UNIVERSITY OF NORTH CAROLINA

SEA GRANT PROGRAM

STRUCTURE AND FUNCTIONING OF ESTUARINE ECOSYSTEMS EXPOSED TO TREATED SEWAGE WASTES, III.

1971 – 1972

Edward J. Kuenzler, Alphonse F. Chestnut and Charles M. Weiss

Principal Investigators

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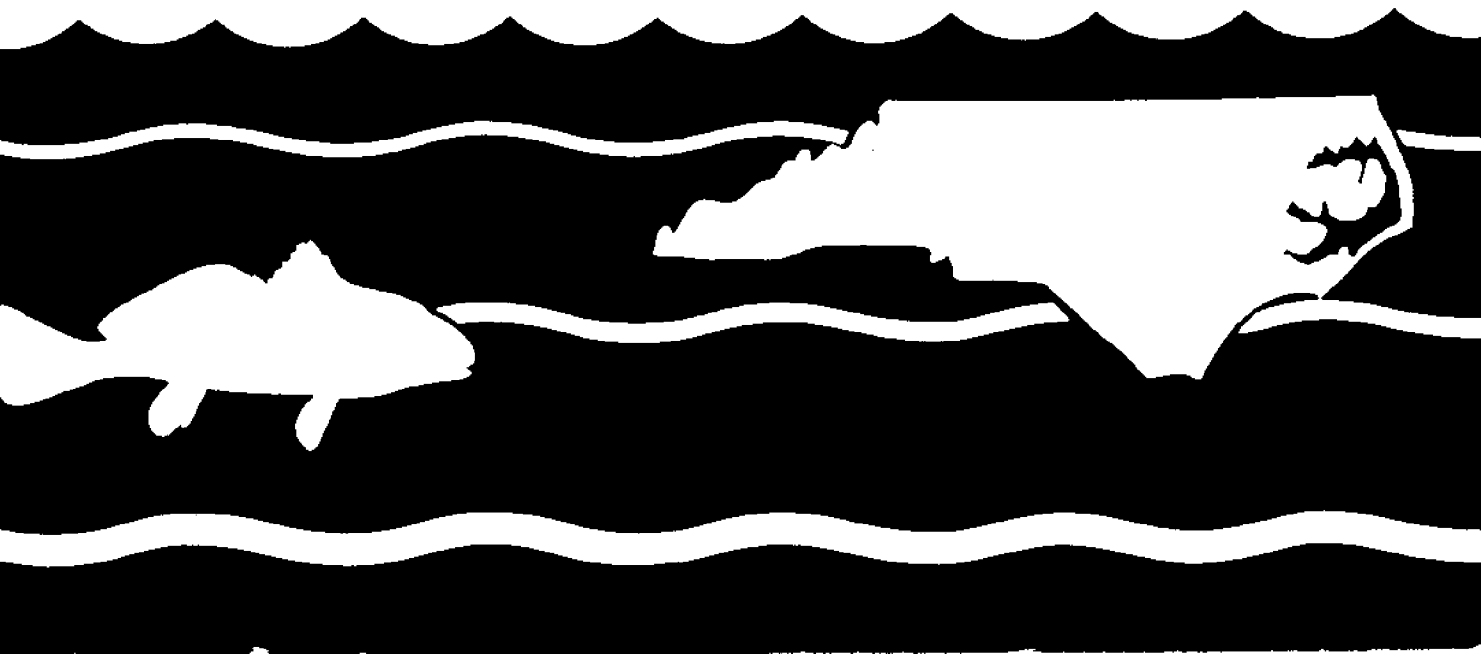

STRUCTURE AND FUNCTIONING OF ESTUARINE ECOSYSTEMS EXPOSED TO TREATED SEWAGE WASTES III. 1971-1972

by
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INTRODUCTION

This is the fifth report of investigations of the ecological systems which develop in small brackish-water ponds enriched with domestic sewage which has received secondary treatment. Faculty and students of the University of North Carolina at Chapel Hill have studied various phases of community structure and metabolism since 1968.

Three of the ponds, designated control ponds (C-1, C-2, and C-3) received a constant flow of water from Bogue Sound (Fig. 1) which was diluted with tap water to reduce the salinity. The three waste ponds (P-1, P-2, and P-3) received an intermittent flow of water from Calico Creek (Fig. 1) mixed with some of the effluent from the Morehead City sewage treatment plant. Pumping of Calico Creek water ordinarily took place during high tide in order to obtain water of relatively high salinity for the ponds.

This research effort endeavored to achieve an understanding of the ponds and of their functioning as ecological systems. Prior reports gave details on populations of phytoplankton, zooplankton, fishes, crustaceans, molluscs, and foraminifera. They also provided information on the carbon budget, the phosphorus kinetics, the nitrogen metabolism, primary productivity, and bacterial heterotrophy in the ponds. There were also reports on populations and processes of nearby estuaries and salt marshes: growth of Ulva and Spartina; hydrography and phytoplankton of Calico Creek; microarthropods and insects; fiddler crabs; and salt marsh snails.

This is the final report of this project. It covers primarily research performed during the period July 1971 - December 1972. During this time most studies have been brought to conclusion.

The list of all of the scientific reports resulting from this research project indicates the level of productivity and the effectiveness of our efforts to get our results out to the public. In addition to the reports shown here, six theses or dissertations which are largely or entirely based on studies supported by this project are within about one year of completion. We also expect a number of additional published papers to be prepared soon.

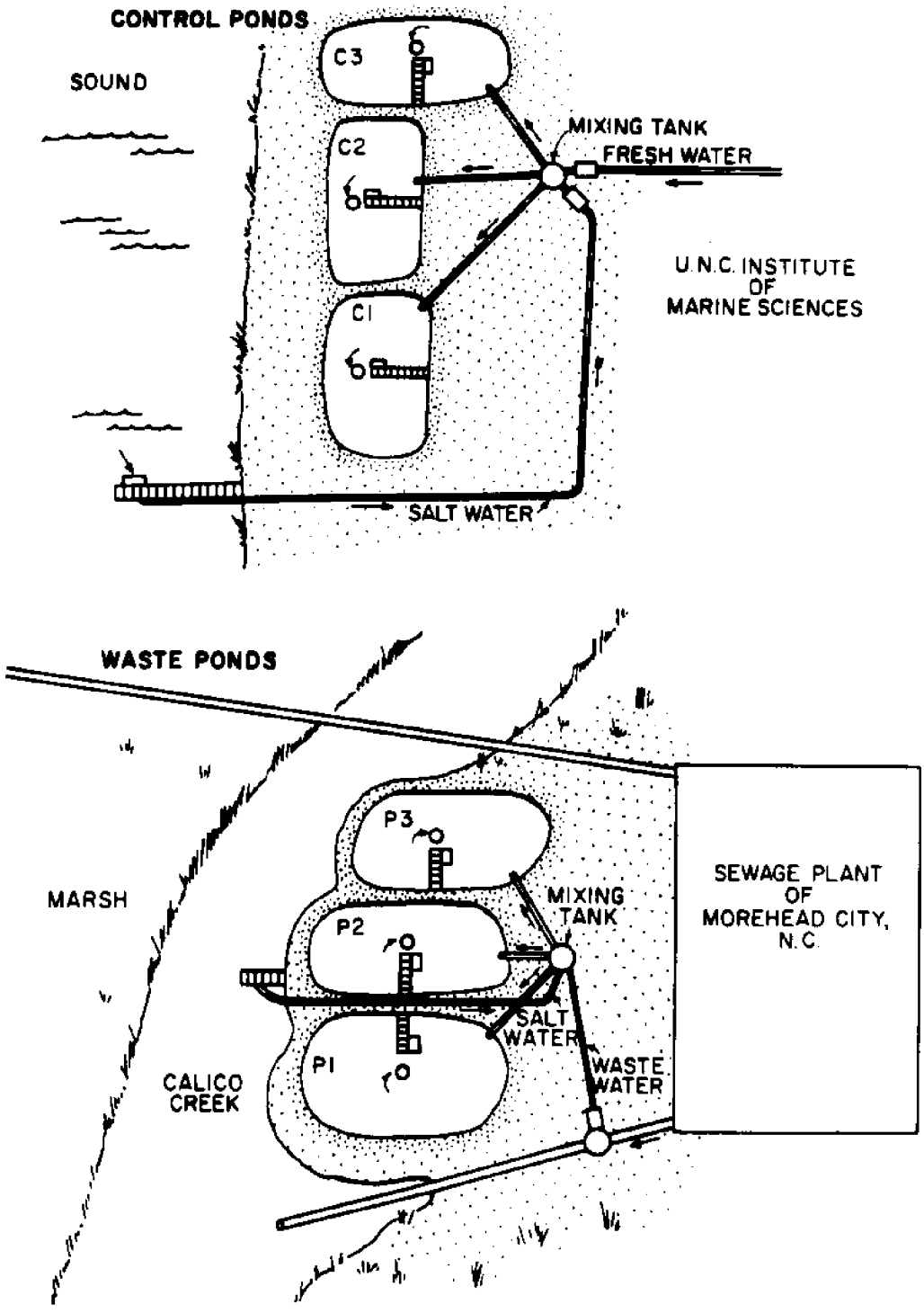

Fig. 1. Diagrams of control(C) and waste(P) ponds showing water flow systems.

