	C-3 wet	P-1 dry & wet	P-2 dry & wet	P-3 dry & wet
# of stems	12	43	13	38	14	47	50	65	79
mean ht. (cms)	77	95	88	97	92	97	99	97	98
cm. of stem	924	4085	1144	3686	1288	4559	4950	6305	7742
weight (g.)	81	359	97	330	117	444	479	631	775
grams/cm.	.09	.09	.08	.09	.09	.10	.10	.10	.10
cms./gram	11.4	11.4	11.8	11.2	11.0	11.0	11.0	10.0	10.0

Parameter	C-1 dry	C-1 wet	C-2 dry	C-2 wet	C-3 dry	C-3 wet
# of stems	31	25	46	45	40	42
mean ht. (cms)	113	119	113	125	133	133
cm. of stem	3503	2974	5200	5625	5310	5586
weight (g.)	244	200	296	362	351	366
grams/cm	.07	.07	.06	.06	.07	.07
cms/gram	14.4	14.8	17.6	15.6	15.2	15.3

P-1 dry	P-1 wet	P-2 dry	P-2 wet	P-3 dry	P-2 wet	P-2 Nov.	P-3 Nov.
27	21	26	28	31	46	17	31
131	147	138	140	138	145	160	142
3537	3087	3584	3918	4278	6670	2717	4402
258	230	234	302	317	460	240	280
.07	.08	.07	.08	.07	.07	.09	.06
13.7	13.4	15.0	13.0	13.4	14.5	11.3	15.7



a.

b.

Figure 57. View of Spartina growing in waste pond P-1 with Calico Creek marsh in background. (a) August, 1969 with one seasons growth after planting; (b) same view in August, 1972 after 4 years growth.

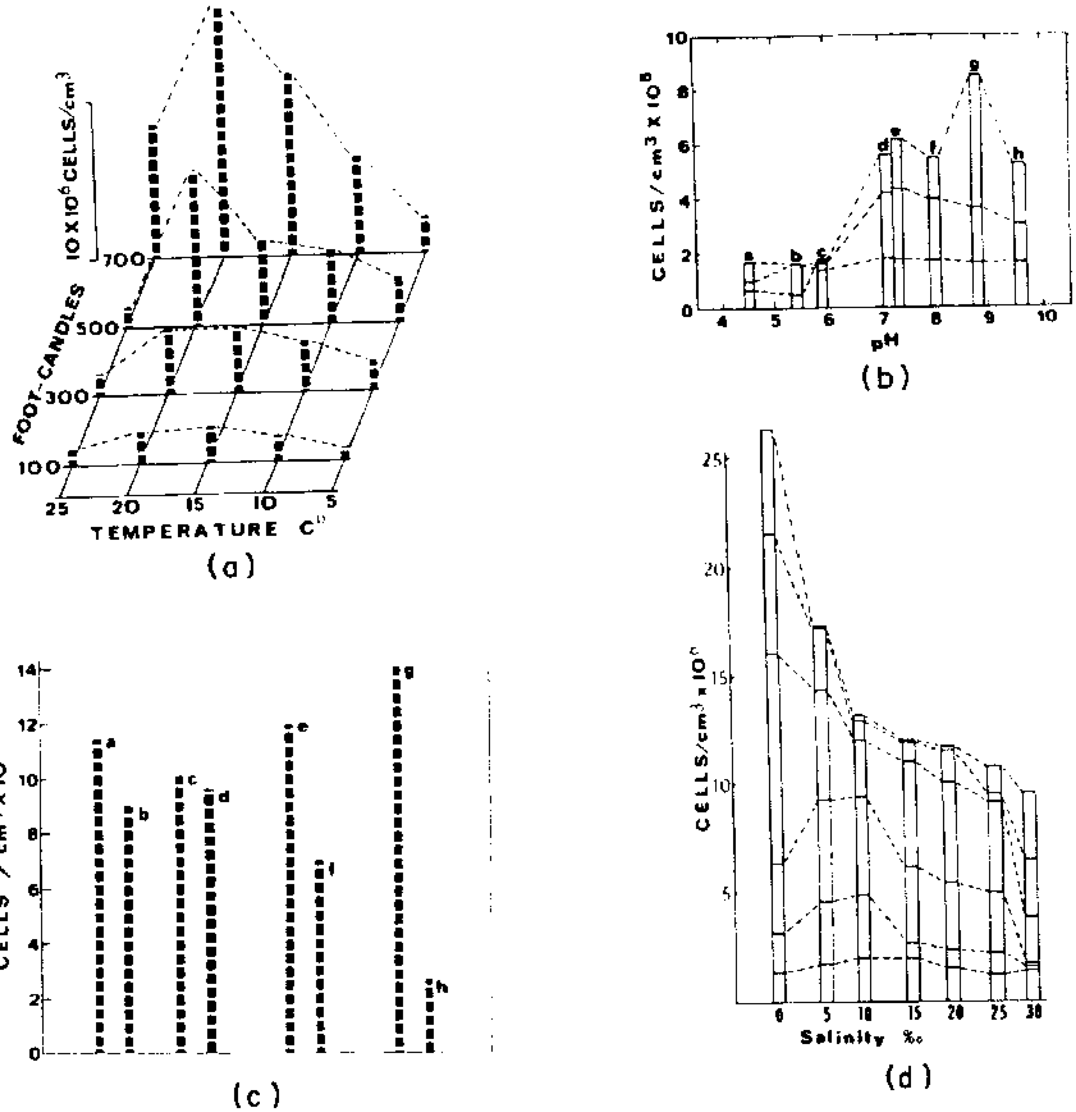


Figure 58. Growth of Monodus in laboratory flasks with sterilized Morehead City treated sewage in Calico Creek water (Hommersand and Talbert, 1971).
(a) growth in crossed gradient apparatus, 6 days, salinity 13 ppt, inoculum 0.5 E6 cells/ml; (b) growth at 700 foot candles, 17 C, and 13 ppt with varying pH; (c) growth in various media:

a-Morehead sewage, Calico Creek water 1:1, 17 C, 750 foot candles, 4 days;
b-Morehead sewage, diluted ocean water 1:1, 26 ppt, 17 C, 700 foot candles, 4 days;
c-Chapel Hill sewage, Calico Creek water, 26 ppt 1:1, 17 C, 700 foot candles, 4 days;
d-Chapel Hill sewage, diluted ocean water, 26 ppt 1:1, 17 C, 700 foot candles, 4 days;
e-Morehead sewage, diluted ocean water 26 ppt 1:1, 17 C, 700 foot candles, von Stoach nutrient supplement (1.0 ml/l), 5 days;
f-seawater 13 ppt, 17 C, 700 foot candles, von Stoach nutrient supplement (1.0 mg/l), 5 days;
g-Morehead sewage, Calico Creek water 1:1, 25 C, 1500 foot candles, gassed with air, 4 days;
h-Morehead sewage, Calico Creek water 1:1, 25 C, 1500 foot candles, gassed with 5% carbon dioxide in air, 4 days;

(d) Daily growth of cells for 6 days in Roux flasks with varying salinity, 100 ml sterilized morehead City Calico Creek water 1:1, 17 C, 700 foot candles, innoculum 1.0 E6 cells/ml gassed with air.

Characteristics of the Acid Period

When control ponds C-2 and C-3 were acid from July-September 1968, before pumping started, phytoplankton characteristics of acid mine drainage were found, especially Ochromonas vallesiaca, and animals included blue crabs, water beetles, copepods and mosquitoes. Kuenzler and Davidson (1968) studied the phytoplankton in the acid ponds with results in Table 24 as compared with pond P-1 at the same salinity. Rapid uptake of phosphate was measured with radioactive phosphorus (Figure 40c). Presence of alkaline phosphatases in phytoplankton suggested low phosphorus condition, but enrichment with phosphorus was not growth stimulating, whereas nitrogen enrichment was (Figure 56). Sewage pumping had not yet started in the waste ponds, and phosphorus was higher in the acid pond. Chlorophyll was high. R. Outen found that grass shrimp, Palaeomonetes pugio, placed in cages in the acid ponds did not survive whereas controls in other ponds did.

Figure 17a shows the diurnal record of oxygen and metabolism of pond C-2 during its acid period compared with non-acid pond on the same day. Figures 17a and 59 have pH records in the two acid ponds (C-2 and C-3). The graph of oxygen metabolism is not unlike the one at the same time in pond P-3 at normal ph (Figure 17b).

At the low pH inorganic carbon storage was very small and carbon-dioxide use by photosynthesis did not shift pH much. J.D. Johnson and J. Day (1968) studied the physical chemistry with potentiometric pH titrations calculating pK values (Table 25). The value for the acid pond suggested 0.12 parts per thousand sulfuric acid as cause of the acidity. Sulfuric acid was also extracted from the muds. Figure 60 shows the difference between acid base titration curves for the low pH pond C-3 and the more normal pond P-2. During this period the pH curves of waste pond P-2 and the other ponds at ordinary pH (P-1, P-3, and C-1) were normal for shallow estuaries. Estuarine water had been added, but pumping had not yet started.

Microbial Consumers and Bottom Sediments

Dense populations of bacterial and other microbial consumers including coliform bacteria were found in the pond waters, bottom oozes and on other surfaces. Meaurements of respiration in free water, bottles, bell jars and sediment core tubes given in previous sections on oxygen and carbon metabolism were in large measure due to microbial consumers as well as microbial algae. Data on microconsumers were obtained from direct examination, agar plating and metabolic measurements.

Direct counts of bacteria in waters by J. Marsh and A.N. Rabin (Figure 61 and Table 26) indicate higher levels in waste ponds (up to 10 million cells/ml), but less in winter (100,000 cells/ml). Plate counts of waters were an order of magnitude less. Predominant colony types "A" and "B" were on plates of control and waste ponds. Plate counts of waters from waste ponds were higher but diversity

Table 24. Comparison of control pond C3 (during its acid period) and waste pond P1, August, 1968 (Kuenzler and Davidson, 1968).

Designation	C-3 Acid condition	P-1 waste
Location	Bogue Sound	Calico Creek
Temperature	25^o-30^o	25^o-30^o
Salinities	25-30‰	25-30‰
pH	3.1-3.5	6.5-7.5
No. of Important species	1	10
Diversity, $d = \frac{S-1}{\ln N}$	0.32	1.9
Phosphorus conc. (ug. at/l)		
PP	0.8-2.4	0.3-1.2
DIP	0.05-0.10	0-.005
DOP	0.06-0.3	0.1-0.6
Phosphatase in algae	acid	alkaline
DIP uptake rate, k (min^{-1})	0.088	0.17

Table 25. Titration data on pond waters in August, 1968.

Pond	pK_1	pK_2
C-2 (Acid)	5.1	8.3
C-3 (Acid)	4.8	9.0
P-2 (Waste)	6.0	9.0

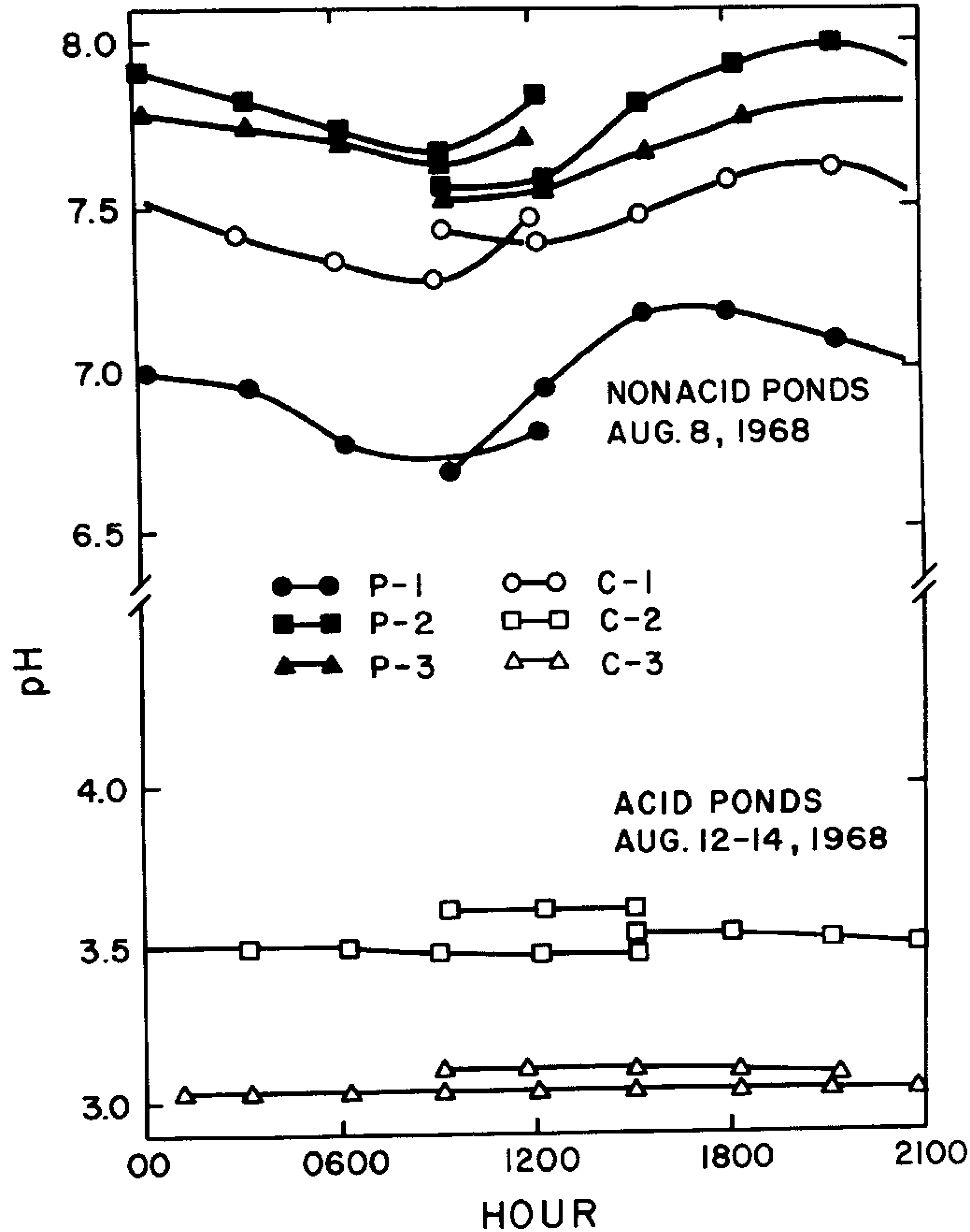


Figure 59. Diurnal record of pH in ponds in August, 1968 when two ponds, C-2 and C-3 were acid. Salinities were: P-1, 28 ppt; P-2, 23 ppt; P-3, 27 ppt; C-1, 32 ppt; C-2, 26 ppt; and C-3, 24 ppt.

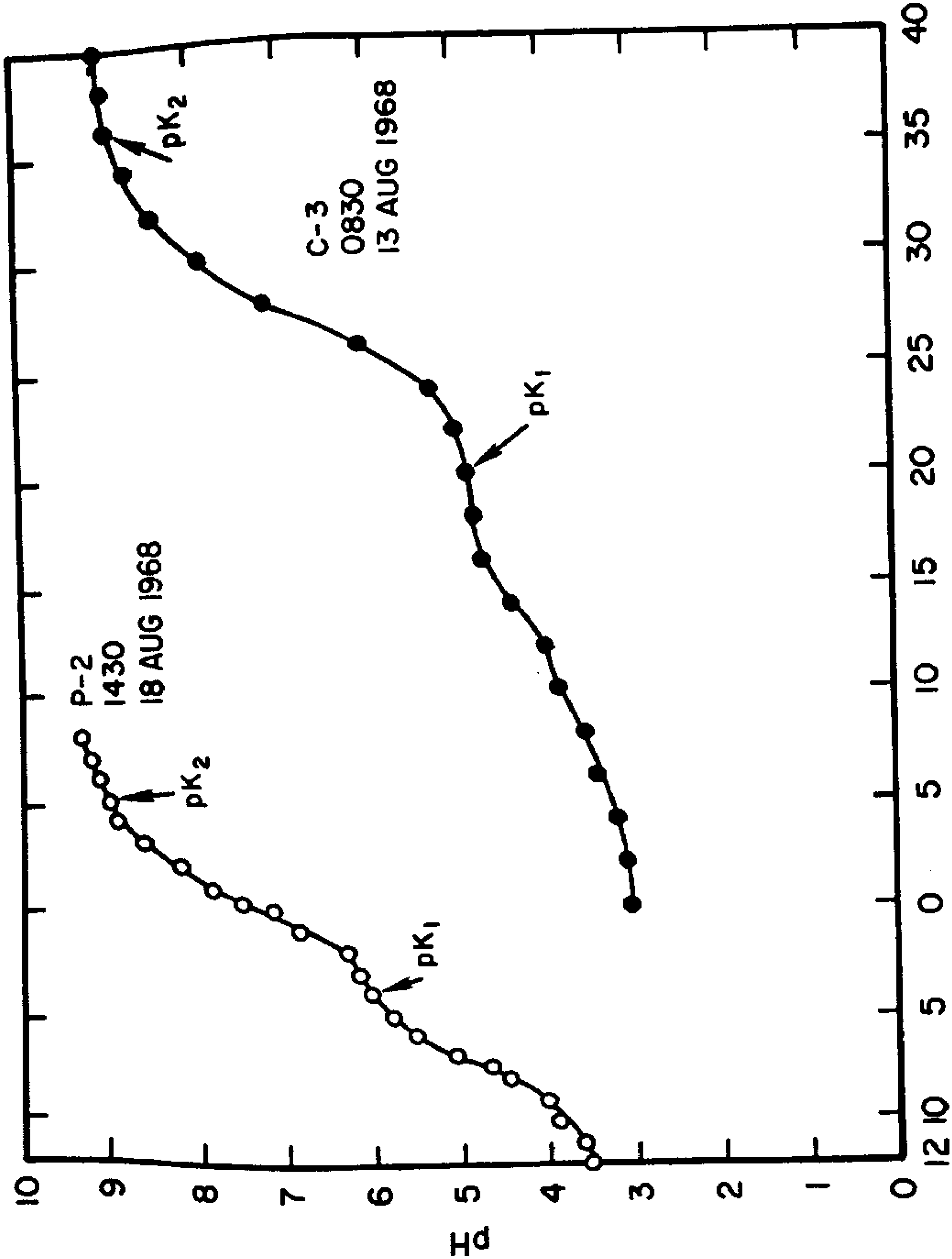


Figure 60. Acid-base titration of water from pond C-3 during its acid period compared with water from pond P-2 recently filled with estuarine water from Calico Creek.

was less with 12 colony types out of 313 colonies counted. Plate counts of waters from control ponds were less but more diverse with 23 colony types out of 310 colonies counted.

Coliform measurements by A. Harvell, M. May, and T.L. Herbert (Table 27 and Figure 62) showed much higher concentrations in waste ponds (200-1100 mpn/ml) than in control ponds (0.5-23 mpn[ml). Most of Figure 62 is for a period with the sewage inflow pumps turned off, but sewage wastes were entering indirectly through the estuarine pump because the sewage plant"s effluent discharges into Calico Creek a few yards from the estuarine pump. Coliform concentrations (20-150 mpn/ml) were less than in 1969 (1100 mpn/ml, Table 27) when direct sewage inflow pump was operating most of the time. With losses possibly due to consumers, coliform counts in waste ponds were less than in inflowing waters. Plating characteristics and identifications are given in Table 28.

Results of the fungal survey (B. Rao and W. Koch, 1970) in Table 29 and Figure 63) were 21 species: 8 phycomycetes, 6 ascomycetes, and 7 fungi imperfecti with more species in control ponds than waste ponds. Phycomycetes were numerous in water samples, but ascomycetes and fungi imperfecti were more in plant materials.

Representative graphs of glucose uptake (Marsh, 1970, 1971) are given in Figures 64-67. Uptake rate was proportional to available sugar (Figure 64) suggesting demand for sugar. Diurnal records of Michaelis-Menten uptake parameters in Figure 65 indicated faster uptake rates and turnovers in waste ponds (smaller turnover time, T=1.5-13; maximum uptake velocity, 0.45-22 ug/l/hr) than in control ponds (turnover time, T=1.5-13 hrs; maximum uptake velocity, 0.13-10 ug/l/hr). There were two- to three-fold diurnal variations, but timing of ups and downs was variable. Uptake by resuspended top sediments was much faster than uptake by waters (Figure 66-67). No uptake was found in water from control pond C-1. Percent of glucose uptake that was respired (Figure 67) was higher in control ponds.

After three years of ecosystem development, the ponds bottoms were covered with an olive-green to brownish-green, detritus-rich, metabolically-active, microbiological ooze (1 cm in control ponds and 2 cm in waste ponds). Loss on ignition of this ooze (Table 15-16) was 7 to 18%, 149-480 g/m2 in a layer one cm thick. Chemical oxygen demand measurements (Table 16) were somewhat lower. In July 1971, waste pond ooze was 10.2% carbon and control ponds 4.4% carbon. Table 22 has estimates of algal fraction of the ooze. Generally oxidized, these sediments were on top of the 20 cm of black, fibrous marsh mud, that had been put on the bottom of all ponds (Figure 5). The interface was recognizable even after 3 years. Below 4 cm, judging by the black color and smell of hydrogen sulfide, this original mud remained reduced. Locally the bottom was frequently disturbed by the many scientists walking through to take samples.

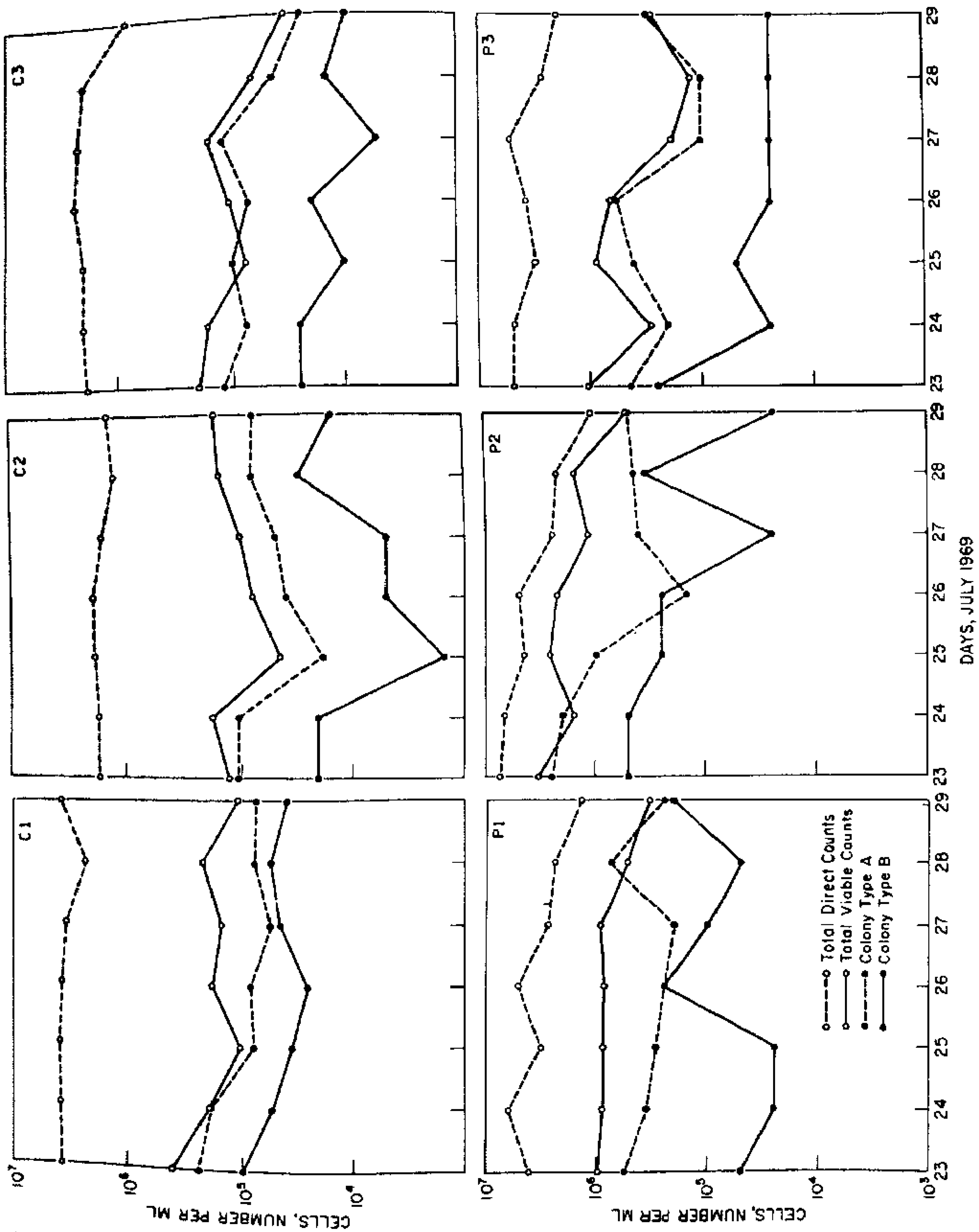


Figure 61. Bacterial populations in ponds in July 1969 (Rabin,1970). "Viable" counts are from plates; colony type A was 2 mm circular, opaque and smooth-edged; colony type B was 2 mm, circular and brick-red.

Table 26. Direct counts of bacteria in ponds 1969-1970 (J.A. Marsh).

	C-1		P-1	
Date	Temp(^{o}C)	Cells/ml	Temp(^{o}C)	Cells/ml
10 Mar 69	10	4.8×10^6	-	-
13 May 69	19.5	5.2×10^6	-	-
14 May 69	-	-	22	8.1×10^6
7 Jun 69	27	2.6×10^6	27	1.1×10^7
25 Jun 69	27	7.5×10^6	-	-
26 Jun 69	-	-	28	2.3×10^6
25 Jul 69	-	-	28	8.1×10^6
24 Sep 69	-	-	21	2.6×10^7
26 Jan 70	9.5	4.6×10^5	-	-
24 Feb 70	11.5	8.0×10^5	-	-
13 May 70	-	-	25	7.4×10^6
16 Jun 70 1240	27	3.6×10^6	28	8.1×10^6
2200	29	3.6×10^6	30	9.6×10^6
17 Jun 70 0750	26.5	2.8×10^6	27	9.6×10^6
26 Jun 70	26	1.4×10^6	26	9.4×10^6
23 Jul 70 0900	27	2.9×10^6	-	-
1500	28	2.7×10^6	-	-
2100	28	2.9×10^6	-	-
24 Jul 70 0300	27	2.7×10^6	-	-

Table 27. Coliform counts in ponds in 1969 (M. May and A. Harvell).