Scientific Reports Resulting from This Project

Papers Presented and Published

1. Kuenzler, E. J. 1968. Cycling of phosphorus in marine ponds. Atlantic Estuarine Research Society. Atlantic Beach, N. C. October 4.

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

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6. Rhyne, C. F. and W. J. Woods. 1971. Field and laboratory observations on the seaweed Ulva curvata. Atlantic Estuarine Research Society. Atlantic Beach, N. C. April 16.

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1. Beeston, M. D. 1971. Decapod crustacean and fish populations in experimental marine ponds receiving treated sewage wastes. Master of Science thesis, Curriculum in Marine Sciences, University of North Carolina, Chapel Hill, under the direction of Dr. A. B. Williams.

2. Day, J. 1971. The Carbon Metabolism of Estuarine Ponds Receiving Treated Sewage Wastes. Ph.D. Dissertation, Curriculum in Marine Sciences, University of North Carolina, Chapel Hill, under the direction of Dr. C. M. Weiss.

3. McKellar, H. 1971. The Phosphorus System of Brackish Water Pond Ecosystems Exposed to Treated Sewage Waste. Master of Science thesis, Curriculum in Marine Sciences, University of North Carolina, Chapel Hill, under the direction of Dr. E. J. Kuenzler.

4. LeFurgey, Ann. 1972. Foraminifera in Estuarine Ponds Designed for Waste Control and Aquaculture. Master of Science thesis, Curriculum in Marine Sciences, University of North Carolina, Chapel Hill, under the direction of Dr. J. St. Jean.

5. Smith, Martha. 1972. Productivity of Marine Ponds Receiving Treated Sewage. Master of Science thesis, Department of Zoology, University of North Carolina, Chapel Hill, under the direction of Dr. H. T. Odum.

6. Muse, Barbara. 1973. The Effect of Effluent from a Secondary Sewage Treatment Plant on Oyster Condition. Master of Science thesis, Curriculum in Marine Sciences, University of North Carolina, Chapel Hill, under the Direction of Dr. A. F. Chestnut.

7. Rapps, Martin. 1973. The Effects of Treated Sewage Effluents on Nitrogen Fixation and Ammonia Diffusion. Department of Environmental Sciences and Engineering, University of North Carolina, Chapel Hill, under the direction of Dr. C. M. Weiss.

8. Rhyne, Charles F. 1973. Field and Experimental Studies on Systematics and Ecology of Ulva curvata and Ulva rotundata. Department of Botany, University of North Carolina, Chapel Hill, under the direction of Dr. M. Hommersand.

RECORD OF EVENTS AND PHYSICAL FACTORS

A. F. Chestnut and E. J. Kuenzler

Record of Events

A log was kept of the major activities, events, and accidents which took place in the control and waste ponds since the project was initiated (Odum and Chestnut 1970; Laughinghouse, Smith, and Kuenzler 1971). The prime value of these notes has been for communication between research projects rather than for their innate value. The events of 1971-72 are listed in Table 1.

Insolation, Salinity, and Turbidity

Prior reports on this project have provided details of the temperature, salinity, turbidity, and turnover rates of water in the ponds and of the insolation incident upon these systems for earlier years (Odum and Chestnut 1970; Laughinghouse, Smith, and Kuenzler 1971; Laughinghouse and Kuenzler 1971).

Insolation was determined with a Mark IV Sol-a-Meter, Talley Industries, Inc. Meter readings were made 6-7 times per week; differences between successive readings were multiplied by 177 to convert to cal cm^{-2}. The weekly pattern (Fig. 1) shows variations of 500-1000 cal cm^{-2} $week^{-1}$ over most of the year; in winter this may result in a 2-fold or greater difference in insolation between successive weeks. The monthly pattern shows the integrated annual pattern of solar energy; there is about three times more insolation in summer than in winter.

The salinities of the control ponds (Fig. 3) were generally in the range 16-26 °/oo, somewhat higher than in prior years. In the waste ponds (Fig. 2) the salinities were generally 13-23 °/oo. Over the years it has been very difficult to maintain the salinities of these ponds even relatively constant by manual manipulations. Even daily attention to the pumping regimes did not suffice to correct for heavy rains, backflooding through the standpipes, or pump failures. The salinities during the recent past (Fig. 3) average slightly higher than in earlier years (Odum and Chestnut 1970; Laughinghouse, Smith, and Kuenzler 1971).

The turbidity of the water closely parallels the density of the phytoplankton populations. As in past years, the turbidity of the control ponds, as indicated by the depth at which a Secchi disc is visible (Fig. 3), is least (Secchi depth is greatest) during the winter. Ponds C-1 and C-2 began to develop their summer phytoplankton populations in April. The phytoplankton in C-3, however, developed late and to a lesser extent, probably because of the dense growth of Ruppia maritima on the bottom which competed with the phytoplankton for nutrients. The harvest of Ruppia in June 1971 may have permitted the phytoplankton to begin growth.

The turbidity of the waste ponds from January through April (Fig. 3) is attributable to the dense population of the xanthophyte Monodus guttula as in previous years. The Monodus bloom appears to have crashed in May in all ponds; the summer populations generally were less dense and resulted in greater Secchi disc depths. This pattern of phytoplankton growth is essentially the same as in prior years (Odum and Chestnut 1970; Laughinghouse, Smith, and Kuenzler 1971; Laughinghouse and Kuenzler 1971).

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Laughinghouse, W., M. Smith, and E. J. Kuenzler.1971. Log of Activities and General Notes. In: Kuenzler, E. J. and A. F. Chestnut Structure and Functioning of Estuarine Ecosystems Exposed to Treated Sewage Wastes. Annual Report to N.O.A.A., Office of Sea Grants. pp. 13-23.

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Table 1. Record of events in ponds, February 1971-September 1972

Date	Activity
2/1/71	Sound pump off this morning - line frozen
	Creek pump off
2/3/71	Creek pump on
2/4/71	Creek pump recorder, tape changed
2/5/71	C-1 - 1 dead pinfish
	Sound pump on
2/8/71	C ponds - salt water flow 1/2 rate
2/9/71	Instruments moved C-1 to P-1
	Creek pump shut off
2/10/71	Sound pump off
2/11/71	Creek pump on
2/12/71	Sound pump on
2/13/71	Wind storm, 74 mph at 1320
2/16/71	Sound pump replaced
2/17/71	Creek pump replaced
2/19/71	Mixing tank for P-pond cleaned out
2/22/71	C-3 - algae floating up from bottom
2/23/71	Sound pump off
3/6/71	Fresh water line cut off
3/10/71	Dredging at C&D pier, water with heavy sediment
3/12/71	C-3 - algae suspended in water
3/22/71	Calico Creek salinity - 28.3 at 1540
3/25/71	Instruments set up in C-1
3/30/71	P-ponds turning a darker shade
	C-1 and C-2 darker green
4/8/71	Instruments moved from C-1 to P-1
4/9/71	Sewer pump off, Creek pump off
4/11/71	Flow rates for Creek and sewer pumps - calibrated with 1 gal. bucket
	P-1 - 163.6 gph
	P-2 - 163.6 gph
	P-3 - 144.0 gph
4/15/71	Sewer pump on
5/5/71	About 2 dozen oysters from Calico Creek put in baskets in each pond
	Ruppia dying in C-3
5/10/71	Flow rate for sewer pump only - calibrated with 1 gal bucket
	P-1 - 124.2 gph
	P-2 - 120.0 gph
	P-3 - 109.1 gph

5/10/71 (cont'd) Flow rates for C-ponds
C-1 - 189.5 gph = 4547.5 gp day
C-2 - 150.0 gph = 3600.0 gpd
C-3 - 133.2 gph = 3196.8 gpd

5/11/71 Sewer pump off, because sewer plant is being worked on and output has high chlorine content

5/12/71 Flow rate for creek pump only - calibrated with 1 gal bucket
P-1 - 156.5 gph
P-2 - 163.6 gph
P-3 - 138.5 gph

5/17/71 C-3 - Ruppia growing well, blooming
5/24/71 P-1 - Oxygen crash has begun
Sewer pump on
5/26/71 P-3 - back flooded

6/7/71 P-3 - 2 dead crabs
6/10/71 P-3 - much algae on surface
Fundulus varigatus starting breeding period
6/14/71 Sewer pump off
6/29/71 Sewer pump on

7/15/71 Aeration pump and hose installed in P-2 and turned on
7/23/71 Aeration pump removed from P-2