Date	Pond	Lactose 10	Lactose 1	Lactose 0.1	Brilliant green broth 10	Brilliant green broth 1	Brilliant green broth 0.1	MPN per 100 ml
June 18	P-1	+++	+--	---				
			++	---	++-	+--		15
	P-2	+++	++-	+--				
			+	--	+++	++-	+	150
	P-3	+++	++-	---				
			+	---	+++	+++		240
	C-1	+--	---	---				
		++			+++			23
	C-2	---	---	---				
		---	---	---				<4
	C-3	---	+--	--				
		+++	--	--	+-+	+		15
June 30	P-1				+++	+++	+-+	1,100
		+++	+++	+-+				
	P-2				+++	++-	---	
		+++	++-	---				93
	P-3	+++		---	+++	+++	-+-	
			+++	+				460
July 10	P-1			--				
		+++	+++	+--	+++	+++	+	460
	P-2	---	---	---				
		+++	---	---	+++			23
	P-3		-++	+--				
		+++	-	--	+++	-++	+	150
	C-P	+++	+++	+++				
					+++	+++	+++	1,100
July 13	C-1	--+	---	---	--+			
						-		4

Table 27 (cont.)

Date	Pond	Lactose 10	Lactose 1	Lactose 0.1	Brilliant green broth 10	Brilliant green broth 1	Brilliant green broth 0.1	MPN per 100 ml
July 13, cont.	C-2	++-	+-+	---	++	-		9
	C-3	-+-	---	---	--			< 0.5
	C-E_s	+--	---	---	-			< 0.5
	C-T	---	---	---				< 0.5
July 18 Nephrocytium	P-1	--- +++	--- ---	--- --+	+++	---	-- T	39
	P-2	--- ++-	--- ---	-- --	+--	---	---	< 0.5
	P-3	--- +++	--- ++-	--- ---	+++	++-	---	93
	C-1	--- -+-	--- ---	--- ---	- - -	---	--	0.5
	C-2	--- +++	--- -+-	--- ---	---	-+-	--	0.5
	C-3	--- +--	--- -+-	--- -+-	+	+		1
	C-E_s	--- +--	--- ---	--- ---	---	---	---	0.5
	C-T	--- ---	--- ---	--- ---				
July 26	P-1	+++	+++	+++	+++	+++	+++	1,100
	P-2	+++	+++	-++	+++	+++	++	1,100
	P-3	+++	+++	+++	+++	+++	+++	1,100
	P-E_s	+++	+++	+++	+++	+++	+++	1,100
	C-1	+--	++-	+ ++	+	++	+++	210
	C-2	+++	+++	+++	+-+	+++	+ +	1,100
	C-3	+++	+--	---	+++	+		43
	C-E_s	-+-	+--	-+-	+	+	+	11

Table 27 (cont.)

Date	Pond	Lactose 10	Lactose 1	Lactose 0.1	Brilliant green broth 10	Brilliant green broth 1	Brilliant green broth 0.1	MPN per 100 ml
Aug. 2	P-1	--- +++	++- +	+-- ++	+++	+++	+++	1,100
	P-2	-+- + +	+++	+-- --	+++	+++	+	460
	P-3	-+- - -	+++	++- -	+++	+++	+++	1,100
	P-E_s	--- +++	+++	+++	+++	+++	+++	1,100
	P-Mix	+++	+++	--- +--	+++	+++	+	460
	P-S	--- ---	--- ---	--- ---				0.5
	C-1	--- +++	--- -++	--- +--	+++	++		93
	C-2	--- +-+	--- -+-	--- ---	+ +	+		15
	C-3	--- +--	--- +--	--- +--	+	+	-	7
	C-E_s	--- +-+	--- +--	--- ---	+ +	-		23

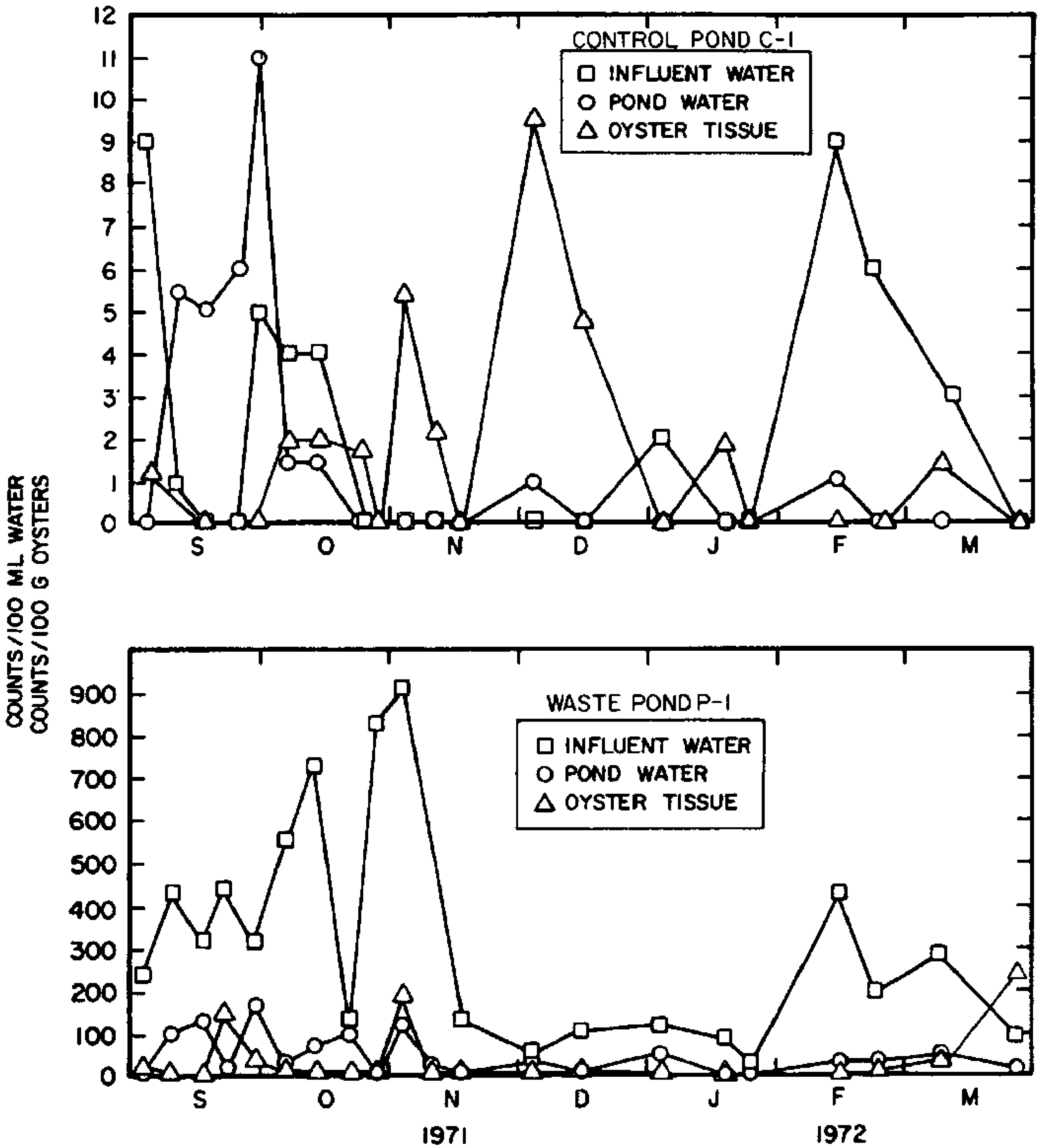


Figure 62. Coliform counts in ponds in 1971-1972 (T.L. Herbert, 1975). Direct in-pumping of sewage stopped Aug. 28, 1971, and resumed March 26,1972, but the influent in this period had sewage waste indirectly received because the intake of the estuarine water pump was near the sewage plant outfall.

Table 28. Characteristics of pond bacteria for identification (T.L. Herbert).

Test	1	2	3	4	5	6	7	8	9
S S Agar	0	+ clear	0	0	0	0	+ pink	+ red	0
Citrate, Simmons	0	+	0	0	0	+	0	+	0
Starch	0	0	0	0	+	0	0	0	+
Methyl red	+	0	+	0	+	0	+	0	+
Vogues proskauer	0	0	0	+	+	+	+	0	0
Gelatin	+	0	0	0	0	0	+	0	0
Urea	0	0	0	0	0	0	0	0	0
Endo agar	+	+	0	0	0	0	0	+	0
EMB agar	0	0	0	0	0	0	0	+	+
Brilliant green bile agar	0	0	+	0	+	0	0	+	0
3-Sugar iron agar Kliglers iron agar	lactose sucr. dext.	glucose dext.	lactose sucr. lactose	lactose sucr. dext.	lactose sucr. lactose	glucose H_2S dext.	lact. sucr. dext.	NC dext.	glucose dext.
Gram stain	0	0	0	0	0	0	+	0	0
Description	cocci, chains tiny	rod single	cocci, short chain yellow	cocci chains	cocci-rod	cocci-rod	rod	rod short, chains yellow when old.	cocci rod chain

KEY: (0) negative reaction
(+) positive reaction
NC no change
(±) both positive and negative results
lact. = fermentation of lactose
sucr. = fermentation of sucrose
dext. = fermentation of dextrose
H_2S = hydrogen sulfide was produced

CONCLUSIONS: #1. Escherichia freundii
#2. Salmonella sp.
#3. Escherichia coli
#6. Proteus mirabilis
#7. Streptococcus liquefaciens
#8. Alcaligenes sp.
#9. Escherichia coli strain?

Table 29. List of fungi in ponds, June and July, 1969 (B. Rao and W. Koch); W, water; P, plant material; S, sediment; F, foam.

		Fungi	C			P		
			1	2	3	1	2	3
PHYCOMYCETES	1	Haliphltroceros sp	++	+				
	2	Laginidium sp	+					
	3	Mucor sp	+			+		
	4	Labyrinthula sp	+++	+	++	++	+	++
	5	Pythium sp	+++	+++	++	++		
	6	Rhizidiomysis	+					
	7	Schizochytrium sp	++++	+++++	+	+	++	+
	8	Thraustochytrium	++++++	++++++	++++++	++++	+++	++
ASCOMYCETES	9*	Didimella sp						+
	10*	Leptosphaeria sp	++	++		+	+	
	11*	Lulworthia sp	++		+		+	+
	12*	Passiriniella discorse	+++	++	+	+	++	+
	13*	Phospora sp		+		++		+
	14*	Sphoerulina sp					+	+
FUNGI IMPERFECTI	15*	Alternaria sp	++	++			+	+
	16	Aspergillus sp	++	+			+	+
	17	Curvuloria sp	++				+	
	18*	Desmidiospora sp	++	+	++	++	++	+
	19	Fusorium sp	+++	++	+++	+++	++	+++
	20	Penicillium sp		+		++		+
	21	Phomo sp	++++	++	+	++	+	+
		Numbers of successful isolations	43	30	19	23	19	18
		Number of fungi recorded	18	14	9	12	13	14

* Fungi recorded by direct isolation method.

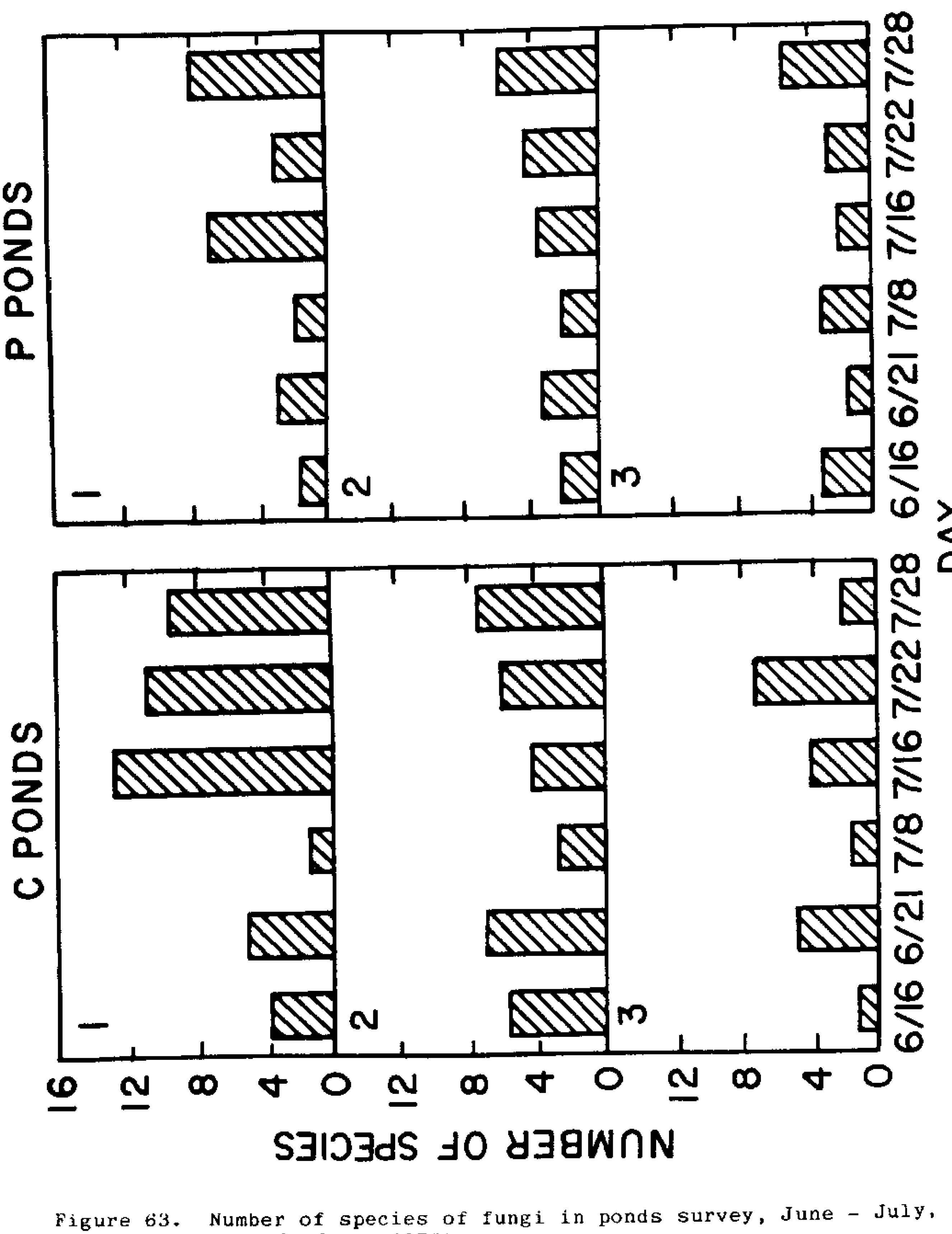


Figure 63. Number of species of fungi in ponds survey, June - July, 1969 (B. Rao and W. Koch, 1970).

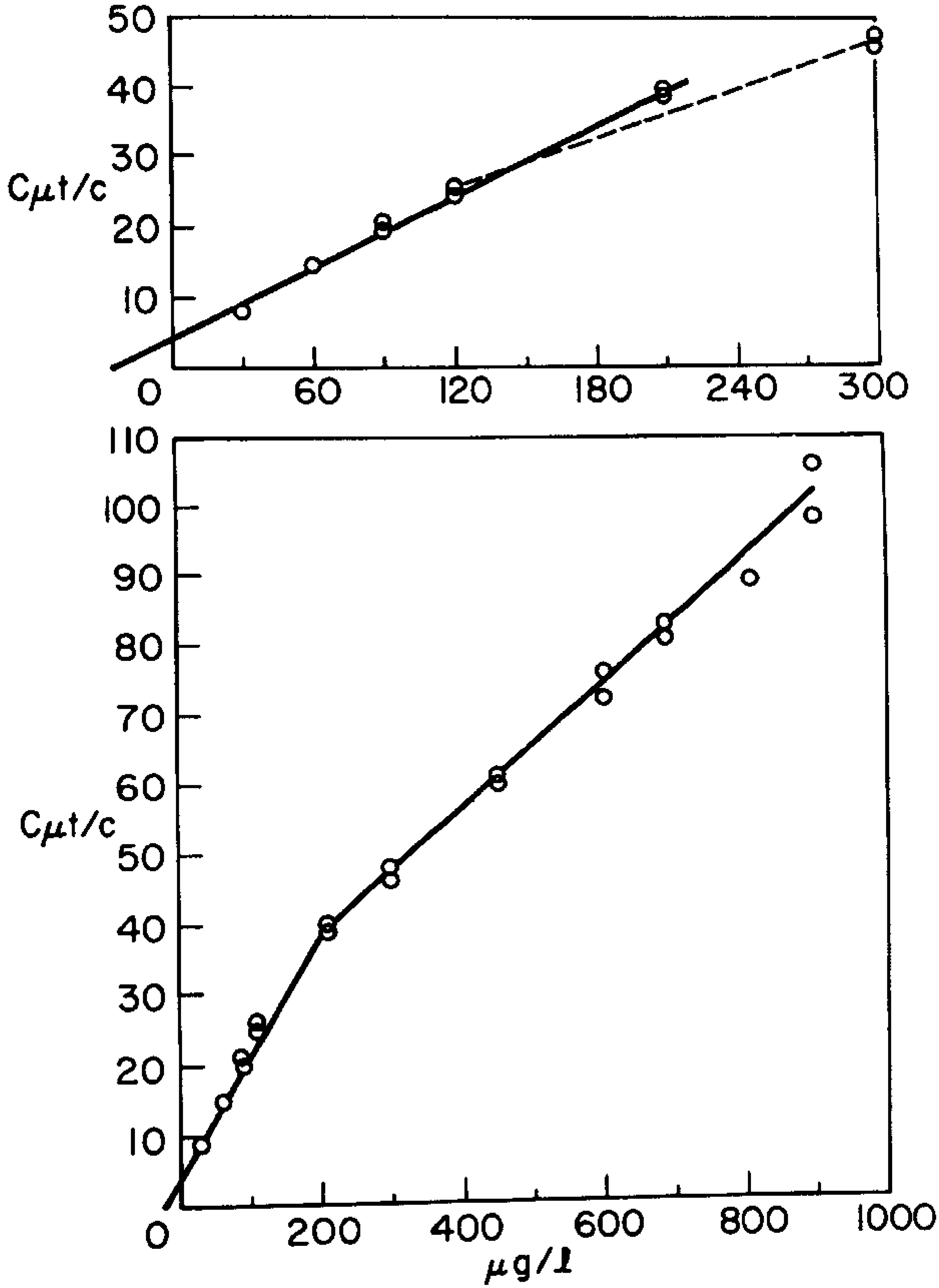


Figure 64. Glucose uptake as a function of added substrate in pond C-1, March 7, 1969 (Marsh, 1970).

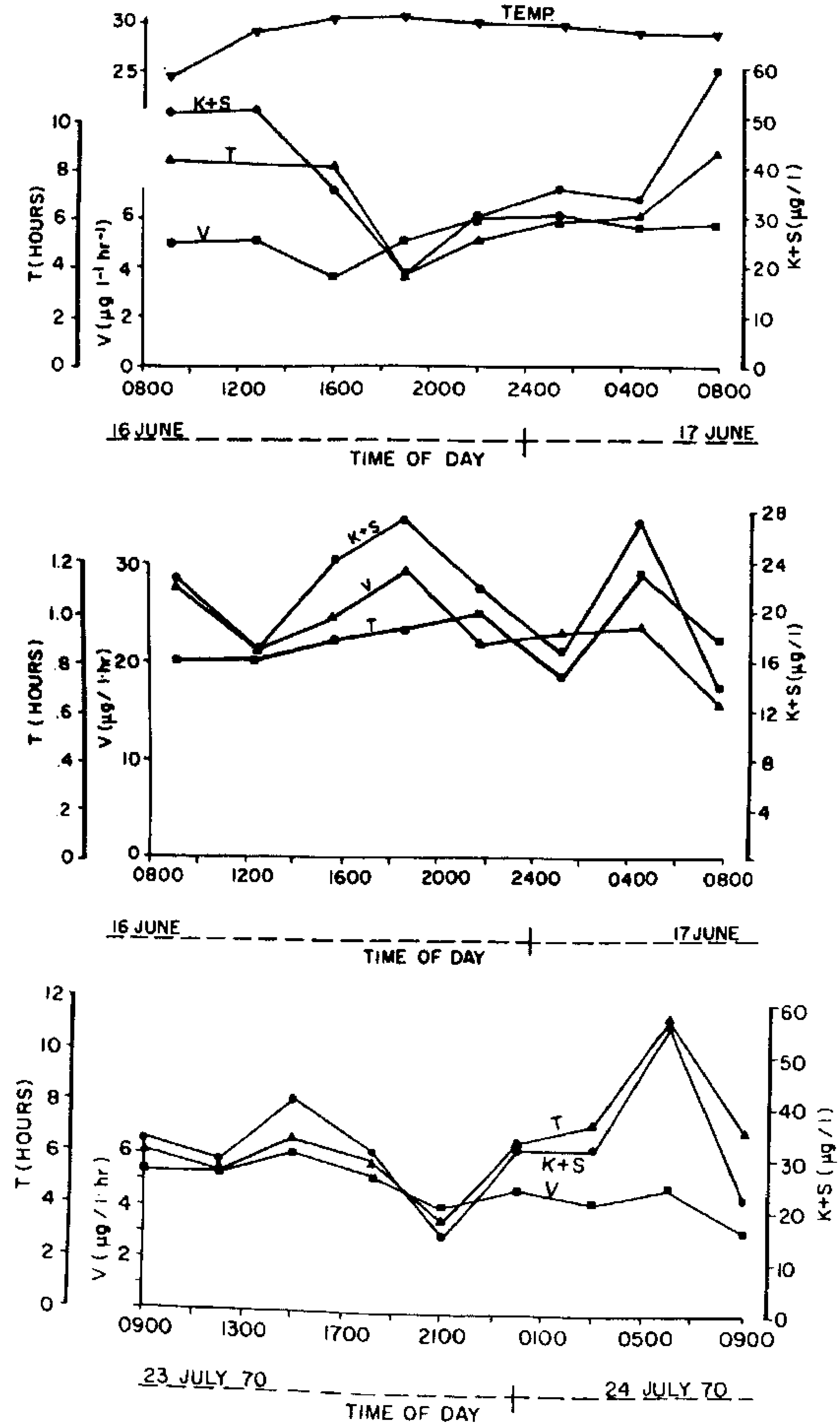


Figure 65. Diurnal variation of organic carbon uptake parameters (Marsh, 1971). (a) Control pond C-1, June 16-17, 1970; (b) waste pond P-1, June 16-17, 1970; (c) Control pond C-1, July 23-24, 1970.

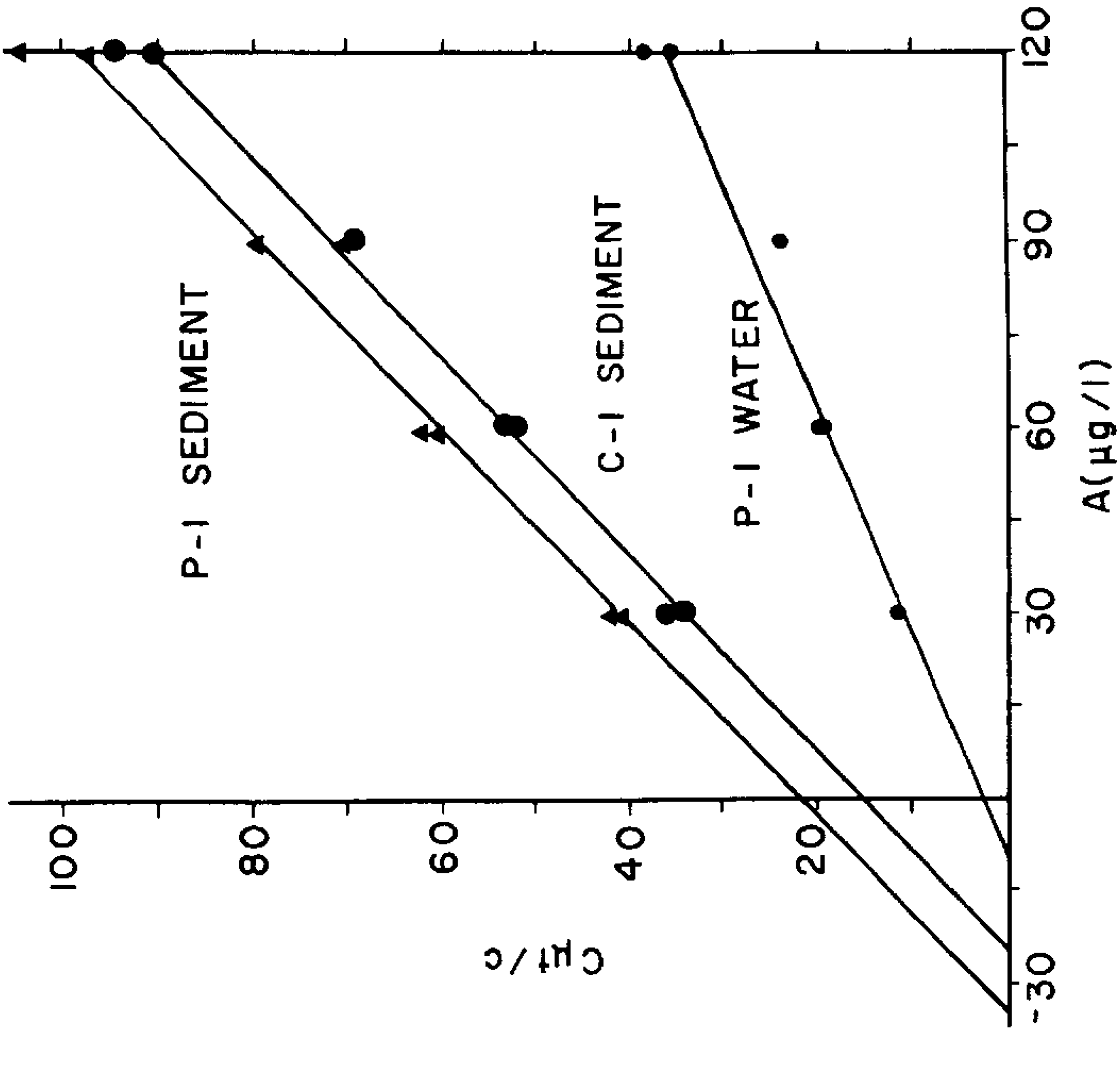


Figure 66. Glucose uptake in pond sediment suspensions, Jan. 20, 1970, at 8.5 C incubation temperature (Marsh, 1971).

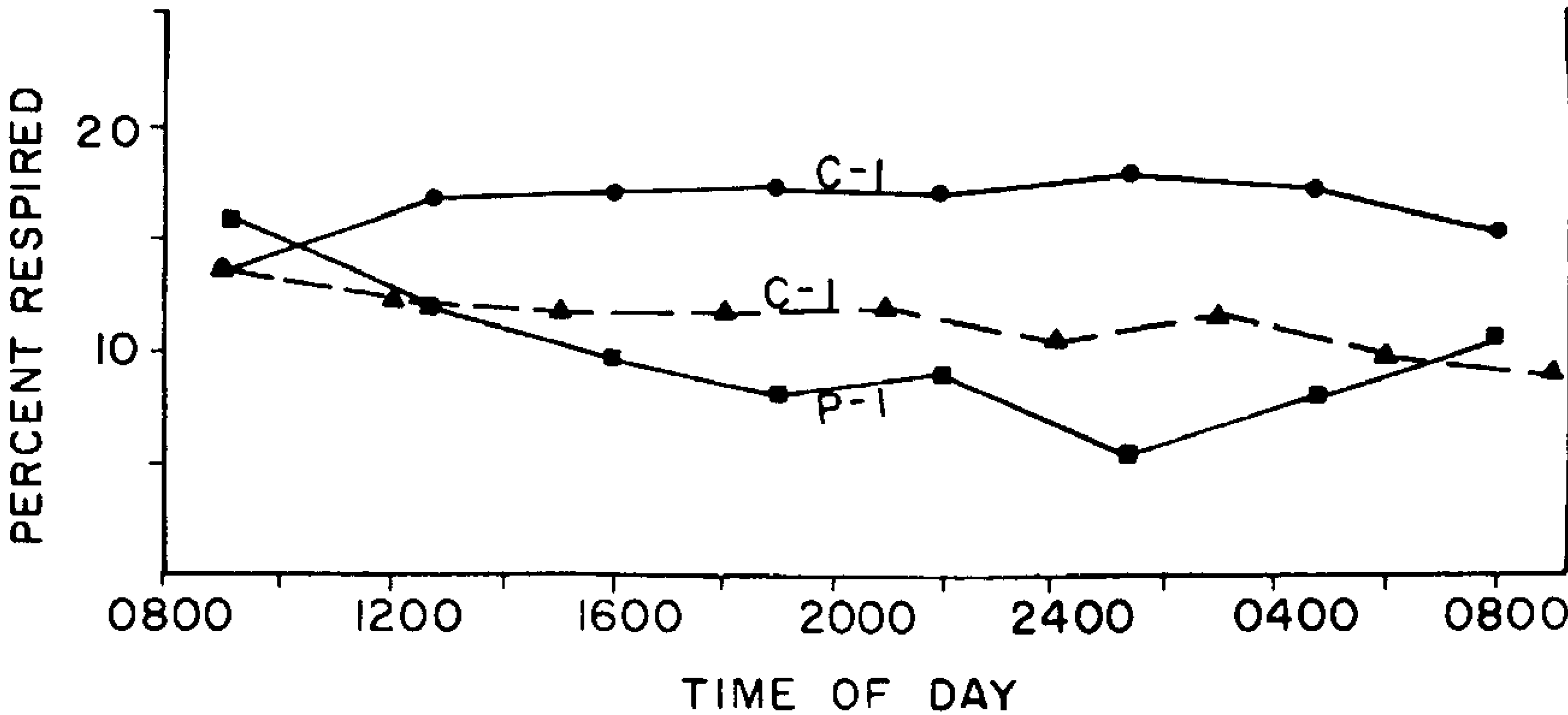


Figure 67. Diurnal pattern of percent respiration (%R) determined as carbon dioxide evolution divided by total glucose uptake (Marsh, 1971). Each point is the mean percent respiration for all concentrations of added glucose at that hour; control pond C-1, June 16-17,1970; waste pond P-1, July 23-24, 1970.

Raps (1973) measured nitrogen fixing activity in the top ooze and in the deeper mud. Light driven nitrogen fixation by plants in the ooze was diurnally sensitive, whereas that in the deep mud was substrate driven. Although nitrogen fixing was greater in the ooze, the deeper mud, because of its volume, contributed more on an area basis.

Sediments particles were studied by A. LeFurgey (1971e) with R. Ingram. More sand was in the periphery and more clay in deeper center. Clay minerals were more uniform (Table 30). Kaolinite, illite, and 14A hydroxy-interlayer mineral occurred in all samples from both sets of pond. Each percentage was calculated from magnesium- treated samples from 1.2 and 0.9 m water depth.

Foraminiferal distributions, electron micrograph structures, diversity, biomass and productivity were studied by A. LeFurgey and J. St. Jean (Lefurgey and J. St. Jean, 1973; 1976). Elphidium clavatum was most common in all ponds; also common were Ammonia becarii, Miliammina fusca, Ammontium salsum and Elphidium tumidum. Foram biomass averaged 0.7 dry g/m2 from 80,000 individuals per m2 in control ponds but only 0.23 g/m2 from 27,000 individuals per m2 in waste ponds. Individual foraminifera were larger in the waste ponds with fewer juvenile populations than in control ponds. Foram production rates were estimated as 112 g carbon per control pond and 28 g carbon per waste pond. Percentage similarities of species between ponds ranged from 25% to 61% but without consistent difference between control and waste ponds. Diversities in species found per thousand individuals counted for control ponds was 24 in summer and 22 in winter; for waste ponds 18 in summer and 16 in winter. Ratio of living to dead forams was 51% to 78% in control ponds compared to 39% to 64% in waste ponds, suggesting a higher sedimentation rate in the latter.

Small bottom animals, principally Capitella, Leonereis, and oligochaetes, were estimated from cores in the third summer by McMahan, Powell, and McCrary (Table 31) as 108 g wet biomass/m2 in waste ponds and 39.7 g wet in control ponds. R. Riedl and J. Hall provided Table 32 and these additional notes: Estimates of animal density, 150-200 individuals per 0.01 m2; nematodes comprising 98% of meiofauna; harpactacoid copepods next in numbers; more individuals in waste pond P-2 but more taxa in control pond C-2.

Table 30. Clay in pond sediments (A. Lefurgey and R. Ingram).

Sample	Pond	Lattice width, Angstroms 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 31. Numbers and biomass of small bottom animals in cores+ June 4, 1941 (E. McMahan, A. Powell, and A. McCrary).

Species	Size Diameter mm	C-Ponds number	C-Ponds wet wt., g	P-Ponds number	P-Ponds wet wt., g
Capitella capitatta	1.05 x 0.37	145	0.54	456	2.11
Leonereis culveri	0.78	57	0.39	113	.49
Oligochaetes species	0.24	49	0.16	32	.09
Other worms	-	6	0.03	6	.07
Amphipod		6	0.02	2	.01
Ostracod	0.63	0	0	113	.36
Palaeomontes		2	0.01	0	0
Squillae lava		1	0.01	0	0
Total biomass, g/290/cm^2		262	1.15	589	3.13
g/m^2		262	39.7	589	108
Number of species		7		9	
Species per 1000#		8.1		9.5	

+ 4 cores for each pond, each 6.77 cm diameter, area 24.3cm^2; 12 cores each in C-ponds and P-ponds; area, 290 cm^2 each.

≠ cm^3 unit volumes sp A, .0038; spB, .0068; sp. C, .0032 ostracods, .0032.

Extrapolated on semilog graph
U. Standard Sieve Series
60 Sieve .240 micon

Table 32. Meiofauna in waste pond ooze, 1970 (R. Riedl and J. Hall).

Group		Numbers	Total
plants	blue-green algae	830	
	diatoms in blue stage	1,760	
	diatoms in green or brown stage	320	
	brown algae stages	270	3,180
protozans	Holotricha	370	370
invertebrates			
	Nematoda*	10,300	
	eggs and embryos, undetermined	910	
	Polychaeta	430	
	Harpacticocoidea+	190	
	Nauplii	160	11,990
			15,540

* Monohysteridae, Oncholaimidae, Cyalholaimidae, Linhomoeidae and Chromadoridae

+ Laophontidae and Tachidiidae

Zooplankton

Zooplankton populations, including larvae and permanent plankters, were quick to develop with seeding by hand (Table 3) and through the estuarine water inflow pumps. Williams (1968) found zooplankton in Clarke-Bumpus tows increasing rapidly after filling. In the first year, especially before pumping, faunal elements included many species usually associated with fresh waters, mosquitoes, tendipedid larvae and corixids. Tables 33-34 show plankton able to pass through the pumps. Chlorine was in tap water going to mixing tanks of control ponds, and chlorine was added to effluent leaving the sewage plant passing to the mixing tanks of the waste ponds.

A. McCrary provided Tables 35-47 showing the sequence of changing populations of zooplankton 1968-70, each pond somewhat different. For example, there were Nereis, cyclops and tendipedid insect larvae in 1969 only; Acartia tonsa were numerous in warm months in control ponds, but died out frequently in control ponds; Pseudodiaptomus coronatus was seasonal in control ponds, but mostly missing after the first year in the waste ponds. Copepod nauplii were abundant in all ponds except few in winter; harpactacoid copepods became abundant in all ponds after ooze developed in the first year; barnacle larvae were numerous in two of the control ponds, but mostly absent in other ponds. Numerically, larvae of Spionid annelids were very abundant seasonally in two control ponds in early spring (Figure 68). These abundant annelid larvae later were observed to be dominant benthos: Laeonereis culveri, Nereis succinea, capitellids, Polydora ligni, and Streblospia. The last three were already in the bottom fauna in pond C-1 in summer 1968. After the first year a dense soup of Oithona dominated the waste ponds in summer, up to 1.7 million copepods per m3.

More larvae of species dominant in waste ponds were maintained in control ponds. Larger plankton (net mesh #2) collected with two methods by A. Powell as part of the summer inventory in 1971 (Tables 48-49) were much more numerous in the control ponds than the waste ponds.

Marsh Animals

Fiddler crab larvae (Uca) were observed in the waste pond plankton immediately after filling (Figures 69-70), and fiddler crab adults and burrows developed in and around the Spartina alterniflora patches soon after (Table 50). Camp, Knight and McMahan (1971) observed Uca minax and Uca pugnax. Burrows were twice as numerous in waste ponds where Spartina growth was larger than in control ponds.

Whereas periwinkles, Littorina irrorata, were prominent in surrounding marshes, they did not develop in the non-tidal pond marshes even though seeded as adults and probably passing through pumps. Stiven and Hunter (1971) and Hunter and Stiven (1973), studying the species in the surrounding tidal marshes, found larval and juvenile populations erratic in the waste-affected Calico Creek area, but the snails there were larger and growing faster.

Table 33. Barnacle larvae passing through pump system into mixing tanks (R. Dowds); larvae per litter.

Date, 1970	C-Ponds	P-Ponds
March 14	239	-
March 15	36	-
April 10	5	1.5
May 5	1	-
May 30	4	.3
June 27	0	.5

- = no data

Table 34. Organisms entering ponds through inflow pipes, 9 pm, July 31, 1969; 15 minute catch with #6 plankton net (McCrary).

Pond C-3	Inflow 9:17 p.m.
4	*Pseudodiaptomus*
1	Harpacticoid
1	*Streblospio* larva
2	Nematodes
2	planula ? larvae

Pond P-2	Inflow 10:17 p.m. (only creek water running; no chlorine)
1184	Spionid larvae (Polydora & Streblospio)
2200	Copepod nauplii
292	*Acartia*
964	*Oithona* (3 species)
40	*Pseudodiaptomus*
8	*Paracalanus*
16	*Saphirella*
16	Harpacticoids (4 species)
4	Megalops
4	Rotifer
60	Nematodes
100	Gastropod Veligers
8	Bivalve Veligers
4	*Crassostrea* Veliger
168	Eggs (molluscan)
4	Egg
8	Barnacle nauplii

Table 34 (cont.)

Pond P-2	Inflow 10:17 p.m. (only creek water running; no chlorine)
4	*Oikopleura*
4	Ostracod
4	Loxosomatid Entoproct
4	Foraminferan

Table 35. Annelid larvae, thousands/m^3 (A. McCrary).

Year		C-1	C-2	C-3	P-1	P-2	P-3
1968							
	Aug.	.001				.033	.464
	Sept.	.007					
	Oct.			.328			
	Nov.		.310		.001		
	Dec.						
1969							
	Jan.						
	Feb.	.15	16.1	2.92	.752	.032	.032
	Mar.	.17	15.2	.524			.032
	Apr.	3.71	117.	15.7			.224
	May	.15	13.4	34.8	.128	.320	
	June	3.42	29.4	21.7	4.0	2.81	.256
	July	4.0	3.7	2.59	2.0		
	Aug.	.064	6.78	8.8	112.		
	Sept.		.19	.26	79.		2.0
	Oct.	.064	1.12	.38	46.		16.1
	Nov.	.003		2.4	.016	.001	36.
	Dec.	.032	2.28	1.2	.004		2.12
1970							
	Jan.		1.57	.91	.064		.004
	Feb.	.032	.004	4.5	.048	.008	.008
	Mar.		2.0	.032	.26	.048	.064
	Apr.	.036		16.8	75.8		.192

Table 36. Nereis sp., thousands/m^3 (A. McCrary).

Year	C-1	C-2	C-3	P-1	P-2	P-3
1969						
Apr.	0	0	.77n	0	0	.128n
May	0	0	.032n	0	0	0
			.128t			
June	1.28t	.77t	.21t	0	0	0
July	0	1.18t	.016t	0	0	0
Aug.	.001n	.064t	1.34t	0	0.26n	0
1970						
Apr.	0	0	1.34t	0.38t	0.64n	0
None in other months						

t: Trochophore

n: Nectochaeta

Table 37. Acartia tonsa, thousands/m^3 (A. McCrary).

Year	C-1	C-2	C-3	P-1	P-2	P-3
1968						
Aug.	.220	.006	0	.004	3.33	3.87
Sept.	1.68	0	0	.192	58.9	10.8
Oct.	.004	.016	.85	6.4	27.6	9.6
Nov.	.010	1.87	2.53	7.4	2.62	27.9
Dec.	.001	.001	.001	0	0	7.2
1969						
Jan.	.002	0	0	.001	0	0
Feb.	0	0	0	.090	0	0
Mar.	0	0	.001	0	0	0
Apr.	1.22	1.79	.128	0	2.46	1.54
May	1.32	78.8	.160	.128	.181	1.49
June	28.9	81.7	63.7	0	21.8	26.0
July	12.0	6.6	3.81	0	0	0
Aug.	56.7	28.4	44.8	0	0	0
Sept.	4.6	8.1	6.3	0	0	.59
Oct.	2.4	0.70	0.224	.51	1.02	20.7
Nov.	.003	0	0	.096	.004	.77
Dec.	.001	0	0	0	0	0
1970						
Jan.	0	0	0	0	0	0
Feb.	0	0	0	.016	.036	.008
Mar.	.016	.039	.012	0	0	.004
Apr.	.080	.001	10.0	0	0	.128
May	.66	.40	0.14	0	0	0

Table 38. Pseudodiaptomus coronatur, thousands/m^3 (A. McCrary).

Year	C-1	C-2	C-3	P-1	P-2	P-3
1968						
Aug.	.048	0	0	0	.014	.011
Sept.	0	0	0	.001	.014	.011
Oct.	0	0	.096	0	.001	.001
Nov.	.047	.001	0	.001	.003	.014
Dec.	.001	.001	0	.001	.001	.003
1969						
Jan.	.001	0	.001	0	0	0
Feb.	0	0	0	0	0	0
Mar.	0	0	0	0	0	0
Apr.	0	0	0	0	0	0
May	0	0	0	0	0	0
June	.45	9.73	1.70	0	0	0
July	1.64	1.65	.080	0	0	0
Aug.	2.56	0.70	.64	0	0	0
Sept.	1.73	1.15	.128	0	0	.032
Oct.	5.5	0	0	0	0	0
Nov.	.010	.002	0	0	0	0
Dec.	.047	.002	.001	0	0	.016
1970						
Jan.	.017	.007	0	0	0	0
Feb.	.002	0	0	0	.016	0
Mar.	0	0	0	0	0	0
Apr.	.068	.001	.048	0	0	0
May	0	0	0	0	0	0

Table 39. Copepod *nauplii*, thousands/m^3 (A. McCrary).

Year	C-1	C-2	C-3	P-1	P-2	P-3
1968						
Aug.	0	0	0	.002	.002	.001
Sept.	.001	0	0	.032	.001	0
Oct.	0	0	.096	0	0	0
Nov.	0	0	.002	.002	.080	.002
Dec.	0	0	0	.001	0	0
1969						
Jan.	0	0	0	0	0	0
Feb.	0	0	0	2.24	.160	.64
Mar.	0	0	0	18.7	.096	1.09
Apr.	1.02	4.86	.256	4.48	4.67	4.7
May	.74	4.16	2.94	6.4	.83	5.8
June	4.45	.98	48.8	467.	251.	.77
July	.50	5.5	2.30	1,096.	527.	612.
Aug.	13.8	61.6	32.3	112.	517.	212.
Sept.	.26	4.6	1.4	75.	203.	80.
Oct.	7.0	10.8	11.3	46.	81.	34.
Nov.	.004	.080	.016	.016	0	22.
Dec.	0	0	0	0	0	.026
1970						
Jan.	0	0	0	.004	0	.084
Feb.	0	0	0	.064	.020	.82
Mar.	.080	.048	.032	.048	.112	.27
Apr.	.232	0	2.11	.256	0	2.43
May	.020	.004	.052	.758	77.	.77

Table 40. Oithona, thousands/m^3 (A. McCrary).

Year	C-1	C-2	C-3	P-1	P-2	P-3
1968						
Aug.	0	0	0	2.6	.008	0
Sept.	.128	0	0	3.9	0	0
Oct.	.001	.014	.46	.192	0	0
Nov.	0	0	0	0	0	0
Dec.	0	0	0	.008	0	0
1969						
Jan.	0	0	0	.005	0	.001
Feb.	0	0	0	0	0	0
Mar.	0	0	0	0	0	0
Apr.	0	2.05	0	0	.192	0
May	0	.43	0	13.7	.373	.085
June	.112	1.02	0	424.	37.9	1,464.
July	.080	0	0	1,720.	807.	290.
Aug.	.128	0	.38	77.5	312.	234.
Sept.	.45	0	0	155.6	149.	74.
Oct.	0	.016	0	68.9	30.	7.2
Nov.	0	0	0	0	.012	.51
Dec.	0	0	.016	0	0	.048
1970						
Jan.	0	0	0	0	0	0
Feb.	.128	0	0	.128	.168	.064
Mar.	.46	.016	.90	.61	.23	.40
Apr.	.044	0	0	12.1	18.5	6.0
May	.24	.26	.012	159.	60.5	379.

Table 41. Harpactacoid copepods, thousands/m^3 (A. McCrary).

Year	C-1	C-2	C-3	P-1	P-2	P-3
1968						
Aug.	0	0	0	.001	0	.001
Sept.	0	0	0	0	0	0
Oct.	0	0	0	0	0	0
Nov.	0	0	0	0	0	0
Dec.	0	0	0	.012	0	0
1969						
Jan.	0	0	0	.040	.001	.006
Feb.	.001	.064	.001	5.20	.288	1.30
Mar.	.035	0	.008	16.3	.096	.67
Apr.	.128	.384	.128	.26	.192	.192
May	.038	.048	.032	.064	0	.043
June	.048	0	.208	.51	9.2	.26
July	0	.001	.016	45.	0	0
Aug.	.26	.64	.192	.77	0	0
Sept.	0	.128	0	0	0	.51
Oct.	.064	.032	0	0	0	.26
Nov.	.030	.032	.016	.096	.024	.29
Dec.	.032	.080	0	.024	.008	.96
1970						
Jan.	2.66	.084	.048	.124	.064	.34
Feb.	15.2	3.90	.320	.45	.152	.016
Mar.	4.0	.224	5.12	.45	1.95	.50
Apr.	.028	.001	0	.45	.384	.45
May	0	0	0	.51	0	.26

Table 42. All copepods, thousands per m^3 (A. McCrary).

Year	C-1	C-2	C-3	P-1	P-2	P-3
1968						
Aug.	1.40	.006	0	2.58	4.50	4.01
Sept.	4.77	0	0	4.15	59.6	12.1
Oct.	.005	.030	1.5	6.61	27.6	9.57
Nov.	.057	1.87	2.52	7.43	2.63	27.9
Dec.	.002	.002	.001	.021	.001	7.17
1969						
Jan.	.003	0	.001	.046	.001	.007
Feb.	.001	.064	.001	5.29	.288	1.30
Mar.	.035	0	.009	16.26	.096	.672
Apr.	1.344	4.22	.256	.256	2.85	1.73
May	2.362	79.3	.192	14.1	.554	1.75
June	29.504	92.4	65.7	425.	68.9	1,490.
July	13.798	1.7	3.9	1,765.	809.	291.
Aug.	73.5	91.5	78.3	191.	831.	446.
Sept.	7.04	14.0	7.81	231.	353.	155.
Oct.	15.0	11.5	11.5	115.	112.	370.
Nov.	.052	.114	.032	.112	.040	20.3
Dec.	.080	.082	.017	.024	.008	1.28
1970						
Jan.	2.67	0.91	.048	.128	.064	.700
Feb.	15.3	3.90	.320	.704	.404	1.68
Mar.	4.54	.288	6.05	1.20	2.32	3.06
Apr.	.952	.002	12.1	12.9	19.0	13.5
May	.912	.004	.052	235.	138.	380.

Table 43. Barnacle <u>nauplii</u>, thousands per m^3 (A. McCrary).

Year	C-1	C-2	C-3	P-1	P-2	P-3
1968						
Aug.	0	0	0	0	0	0
Sept.	0	0	0	0	0	0
Oct.	0	0	.032	0	0	0
Nov.	0	0	0	0	0	0
Dec.	0	0	0	0	0	0
1969						
Jan.	0	0	0	0	0	0
Feb.	0	.016	0	0	0	0
Mar.	0	0	0	0	0	0
Apr.	0	1.15	0	0	0	0
May	0	0	.51	0	0	0
June	0	2.05	.59	0	.26	0
July	0	.032	.57	0	0	0
Aug.	0	0	1.22	0	0	0
Sept.	0	0	.064	0	0	0
Oct.	0	.048	.016	0	0	0
Nov.	0	.112	.064	0	0	0
Dec.	0	.112	0	0	0	0
1970						
Jan.	0	.048	.016	0	0	0
Feb.	0	0	0	0	.012	0
Mar.	0	0	0	0	0	0
Apr.	.004	0	2.1	0	0	0
May	0	0	.052	0	0	0

Table 44. Small Palaemonetes, individuals/m^3 (A. McCrary).

Year	C-1	C-2	C-3	P-1	P-2	P-3
1968						
Aug.	3	0	0	71	347	85
Sept.	1	0	0	32	97	95
Oct.	0	0	1	11	3	140
Nov.	0	0	0	11	1	5
Dec.	0	0	0	1	2	1
1969						
Jan.	0	0	0	0	1	0
Feb.	0	0	0	2	0	0
Mar.	0	0	0	10	13	23
Apr.	3	0	0	23	29	28
May	331	16	0	1	24	9
June	528	16	4	280	110	89
July	105	18	5	71	102	103
Aug.	16	3	0	22	97	20
Sept.	5	0	4	20	23	7
Oct.	0	0	0	10	8	5
Nov.	0	0	0	0	1	0
Dec.	0	0	0	1	0	0
1970						
Jan.	0	0	1	3	13	0
Feb.	2	0	0	3	3	3
Mar.	0	0	0	1	1	1
Apr.	4	1	3	1	0	10
May.	0	2	0	1	0	0

Table 45. Palaemonetes larval stages #1 and #2, individuals/m^3 (A. McCrary).

Year	C-1	C-2	C-3	P-1	P-2	P-3
1968						
Aug.	0	0	0	42	0	15
Sept.	0	0	0	1	8	9
Oct.	0	0	0	2	0	1
Nov.	0	0	0	0	0	0
Dec.	0	0	0	0	0	0
1969						
Jan.	0	0	0	0	0	0
Feb.	0	0	0	0	0	0
Mar.	0	0	0	0	0	0
Apr.	0	0	0	0	0	0
May	280	16	0	0	15	0
June	208	0	4	256	64	32
July	50	11	4	32	20	64
Aug.	124	0	4	115	130	58
Sept.	11	12	0	0	52	58
Oct.	2	0	0	0	18	1
Nov.	0	0	0	0	0	0
Dec.	0	0	0	0	0	0
1970						
Jan.	0	0	1	0	0	0
Feb.	0	0	0	0	0	0
Mar.	0	0	0	0	0	0
Apr.	10	3	6	0	0	0
May	38	57	0	4	0	6

Table 46. Corixid water bugs, individuals/m^3 (A. McCrary).

Year	C-1	C-2	C-3	C-4	C-5	C-6
1968 Aug.	0	3	41	12	32	5
Sept.	1	4	34	Obsv.*	9	1
Oct.	1	18	5	0	Obsv.*	0
Nov.	0	2	2	0	0	0
Dec.	11	33	41	0	1	0
1969 Jan.	4	4	13	Obsv.*	0	0
Feb.	0	16	3	0	0	0
Mar.	1	1	3	0	0	0
Apr.	0	0	35	0	0	0
May	0	0	0	0	0	0
June	0	0	0	0	0	0
July	0	0	0	0	0	0

* Observed in pond but not taken in sample.