8/4/71 P-3 turning red brown
8/14/71 Aeration pump and hose installed in P-2
8/18/71 Heavy rains 16-18 Aug.
8/19/71 Air hose relaid in cloverleaf pattern
8/21/71 C-2 - seeded with 212 juvenile Penaeus setiferus
P-2 - seeded with 208 juvenile Penaeus setiferus
8/27/71 Tropical storm came through (Doria) - sound pump off - tide flowed over banks of P-1 and P-2, all P-ponds flooded - Cage in C-2 with 100 shrimp in it blown over
8/28/71 Sewer pump off
8/31/71 Sound pump on and pumping muddy water from dredge

9/3/71 Creek pump off because sewer plant putting out high chlorine water
9/7/71 Creek pump back on
9/13/71 P-2 - seeded with 76 shrimp seined from Hoop Hole Creek - 2 water samples taken at 2045 to determine oxygen content
9/14/71 Oysters from Calico Creek placed in baskets in P-ponds - 2 water samples taken at 2145 for oxygen determination from P-2
9/15/71 P-2 - 2 water samples taken at 0530 for oxygen determination
P-2 - 2 water samples taken at 2220 for oxygen determination
Crab pots placed in P-ponds
9/16/71 P-2 - 2 water samples at 0545
P-2 - 2 water samples at 2050
9/17/71 P-2 - 2 water samples at 0535
Crabs from crab pots - P-1 - 4 males - 1 female
P-2 - 3 males
P-3 - 1 male - 4 females

9/20/71 Crabs from P-ponds - P-1 - 1 male
P-2 - 3 males - 1 female
P-3 - 2 males

9/21/71 P-2 - 1 male crab

9/22/71 Crabs from P-ponds - P-1 - 2 males
P-2 - 4 males - 1 female
P-3 - 4 males - 1 female

9/28/71 Creek pump off

9/29/71 Crabs from P-ponds - P-1 - 0
P-2 - 1 male - 1 female
P-3 - 1 female

P-ponds - all power turned off and electrode float removed from P-2 due to approaching hurricane
Sount pump removed from dock

10/1/71 Salinities low due to heavy rains, high tides and pumps shut down during hurricane
Creek pump turned on
Crabs from P-ponds - P-1 - 2 males
P-2 - 1 male - 2 females
P-3 - 1 male - 2 females

10/4/71 Sound pump replaced on dock and in operation

10/5/71 P-1 and P-2 back flooded
Crabs from P-ponds - P-1 - 0
P-2 - 1 male
P-3 - 2 females

Excessive high tides flooded over banks of P-1, P-2, and P-3

10/7/71 Crabs from P-ponds - P-1 - 1 female
P-2 - 1 male
P-3 - 0

10/8/71 Crabs from P-ponds - P-1 - 0
P-2 - 0
P-3 - 1 male - 1 female

10/12/71 Crabs from P-ponds - P-1 - 0
P-2 - 1 male
P-3 - 3 females

Approximately 210 oysters from C-1 placed in baskets in P-ponds

10/14/71 Crabs from P-ponds P-1 - 2 males
P-2 - 0
P-3 - 1 male - 1 female

10/18/71 Crabs from P-ponds P-1 - 1 male
P-2 - 0
P-3 - 0

10/20/71 Crabs from P-ponds P-1 - 1 male - 2 females
P-2 - 2 males
P-3 - 1 male

10/21/71 Crabs from P-ponds P-1 - 2 females
P-2 - 2 females
P-3 - 0

Sewer plant fence being painted with aluminum paint, noted that paint had covered P-1 with thin film, also P-2 and P-3 partially covered with paint.

10/28/71	Crabs from P-ponds	P-1 - 1 male
		P-2 - 2 males - 2 females
		P-3 - 2 males - 1 female
10/29/71	Crabs from P-ponds	P-1 - 0
		P-2 - 0
		P-3 - 1 female
	Approximately 100 oysters from Calico Creek put in basket in C-1	
10/31/71	Heavy rains (10.66 inches) this month caused low salinities	
11/1/71	Crabs in P-ponds	P-1 - 0
		P-2 - 2 males
		P-3 - 1 male
	Creek pump off	
11/2/71	Crabs in P-ponds	P-1 - 2 males
		P-2 - 0
		P-3 - 1 female
11/3/71	P-pond back flooded, creek pump back on	
11/8/71	Crabs in P-ponds	P-1 - 1 female
		P-2 - 0
		P-3 - 1 male
11/9/71	Crabs in P-ponds	P-1 - 1 female
		P-2 - 0
		P-3 - 0
	P-2 - Aeration pump turned off - pump and hose removed from pond	
11/10/71	Shrimp seined - 60 out of C-2, 33 out of P-2	
11/11/71	Crab pots removed from P-ponds and placed in C-ponds	
	P-1 and P-2 - water samples taken for oxygen determination	
11/12/71	P-2 and P-3 - water samples taken for oxygen determination	
	Crabs from C-ponds -	C-1 - 2 males - 2 females
		C-2 - 1 male
		C-3 - 3 males - 1 female
11/15/71	Crabs from C-ponds	C-1 - 1 female
		C-2 - 1 female
		C-3 - 0
	Approximately 80 oysters from Calico Creek put in basket in C-1	
11/16/71	Crabs from C-ponds	C-1 - 1 female
		C-2 - 0
		C-3 - 0
11/29/71	Crab pots removed from C-ponds and placed in P-ponds	
11/30/71	Crabs from P-ponds	P-1 - 2 males
		P-2 - 0
		P-3 - 2 males - 2 females
	Salinometer out of service, salinities being read with refractometer	
12/10/71	Crab pots removed from P-ponds because no yield	

1/10/72 Oysters from C-1 put in P-1
1/17/72 P and C ponds covered with about 1/2 inch ice
1/19/72 Creek pump off
1/24/72 Creek pump in operation

2/5/72 Creek pump frozen
2/8/72 115 oysters from Calico Creek placed in C ponds
2/14/72 about 4 inches rain since 9 Feb., salinities dropp ed
2/24/72 175 oysters from Calico Creek put in C ponds
2/25/72 100 oysters from Calico Creek put in P-1
2/28/72 100 oysters from Calico Creek put in P-1

3/6/72 green algal growth starting in P-3

4/6/72 100 oysters from Calico Creek placed in C-3
4/10/72 P-3 water clear along shore, large clump algae below surface
4/17/72 Sewer pump off
4/19/72 Sewer pump on
4/26/72 P-3 water clear, bottom visible

5/5/72 Heavy rain (@ 1"), filamentous algal mat along shore of P-3
5/11/72 Palaemonetes seined in P-3, marked with blue stain and returned
5/12/72 Algal growth starting along shore of C-3
Palaemonetes seined in C-3, stained blue and returned

6/5/72 Postlarval fishes in P-2, Ruppia blooming in C-2 and C-3
6/9/72 Fresh water flow cut off in C-1 and C-2
6/12/72 Numerous small Palaemonetes at inflow in C-1 and C-2
6/13/72 About 200 Palaemonetes stained and released in P-3
6/14/72 About 200 Palaemonetes stained and released in C-3
Flow rate calculated in C-3 at 180 g.p.h.
6/19/72 7 sponge crabs (Callivictes) placed in C-1
175 clams placed in meter square in C-1
6/20/72 225 clams planted in C-1

7/5/72 2 sponge crabs placed in C-1
7/7/72 Sewer pump and creek pump off, sewage plant renovations.
7/19/72 about 150-200 Palaemonetes stained and released in P-3
7/20/72 about 150 Palaemonetes stained and released in C-3

8/8/72 oxygen samples from C and P ponds, creek pump on
8/9/72 oxygen samplings started, to be continued
8/11/72 Creek pump off
8/15/72 Creek pump on, replaced

9/12/72 Shore birds (sandpipers) feeding in P-2
9/13/72 Records of shore birds commenced.