Table 47. Other plankters, thousands/m^3 (A. McCrary).

Year		C-1	C-2	C-3	P-1	P-2	P-3
1968	Aug.					.001	.003
	Sept.						
	Oct.			.032		.001	.001
	Nov.				.004	.001	.001
	Dec.			.002	.002	.004	.008
1969	Jan.	.003	.001		.005	.011	.018
	Feb.	.009	.048	.002	.490	.384	1.568
	Mar.	.141			1.152	.096	.352
	Apr.		1.152	.768			
	May	.011	.560	2.388	.192	.128	
	June		2.304	.742	.256		.016
	July	.004	.048	.576	3.072		
	Aug.	.264	.193	.192	85.0	653.	842.
	Sept.	0	0	.001	184.	95.0	42.0
	Oct.	.066	.032	.80	786.	420.	1.54
	Nov.	.002	.032	.032	.416	.005	0
	Dec.	.036	.033	.017	0	0	.048
1970	Jan.	.004	.002	.016	.004	0	0
	Feb.	.073	.026	.064	.016	.028	0
	March	.046	.050	.001	.416	0	0
	April	.101	.371	.011	5.06	1.73	1.15
	May	.004	.008	.005	663.	356.	0

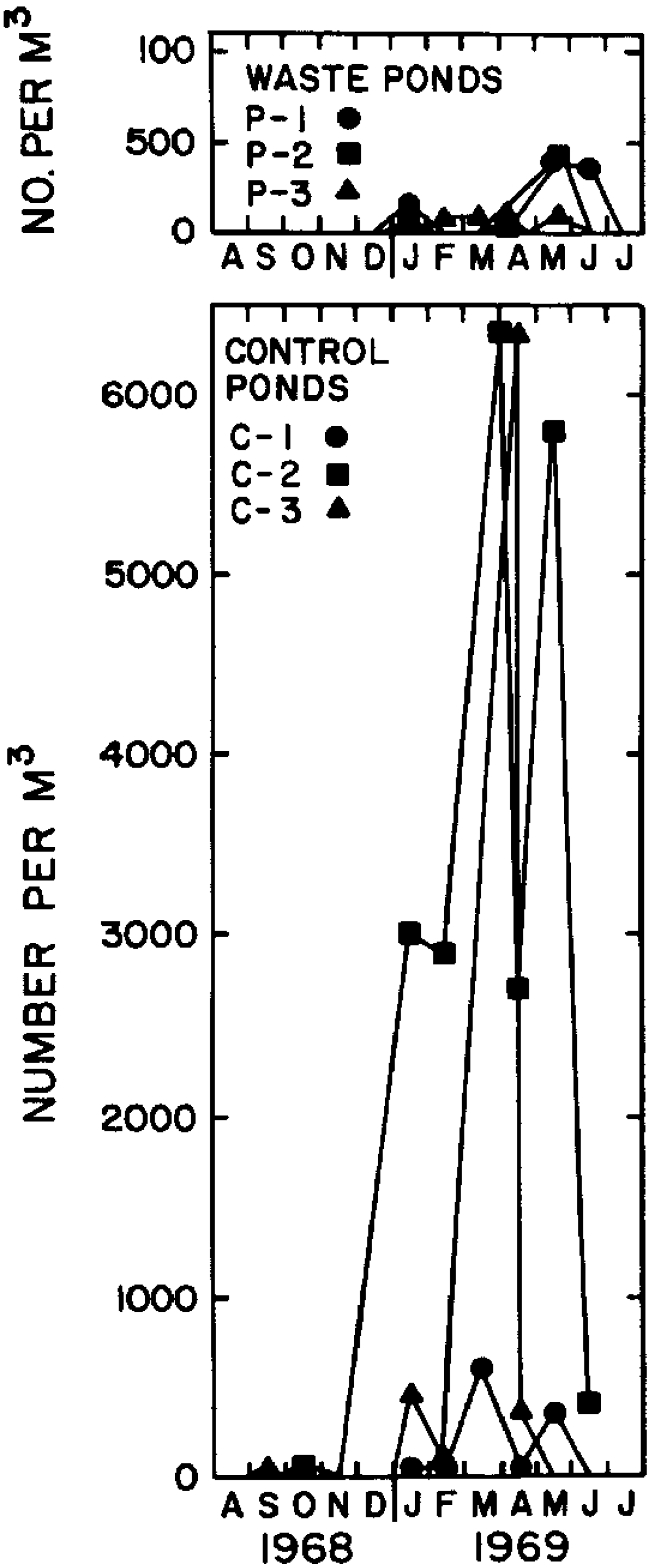


Figure 68. Spionid annelid larvae in 6 ponds, 1968-69 (A. McCrary).

Table 48. Larger zooplankton in ponds July 6, 1971, bucket and net+ (A. Powell).

Species	Numbers C-1	C-2	C-3	Total	Numbers P-1	P-2	P-3	Total
Mysidopsis bigelowri	217	14	4	235	1		5	6
Copepods	550	37	31	618	15	0	28	43
Insects		1	1	2		1		1
Paleomonetes pugio	38	9	6	53		17	6	23
Snapping shrimp	1			1				
Gammarid F		1		1				
Uca larvae zoea					3	12	36	51
Fundulus							1	1
Number Ind.	816	62	42	920	19	40	76	135
Wet wt., mg								
Volume liters	9	9	9	27	9	9	9	27
Species found, #								

+ Buckets into nets at night #2 net, .366 mm.

Table 49. Larger zooplankton in pond July 6, 1971, Clarke Bumpus sampler[+] (A. Powell).

Species	Numbers C-1	C-2	C-3	Total	Numbers P-1	P-2	P-3	Total
Mysidopsis	9		3	12	2			2
Copepods	3052	1	172	3125	158	1	33	192
Annelids		4	2	6			20	20
Insects, mixed					11			11
Diptera						4		4
Corophium lacustre	1	62	30	93	10	6	1	17
Gammarid						26		26
Paleomonetes pugio	13	8	11	32	22	19	2	43
Ostracods						2		2
Uca			6	6	125	85	10	220
Fundulus							2	2
Numbers of Ind.				3274				539
Wet wt. mg								
Volume liters	223	87	161	377	244	190	194	628

+ Clarke Bumpus, tow #2 net.

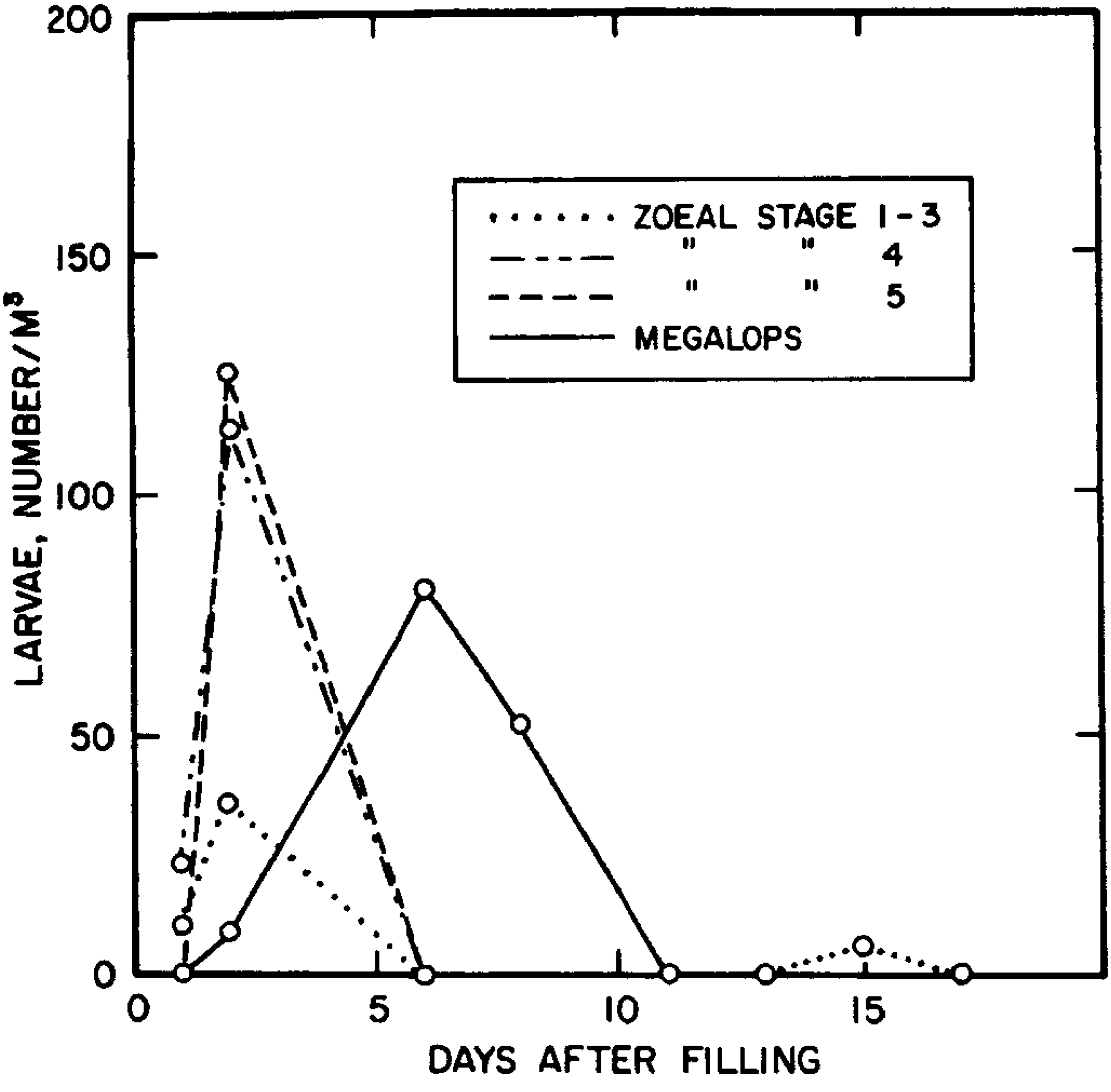


Figure 69. Larval Uca stages in pond P-1 after filling in July, 1968 (R. Hyle (Williams, 1968).

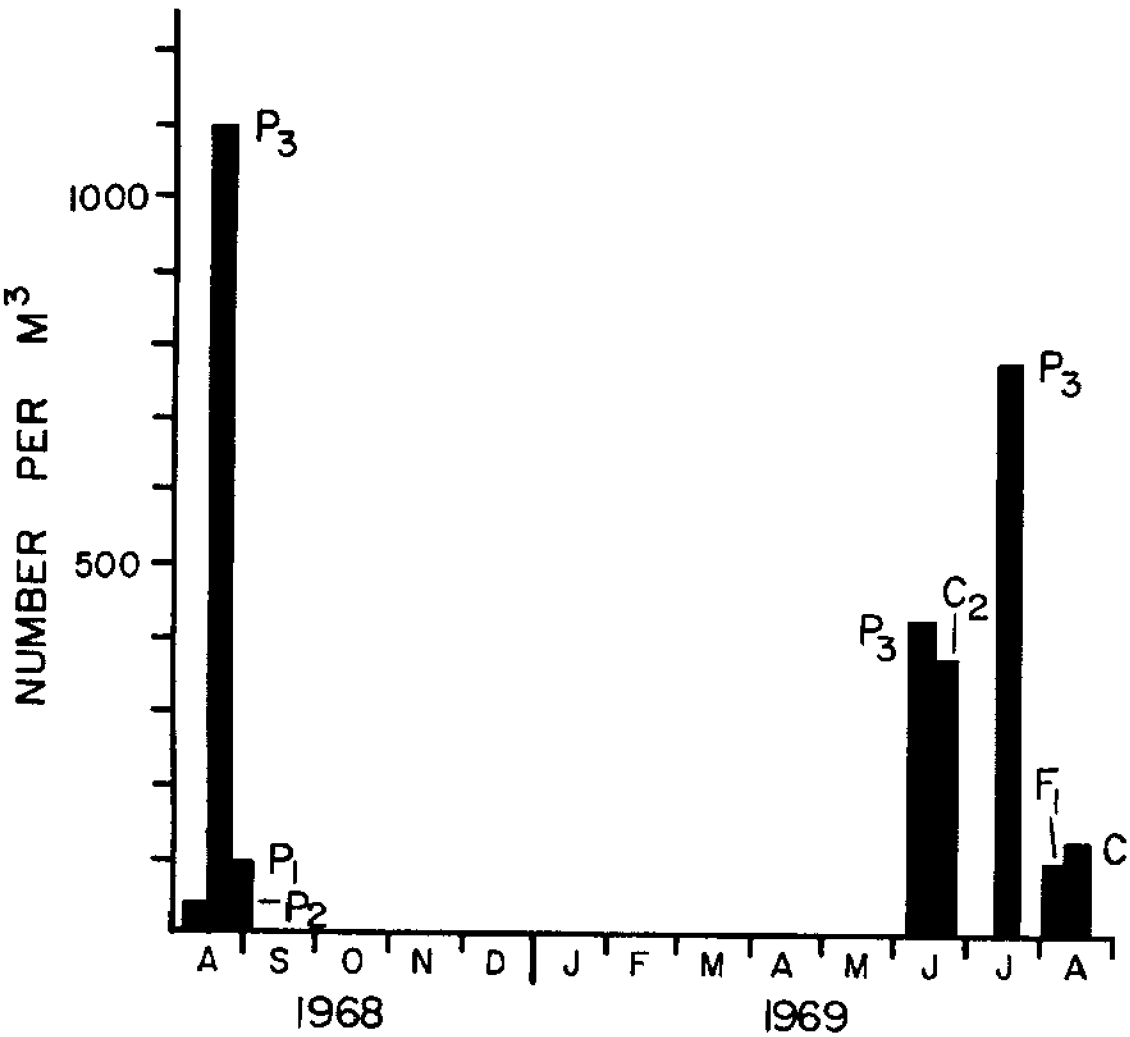


Figure 70. Zoea larvae of Uca in ponds in 1968-69 (A. McCrary).

Table 50. Uca burrows around comparable clear margins[f] of Spartina in ponds, 1970[1]-1971[2] (Camp, Knight, and McMahan, 1971).

Pond margin	1970	1971	Difference
P-1	402	642	+240
P-2	224	201	- 23
P-3	168	154	- 14
	794	997	+203
C-1	254	320	+ 66
C-2	233	322	+ 89
C-3	194	247	+ 53
	681	889	+208

[1] Data from Camp, Knight, and McMahan, 1971.

[2] Data from Miller and McMahan, 1972, unpublished report.

[f] Spartina growth was so dense as to make burrow detection extremely difficult in certain areas. Comparisons are made of relatively clear areas that were counted on both occasions. Also about 20 meters of P1 margin had been eliminated for burrow counts by walkway excavations in 1971.

Enormous numbers and variety of microarthropods (Tables 51-52) were sampled by vacuum suction from the pond marshes and from the surrounding tidal marshes by McMahan, Knight, and Camp (1971,1972). Densities in the non-tidal pond marshes was considerably higher than in the tidal marshes. Diversities in the non-tidal pond marshes was considerably higher than in the tidal marshes. Diversity was less in the waste pond marsh than control pond marsh although quantities of arthropods were comparable (Table 53).

Animals of Encrusting Surfaces and Reefs

The various wood and masonry surfaces that were introduced (Figure 2, Table 3) developed encrusting, reef-like communities borers, ectoproct bryozoa, small anemones, a few oysters, etc. (Table 54). Dowds and Williams (1971) conducted four surveys comparing events in the estuaries with those in the ponds. In the waste ponds, fouling surfaces were overgrown with blue-green algal mats. On these there were seven animal phyla and 10 species. One plate had 59.5 dry g alga (791 g/m2 of plate area). At the time of the pond inventory in 1971 a small striped anemone (Diadumene luciae) was covering all the solid surfaces in pond P-2. Plates from the control ponds held fewer algae with seven animal phyla and nine species. Diversity in plates from Bogue sound was higher, with nine animal phyla and 26 species, and lower in Calico Creek with four animal phyla and five species.

In both sets of ponds sparse populations of barnacles, Balanus eburneus and a few Balanus amphitrite (Tables 55-57) grew from a set observed only in the first year when larvae were also observed in the ponds (Table 43). On the pier posts, the side facing the shaded underside of the pier had the highest density. Populations in the ponds were less than those in surrounding estuaries where currents were stronger and larval populations may have been greater.

Borers, Limnoria tripunctata, found in stakes in surrounding Bogue Sound did not enter and colonize wood in the ponds, but populations already present in introduced boards survived a 4.5-month period of study (Table 58).

An association of encrusting organisms and associated swimming animals developed on the concrete-block rubble reefs marked in Figure 2. Ivory barnacles and oysters set in the first year, partly covered with blue-green algae in the waste ponds. Ulva and other macrophytic algae encouraged to grow on these reefs did not develop. Walton and Williams (1971) in 1970 found reef-associated populations of snapping shrimp were abundant in pond C-1 (Table 59) and a few in pond C-2. Xanthid crabs (Table 60) were abundant on control pond reefs in 1970 and in the general pond inventory in 1971, but only two were found in waste pond reefs. Of the nine xanthid crabs in similar habitat in Bogue Sound, three were found in the ponds (Eurypanopeus depressus), the most numerous with a few Panopeus herbstii and Neopanope texana savi. For 19 crabs measured and dried, dry weight was 41.8% of wet weight with range 31% to 46%.

Table 51. Taxonomic grouping of microarthropods from Spartina marsh in ponds in 1970 (McMahan, Knight, and Camp, 1971).

Order or Group	P Pond Specimens	P Pond Species	C Pond Specimens	C Pond Species
Collembola	0	0	31	3
Psocoptera	8	1	0	0
Homoptera	312	7	166	5
Heteroptera	229	3	94	3
Thysanoptera	17	3	101	5
Lepidoptera	1	1	1	1
Coleoptera	23	8	10	5
Hymenoptera	103	10	94	23
Diptera	253	29	304	35
Mites	9	2	12	3
Spiders	78	9	52	5
Total	1033	73	865	88
Diversity Index:				
No. species / 1000 specimens	71		102	

Table 52. Comparison of microarthropods in pond marshes, 1970 (McMahan, Knight, and Camp, 1971).

	P Pond		C Pond	
Sample, 1970	Specimens	Species	Specimens	Species
1. March 29	190	18	169	19
2. April 25	461	33	409	47
3. June 4	382	51	287	46
Total	1033	73*	865	88*

* Total number of species does not equal the sum for the three collecting dates because of species overlap in the 3 samples.

Table 53. Diversity measurements in summer.

	Investigator	Pond	Species per 1000 individuals counted			
			1968[a]	1969[b]	1970	1971
Phytoplankton	M. May*	P-1	33	17	18	
		P-2	6[c]	27	12	
		P-3	29	13	24	
		C-1	9	5	8	
		C-2	10	6	21	1
		C-3	10	11	30	
			Top	Bottom		
Algae, on glass slides one month	M. May	P-1	42	26	19	
		P-2	23	-	15	
		P-3	22	25	13	
		C-1	26	22	24	
		C-2	37	46	14	
		C-3	40	33	15	
					Aug 24	
Zooplankton	M. May	P-1	8		8	
		P-2	8		7	
		P-3	10		8	
		C-1	10		14	
		C-2	9		18	
		C-3	12		8	
Seined animals	Team	P-1				5.9
		P-2				8.1
		P-3				8.8
		C-1				17.9
		C-2				12.2
		C-3				14.8
Insects in pond *Spartina*	E.A. McMahan**	P-1			22	
		P-2			37	
		P-3			63	
		C-1			24	
		C-2			59	
		C-3			60	

a, July 15, 1969; b, July 25, 1969; c, bloom of 10^6ml *Oocystis parva*; d, Aug 23.

* NSF Research trainee with H.T. Odum, Dept. of Botany.

**E.A. McMahan (1971).

Table 53 (cont.)

Insects in pond pcSpartina patches ies	E. McMahan et al. (1971)	**Species per 1000 individuals** 1970	
		P1	22
		P2	37
		P3	63
		C1	24
		C2	59
		C3	60
Bottom microfauna		C	8.1
		P	9.5
Living foraminifora Winter, 1970	A. LeFurgey		
		C1	24
		C2	18
		C3	18
		P1	24*
		P2	16*
		P3	16*
Summer, 1971		C1	26
		C2	19
		C3	13
		Species per 1000 individuals 1970	
Summer, 1971		P1	24
		P2	23
		P3	25

* Extrapolated from fewer than 100 specimens; see Fig. 27b.

Table 54. Animals encrusting on fouling plates,[a] 1968-1970.

		June, 1969		June, 1971	
		Barnacles[f]	Oysters[g]	Barnacles[f]	Oysters[g]
Control Ponds	C1	17[b]	6[c]	43	4
	C2	46[d]	0	60	3
	C3	11	0	24	2
Waste Ponds	P1	84[e]	0	172	0
	P2	3	0	160	0
	P3	6	0	165	0

Footnotes:

a Four plates planted in each pond June, 1968 with dimensions
b 31 x 25 x 5 m;-area 0.211 m^2; total area per pond, 0.8 15-20 mm diameter; a set observed autumn 1967.
c 45 mm diameter
d 5 - 10 mm diameter
e 2.5 mm diameter
f Balanus eburneus, barnacles in two sizes
g Crassostrea virginica

Table 55. Barnacle larvae and set, 1970 (R. Dowds).

	14 Mar	15 Mar	10 Apr	5 May	30 May	27 June
C Seawater Spigot	238.5	36.0	4.8	0 8	3.5	0.0
P Seawater Spigot	--	--	1.5	--	0 3	0.5

Mean number of barnacle nauplii per liter in all study areas on April 10, 1970.

	Sound	C Spigot	C_1	C_2	C_3	Calico Creek	P Spigot	P_1	P_2	P_3
Mean barnacle nauplii Per liter	.1	4.8	0	0	0	.2	1.5	0	0	0

Barnacles per 100 cm^2 on plexiglass plates.

	Sound	C_1	C_2	C_3	Calico	P_1	P_2	P_3
Barnacles per 100 cm^2	39.8	0	0	0	20.6	0	0	(
% live	10				97			

Table 56. Barnacle fouling on wood and concrete, 1968-1970 (Dowds and Williams, 1971).

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
C-2	182,300	0.83	1.80	57.5	88.5	5.7	0	6.8
P-2	175,228	1.33	1.61	77.4	96.3	0.8	0	2.8

* Indeterminate due to extremely small size of some individuals.

Table 57a. Barnacle fouling on sides of pier posts, 1970 (Dowds and Williams, 1971).

	Barnacles per 100 cm^2	% live	Mean diam. (cm)
Pond C-2			
Outermost	0.2	0	1.4
Lateral	0.3	66.7	1.8
Lateral	1.3	64.3	2.2
Innermost*	1.7	60.9	2.1
Pond P-2			
Outermost	1.2	50.0	1.7
Lateral	3.2	68.6	1.9
Lateral	3.0	65.6	1.7
Innermost*	3.8	64.3	1.9

* in shadows

Table 57b. Barnacle fouling on stakes, 1970 (Dowds and Williams, 1971).

Location	Barnacles per 100 cm^2		Mean diam. (cm)
Sound	92.6		0.6
Creek	53.2		0.5
C-1	0		-
C-2	0		-
C-3	0		-
P-1	0.52	(Approx.)	1.1
P-2	0.65	(Approx.)	1.2
P-3	0.52	(Approx.)	1.3

Table 58. <u>Limnoria</u> survival in transplanted boards (Dowds and Williams, 1971).

	Initial 6 Aug	7 Sept	14 Dec.
Sound	5.3	20.1	4.9*
C-2	7.6	0.8	2.6
Creek	9.2	1.9	0.6
P-2	8.5	9.2	3.1

* This sample heavily infested with <u>Bankia</u> sp; therefore not all the volume sampled was available for <u>Limnoria</u> infestation; consequently this figure may be an underestimate.

Table 59. Snapping shrimp, *Alpheus heterochaelos* in shell reef of pond C1, 1970 (Walton and Williams, 1971.

Date	Total N	Mean total length,mm	Total wet wt., g	Estimated pop.	Calculated total wet weight, g
June 30	24	18.9	-	1,080	-
July 7	32	18.8	-	1,440	-
July 14	47	16.5	-	2,120	-
July 22	39	15.1	4.5	1,760	202
July 28	61	14.4	4.9	2,740	220
Aug. 5	45	15.9	4.7	2,020	212
Aug. 11	40	15.1	3.7	1,800	166
Aug. 15	34	14.5	3.2	1,530	144
Aug. 16	32	12.8	2.4	1,440	108
Aug. 20	42	15.5	5.1	1,890	230
Aug. 28	59	14.3	-	2,660	-
Sept. 7	35	15.5	3.7	1,580	166
Sept. 15	55	15.6	5.7	2,480	256
Sept. 22	45	16.0	5.2	2,020	234
Sept. 29	28	15.9	4.1	1,260	184
Oct. 6	36	14.3	3.2	1,620	144

Table 60a. Xanthid crabs *Eurypanopeus depressus* in Screen A shell reef of pond C2, 1970 (Walton and Williams, 1971).

Date	Total N	Mean ♂	width ♀	carapace, mm ♂+♀	Total wet weight, g	Estimated pop.	Calculated total wet weight, g	Ratio ♂/♀	% ♀ gravid
June 20	101	11.1	9.1	9.8	-	3,983.4	-	.6	19
June 30	61	11.5	9.0	9.9	-	2,405.8	-	.5	15.4
July 7	62	12.2	9.4	10.4	21.8	2,445.3	858.2	.4	6.9
July 14	36	12.3	10.0	10.4	12.3	1,419.8	483.5	.2	7.5
July 22	56	11.9	9.0	9.7	16.3	2,208.6	641.3	.2	16.3
July 28	41	12.0	9.1	10.0	18.7	1,617.0	739.5	.5	0
Aug. 5	55	12.1	9.6	10.4	21.3	2,169.2	840.1	.5	2.7
Aug. 11	27	15.4	9.6	9.9	9.3	1,063.8	366.4	.9	14.3
Aug. 20	10	13.0	9.1	10.3	4.1	394.0	161.2	.4	16.7
Aug. 28	7	8.0	9.7	8.2	2.4	275.8	94.6	.7	50
Sept. 7	11	11.4	9.0	10.1	4.5	433.4	179.3	1.2	0
Sept. 15	8	10.2	12.5	10.9	2.8	315.2	110.3	3.0	50
Sept. 22	6	11.8	13.0	12.0	2.4	264.0	94.6	5.0	0
Sept. 29	1	-	8.0	8.0	.2	39.4	7.9	-	0
Oct. 6	1	8.0	-	8.0	.2	39.4	5.9	-	-

Table 60b. Xanthid crabs Eurypanopeus depressus in Screen B shell reef of control pond C2, 1970 (Walton and Williams, 1971).

Date	Total N	Mean ♂	width ♀	Carapace, mm ♂+♀	Total wet weight, g	Estimated pop.	Calculated total wet weight, g	Ratio ♂/♀	% ♀ gravid
July 16	64	10.3	9.4	9.7	20.6	2,542.2	811.3	.6	17
July 22	30	11.9	9.0	10.8	13.2	1,183.2	519.8	.8	0
July 28	34	11.5	9.3	9.7	11.3	1,340.9	443.7	.4	12.5
Aug. 5	33	11.4	9.8	10.3	13.6	1,301.5	536.4	.5	18.2
Aug. 11	33	11.5	9.9	10.6	15.1	1,300.2	594.9	.8	11.1
Aug. 20	10	12.0	9.2	10.3	6.0	394.0	236.4	.7	16.7
Sept. 7	2	12.5	-	12.5	2.1	78.8	81.9	-	-
Sept. 15	5	9.7	9.0	9.4	2.1	197.0	82.7	1.3	0
Sept. 22	1	-	8.0	8.0	.2	39.4	6.3	-	-
Sept. 29	4	11.0	10.0	10.7	1.6	157.6	63.0	1.0	0
Oct. 6	0	-	-	-	-	-	-		-

Table 60c. Xanthid crabs *Eurypanopeus depressus* from screen sampling of shell reef of control pond C1, 1970 (Walton and Williams, 1971).

Date	Total N	Mean width carapace; mm ♂	♀	♂+♀	Total wet weight, g	Estimated pop.	Calculated total wet weight, g	Ratio ♂/♀	% ♀ gravid
June 30	12	15.8	12.3	14.1	-	540.0	-	1.0	50
July 7	21	14.5	11.9	13.4	18.0	945.0	811.4	1.3	67
July 14	17	15.5	12.3	14.4	18.4	765.0	828.0	1.8	16.7
July 22	12	16.4	13.6	15.3	14.2	540.0	639.0	1.4	40
July 28	16	16.0	13.0	14.3	15.5	720.0	697.5	.8	33
Aug. 5	17	17.6	13.1	15.8	21.0	765.0	945.9	1.4	42.9
Aug. 11	13	17.8	12.8	16.2	17.2	585.0	774.0	2.3	0
Aug. 15	8	19.0	13.0	16.0	10.1	360.0	454.5	1.0	25
Aug. 16	7	17.5	13.7	15.9	8.0	315.0	360.0	1.3	0
Aug. 20	6	17.5	13.5	14.8	6.6	270.0	297.0	.5	50
Aug. 28	8	19.0	13.3	14.8	12.2	360.0	549.0	.3	50
Sept. 7	8	20	13.4	14.5	6.2	360.0	279.0	.2	80
Sept. 15	7	18.0	14.5	16.0	7.0	315.0	315.0	.7	25
Sept. 22	6	17.7	14.3	16.0	6.5	270.0	292.5	1.0	0
Sept. 29	6	17.5	14.3	15.3	6.2	270.0	270.0	.5	.25
Oct. 6	4	18.7	13.0	17.3	5.8	180.0	216.0	3.0	0

Table 60d. Xanthid crabs *Eurypanopeus depressus* from screen sampling of shell reef in control pond C3, 1970 (Walton and Williams, 1971).

Date	Total N	Mean ♂	width ♀	carapace,mm ♂+♀	Total wet weight, g	Estimated pop.	Calculated total wet weitht, g	Ratio ♂/♀	% ♀ gravid
June 30	4	-	9.7	9.7	-	220.8	-	0	0
July 7	14	16.0	12.8	12.8	9.7	772.8	535.4	.4	40
July 14	6	14.3	12.3	13.3	3.8	331.2	209.8	1.0	33
July 22	10	17.0	12.0	14.5	11.0	552.0	607.2	1.0	80
July 28	7	12.7	14.5	13.7	5.4	386.4	298.1	.7	75
Aug. 5	7	14.6	15.0	14.3	7.4	386.4	408.5	6.0	100
Aug. 11	11	17.5	13.9	15.2	12.5	607.2	690.0	.6	14.3
Aug. 20	10	18.0	13.9	15.1	11.8	552.0	651.4	.4	86
Aug. 28	8	16.0	14.3	15.1	10.5	441.6	579.6	1.0	75
Sept. 7	5	14.5	14.3	14.4	4.8	276.0	264.9	.7	67
Sept. 15	5	13.0	13.8	13.6	3.8	276.0	209.8	.3	100
Sept. 22	4	-	16.0	16.0	3.6	220.8	198.7	-	25
Sept. 29	6	17.5	14.3	15.3	6.2	270.0	279.0	.5	25
Oct. 6	1	20.0	-	20.0	1.8	55.2	99.4	-	-

Reproduction was observed. Decline in populations was observed starting in mid-summer, a time when these crabs normally migrate to deeper water. Ten meter movements of tagged crabs were observed.

Oysters and Clams

Although some recruitment from the larval set was observed, studies of survival and growth were primarily made of previously introduced and measured oysters (Chestnut, 1970; Muse, 1971; Muse and Chestnut, 1973). Rangia clams and oysters introduced as spat on shell reef in 1968-69 were measured during the 1971 pond inventory. Table 61 summarizes survival and growth of these and the oysters introduced in trays and on strings. In pond C-1 a reef of 298 live oysters developed, whereas there were few alive on the Ruppia-covered bottoms in ponds C-2 and C-3, and none of the original oysters in the waste ponds Surviving from a recent addition were 80 live oysters in waste pond P-3, four in pond P-2 and none in pond P-1.

Oyster growth studies (Figures 71-73) and mortality rates (Figure 74) by A.F. Chestnut and B. Muse, 1968-70, showed substantial growth rates in control ponds and some growth in oysters surviving in the waste ponds. However, oysters from Newport River estuary had 35% more glycogen than those in ponds. Mortality of one year oysters in waste ponds was 50% from August to December 1969 compared to about 10% in control ponds. In the waste ponds during the winter Monodus bloom, unfavorable factors for oysters were high pH, excessive oxygen, and absence of plant cells of suitable size and texture for oyster nutrition. During this time condition of oysters (dry weight, glycogen) in waste ponds was not as heavy as in control pond. After one year, in August, 1970, no oysters or spat survived in waste pond P-2, but those surviving in Ponds P-1 and P-3 gained 75% in weight.

Coliform measurements in oysters (Figure 62) by T.L. Herbert and C.M. Weiss (Herbert, 1975) were made for 1971-1972 when sewage waste input was less than in prior years. Coliform counts per gram of oyster were less than counts per ml of water and below safe limit of U.S. Public Health Service (230 fecal colonies per 100 g). After direct pumping of treated sewage was resumed on March 26,1972, shellfish bacterial counts increased above acceptable levels. Oysters with coliforms required 12-15 days to lose their main coliform contents when transferred to a control pond.

Introduced Rangia clams did not survive in waste ponds but survived and grew in control ponds (Table 61) which had a higher salinity range than the normal habitat for Rangia. Growth was greatest in pond C-1 without the Ruppia vegetation. Twenty four of the harvest clams weighed 47.1 g, 12.5% fresh meat and 87.5% shell. Eight small Rangia (8.5 to 13 mm total length) were found in C-1, June 23-25, 1971, possibly indicating reproduction in the ponds since the Bogue Sound waters are far from Rangia habitat.

Table 61. Growth of surviving *Rangia*, October 17, 1968 to June 23, 1971.

Pond	Group	Mean length at end of period		Mean growth increment	Mean weight increment
		mm	Number	mm	g(dry)[b]
C-1	planted plot	49.6	6	7.4	
	pier group	49.2	29	7.7	
C-2	lot 1	47.3	26	5.4	
	lot 2	46	21	3.7	
C-3	planted plot	44.1	9	3.7	
	pier group	44.4	65	3.4	
P-1	none surviving				
P-2	none surviving				
P-3	none surviving				

[a] measurement in axis of notch, located on shell margin equidistant from ends.

[b] dry-weight increment calculated from length-weight graph.

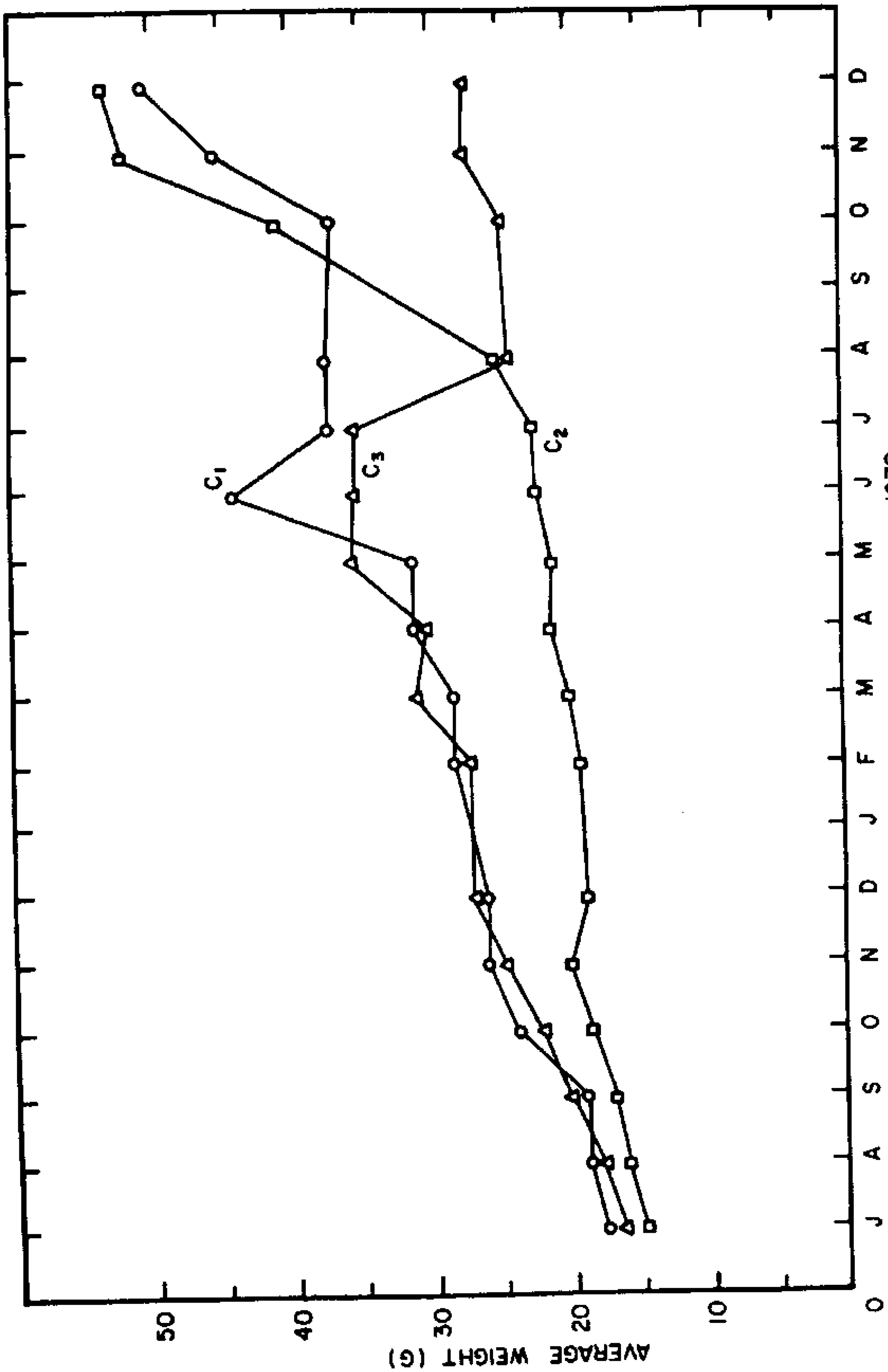


Figure 71. Oyster growth in control ponds in 1969, damp dry (B. Muse and A.F. Chestnut).

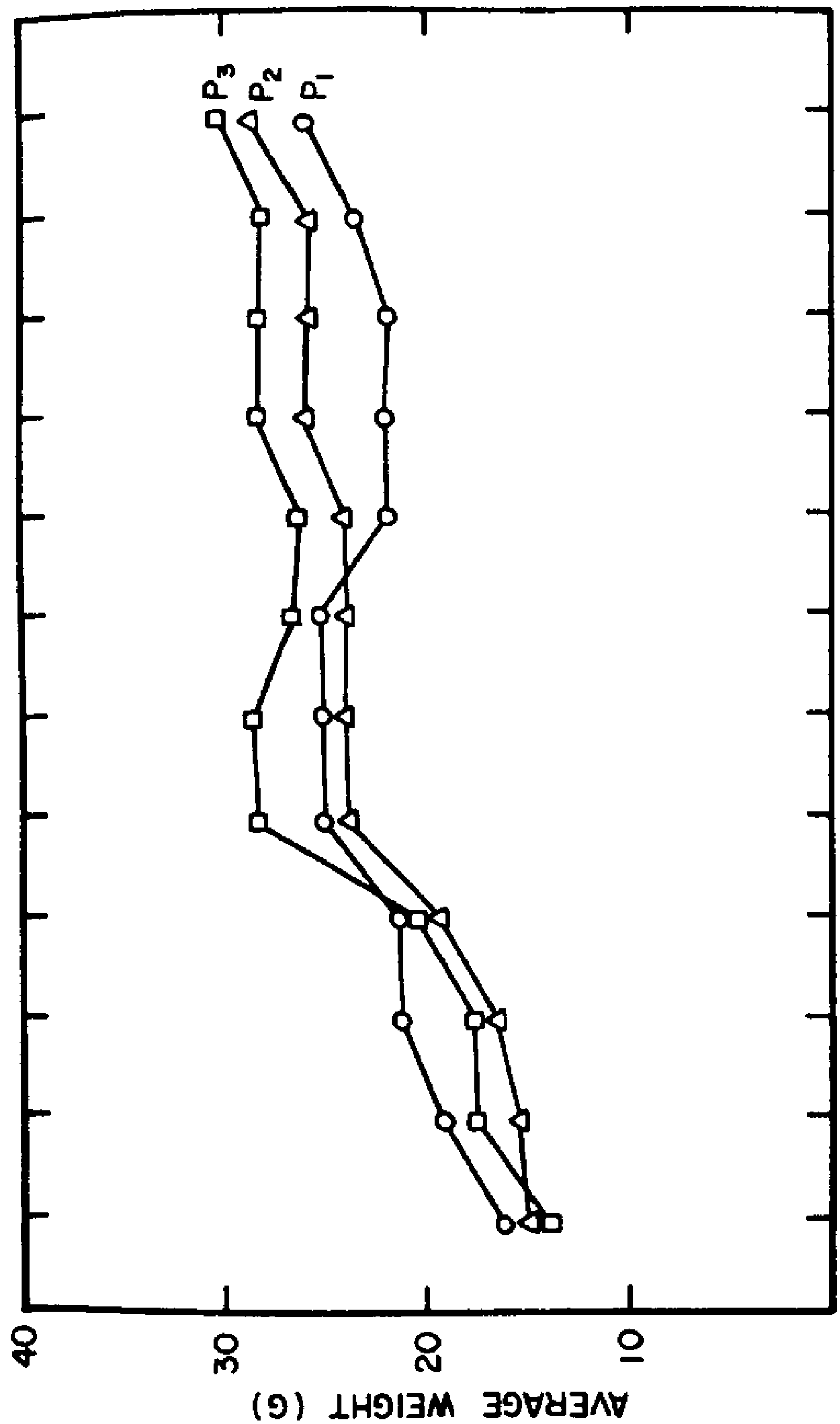


Figure 72. Oyster growth in waste ponds in 1969, damp dry (B. Muse and A.F. Chestnut).

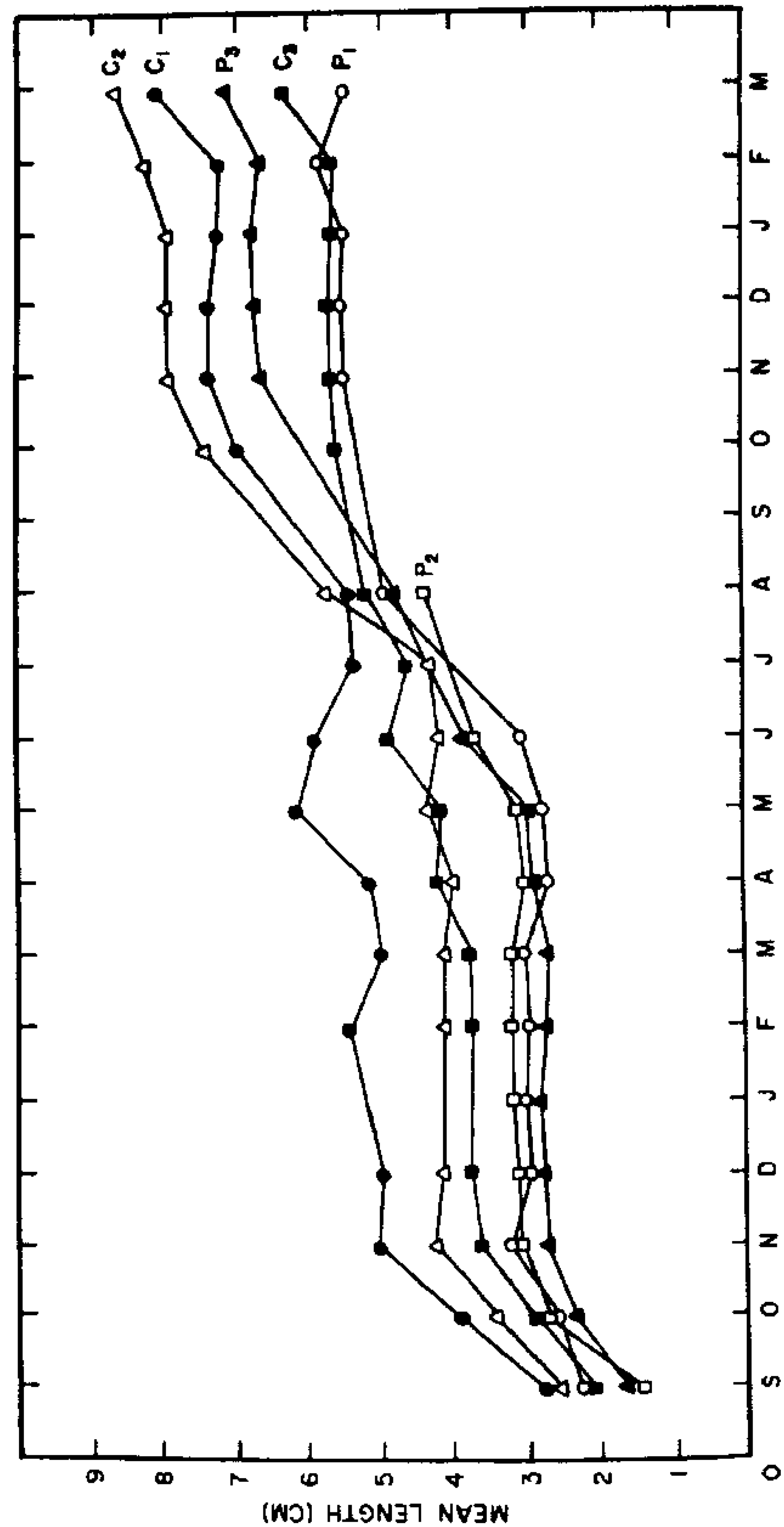


Figure 73;. Growth of oyster spat in control (C) and waste (P) ponds in 1969 (B. Muse and A.F. Chestnut).

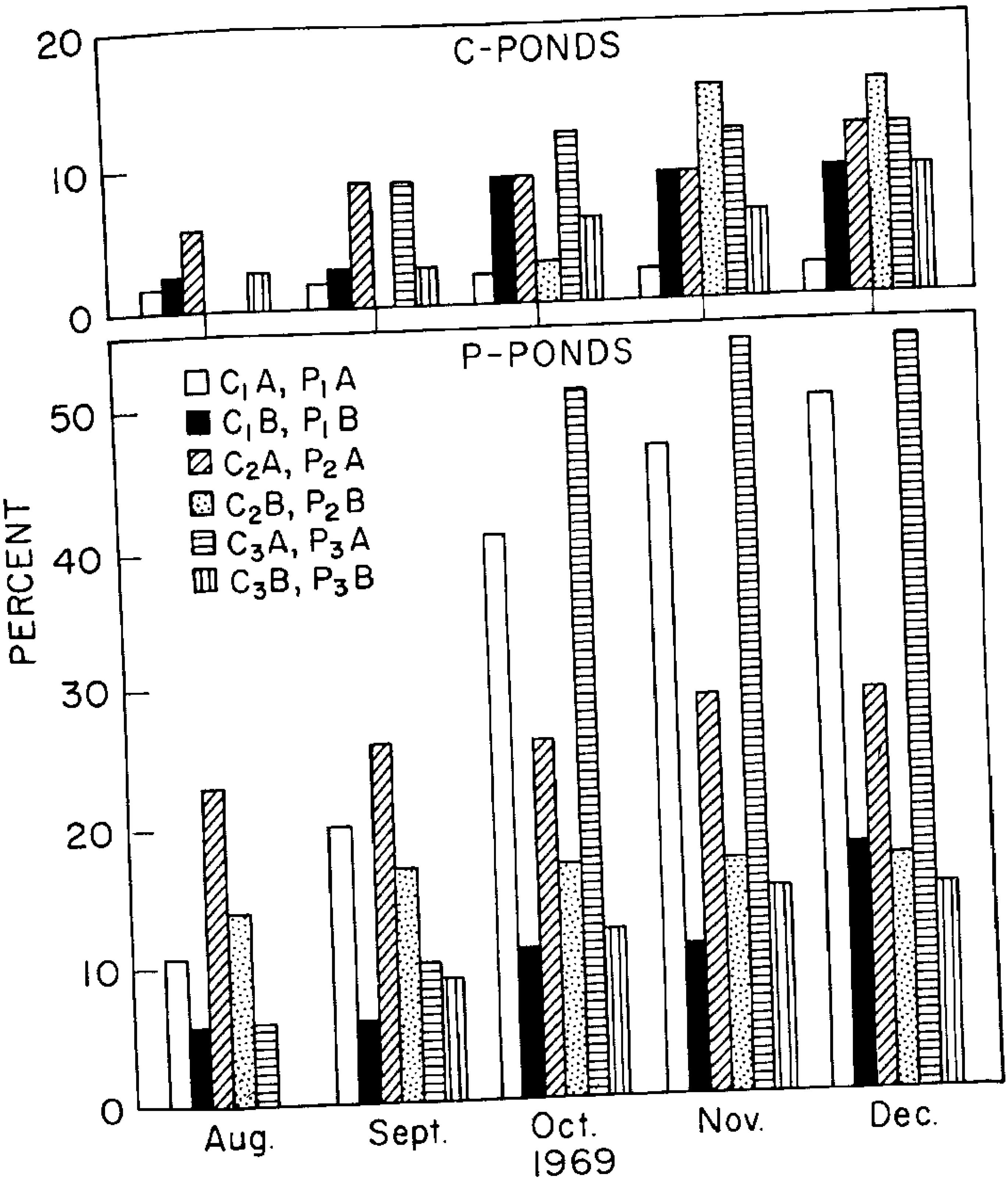


Figure 74. Cumulative mortality in ponds; A, near shore; B, in pond center (B. Muse and A.F. Chestnut).

Larger Swimming Animals, Water Birds

Populations of fishes, blue crabs and shrimp developed from the springtime seeding with larval fishes (caught in coarse meter nets from the sound) and the seeding by backflows up the stand-pipes when tides were unusually high (especially into pond P-3 with slightly lower standpipe). Smaller fish and shrimp species reproduced within the ponds. Fish mortality was observed on several of the coldest winter days and sometimes when oxygen was lowest at dawn in waste ponds. One kill in pond P-2 July 7, 1969, occurred when the main sewage plant failed and some raw sewage went into the ponds.

Especially in the early morning, water birds were observed removing stressed fish. Data on presence of feeding waterfowl are given in Table 62 and Figure 75, with large numbers often present in waste ponds. A large family of semi-domesticated mallard ducks were residents in the ponds in May, 1970 and 1971. In order of abundance, species seen feeding were: Mallards, American Egrets, Snowy Egrets Least Terns, and Green Herons, plus a dozen others. In the control ponds, only a green heron was usually seen, but this site was more disturbed by people than the waste pond area.

In the first three years, non-destructive tag, release and recapture methods were used to estimate swimming populations (directed by F. Schwartz and A. Williams: R. Field, 1968-1969; M. Beeston 1969-70; R. Hyle, 1970-71. Then in midsummer 1971 ponds were seined with a large seine pulled repeatedly until few animals were remaining. This inventory (Tables 63-69), confirming tagging studies in 1979, found a large biomass of air-breathing killifish, blue crabs and mullet in the waste ponds and fewer but more diverse nekton in the control ponds, including croakers, pinfish, spots, anchovies and flounders.

Diversity indices (Figure 76 and Table 53) are low in the waste ponds and moderate in the control ponds. The killifish, Fundulus heteroclitus, was the most abundant fish in all P ponds. Its dry weight was 24.8% of fresh weight. Jumping mullet were common in waste pond P-3 with 18 individuals and 0.5 g/m2 dry weight estimated in 1969. The spot Leiostomus xanthura was the most abundant fish in control ponds. Estimated population was 190 individuals and 1.45 g/m2 dry weight in pond C-2 and C-3.