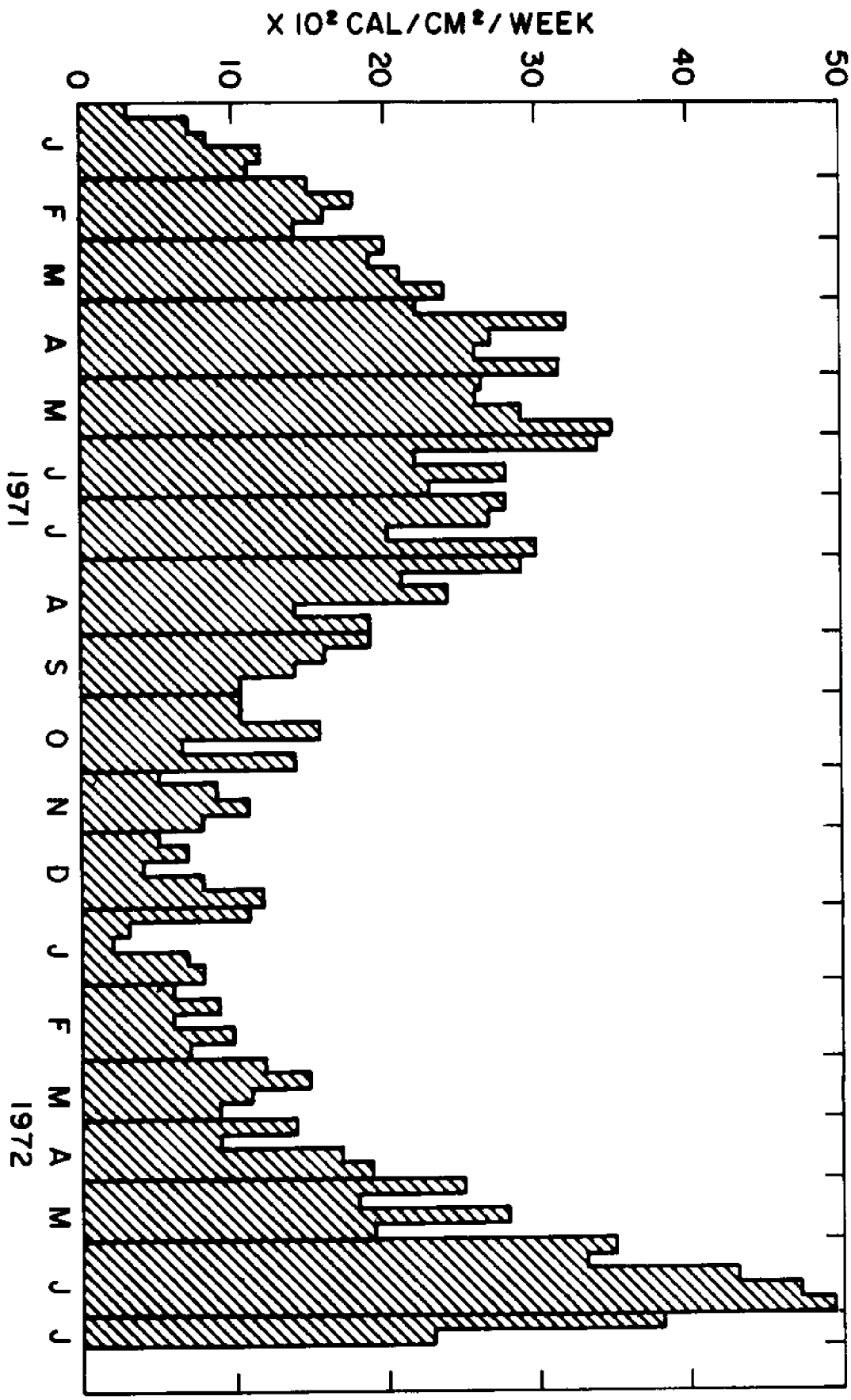

Fig. 1. Weekly amounts of insolation at Morehead City, North Carolina, during 1971-72.

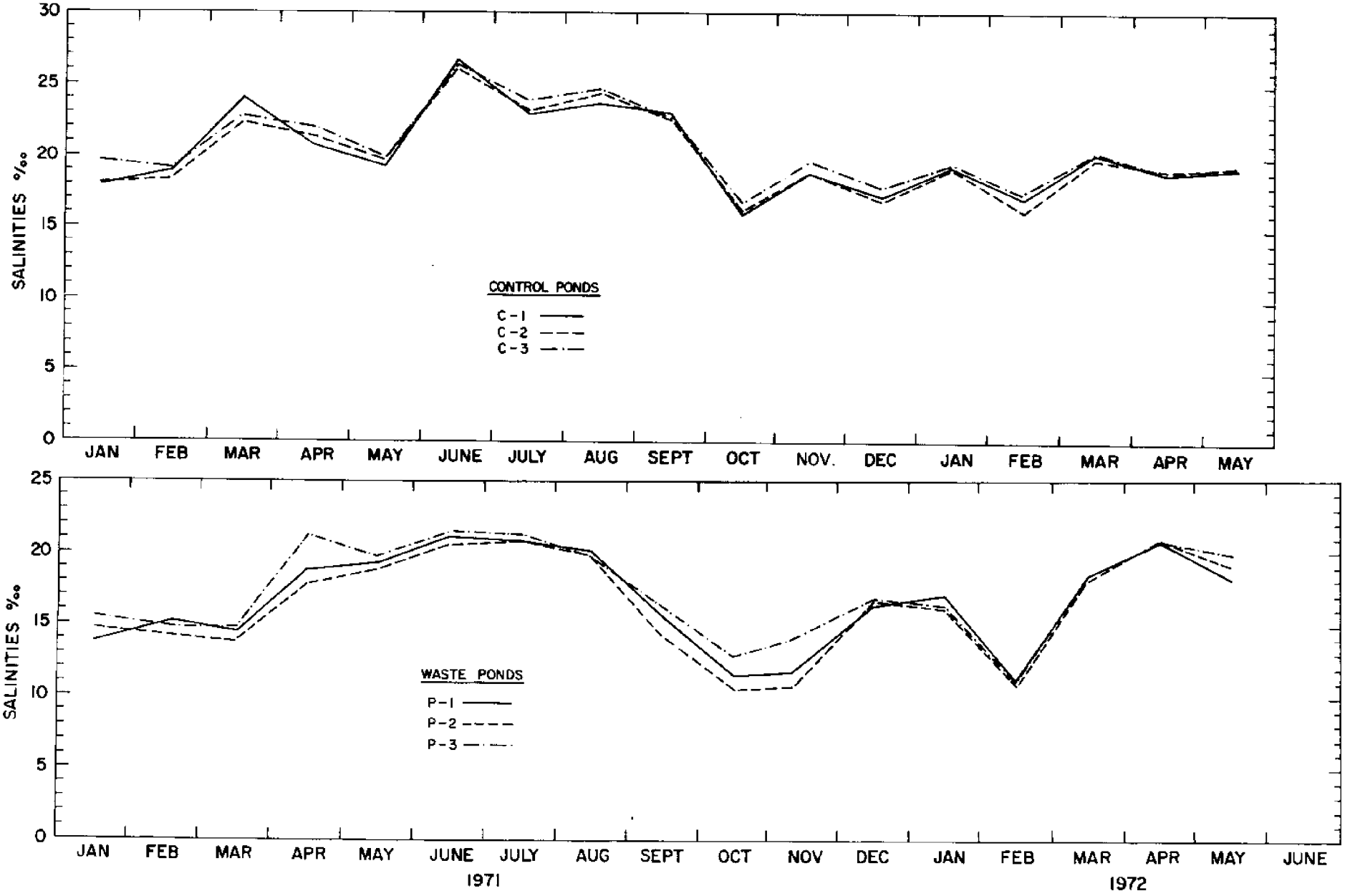

Fig. 2. Salinities (‰) in control and waste ponds during 1971-72.