The contrast between a few individuals of many species of typical estuarine fishes in control ponds and a few oxygen-tolerant species in large numbers in the waste ponds was established by summer 1969 - the end of the first year (Tables 70-73). Estimates for biomass of Fundulus were: P-1, 19.5 g/m2; P-2, 1.4 g/m2 (following a fish kill) and P-3, 37.6 g/m2. Populations were similar in 1970 (Tables 74-75) and 1971 (Tables 63-69). For comparison, Hyle (1971) found Lagodon, Leiostomus and anchovies dominant in Calico Creek where circulation keeps oxygen levels higher than in waste ponds.

Blue crabs were a dominant carnivore in all ponds in all years: Tables 76-77 from 1969-70 and Tables 63-69 for 1971. Less susceptible to water birds than the fishes, the blue crabs dealt

Table 62. Bird observations in 1970-71 (C.J. Spears and Williams, 1973).

	P-Ponds	C-Ponds
Total number of visits	77	14
Number of species seen	17	1
Number of individuals	124	1
Total observation time	36 hrs.	3 hrs. 15 min.
Birds per hour	3.4	0.3

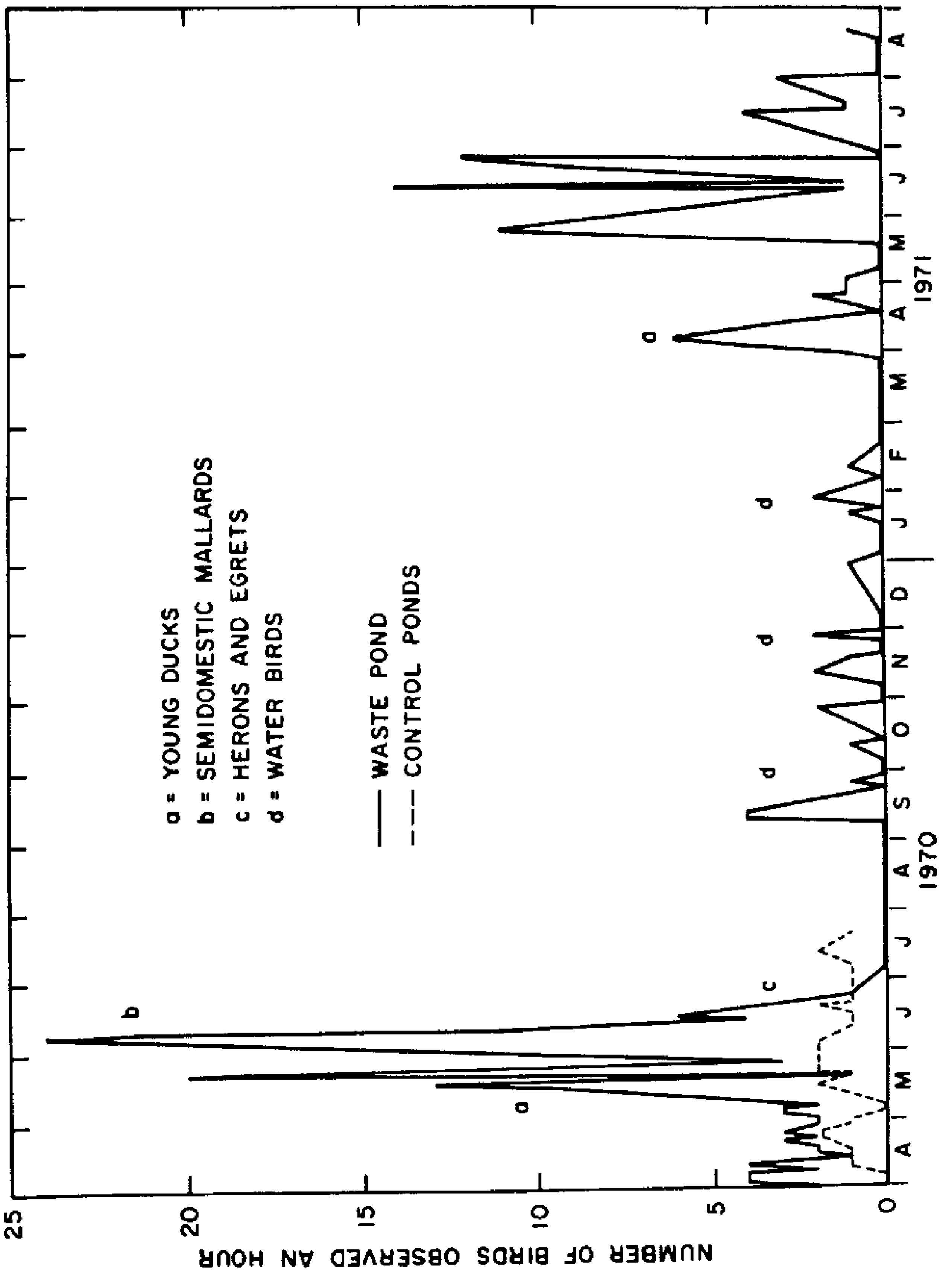


Figure 75. Water birds observed in P ponds in 1970 by W. Smith and others and in 1971 by C.J. Spears.

Table 63. Inventory of Pond C1,[a] June 23, 1971, size over one cm.

Name	Number	Individual weight (wet) g	Wet Weight g
Clams			
Rangia cuneata			
Large plot[b]	6	65.5	393
Pier Group[c]	29	74.96	2174
Young	8	.25	2
Modiulus demissus	3	2	6
Crassostrea virginica			
Shell reef	298	94.07	28033
Trays[e]	176	54.71	9630
Strings[e]	147	93.33	13720
Crustacea			
Callinectes sapidus	3	244.7	734
Palaemonetes pugio	9617	0.35[f]	2885
Alpheus heterochelis	1086	1.17	1273
Penaeus duroarum	7	32.4	227
Balanus eburneus	48		
Penopeus herbstii	5	7.4	37
Eurypanopeus depressus	11	2.45	27
Clibinarius vittatus	1	3	3
Fishes			
Fundulus heteroclitus	3	3	9

Table 63 (cont.)

Name	Number	Individual weight (wet) g	Wet weight g
Menidia menidia	14	4.14	58
Mugil cephalus	19	5.84	111
Leiostomus xanthurus	6	45.5	273
Lagodon rhomboides	7	62.85	440
Myrophis punctatus	1	11	11
Anguilla rostrata	1	25	25
Paralichthys dentatus	1	196	196
Paralichthys lethostigma	10	267.3	2673
Paralichthys albigatta	4	177.25	709

Footnotes:

a Pond area, 500 m^2.
b planted in 1968
c planted in
d grew in pond from spot added with shell or spat
e aquaculture population on trays and strings
f mean of three

Diversity of species larger than 1.3 cm was 24 species/11,212 individuals counted.

Table 64. Inventory of Pond C2,[a] June 21, 1971, size over one cm.

Name	Number	Individual weight g	Wet weight g
Total Animals larger than 1/2 inch			
Clams			
Rangia cuneata			
Large plot[b]	24		1384
Pier Group[c]	18		1098
Modiolus demissus	0		
Crassostrea virginica			
Shell reef[d]			
Trays[e]	90	58.44	5260
Strings	127	93.93	11930
Crustacea			
Palaemonetes pugio	35390	0.30[b]	9617
Alpheus heterochelis	29	1.206	35
Penaeus duorarum	19	35.84	681
Balanus eburneus			
Eurypanopeus depressus	23	2.695	62
Callinectes sapidus	1		87
Clibinarius vittatus	1		2
Fishes			
Fundulus heteroclitus	89	1.74	155
Menidia menidia	8	2.75	23
Mugil cephalus	49	14.91	731

Table 64 (cont.)

Name	Number	Individual weight g	Wet weight g
Leiostomus xanthurus	13	118.76	1544
Lagodon rhomboides	11	95.63	1052
Anguilla rostrata	9	56	336
Paralichthys dentatus	1	188	188
Paralichthys lethostigma	7	346.71	2427
Paralichthys albigutta	4	190	760
	35894		
Plants			
Spartina alterniflora		11577[c]	
Underwater plants			
Ruppia marilima	425057[c]		
Algal mat- Chaetomorpha linum	42562[c]		

Footnotes:

- a Area of Pond, 559 m^2
- b 100 weigh 30, 32, and 29 g wt
- c removed and weighed
- d grew in pond from spot added with shell or spat
- e aquaculture population on trays and strings

Diversity of animals larger than 1.3 cm was 18 species/35,894 individuals counted.

Table 65. Inventory of Pond C3,[a] June 22, 1971, size over one cm.

	Number	Individual weight g	Wet Weight g
Clams			
Rangia cuneata			
Large plot[b]	9	50.77	457
Pier Group[c]	65	52.69	3425
Modiolus demissus			
Crassostrea virginica			
Shell reef[d]			
Trays[e]	159	58.93	9370
Strings[e]	139	93.88	13050
Crustacea			
Palaemonetes pugio	827	0.58	479
Alpheus heterochelis	1	4.0	4
Penaeus duorarum	17	20.83	354.12
Balanus eburneus	126		
Eurypanopeus depressus	38	4.0	152
Callinectes sapidus	1		94
Fishes			
Fundulus heteroclitus	473	1.35	641
Menidia menidia			
Mugil cephalus	29	6.41	186
Leiostomus xanthurus	7	103.28	723

Table 65 (cont.)

Name	Number	Individual weight g	Wet weight g
Lagodon rhombiodes	21	157.61	3310
Auguilla rostrata	8	91.25	730
Paralichtys albigotta	6	179.83	1079
Paralichtys lcth-ostigma	4	567.5	2270
	1929		
	Area m^2:		
Plants			
Spartina alterniflora	16684[b]		
Spartina patens			
Distichlis			
Underwater plants			
Ruppia mari-tima	164688[b]		
Algal mat-Chaetomorpha linum	36887[b]		

Footnotes:

a Pond area, 525 m^2
b Removed and weighed
c removed and weighed
d grew in pond from spot added with shell or spat
e aquaculture population on trays and strings

Diversity of species larger than 1.3 cm = 16 species/192 individuals counted.

Table 66. Inventory of Pond P1,[a] June 24, 1971, size over one cm.

Name	Number	Wet Weight g
Anemone	-----	-----
Clams		
Crassostrea virginica[b]		
in shell reef	0	0
trays	83	7810
strings	35	3300
Rangia cuneata	0	0
Crustacea		
Palaemonetes pugio	16,420	5768
Balanus eburneus	1,052	
Callinectes sapidus	63	5435
Eurypanopeus depressus	3	10
Fishes		
Fundulus heteroclitus[f]	341	2399
Cyprinodon variegatus[e]	12,083	20,580
Mugil cephalus	3	89
	30,083	
Plants		
Spartina alterniflora		
Spartina patens		
Juncus roemeriana		

Footnotes:

a Area of Pond, 490 m^2; 4 seine hauls.
b using 94.1 g wet per individual
c Mean of 17 female, 117.7 g; mena of 38 males, 78.0 g.
d 100 individuals weighed 36, 35, 35 g wet.
e 352 individuals weighed 624 g wet; 1.77 g wet; 1.77 g wet/ individual.
f Females in 1.7:1 ratio to males by number; 2.0 ratio by weight.

Table 67. Inventory of Pond P2,[a] June 25, 1971, size over one cm.

Name	Number	Wet weight g
Clams		
Crassostrea virginica[b]		
in shell reef	4	1816
trays	34	3192
Crustacea		
Palaemonetes pugio[c]	5455	2111
Balanus eburneus	822	
Callinectes sapidus	47	3154
Fishes		
Fundulus heteroclitus	481	3669
Gambusia holbrooki	138	208
Cyprinodon variegatus	703	2426
Mugil cephalus	5	95
Gobiosoma bosci	1	0.5
Anguilla rostrata	1	442
	7691	
	Area:m^2	

Footnotes:

a Area of Pond, 510 m^2; 4 seine hauls

b using 94.1 g wet per individual

c weight per individual 0.31, 0.38, 0.36, 0.54 g

Diversity of species larger than 1.3 cm = 10 species per 7,691 individuals counted.

Table 68. Inventory of Pond P3,[a] June 25, 1971, size over one cm.

Name	Number	Wet Weight g
Clams		
Crassostrea virginica[b]		
in shell reef	80	7530
trays	156	14650
Rangia cuneata	1	75
Crustacea		
Palaemonetes pugio	16920	5255
Balanus eburneus	1179	
Callinectes sapidus	2017	39
Eurypanopeus depressus	1	2
Fishes		
Fundulus heteroclitus	624	4725
Gamiosoma bosci	1	1
Anguilla rostiata	5	468

Footnotes:

a Area of Pond, 480 m^2; 5 seine hauls
b using 94.1 g wet/ individual

Diversity of species larger than 1.3 cm = 12 species per 15,000 individuals counted.

Table 69. Weight (kg) of the most abundant macrofauna seined out of the ponds in June, 1971.

Species	Control Ponds 1	Control Ponds 2	Control Ponds 3	Waste Ponds 1	Waste Ponds 2	Waste Ponds 3
Molluscs						
Crassostrea virginica	4.1	1.4	1.8	0.9	0.4	1.8
Crustaceans						
Callinectes sapidus	0.7	0.1	0.1	5.4	3.1	2.0
Palaemonetes pugio	2.9	9.6	0.5	5.8	2.1	5.3
Alpheus heterochelis	1.3	P	P	0	0	0
Fishes						
Fundulus heteroclitus	P	0.2	0.6	2.4	3.7	4.7
Cyprinodon veriegatus	0	0	0	20.6	2.4	0
Paralichthys lethostigmus	2.7	2.4	2.3	0	0	0
P. albiguttus	0.7	0.8	1.1	0	0	0
Leiostomus xanthurus	0.3	1.5	0.7	0	0	0
Lagodon rhomboides	0.4	1.1	3.3	0	0	0
Total	13.1	17.1	10.4	35.1	11.7	13.8

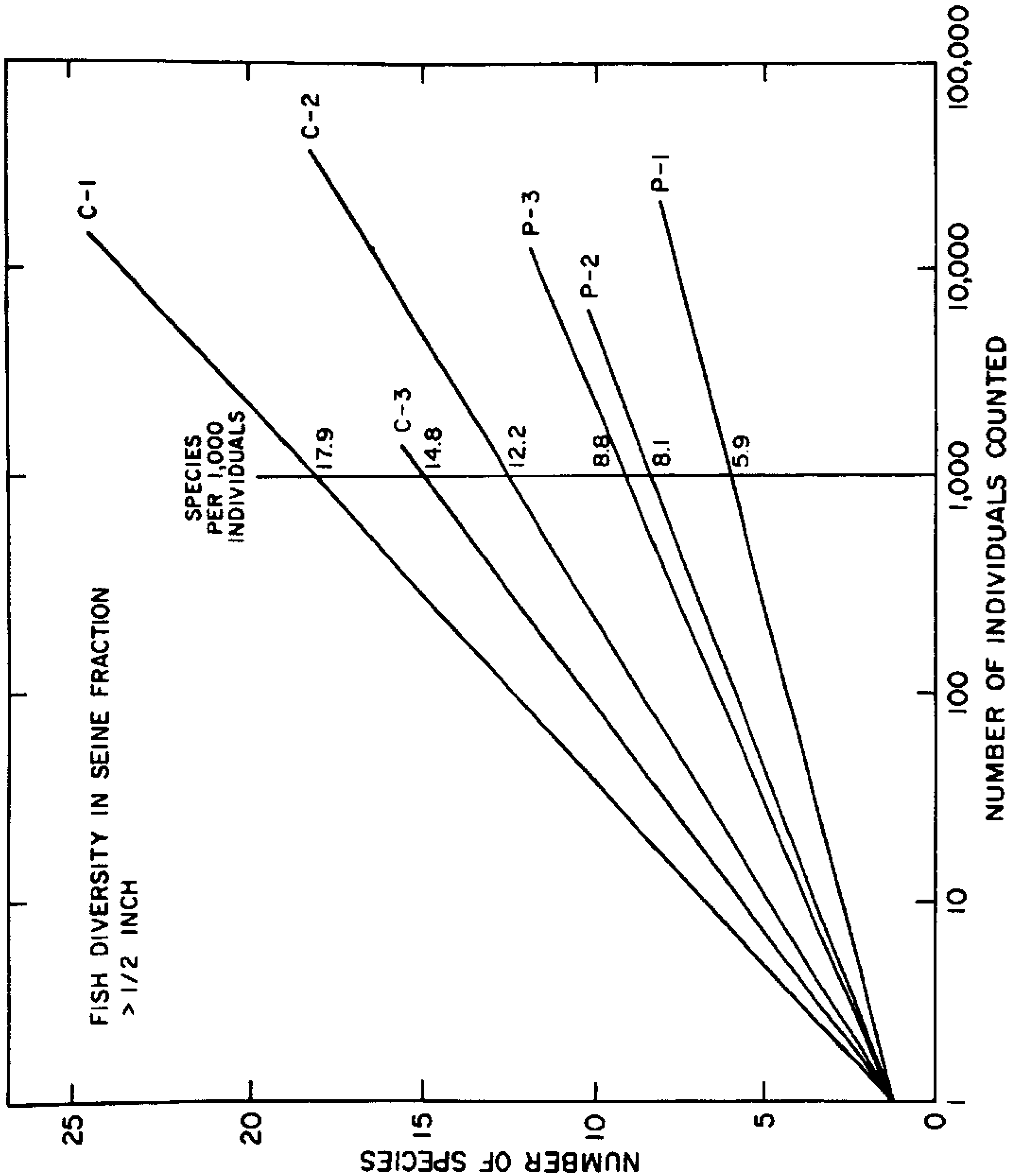


Figure 76. Diversity of Fish seined in inventory, June 23-July 1, 1971; graph indicated method of extrapolating to obtain diversity index.

Table 70. Species of fishes in ponds in summer, 1969 (Beeston, 1970).

	C-1	C-2	C-3	P-1	P-2	P-3
Aegathoa oculata (parasitic isopod)			+			
Callinectes spidus (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 rhombodies (pinfish)	-	-	-			
Leiostomus xanthurus (spot)	+	+	+			
Membras martinica (silverside)	-					
Micropogon undulatus (croaker)	-	-				
Mugil cephalus (mullet)			-			+
Paralichtys dentatus (flounder)	+		-			

+ indicates relatively abundant population.

- indicates only one or several individuals observed.

Table 71. Fishes trapped in summer, 1969 (R. Field).

Species	P-1	P-2	P-3	C-1	C-2	C-3
Killifish:						
*Fundulus heteroclitus**	750	118	2036	-	-	-
Cyprinodon variegatus	69	-	2	-	-	-
Fundulus majalis	1	-	-	-	-	-
Carnivores:						
*Leiostomus xanthurus**	-	-	-	6	3	22
Lagodon rhomboides	-	-	-	1	-	1
Micropogon undulatus	-	-	-		1	-
*Paralichthys sp.**	-	-	-	6	2	2

* Calculations of number in pond by trap, clip fin, and recapture.

Table 72. Biomass of *Leiostomus xanthrus*, 1969 (Beeston, 1970).

Pond	Estimated Population	Dry Weight g/m^2
C-1	-	-
C-2	190	1.4
C-3	200	1.3

Table 73. Biomass of *Fundulus heteroclitis*, 1969 (Beeston, 1970).

Pond	Estimated Population	Dry Weight g/m^2
	September 1	
P-1	466	0.80
P-2	34	0.06
P-3	725	1.9
	October 25	
P-1	3320	4.2
P-2	1050	1.3
P-3	1235	1.6

Table 74. Fish species in ponds 1969-71.

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
Paralichthys 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 75. Biomass of *Fundulus heteroclitus* and *Cyprinodon variegatus* in 1970 (Hyle, 1971).

	Fundulus		Cyprinodon	
	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 76. Populations and biomass of Calinectes sapidus in ponds, 1969-1970 (Beeston, 1971).

Pond	July 1969 Estimated population	July 1969 Biomass g/m^2 dry weight	July 1970 Estimated population	July 1970 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 77. Dry weights and population sizes of *Callinectes sapidus* and *Penaeus aztecus* in ponds, 1969-1970 (Beeston, 1971).

	Date	Dry Weight g/m^2	Estimated Population
	Callinectes sapidus (blue crab)		
C-1	July 1969	4.1	65
	Mar 25 - 31 1970	0.92	33
	June 9 -15 1970	1.89	38
C-2	July 1969	2.7	43
	Mar 25 - 31 1970	0.64	8
	June 9 - 15 1970	1.14	11
C-3	July 1969	2.0	39
	Mar 25 - 31 1970	1.01	15
	June 9 - 15 1970	0.73	10
P-1	July 1969	4.5	53
	Mar 25 - 31 1970	1.13	12
	June 9 - 15 1970	1.28	16
P-2	July 1969	3.5	43
	Mar 25 - 31 1970	1.46	18
	June 9 - 15 1970	0.89	14
P-3	July 1969	2.7	37
	Mar 25 - 31 1970	0.90	11
	June 9 - 15 1970	0.73	10
	Penaeus aztecus (shrimp)		
C-1	Aug 1969	0.47	29
	June 18 1970	0.03	3
C-2	Aug 1969	0.75	52
	June 18 1970	0.06	10
C-3	Aug 1969	0.26	21
	June 18 1970	0	0

None in waste series

with low summertime late night oxygen shortages by moving to the shallow pond margins where depth was only one or two centimeters. Here they were observed at dawn, flushing water over their gills.

Grass shrimp Palaemonetes was a principal intermediate in the food chain with a full life cycle in the ponds (Table 78 and Figure 77). They did not get established in the control ponds until spring of 1969. Paleomonetes pugio was abundant in both control and waste ponds; P. vulgaris was less abundant in control ponds and absent from waste ponds. Populations declined in fall and winter. Biomass in waste pond P-2 Oct. 25, 1969 was 5.3 g dry/m2, the highest level measured. Egg deposition by large over-wintering females was in late march, peak egg production in May, juvenile recruitment in early June, with rapid summer growth. Life cycle was similar to that in open estuaries. Sex ratios varied with females, 85-90% of the waste pond population in early spring, whereas males and females were more balanced in control ponds.

Large penaeid shrimps (larger than 1.25 cm stretched mesh) occurred in 1969 only in control ponds; brown shrimp (Penaeus aztecus) in three ponds in summer 1969; and a few pink shrimp (Penaeus duorarum) in control ponds C-2 and C-3. Some pinks and browns were caught in control ponds in 1970 and 1971 also. See population and biomass estimates (Table 79). Dry weight was 27.3 % of fresh weight

Regime with Aeration and Less Sewage Waters

Objectives of the pond program changed in September 1971, to maintain higher minimum levels of oxygen in the waste pond P-2 by adding an aeration pump (Rickards and Williams, 1973) and by cutting off direct sewage pumping to improve survival of shrimp. Coliforms bacteria were less (Figure 62). The Monodus bloom did not develop. Prior to aeration, in the summer, dissolved oxygen ranged diurnally as wide as 0.0 mg/l to 13.5 mg/l (Figures 21-22) and pH 7.5 to 9.8 (Figures 31). With aeration pumps operating (3/4 horsepower oil-less compressor through 200 feet of perforated plastic air hose in a four-leaf clover pattern), the pH range was less, 7.6 to 8.3 and oxygen minimum was 2 mg/l. As given in Table 80, white shrimp, Penaeus setiferus, were introduced into waste pond P-2 and into control pond C-2, and growths measured after 70 days to test aquaculture potential (Rickards and Williams, 1973). Survival was less but the survivors were commercial size (30 heads-on shrimp per pound), larger than those grown in C ponds (50 per pound).

Part of the top minnows seined in summer 1971 were returned to the pond to seed the growth in 1971-72. In 1972 Hyle (1973) described harvested wet weights in waste pond P-1: Fundulus, 4,574 g; Cyprinodon, 5,5550 g; and Mullet, 470 g; In pond P- , Fundulus, 4,614 g, Cyprinodon, 1,440 g, Mullet, 934 g, and eels (A. rostrata). These numbers were similar to the harvest the previous summer of 1971. Hyle estimated annual market retail value of the top minnow yield as bait as $511 (1972 dollars) per acre/yr based on one dollar per dozen at the wharf at Morehead City.

Table 78. Dry weights of Palaemonetes in ponds, 1969-70 (Beeston, 1971).

Date		C-1	C-2	C-3	P-1	P-2	P-3
		Palaemonetes pugio			P. pugio		
		P. vulgaris					
30 Aug.	1969	0.40	0.09	0.01	2.0	5.3	1.3
25 Oct.	1969	0.10	0.03	0.01	0.48	9.1	2.6
29 Mar.	1970	.50	.08	.28			
10 Apr.	1970				.77	.69	.71
17 Jun.	1970	.41	.43	.12			
23 July	1970				.48	.49	1.42
14 July	1970	.50	.75	.16	1.54	1.89	2.49
1 Aug.	1970	.37	.73	.07	2.69	3.71	2.03

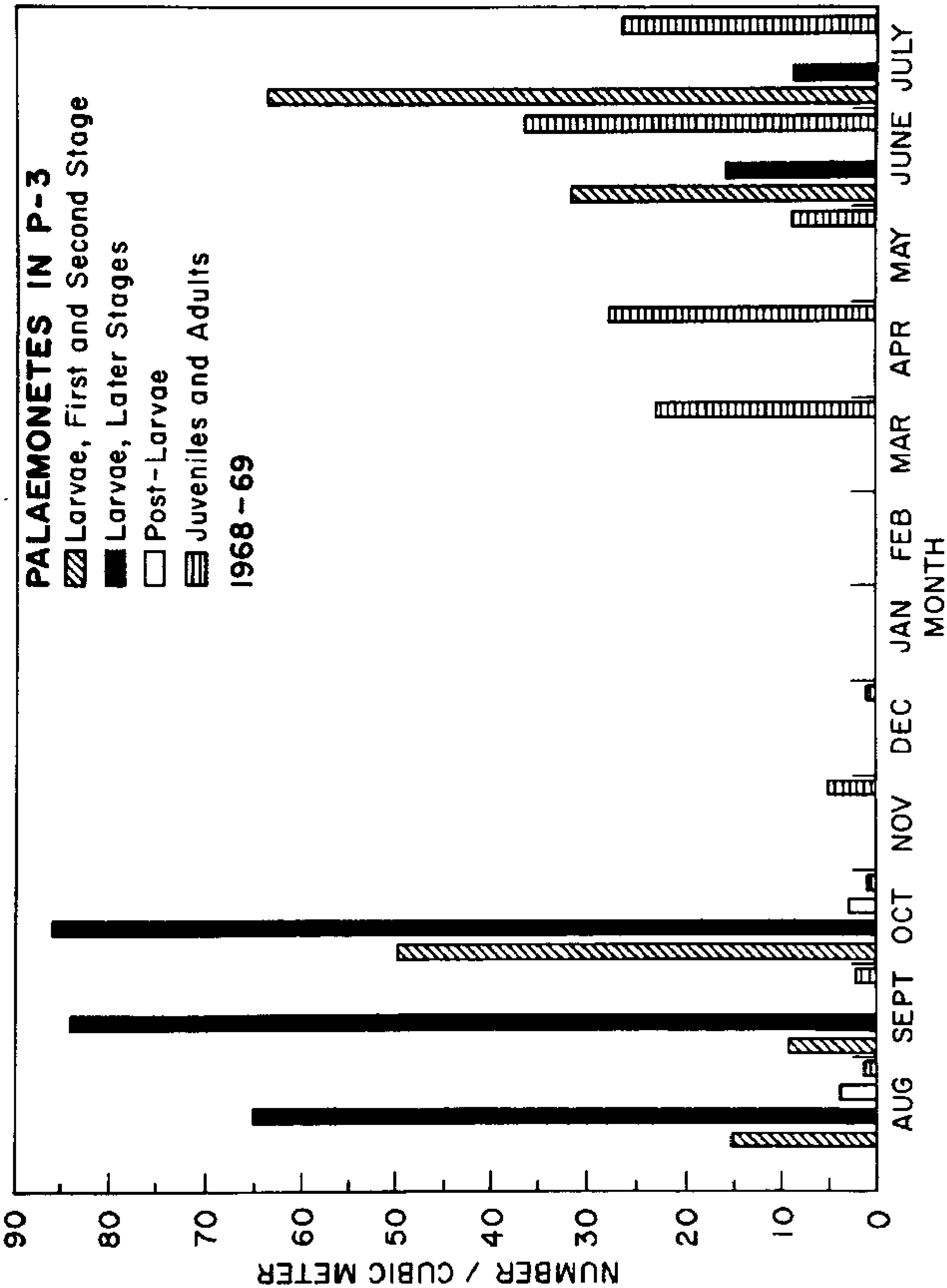


Figure 77. Palaemonetes population in waste pond P-3, 1968-69 (McCrary, 1970).

Table 79. Population and biomass of *Penaeus aztecus* in ponds, 1969-1970 (Beeston, 1971).

	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 80. Growth of <u>Penaeus setiferus</u> in waste pond P-2 and control pond C-2 during 1971-72 with reduced sewage and aeration (Rickards and Williams, 1973).

Pond	Number Stocked	Days in Pond	Number Recovered	Initial Weight g	Initial Length mm	Final Weight g	Final Average Length mm
P2	287	70	33	1.4	57	14.77	121.5
C2	321	70	60	1.4	57	13.45	113.3

DISCUSSION AND SUMMARY

Summary of the Sequence of Events

After pond construction and filling, following an acid phase in control ponds, there was a period of build-up of larger components during which the smaller ones were variable and changing. A heavy fringe of Spartina, an enormous winter stock of Monodus and a substantial population of killifish developed in the waste ponds; wheras a cover of Ruppia and a larger diversity of fishes developed in the control ponds. Variation in the smaller components was augmented by external variations in nutrient pumping, input salinities and weather. Something of a repeating pattern had developed by the third year after which pumping regimes were changed causing ecosystem changes.

Climax Regime in the Waste Ponds

Figure 78 is a graphical summary of the seasonal sequence that emerged in the waste ponds and was repeated to varying degrees for three years until sewage input pumping was stopped. A winter Monodus regime developed in the fall building up an enormous chlorphyll and plankton biomass, high pH and oxygen in early spring. With a crash in early May, the bloom was sedimented with a surge of excess respiration followed by the onset of a more photosynthetic regime of diverse phytoplankton, rise in microbial actions and temperature, seasonal increases in the animals, followed by gradual decline in stocks and process with decline of sunlight and temperature in the fall.

Summary Diagram of Pond Ecosystems

To understand the self-organizational processes that developed estuarine ecosystems in the ponds, examination of the results may be made as a system. Figure 79 is an aggregated overview of the main components and processes. Any simplification of nature must also be an approximation with some arbitrariness in what is shown and what is aggregated. Items included at one scale of view may become too small to stand out at the scale of next larger size. Figure 79 is a composite view that includes most of the categories identified in the research when viewed in the larger scale.

Figure 79 was drawn with energy systems language in which symbols and pathways have precise energetic, mathematical, and hierarchical meaning (Odum, 1967, 1983). One may read the diagram by starting with the factors outside the boundaries such as sun and inflows of waters and follow pathways in, around and through the system noting main components, production, consumption and other process intersections. Hierarchical relationships are represented by positioning items large in quantity such as the sunlight on the left and items small in quantity but important in control actions such as fishes on the right. The diagram includes components that developed in both sets of ponds. The set dominant for waste ponds was different from that for control ponds.

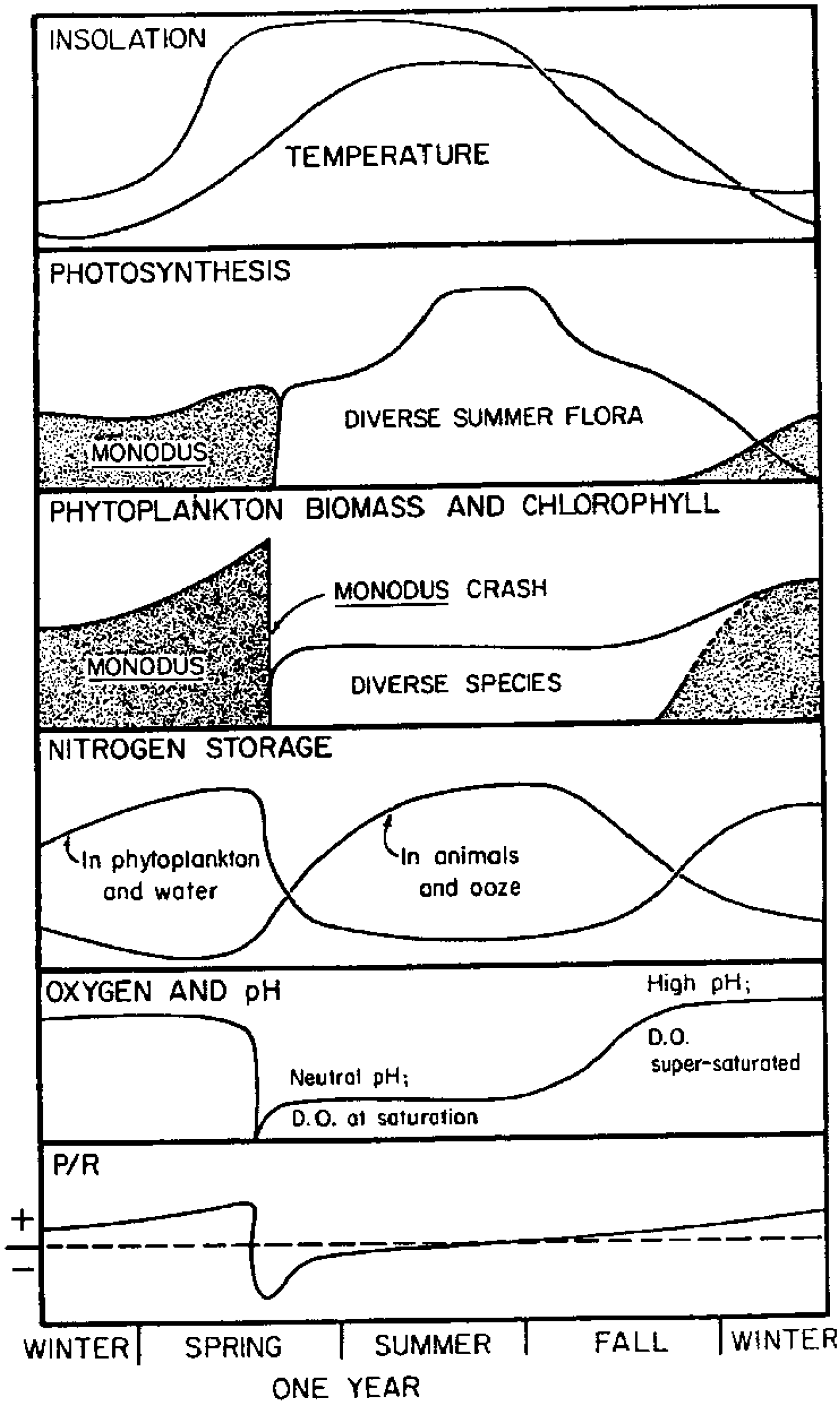


Figure 78. Idealized seasonal pattern of various characteristics of the waste ponds with treated sewage inflowing.

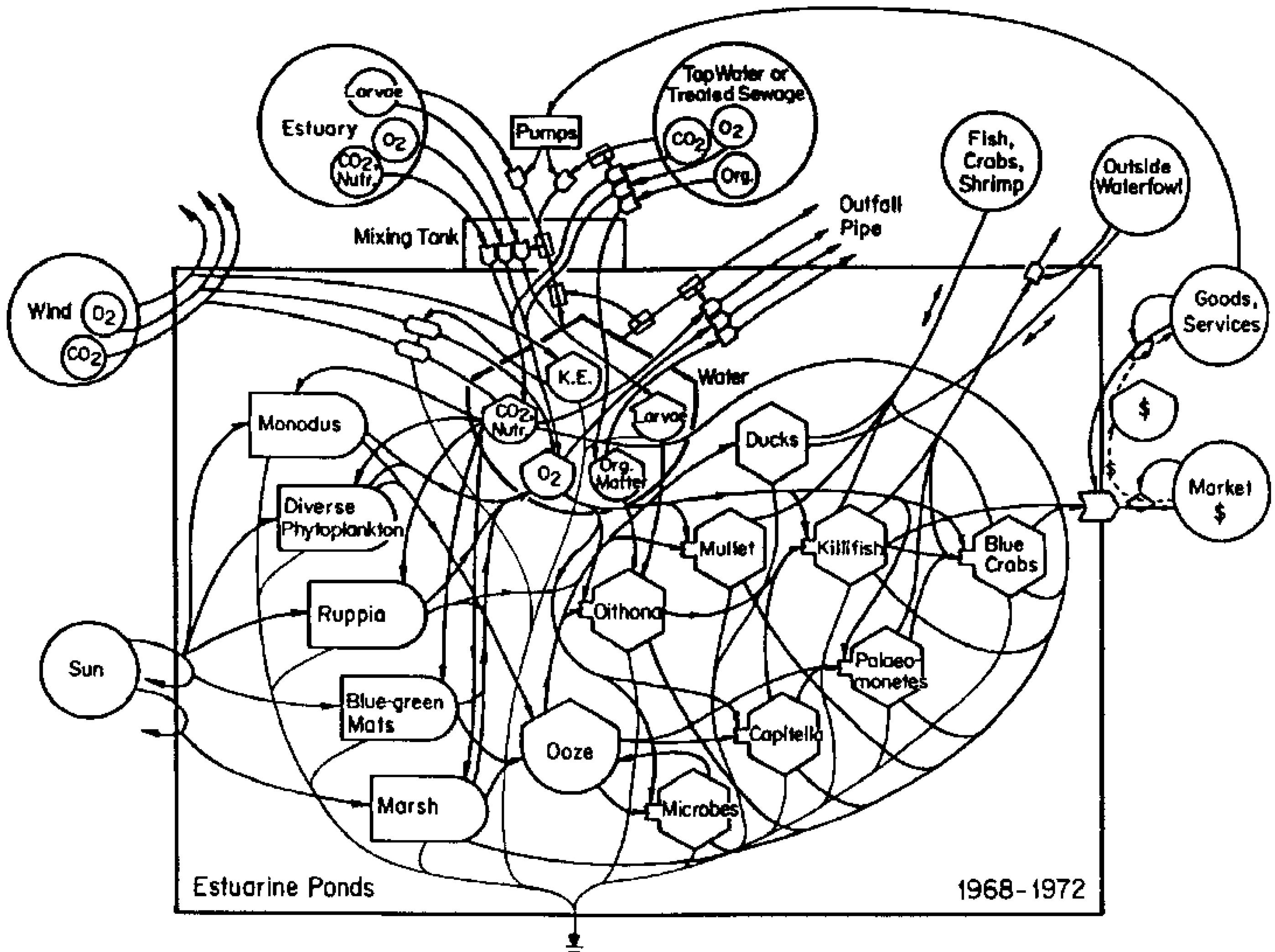


Figure 79. Descriptive model of main features of the pond ecosystems after three years of self organization. (a) Waste ponds receiving treated sewage; (b) control ponds.

Simulation Model of Pond Succession

Whether one's aggregated overview of a system is a good one or not depends on whether it is consistent with the mechanisms known to operate and the main events observed. A simulation model tests the consistency between ideas about relationships and observed events. In other words, a simulation can determine if the relationships represented in the overview ecosystem diagram (Figure 79) explain the events summarized in Figure 78.

Starting with some further aggregation of Figure 79, a simulation model of the ecosystems of the ponds was drawn (Figure 80). Next, a mathematical translation of the ecosystem model was written in the form of differential equations (Table 81). These relationships were included in a BASIC computer program, and coefficients were calibrated with data from the study. Simulations were run generating graphs of pond components with time.

At first, simulation graphs had major differences from the observed details. For example, one preliminary model on analog computer (Odum, 1972) generated broad features of production and respiration during successional build-up of organic matter, but did not generate characteristics of the Monodus regime. Temperature, not included at first, was found necessary to generate the Monodus behavior found by Hommersand's laboratory study. The version presented here was developed on a compucolor microcomputer and later refined in an Apple microcomputer.

To be successful, an overview model should be able to generate behavior in both control and waste ponds, generating curves of the one or the other when the nutrients are varied. Therefore, the model was supplied with pathways for both regimes, thus representing multiple seeding. This was a way of introducing the self-organization observed in nature by which some pathways are reinforced and prevail over others.

The final version of the simulation model is given in energy language in Figure 80. Figure 80a has the designations of variables and pathways as represented also in differential equations in Table 81. The values of storages (state variables) and flows were written on the diagram (Figure 80b) and used to calibrate the coefficients (Table 82). By inspection of Figure 80b, a reader may compare the flow in or out of a storage tank with the storage number in the tank to get a turnover time. In this way the diagram helps visualize which compartments are rapid and which are slow.

The computer program in BASIC, written for Apple microcomputer, is given as Table 83. The changes for running the control pond simulation are included in the program (Table 83) as 6 changes of coefficients that are substituted when XX is set to 1. The main change is nutrient (inflow, NO and water content, N) in Figure 81). The calibration of the model for the waste ponds (P ponds) used phosphorus for N and NO. The calibration of the model for the control ponds (C ponds) used nitrogen for N and NO.

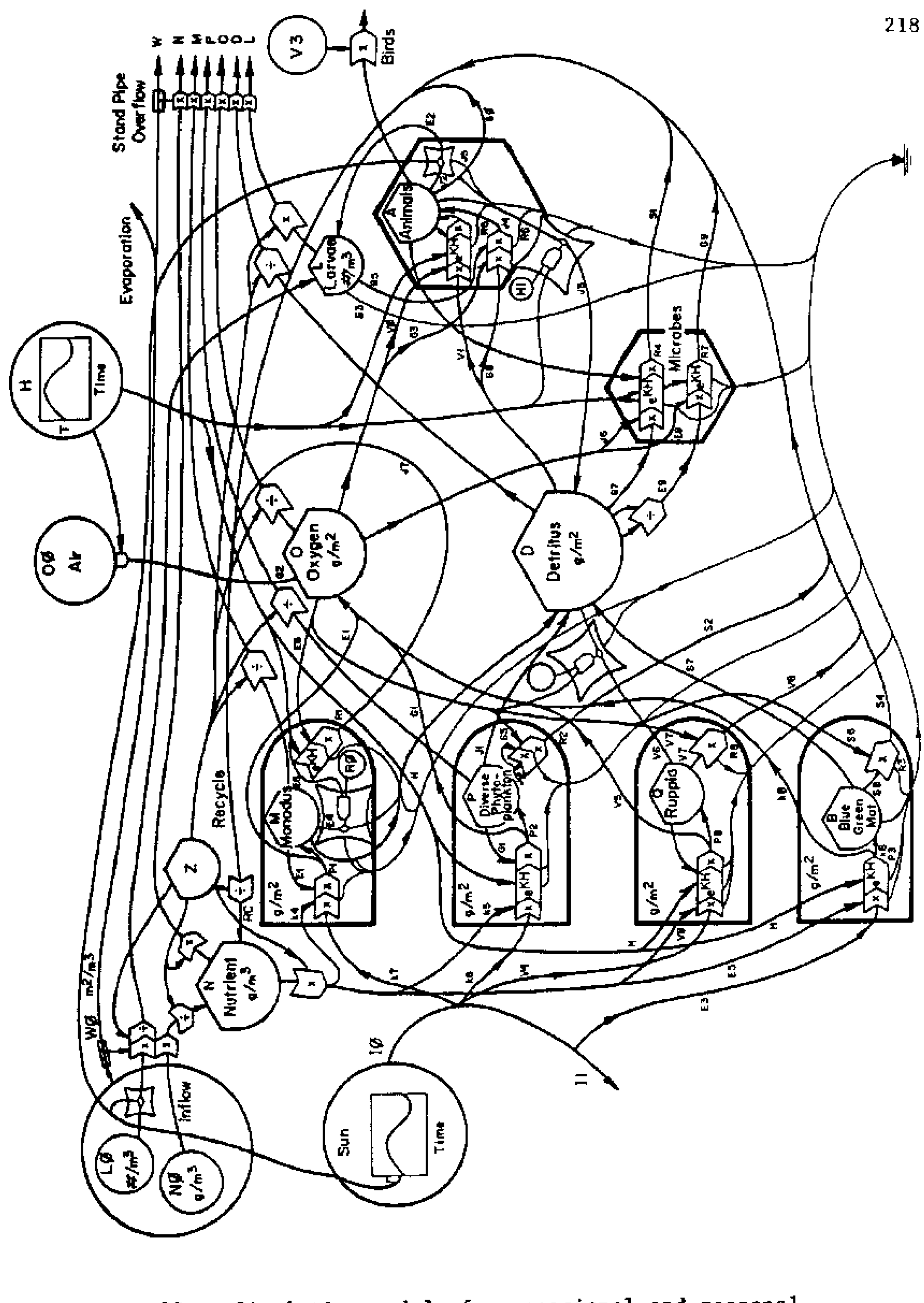


Figure 80a. Simulation model of successional and seasonal changes in ponds; differential equations are listed in Table 81. Here, designations of variables, coefficients and driving sources (forcing functions)

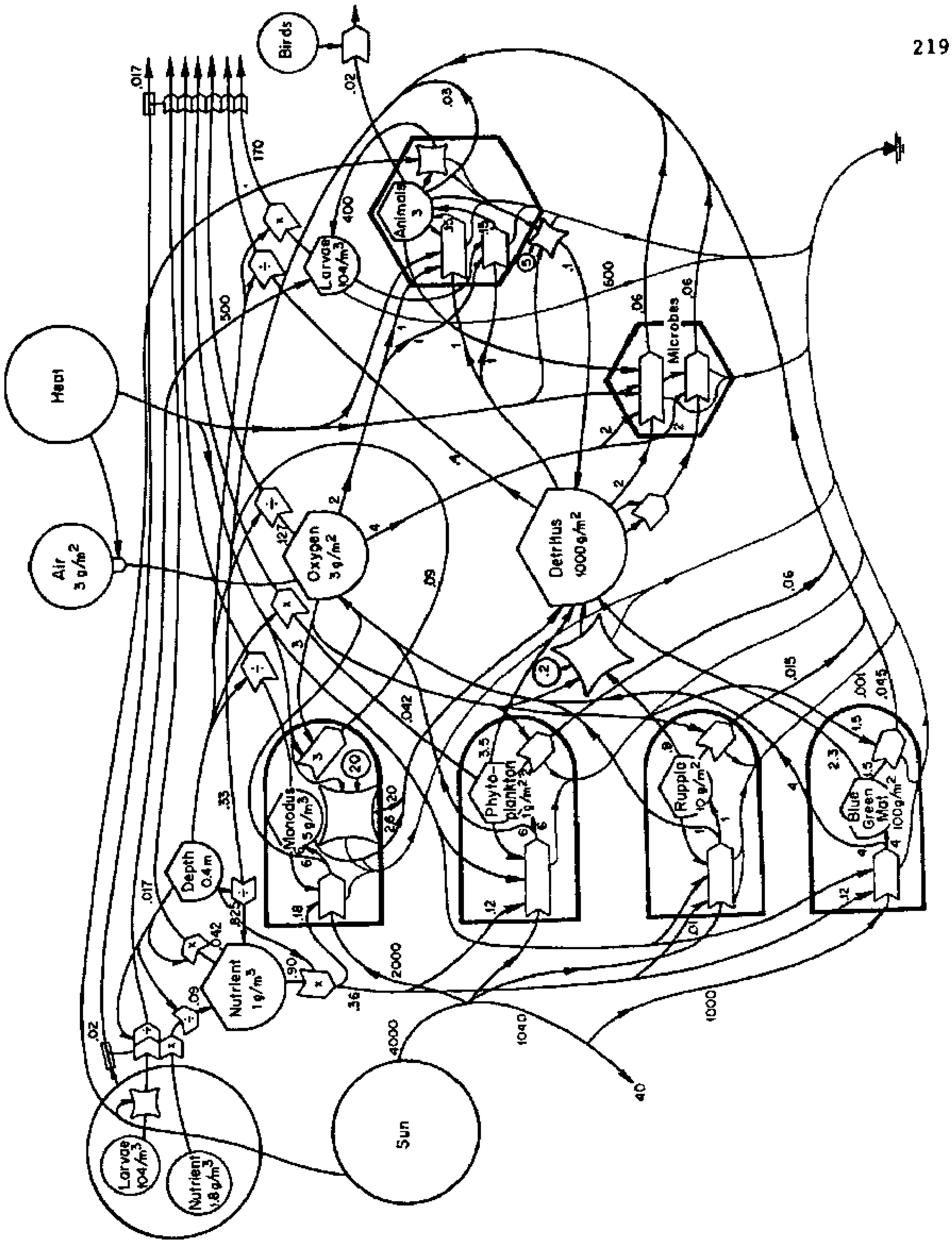


Figure 80b. Values of storages and pathways used to estimate coefficients in Table 82

Table 81. Differential equations of the pond model in Figure 82a.

$$\dot{N} = \frac{W_o N_o}{Z} + R_e - \frac{k_4}{Z} INM - \frac{k_5}{Z} IN(e^{KH})P - V_9 IN(e^{KH})Q - E_5 I_1 N(e^{KH}) - N\frac{W}{Z}$$

$$\dot{M} = E_1 INM - \overbrace{E_4 M}^{IF\ H > R_o} - M(e^{KH})O - \frac{W_o M}{Z}$$

$$\dot{P} = G_1 IB(e^{KH})P - J_1 P - J_o P^2 O$$

$$\dot{Q} = V_5 IN(e^{KH}) - V_6 Q - V_7 QO$$

$$\dot{B} = k_8 I_1 N(e^{KH}) - S_8 BO - S_7 B$$

$$\dot{D} = \overbrace{E_4 M}^{IF\ H > R_o} + J_1 P + \overbrace{V_6 Q}^{IF\ O > .2} + S_7 B - E_9 D^2 O(e^{KH}) - G_7 DO(e^{KH})A + \overbrace{J_3 A}^{IF\ H > H_1} - V_1 DO(e^{KH})A - G_8 DOL$$

$$\dot{O} = G_2(O_o - O) + E_1 I\frac{N}{Z}M + G_1 IN(e^{KH}) + k_8 I_1 N e^{KH} + V_5 IN(e^{KH})Q$$

$$- E_8 M(e^{KH})O - S_5 P^2 O - V_7 QO - S_6 BO - E_9 D^2 O(e^{KH})$$

$$- I_6 DO(e^{KH})A - V_o DO(e^{KH})A - G_3 DOL$$

$$\dot{A} = S_9 DO(e^{KH})A + J_4 DOL - \overbrace{J_3 A}^{IF\ H > H_1} - J_5 A - \overbrace{V_2 A}^{IF\ G_4 = 1} - V_3 A$$

$$\dot{L} = \overbrace{E_2 A}^{IF\ G_2 = 1} + L_o \frac{W_o}{Z} - S_3 L - G_5 DOL - L\frac{W}{Z}$$

$$R_c = J_7 M(e^{KH})O + S\emptyset A + S_1 DO(e^{KH})A + G_9 DO(e^{KH}) + S_2 P^2 O + S_4 BO + V_8 QO$$

Table 82. Calculations of coefficients for the model in Figure 82a with equations in Table 81 and data in Figure 82b.

Coefficient see Fig. 80	Equation for pathway	Pathway flow	Value of coefficient
k_3	$k_3 0 \frac{W}{Z}$	.012	2.4
k_4	$k_4 INM$	.18	9E-5
k_5	$k_5 NI(e^{k_9 H})$	1.5	9.3E-4
k_6	$k_6 NI(e^{k_9 H})P$	2000	2.5
k_7	$k_7 INM$	2000	1
k_8	$k_8 I_1 Ne^{(k_9 H)}$	4	2.5E-3
k_9			.046
E_1	$E_1 NIM$	6	3E-3
E_2	$E_2 A$	400	130
E_3	$E_3 I_1 Ne^{(k_9 H)}$	1000	6.25
E_4	$E_4 M$	20	4
E_5	$E_5 I_1 Ne^{(k_9 H)}$	.12	7.5E-4
E_7	$E_7 M$	2.6	.5
E_8	$E_8 MOe^{(k_9 H)}$	3	.05
E_9	$E_9 DOE^{(k_9 H)}$	2	.16
GØ	G		
G1	$G_1 IN\, e^{(k_9 H)} P$	6	3.8E-3
G2	$G_2(D_0 - 0)$	2.4	.8
G3	$G_3 LDO$	1	3.3E-8
G5	$G_5 DOL$	10^2	3.3E-6
G7	$G_7 ADOe^{(k_9 H)}$	2	6.54E-5
G8	$G_8 DOL$	1	3.3E-8
G9	$G_9 DOe^{(k_9 H)}$	.06	5E-3
J0	$J_0 P^2 0$	2	.66

Table 82 (cont.)