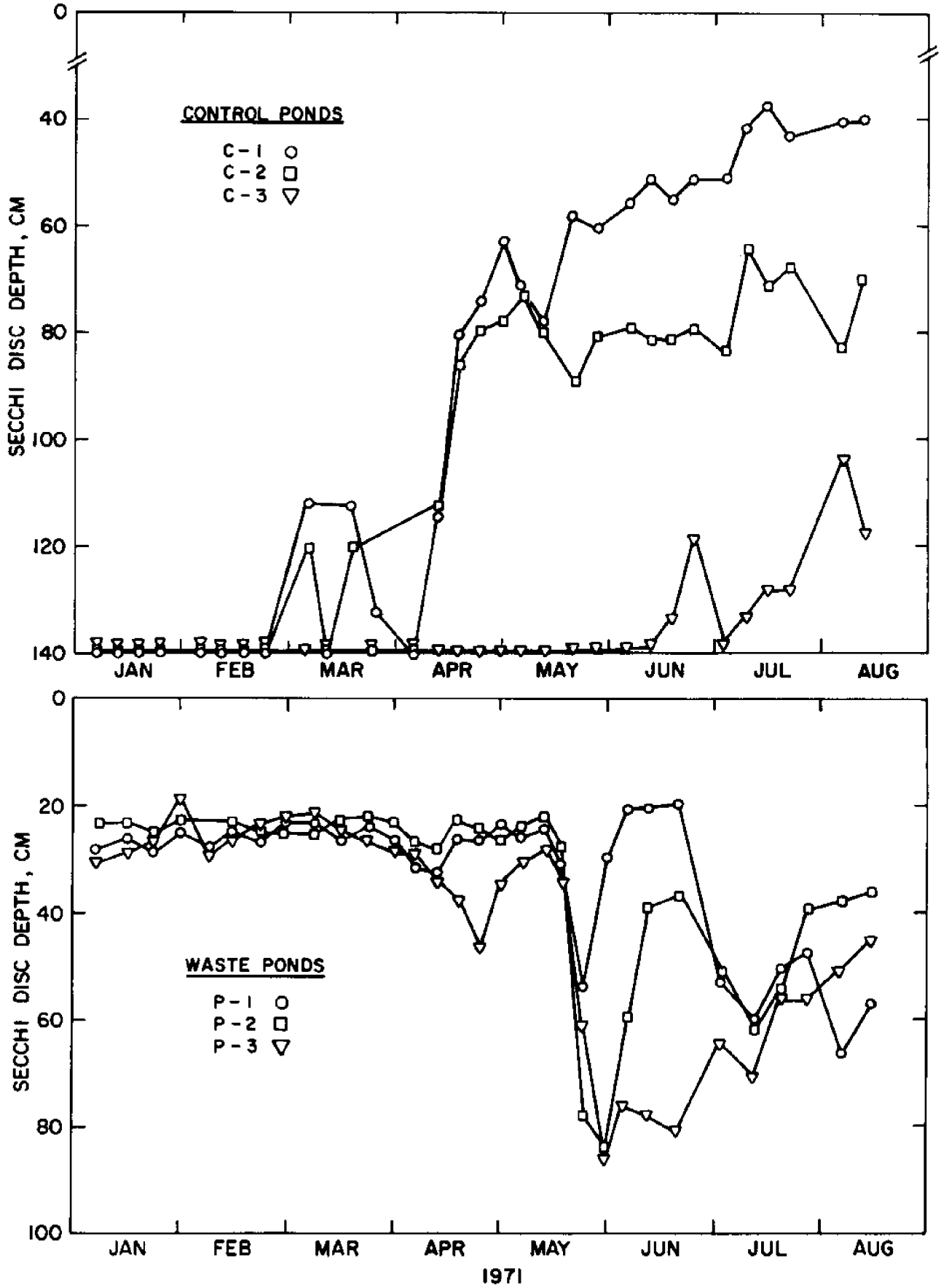

Fig. 3. Turbidity of the control and waste ponds during Jan. - Aug. 1971, expressed as the Secchi disc depth.

CYTOLOGICAL AND BIOCHEMICAL STUDIES ON MONODUS FROM THE TREATED SEWAGE PONDS IN MOREHEAD CITY

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INTRODUCTION

Until this past year a small unicellular yellow-green alga belonging to the genus Monodus has been the dominant algal species in the waste ponds (p-ponds) during the winter months from October until April. In last year's annual report Hommersand and Talbert (1971) demonstrated through laboratory studies that the density and time of blooms of Monodus in the p-ponds could be accounted for as a response to physical and chemical parameters that were characteristic of the ponds. In both the ponds and the laboratory the availability of nitrogen as ammonium ion (NH_4^+) or nitrate (NO_3^-) limited the maximum cell density to about 10^7 cells/ml. Addition of still higher nitrogen concentrations gave greater yields. The time of appearance of the blooms in the p-ponds from October through April corresponded with the requirement that the temperature be below 22°C for normal growth at high densities under stationary conditions. The rapid death and "crash" of Monodus early in May that led to temporary anoxia in the p-ponds was probably due to rising temperatures. The photosynthesis of Monodus was responsible for the high pH (10-10.5) characteristic of the p-ponds during the winter months. This same response of pH was seen in the laboratory. The ready adaptation of Monodus to wide ranges of salinity as seen in the laboratory accounts for the stability of populations of this alga in the face of fluctuating salinities in the p-ponds. This year Monodus did not bloom and did not become the dominant alga in the p-ponds. This difference can be readily accounted for, since the influent of treated sewage was turned off late in the fall and remained off during most of the winter months. Since the density of the Monodus population is critically dependent on nitrogen availability it would not be expected to bloom in the absence of continuing influx of the nitrogen-rich treated sewage. Because Monodus is the dominant organism in the p-ponds under the conditions originally specified, we felt that it was important to attempt to identify this alga and characterize it with respect to its cytological and biochemical properties.

DESCRIPTION OF MONODUS

The Monodus in the waste ponds has been equated with M. guttula Pascher, a freshwater species from central Europe (Campbell 1971, p. 156, pl. 3, figs. 29a-i). Since our material is found primarily in brackish

and marine waters it is probably a distinct, unnamed species. Observations on growth rates of Monodus (Hommersand and Talbert, 1971) demonstrate that our species grows rapidly in freshwater. It is reasonable to speculate that it originated as a freshwater entity and has evolved a tolerance for the marine environment rather than the other way around.

Monodus sp. is a non-motile unicellular alga. Adult cells are typically comma shaped, but may be ellipsoidal to subsphaerical measuring 3-5 x 2-4u. In nature the wall at one end of the cell is tipped with a short spine or beak, while the other end is broadly rounded. Cells typically have 1-2 pale green chloroplasts in the center and several refractive globules at either end, some of which may be oil droplets. Sometimes a dark orange refractive body can be seen associated with the chloroplast which may be a pyrenoid. Reproduction is by autospores. Each cell ordinarily forms two autospores which are released by a longitudinal split in the mother wall (Campbell 1971, pl. 3, fig. 24). Cells grown on agar in the laboratory commonly produce 2, 4, 6 or 8 autospores (fig. 1 a-d). No motile stages have been seen, nor is there any evidence of sexuality.

The cultures of Monodus as they were originally isolated were contaminated by a long rod-shaped bacterium that regularly attached by one end to the surface of the Monodus cell wall. Ordinarily there were one or two such bacterial ectoparasites per Monodus cell (fig. 2 a-c) though as many as eight have been seen attached to a single cell. Cells that were free of bacterial contamination were re-isolated from the original stock by repeated centrifugation, washing and sonication in sterile medium. The bacteria and detritus from two to three washings were removed and the cells diluted and sprayed from a microcapillary under a jet of air onto agar plates. Axenic cultures were isolated and maintained on modified ASP-2 medium (Provasoli et al. 1957) containing the following substance in 1000 ml solution: 5 gm $MgSO_4.7H_2O$, 11.1 gm NaCl, 0.6 gm KCl, 0.27 gm $CaCl_2$, 0.05 gm $NaNO_3$, 0.03 gm $K_2HPO_4.3H_2O$, 0.015 gm $Na_2SiO_3.9H_2O$, 1 gm TRIS, 0.05 gm NH_4Cl, 0.312 mg $ZnCl_2$, 4.32 mg $MnCl_2.4H_2O$, 0.012 mg $CoCl_2.6H_2O$, 0.00322 mg $CuCl_2.2H_2O$, 3.87 mg $FeCl_3.6H_2O$, 30 mg Sodium EDTA. Bacterized cells stayed greener longer, tended to aggregate and also showed phototaxis, moving toward the lighted side of the culture tube. The bacteria could not be grown or maintained separately from the host Monodus cultures.

Growth rates of both original and reisolated axenic cultures were measured in liquid culture containing ASP-2 medium at 13‰ salinity, 17°C, 750 ft candles cool white fluorescent light, gassing with air, and constant shaking. The reisolated axenic cultures grew at least as well as or better than the original bacterized stocks and reached the maximum concentration earler (Table I).

Table I. Comparison of cell count between original and reisolated cultures.

days / culture	2	3	5	6
original	6×10^5	9×10^5	46.5×10^5	50×10^5
reisolated	6×10^5	12×10^5	84.5×10^5	102×10^5

ULTRASTRUCTURE

A platinum shadow cast carbon replica of the cell surface shows the wall to be rough with furrows and wrinkles (fig. 3). The unevenness of the cell surface is also seen in cross-section (figs. 4, 5, 6). The beak-like protrusion at one end of the cell is confirmed by electron microscopy (fig. 5 a,b). The cell wall and plasma membrane are continuous with cytoplasm underlying the raised area. In sectional view the Monodus cell contains 2-4 large mitochondria, a single nucleus, one chloroplast usually without a pyrenoid, one dictyosome and a few lipid droplets. The nucleus lies to one side of the chloroplast and possesses a double membrane and typical nuclear pores (fig. 6). The chloroplast is enclosed by a double membrane that is also covered by an envelope of periplastidial endoplasmic reticulum. It is not clear whether the nuclear envelope is continuous with the chloroplast endoplasmic reticulum. Each chloroplast contains 5-9 lamellae with each lamella apparently composed of three thylakoids with little or no interlamellar thylakoid exchange (figs. 4, 6, 7). Chloroplast lamellae are evenly spaced and run the whole length of the plastid. Most thylakoid pairs terminate near the inner surface of the plastid envelope, although some terminate in the interior of the chloroplast (fig. 7). Girdle lamellae were absent. A single pyrenoid-like body which was not penetrated by chloroplast lamellae was occasionally seen situated on one side near the center of the chloroplast (fig. 8).

PIGMENTATION

Analysis of the pigment extracts of Monodus yielded chlorophyll a, β-carotene and three distinct xanthophylls. The algal cells were harvested by centrifugation (10,000 g.) for 10 minutes and the pellets dispersed by grinding and extracted 2-3 times with absolute methanol with the aid of a grinder. Extracted pigments were transferred to diethyl ether by the addition of a saturated solution of sodium chloride. A small amount of hexane (5%) was added and the ether fraction washed several times with distilled water and over anhydrous Na_2SO_4 and concentrated by evaporation under reduced pressure to 3-5 ml in the dark.

Pigment extracts were chromatographed on 5 x 40 cm columns containing packed 10x powdered confectioner's cane sugar using a solvent system containing 0.5% N-propanol in light (30-60°) petroleum in the dark at 10°C for one hour during which time the solvent developed 25-30 mm in the column. Pigment bands were cut out from the column and eluted with diethyl ether. Each pigment was transferred into a small volume of hexane and re-chromatographed with the same solvent, or with ether:light petroleum at a ratio of 7:3. After re-chromatography each pigment was eluted into ethanol, methanol or ether and spectra were taken in these solvents and in hexane measured with a Cary 14 recording spectrophotometer. The chromatographic behavior of the pigment extracts from Monodus following initial separations in the sugar column is shown in fig. 9. The major pigment fractions are recorded in Table II along with pertinent physical data.

TABLE II. Carotenoids of Monodus

(Pigments listed in the order of increasing adsorptive power on the confectioner's sugar.)

Fraction number	Range of Rf	Relative abundance % of total	Experimental adsorption maxima (nm)	Solvents	Identification	Published data
I	80-98	25	452;479	Ethanol	β-Carotene	452; 480 (1)
			448; 474	Hexane		451; 482 (5)
II	68-70	5	475	Ethanol	Canthaxanthin	477 (2)
			464	Hexane		466 (3)
III	48-65	25	617; 664; 416; 432	Ethanol	Chlorophyll a	
			612; 660; 408; 425	Hexane		
IV	10-30	25	420; 443; 472	Ethanol	Vaucheriaxanthin	419;443; 471 (4)
			402; 425; 452	Ethanol + HCl		400;423; 450 (4)
			417; 441; 470	Hexane		
V	0-30	20	417; 440; 470	Ethanol	Violaxanthin	420;441; 471 (5)
V						419;440; 478 (4)
			379; 401 427	Ethanol + HCl		425;440 480 (4)
			442; 471	Hexane		443; 472 (5)
Crude extract (control)			474; 565 663 416;436; 470	Hexane		

(1) Thomas and Goodwin (1965)
(2) Petracek and Zechmeister (1956)
(3) Isler and Schudel (1963)
(4) Kleinig, H. & Egger, K. (1967)
(5) Goodwin (1955)

The least absorbed pigment was eluted in ethanol or hexane and was identified as β-carotene from its Rf and absorption spectra (fig. 10). This pigment was compared with β-carotene extracted from Vaucheria litorea which had absorption maxima at 421, 450, 474 nm. In mixed chromatography the two pigments failed to separate on the sugar column with either light petroleum (30°-60°) or diethyl ether:light petroleum, 7:3 solvent systems. There has been some confusion concerning the spectra of β-carotene in the literature. Thomas and Goodwin (1965) have given the peak in ethanol as 452, 480nm, while Kleinig and Egger (1967) report the peaks as 424, 448, 474nm based on extracts from Vaucheria and Botrydium. It is interesting that Monodus gave a two-peaked absorption spectrum resembling the standard data of Thomas and Goodwin and that our Vaucheria material gave a three peak spectrum closely resembling the spectra published by Kleinig and Egger.

The orange colored fraction II gave an absorption spectrum that was very symmetrical with only one peak. It was identified tentatively as canthaxanthin by its Rf and absorption spectra (fig. 10). Like canthaxanthin our pigment is hypophasic in 90% aqueous methanol and light petroleum. Also it forms a pink band just below chlorophyll a which is thought to be an interaction between the xanthophyll and chlorophyll a (Whittle and Casselton, 1969). The pink contaminant goes to the hypophase when transferred to light petroleum and washed with saturated sodium chloride solution where the carotenoid is released from its association with chlorophyll and regenerated.

The yellow colored fraction IV was mixed with the yellow-orange colored fraction V in the initial chromatogram. They could be separated by rechromatographing on sugar with ether:light petroleum 7:3. Both fractions could be easily eluted from the sugar with ethanol or diethyl ether but not with hexane or light petroleum. In partition tests both stayed in the hypophase in 90% aqueous methanol and light petroleum. Fraction IV was identified as vaucheriaxanthin by its Rf and absorption spectra in ethanol and hexane. Addition of one drop 1N HCl to an ethanolic solution resulted in a spectral shift of 18 nm (fig. 11). The hydrochloric acid treatment converts the 5,6 epoxy group to a 5,8 epoxide resulting in a bathochromic displacement of about 18 nm in the absorption spectrum for each 5,6 epoxy group present (Davies, 1965). Vaucheriaxanthin is the major carotinoid present in Monodus. It is a 5,6 monoepoxide as evidenced by the bathochromic shift of 18 nm in the presence of acid. Additional experiments showed that the vaucheriaxanthin could be saponified by adding 1 ml of 60% KOH into 10 ml of a methanolic solution. This was evidence that the pigment is esterified as has been described by Kleinig and Egger (1967). Further proof of the identity of vaucheriaxanthin comes from the failure of a mixture of the Monodus preparation to separate from an authentic sample of vaucheriaxanthin prepared from Vaucheria litorea on a sugar column developed by either light petroleum with 0.5% propanol or by ether: light petroleum 7:3 solvents.

Fraction V was identified as violaxanthin by the same methods described above. It was very strongly adsorbed on the sugar column and could be separated from vaucheriaxanthin only with the more polar ether:light petroleum, 7:3 solvent system. The absorption spectrum gave a bathochromic

shift of about 37nm upon the addition of 1N NaCl in ethanol solution (fig. 12) owing to the conversion of a 5,6, 5', 6'-diepoxide to a 5, 8, 5', 8'-diepoxide. Violaxanthin has been characterized from several groups of algae and from higher plants and its spectral properties are well known.

PHOTOSYNTHETIC ACTION SPECTRUM

In order to determine the relative effectiveness of carotenoid and chlorophyll pigments in photosynthesis an action spectrum for the evolution of oxygen in different wavelengths of light from 400nm to 720nm was run and compared to the in-vivo absorption spectrum. This work was carried out by David M. Talbert.

Cells were grown in Roux flasks on a shaker in modified Provasoli's ASP-2 medium as before. Cell suspensions were concentrated by centrifugation in a clinical centrifuge and the pellet was resuspended by bubbling gently with air at an optical density of about 0.4 at 675nm as measured with a Bausch and Lomb Spectronic 20.

The rate of oxygen evolution was measured polarographically according to the methods of Haxo and Blinks (1950) as modified by Haxo (1960) and Hommersand (unpublished). The cell suspension was pressed into a thin film over a stationary platinum surface (15mm x 3mm) that was recessed into a black plastic base to form a shallow well by a layer of dialysis tubing held down with a plexiglass ring. The platinum was connected by means of a salt-agar bridge to a mercury-calomel reference electrode. A potential of 0.6 volts was maintained across the two electrodes. The assembly containing the cell suspension was threaded into a clear plexiglass chamber through which a modified nutrient medium containing 15.0 g $MgSO_4.7H_2O$, 33.3 g NaCl, 1.8 g KCl, 0.09 g $K_2HPO_4.3H_2O$, 0.15 g NH_4Cl, 3.0 g TRIS per liter and diluted to give 13‰ salinity was circulated at a rate of 1100 ml/minute by a Cole Parner model 7012 centrifugal pump. 0.1% $NaHCO_3$ was added to the medium and the pH adjusted to 8.0. It should be noted that a net relative rate of oxygen production by the cells is measured since the oxygen consumed by respiration is not seen by the electrode.

Monochromatic light was provided by a Bausch and Lomb grating monochromator (3000Å blaze) using a tungsten light source which was focused by a series of lenses from the monochromator exit onto the cell layer. By regulating the operating voltage of the tungsten lamp with a powerstat the cell suspension was provided with equal incident energies throughout the visible spectrum that developed an e.m.f. of 6μV (92.4μ-watts cm^{-2}) on an Eppley thermopile as measured by a Keithley 150A microvolt ammeter. Illumination of the cell suspension on the electrode was switched on and off by a shutter placed in the light path. The usual regimen was 2.5 minutes of light followed by 2 minutes of dark.

The in-vivo absorption spectrum of a thin film of cells like that on the electrode was measured in a Cary 14 spectrophotometer provided with a Scatter Transmission Accessory. A special cell was constructed to hold the cells by gluing a plastic cover-slip cut to the same dimensions as the

recessed platinum electrode onto a glass microscope slide. Vaseline around the edges of the glass cover slip formed a temporary seal.