Coefficient see Fig. 80	Equation for pathway	Pathway flow	Value of coefficient
J1	J_1P	3.5	3.5
J2	$J_2P\frac{W}{Z}$	.042	2.47
J3	J_3A	.1	.033
J4	J_4DOL	.15	3.3E-9
J5	J_5A	.03	.01
J6	$J_6ADOe^{(k_9H)}$	2	5.55E-5
J7	$J_7MOe^{(k_9H)}$	.09	7.5E-3
J9	J_9DW	.4	.023
S0	S_0A	.03	.01
S1	$S_1ADOe^{(k_9H)}$	.06	1.66E-6
S2	S_2P^2O	.06	.02
S3	S_3L	600	.06
S4	S_4BO	.045	1.5E-4
S5	S_5P^2O	2	.66
S6	S_6BO	1.5	5E-3
S7	S_7B	2.3	.023
S8	S_8BO	1.5	5E-3
S9	$S_9AOD\ exp^{(k_9H)}$	.1	2.76E-6
V0	$V_0AODexp^{(k_9H)}$	1	2.7E-7
V1	$V_1AODexp^t$	1	2.7E-5
V2	V_2A	.01	.003
V3	V_3A	.2	.007
V4	V_4INO	2000	.714
V5	$V_5INQe^{(k_9H)}$	1	8.9E-5
V6	V_6Q	.9	.0128

Table 82 (cont.)

Coefficient see Fig. 80	Equation for pathway	Pathway flow	Value of coefficient
V7	V_7O^2	.1	4.8E-4
V8	V_8OQ	.015	.4E-5
V9	V_9INQ	.03	3.57E-6

Table 83. Simulation program in Basic for Apple II computers. Changing XX=Ø XX=1 in the program for successive runs changes the nutrient and depth properties from waste ponds to control ponds. Output graphs were generated using an iteration time interval of 0.1 day.

```
4  REM  N.C. PONDS
5  HGR : HCOLOR= 3
6  HPLOT 0,0 TO 0,159 TO 279,159 TO 279,0 TO 0,0
26  IF M < .01 THEN M = .01
50  HPLOT 0,35 TO 278,35
60  HPLOT 0,60 TO 278,60
70  HPLOT 0,80 TO 20,80
75  HPLOT 258,80 TO 278,80
85  HPLOT 0,100 TO 278,100
88  HPLOT 0,130 TO 278,130
90 XX = 0: REM  XX=0 IS FOR WASTE POND CONDITIONS; TYPE IN 91 XX=1 AND RUN
91 XX = 0                                                  FOR CONTROL POND
92 I = .3
94 T0 = 5.2
96 T = 1
105 K4 = 9E - 5
106 K5 = 9.3E - 4
107 K6 = 2.5
108 K7 = I
109 K8 = 2.5E - 3
110 K9 = .046
111 E0 = 1
112 E1 = 3E - 3
113 E2 = 120
114 E3 = 6.25
115 E4 = 4
116 E5 = 7.5E - 4
118 E7 = .5
119 E8 = .05
120 E9 = .16
121 G0 = .067
122 G1 = 3.8E - 3
123 G2 = .8
124 G3 = 3.3E - 8
126 G5 = 1.6E - 6
128 G7 = 5.54E - 5
130 G8 = 3.3E - 8
131 G9 = 5.0E - 3
132 J0 = .66
133 J1 = 3.5
135 J3 = .033
136 J4 = 3.3E - 9
137 J5 = .01
138 J6 = 5.55E - 5
139 J7 = 7.5E - 4
141 J9 = .023
142 S0 = .01
143 S1 = 1.66E - 6
144 S2 = .02
145 S3 = .1
146 S4 = 7.5E - 5
147 S5 = .06
148 S6 = 2.5E - 3
149 S7 = .023
```

```
150 S8 = 2.5E - 3
151 S9 = 2.76E - 6
153 V0 = 2.7E - 5
154 V1 = 2.7E - 5
155 V2 = .003
156 V3 = .007
157 V4 = .714
158 V5 = 6.9E - 5
159 V6 = .0128
160 V7 = 9.6E - 4
162 V8 = .4E - 5
164 V9 = 3.57E - 6
170 W0 = .02: IF XX = 1 THEN W0 = .024
171 I0 = 3000
172 O = 3
173 D = 300: IF XX = 1 THEN D = 10
174 L0 = 1E4
175 L = 1E4
176 A = 1
177 P = .1
178 H0 = 20
179 H1 = 5
180 M = 1
181 N0 = 1.8: IF XX = 1 THEN N0 = .09
182 O0 = 3
183 N = 1: IF XX = 1 THEN N = .01
184 Z = .4: IF XX = 1 THEN Z = .46
185 W = .0185: IF XX = 1 THEN W = .0225
186 B = .1
188 Q = 1
200  GOSUB 500
210 I0 = 2300 + 1500 *  SIN (T / 58)
220 H = 15 + 15 *  SIN (T / 58 - .5)
225 O0 = 12 - 2 * ( EXP (K9 * H) - 1)
240 II = I0 / (1 + K7 * N * M + K6 * N *  EXP (K9 * H) * P + V4 * N * Q *  EXP (K9 * H))
250 I1 = II / (1 + E3 * N *  EXP (K9 * H))
255  IF H > H0 THEN E4 = 4: GOTO 262
260 E4 = 0
262 HM =  EXP (K9 * H)
264 P1 = II * N * M
266 P2 = II * N * HM * P
268 P3 = I1 * N * HM
269 P8 = II * N * Q * HM
270 R1 = M * O * HM
272 R2 = P * P * O
274 R3 = B * O
276 R4 = D * O * A * HM
278 R5 = D * O * A * HM
280 R6 = D * O * L
282 R7 = O * HM
283 R8 = Q * O
284  IF R > 0 THEN P8 = 0:V6 = 100
```

```
310  IF I0 < 1000 THEN X = 0:Y = 0
324  IF I0 > 1000 THEN X = 1
330  IF I0 > 3200 THEN X = 0:Y = 1
340  IF X = 0 THEN G4 = 0:E2 = 0:V2 = 0
350  IF X = 1 AND Y = 0 THEN G4 = 1:EX = E2:V2 = .003
370  IF H > H1 THEN J3 = 0: GOTO 376
375 J3 = .033
376 QA = V5 * P8
377 QB = V6 * Q + V7 * R8
380 MA = E1 * P1
382 MB = M * W / Z + E8 * R1 + E4 * M
384 PA = G1 * P2
386 PB = P * W / Z + J1 * P + J0 * R2
388 BA = K8 * P3
390 BB = S7 * B + S8 * R3
392 AA = S9 * R5 + J4 * R6
394 AB = V3 * A + J5 * A + J3 * A + V2 * A
396 DA = E7 * M + E4 * M + J1 * P + S7 * B + J3 * A + V6 * Q
398 DB = J9 * D * W + V1 * R5 + G8 * R6 + G7 * R4 + E9 * R7
400 OA = E1 * P1 + G1 * P2 + K8 * P3 + G2 * (OO - O) + V5 * P8
402 RX = E8 * R1 + S5 * R2 + S6 * R3 * V0 * R5 + G3 * R6 + J6 * R4 + E9 * R7 + V7 * R8
403 R = RX
404 OB = O * W / Z + R
406 LA = G4 * L0 * W0 / Z + EX * A
408 LB = S3 * L + G5 * R6 + E0 * L * W
410 RS = J7 * R1 + S2 * R2 + S4 * R3 + S1 * R4 + S0 * A + G9 * R7 + V8 * R8
411 RC = .976 * RS / Z
412 NA = N0 * W0 / Z + RC
414 NB = N * W + K4 * P1 + K5 * P2 + E5 * P3 + V9 * P8
416 RM = E9 * R7 + G7 * R4
420 N = N + (NA - NB) * I
422  IF N < 0 THEN N = 0
424 M = M + (MA - MB) * I
426  IF M < .01 THEN M = .01
428 P = P + (PA - PB) * I
430  IF P < .1 THEN P = .1
432 B = B + (BA - BB) * I
434  IF B < 0 THEN B = 0
436 D = D + (DA - DB) * I
438  IF D < 0 THEN D = 0
440 OX = O + (OA - OB) * I
441 O = (OX + O) / 2
442  IF O < .01 THEN O = .01
446 L = L + (LA - LB) * I
448  IF L < 1 THEN L = 1
450 A = A + (AA - AB) * I
452  IF A < .01 THEN A = .01
454 C = 50 * P + 200 * M + 10 * B + 3 * Q
456 PH = PA + MA + BA + QA
458 Q = Q + (QA - QB) * I
460  IF Q < .1 THEN Q = .1
480 T = T + I
484  IF T / T0 < 279 GOTO 200
490  END
```

```
500 OS = .8
501 RR = 2
502 HS = 1.6
503 IS = 130
504 LS = 3000
505 PS = 1
506 MS = 5
507 QS = 2
508 DS = 400
509 BS = 3
510 NS = 3
511 ZS = 1
512 CS = 500
513 AS = 4
514  HCOLOR= 5
515  HPLOT T / TO,80 + R / RR: REM  RESPIRATION PLOTTED FROM LINE 89 DOWNWARD
516  HCOLOR= 1
518  HPLOT T / TO,35 - H / HS: REM              TEMPERATURE
520  HCOLOR= 3
525  HPLOT T / TO,35 - IO / IS: REM             INSOLATION
528  HPLOT T / TO,160 - L / LS: REM             LARVAE
530  HCOLOR= 1
533  HPLOT T / TO,80 - PH / PS: REM  PHOTOSYNTHESIS
534  HCOLOR= 2
535  HPLOT T / TO,130 - M / MS: REM             MONODUS
540  HCOLOR= 3
541  HPLOT T / TO,130 - Q / QS: REM             RUPPIA
542  HCOLOR= 5
545  HPLOT T / TO,60 - D / DS: REM  DETRITUS
546  HCOLOR= 5
547  HPLOT T / TO,160 - RM / RR: REM            MICROBE RESPIRATION
555  HPLOT T / TO,130 - P / ZS: REM                       DIVERSE PLANKTON
557  HCOLOR= 6
558  HPLOT T / TO,130 - B / BS: REM             BLUE-GREEN MAT
559  HPLOT T / TO,35 - N / NS: REM              NUTRIENT
565  HPLOT T / TO,60 - O / Z / OS: REM    OXYGEN
567  HCOLOR= 1
568  HPLOT T / TO,130 - C / CS: REM             CHLOROPHYLL
570  HCOLOR= 2
575  HPLOT T / TO,160 - A / AS: REM  ANIMALS
600  RETURN
```

With high concentrations of nutrient inflow, simulation as shown in Figure 81a and 82a generated a Monodus bloom that dominates the winter, switching to diverse summer regimes in spring.

With low concentrations of nutrient inflow, simulation as shown in Figures 81b and 82b did not develop Monodus blooms, but instead generated Ruppia beds in three years. Some proof of system understanding was demonstrated by the ability of the model to generate the main features of the pond's annual cycle and succession by combining the components and mechanisms found by those studying ecosystem parts and processes.

Summarizing Observations

1. Estuarine ecosystems can develop main components in typical organization of food chains, nutrient cycles and seasonal regimes in three years.

2. Blooms and surges of the smaller, high-turnover phytoplankton, microzoa, and bacteria probably represent variable seeding, competition, selection, waves of homeostasis in the coupling of production and respiration, prey-predator oscillations, that collectively provide the choices and noise to facilitate a continuous process of adaptation and self-organization.

3. In one sense there was a succession of species when populations and patterns appeared briefly, that were not continuing later. Some of these intermediate components were different in each pond, representing varying initial conditions and input transients. The dominance of one species at one time in one pond through population interaction mechanisms may be the means for fine-tuning the maximum performances of the various guilds of the ecosystem.

4. Self organization was accompanied by gradual build up of storages of detritus, animal and plant biomass, productivity, total respiratory metabolism, and diversity. These may be the stabilizing, species-independent design criteria of successful ecosystems that are the end result of and system stabilizer of the noisy surges of populations.

5. Nutrient storage were developed in biomass in all ponds. In control ponds C-1, storages were in shellfish; in control ponds C-2 and C-3, storages were in macrophyte beds of Ruppia and associated grass shrimp; and in waste ponds, storage was in the ooze, in Spartina beds and alternating seasonally between the winter Monodus stock and the summer animals.

6. Diversity in the ponds was less than in the comparable environments outside, except for microarthropods in the marsh grass, which were more diverse within the ponds.

7. Diversities of most groups of organisms was less in the eutrophic ponds than in the more oligotrophic control ponds, partly because of the greater range of oxygen, pH and other variables.

HIGH NUTRIENT

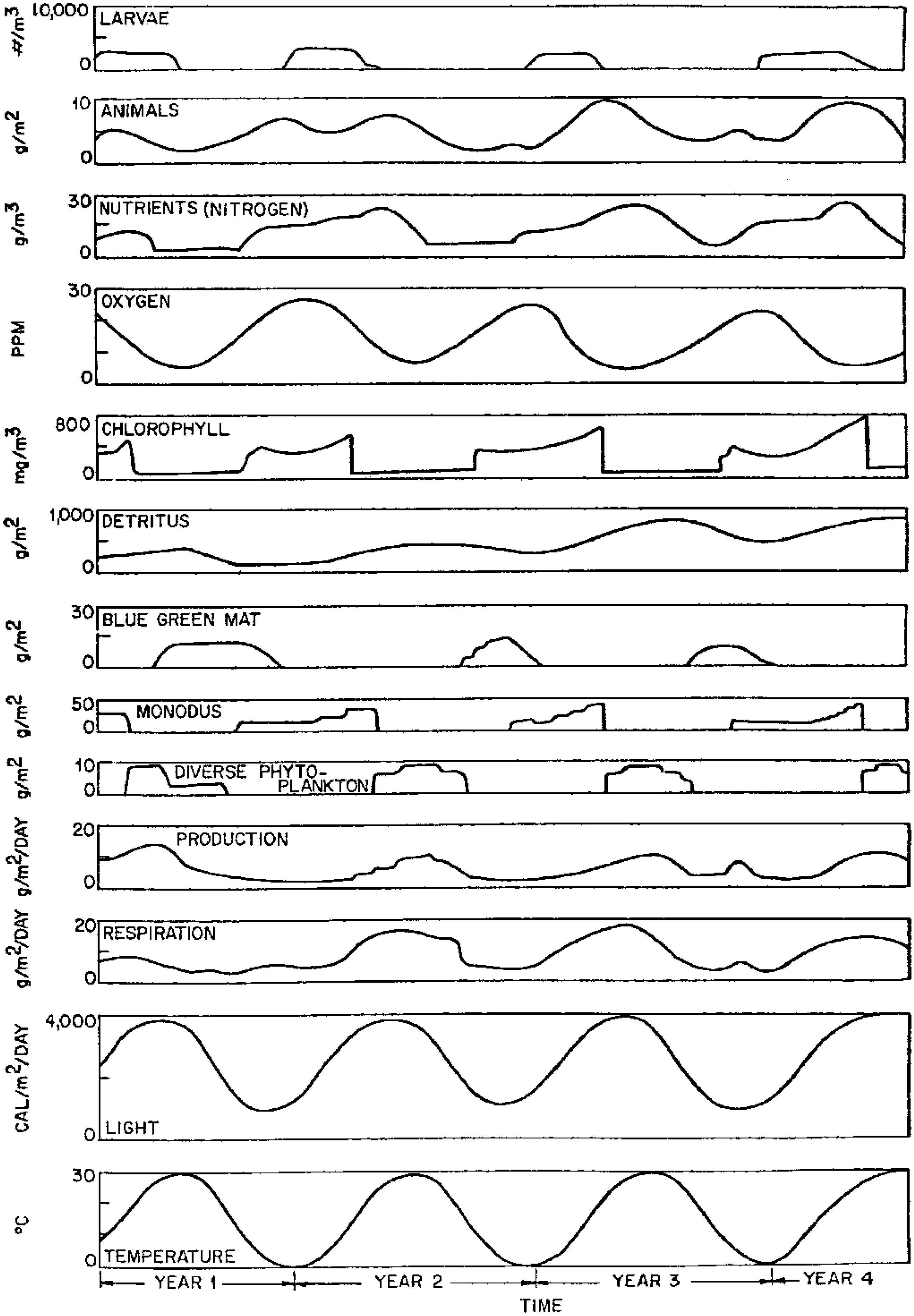


Figure 81. Results of simulation of the successional and seasonal model of the ponds in Figure 80 and Table 81. (a) simulation with high nutrients as in Table 83 with XX set = 0

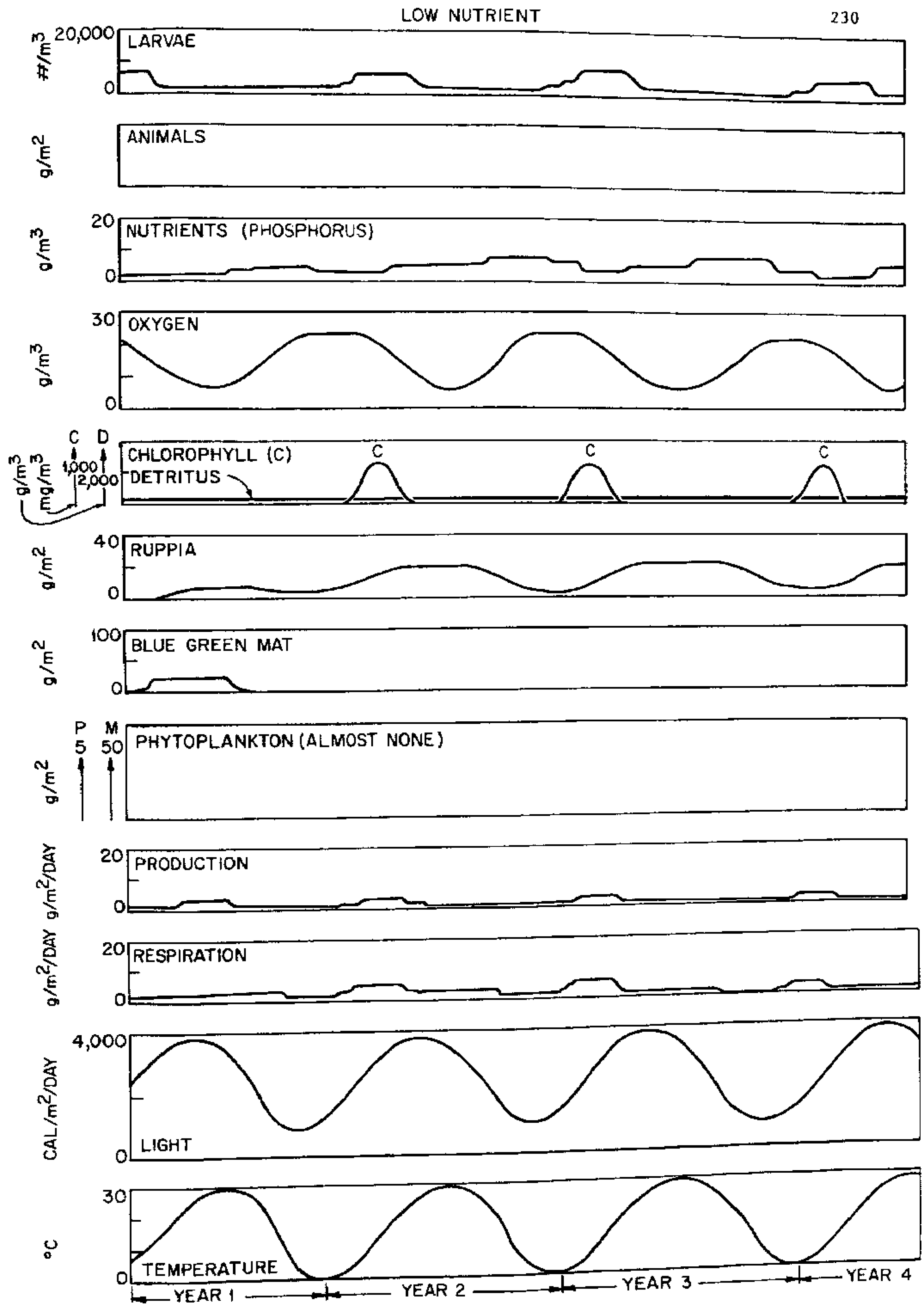
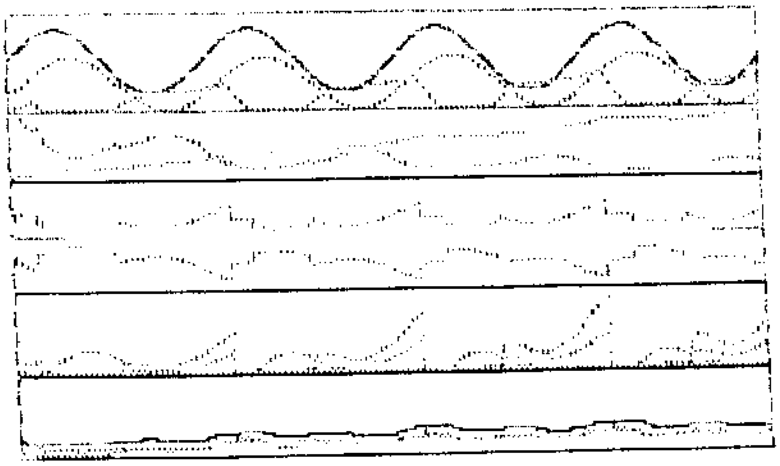
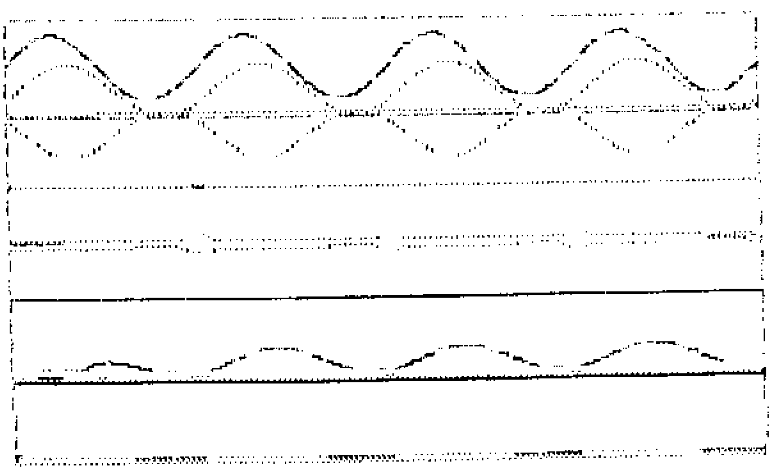


Figure 81b. simulation with low nutrients as in Table 83 with XX set = 1



(a)



(b)

Figure 82. Graphs resulting from simulation of the Apple Basic program (Table 83) of the N.C. ponds model (Figure 80). (a) Simulation of waste ponds by setting XX = 0 in program in Table 83; (b) simulation of control ponds by setting XX = 1 in program in Table 83. For both runs colors on color monitor are as follows. For the top set of curves, insolation, white; temperature, green; nutrients, blue. For the second set of curves: dissolved oxygen, blue; detritus accumulation, red. For the third set of curves: photosynthesis, green; respiration, red (plotted downward as mirror image comparison with photosynthesis plotted upward). For the fourth set of curves: plankton chlorophyll, green; Monodus, violet; blue-green algal mats, blue; benthic Ruppia beds, white; diverse phytoplankton regime, red. For the last set of curves: animals, violet; larvae, white; microbial respiration, red. These curves were separated for clarity in Figure 82.

8. Non-tidal estuarine pond microcosms can develop many of the characteristics of oligotrophic and eutrophic estuaries, which may make them suitable for experimental estuarine study for many purposes.

9. Except during the initial filling time before sewage pumping started, nitrogen was more limiting to photosynthesis than phosphorus, especially in waste ponds as might be expected since a low nitrogen to phosphorus ratio is characteristic of treated sewage.

10. Eutrophic estuarine ponds, because of their oxygen fluctuations, develop a channeling of food chains into a few species, a useful ecological engineering aquaculture technique requiring no dollar management costs.

11. The high productivity of small fish and shrimp in estuarine eutrophic ponds has potential as an inexpensive means of supporting waterfowl and other wildlife. Or with suitable arrangement for outmigration in late summer, the ponds could enrich higher food chains outside.

12. The use of eutrophic estuarine ponds for bait fish aquaculture will require screening from waterfowl to maximize the net yield for economic purposes.

13. The estuarine ponds fertilized with waste had the typical characteristics of cultural eutrophy that are sometimes regarded as undesirable when they develop in natural waters (intensely green with plankton algae, low diversity, high and low extremes of oxygen and pH, missing game fish). Sometimes cultural eutrophy is regarded as pathological, unregulated, unstable and undesirable. However, this systems study found a well organized ecosystem whose perfomance in generating overall production and protein was better than that of the control ponds or the normal surrounding estuaries.

14. The pond system was a fertile interface between waste waters and public waters, a means to carry out and control tertiary treatment at low cost, while reducing and stabilizing effluent effects on outside public waters. A strip of such ecosystems between society (with its effluents from sheet sources and point sources) may be a useful design for a coastal zone that reduces open water eutrophication while utilizing the enrichment benefits. After fish were added there were no mosquitoes.

15. The ecological engineering technique of setting boundary conditions (inflows, turnover times, and controlling actions) and providing massive multiple seeding of species may generate rapidly new ecosystems for new conditions that are useful for the developing harmony between humanity and nature.

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