The in-vivo absorption spectrum is plotted along with the photosynthetic action spectrum in Figure 13. The two spectra are superimposed to coincide in the red wave length region where only chlorophyll absorbs. Two corrections have been applied to the data from the action spectrum. First, the rates of oxygen evolution were recalculated in terms of equal incident quanta rather than equal incident energies as measured. Second, a correction was made for the added photosynthesis due to light reflected by the platinum electrode and absorbed during its second passage through the cell suspension as described by Haxo and Blinks (1950). A second action spectrum (not shown) was run five hours after the first using the same cell suspension. An over all drop of only 8% was ob served in the response and the character of the spectrum was unchanged indicating the great stability of these cells on the electrode.

A proportionally higher rate of oxygen evolution is seen in the red region where light used in photosynthesis is absorbed by chlorophyll alone. It is characteristic of yellow-green algae that carotinoids are present in the cell in excess of chlorophyll. If the carotinoid pigments were inactive in photosynthesis there would be a marked decline in the relative effectiveness of light in the region of carotinoid absorption between 400 and 550nm. The action spectrum in figure 13 does indicate some possible carotinoid inactivity; however, a peak is clearly seen at the 490nm absorption shoulder which can be attributed only to carotinoid absorption.

It would be of interest to learn which of the carotinoid pigments present are active in photosynthesis. The major carotinoid component, vaucheriaxanthin, is known to be a complex molecule that is esterified with short chain fatty acids (Kleinig & Egger, 1967). It may well be that this pigment, like the fucoxanthin of brown algae and diatoms, functions primarily in system II of the two light reactions postulated to occur in photosynthesis. Violaxanthin might then be the 'inactive' constituent. Alternatively, there may be some complex distribution of inputs from carotinoid pigments into systems I and II of photosynthesis which would show up in an enhancement spectrum. A study of photosynthetic enhancement in Monodus is planned for the future. Since Monodus contains so few different kinds of pigments the role of pigment composition in the photosynthesis of this species may prove to be particularly interesting.

TAXONOMIC POSITION OF MONODUS

The genus Monodus Chodat has been placed by Pascher (1939) in the tribe Ellipsoideae, family Pleurochloridaceae, order Heterococcales of the Xanthophyceae, a class of yellow-green algae which lack chlorophyll b, chlorophyll c and fucoxanthin. In a recent work Hibberd and Leedale (1972) have re-examined the classification of coccoid Xanthophyceae based on their comparative ultrastructure. Genera thought to be closely related to Monodus such as Ellipsoidion, Pleurochloris, Vischeria, and Polyedriella have been found to contain species belonging to a distinct new class, the Eustigmatophyceae. Other species presently placed in the Pleurochloridaceae were

found to be true members of the Xanthophyceae.

Eustigmatophyceae were found to have elongate, flask-shaped zoospores with a single anteriorly directed flagellum bearing mastigonemes and a proximal swelling next to the eyespot and an anterior eyespot composed of individual pigment droplets that are not enclosed within a membrane. The vegetative cells are coccoid with a wall composed of one piece. The chloroplast and nucleus are not enclosed in a common sheath of endoplasmic reticulum. A pyrenoid is present in vegetative cells that is large, polyhedral and attached to the inner face of the chloroplast. It is free from chloroplast lamellae. Girdle lamellae are absent. The pigments include chlorophyll a, β-carotene, esterified vaucheriaxanthin and violaxanthin.

Xanthophyceae are characterized by having oval, bilaterally symmetrical zoospores with two unequal flagella, the longer one directed anteriorly and bearing mastigonemes and the shorter one smooth with a basal swelling that is closely pressed against the eyespot region. The nucleus is intimately associated with the chloroplast and normally surrounded by a layer of endoplasmic reticulum enclosing both organelles especially in the zoospore. There are typically two chloroplasts present in the zoospore and the eyespot consists of a single sheet of droplets contained within the anterior tip of the ventral chloroplast. In the vegetative cell pyrenoids are absent from the chloroplast or are semi-immersed within the chloroplast and are usually penetrated by thylakoid lamellae. Girdle lamellae are usually present. The pigments consist of chlorophyll a, β-carotene, esterified vaucheriaxanthin or heteroxanthin and diadinoxanthin.

Unfortunately, Monodus has never been shown to possess a zoosporic or flagellated stage. Since the most critical characters for distinguishing Eustigmatophyceae from Xanthophyceae reside in the zoospore stage, a definitive conclusion can not be drawn concerning its affinities. If one considers only vegetative characters, Monodus shares more features in common with Eustigmatophyceae than with Xanthophyceae. Most significant is the presence of violaxanthin instead of diadinoxanthin (see Whittle and Casselton, 1969). Girdle lamellae are absent in the chloroplast. While the nucleus is pressed against the chloroplast there is no evidence that both are enclosed in a common sheath of endoplasmic reticulum (see fig. 6). A pyrenoid is not usually present, and if one indeed does occur it is atypical and not representative of either group. An interesting feature not mentioned until now is the presence of lamellate vesicles (fig. 7, 7a) of a type commonly seen in zoospores of Eustigmatophyceae (Hibberd and Leedale, 1972). On the balance Monodus would appear to represent an addition tothe new but growing list of Eustigmatophyceae, a group of coccoid organisms whose physiology and ecological adaptations are largely unknown.

IMPORTANCE OF MONODUS

The potential importance of Monodus to estuarine systems receiving treated sewage lies in the fact that it is evenly dispersed in a shallow pond without agitation or circulation. It is tolerant to wide ranges of

temperature and salinity and it requires and consumes large amounts of nitrogen and phosphorous from treated sewage. Our experiments using Chapel Hill sewage as well as Morehead City sewage (Hommersand and Talbert, 1971) indicate a tolerance for still other variables than the ones mentioned.

If means could be found for harvesting Monodus from shallow ponds like the p-ponds this species could prove to be valuable in the removal of nitrogen and phosphate in a tertiary treatment of sewage. The harvest could then, perhaps, be fed to bivalves or other marine organisms or used as fertilizer. Experiments are being initiated to determine the rates of NH_4^+, NO_3^- and PO_4^- uptake and assimilation from different concentrations of these nutrients in the medium. Harvesting and feeding experiments are also planned or underway here and at the Institute of Marine Sciences in Morehead City. Although Monodus appeared in the ponds in a most unexpected way and seemed to present problems for the survival and growth of other organisms its presence and behavior may be fortuitous indeed, as its growth and productivity are very high and its usefulness, especially in the tertiary treatment of sewage, seems promising.

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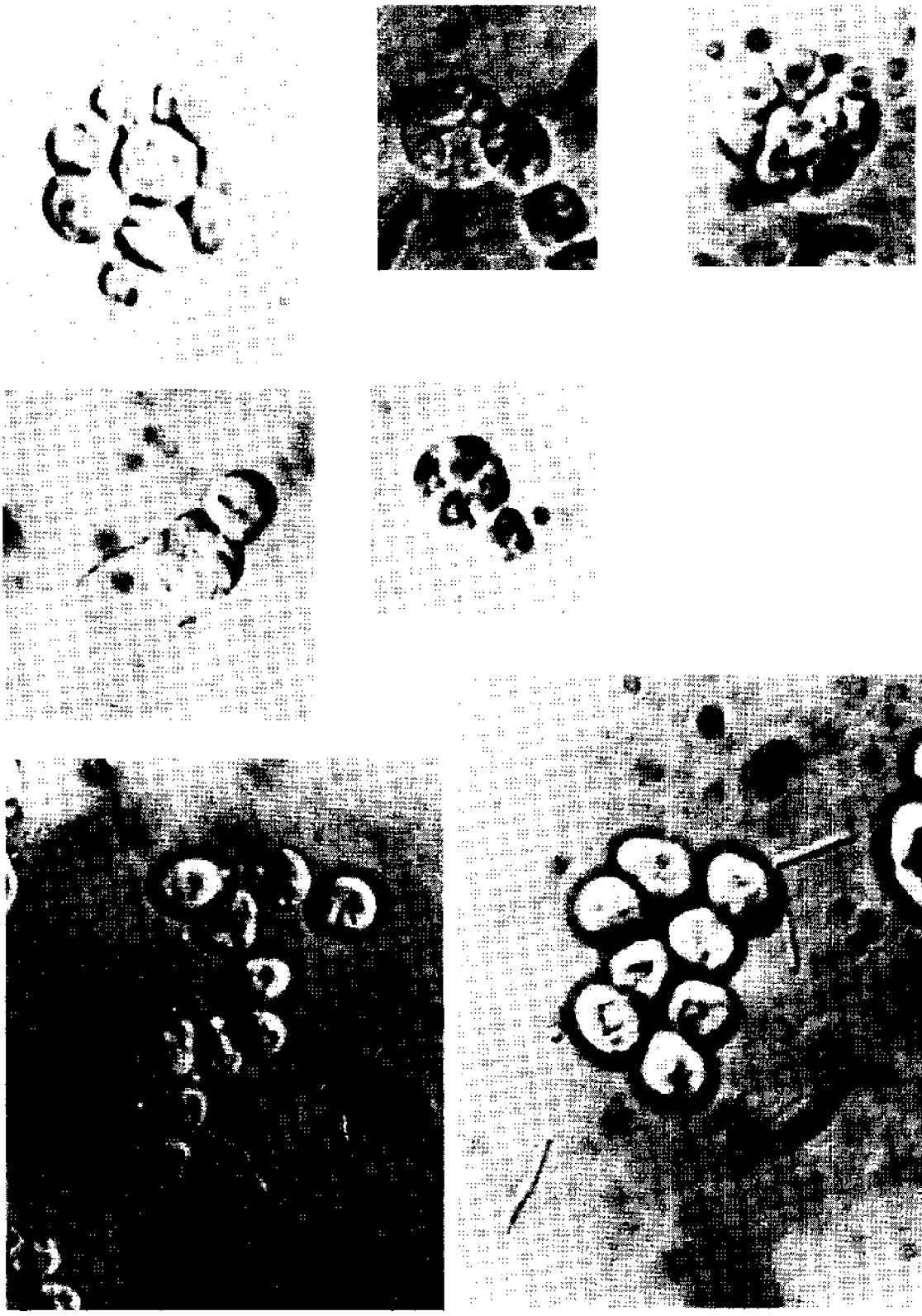


Fig. 1, a-d. Division of Monodus cells on ASP-2 agar plates with the formation of 4-8 autospores.

Fig. 2, a-c. Rod-shaped bacteria attached to the surface of Monodus.

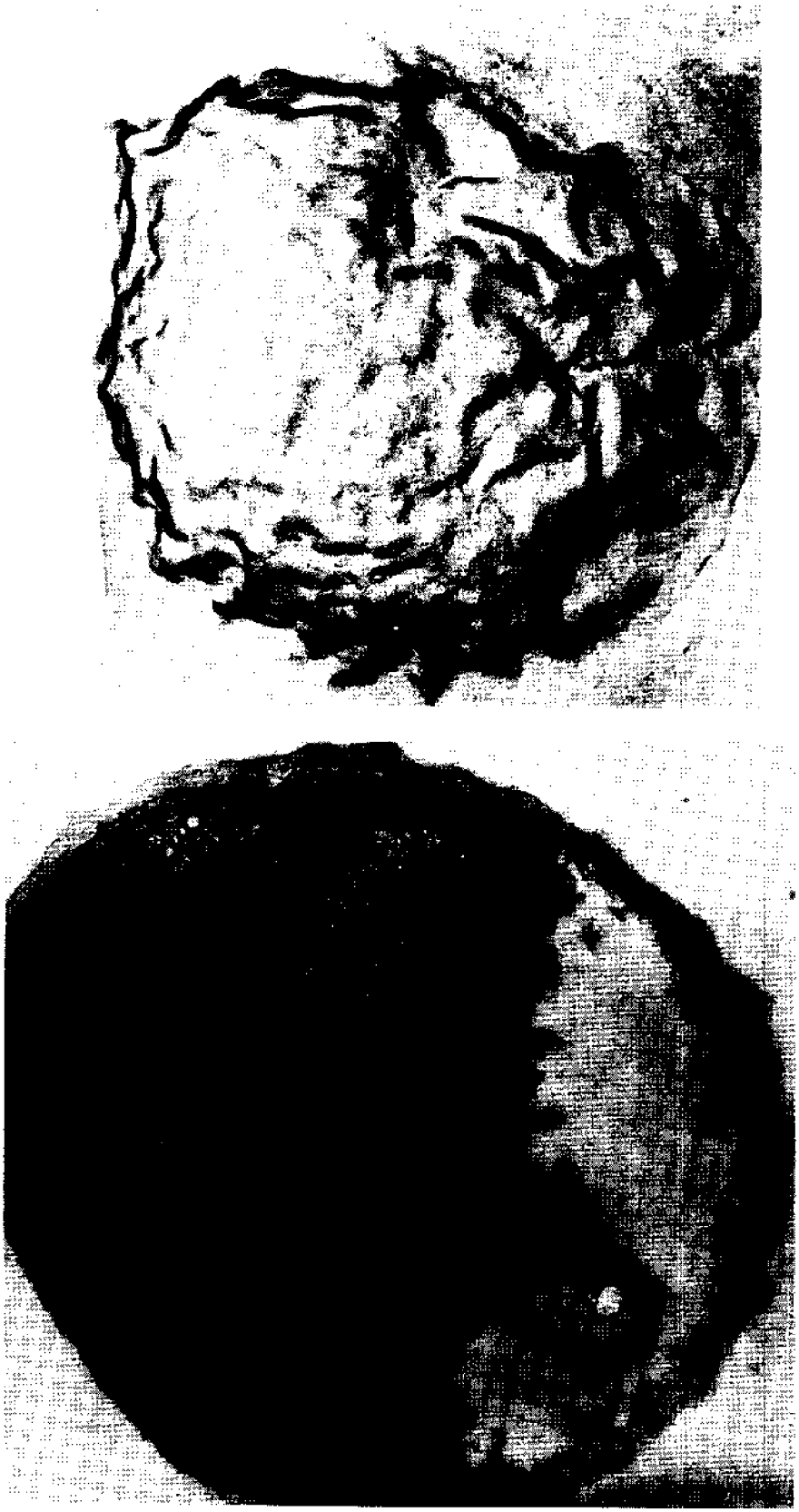


Fig. 3. Carbon replica of the wall surface of Monodus.

Fig. 4. Cross section of Monodus showing the wrinkled wall, the plastid with straight, parallel thylakoids and a membranous inclusion

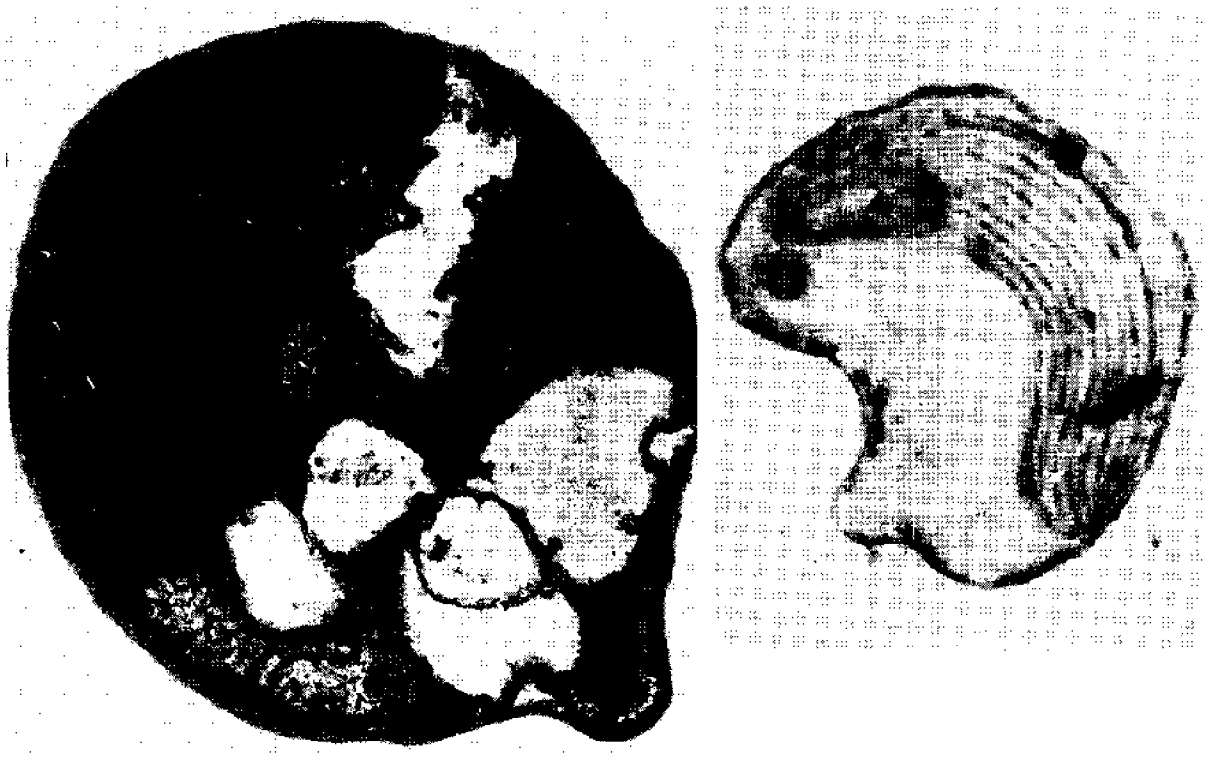


Fig. 5, a-b. Longitudinal section of a cell showing the beak.

Fig. 6. Oblique section showing the two mitochondria, lipid bodies, the chloroplast and thylakoids and the nucleus just beneath the chloroplast. The periplastidial endoplasmic reticulum does not appear to encompass the nucleus.

Fig. 7a. Cross section showing lamellated vesicles.

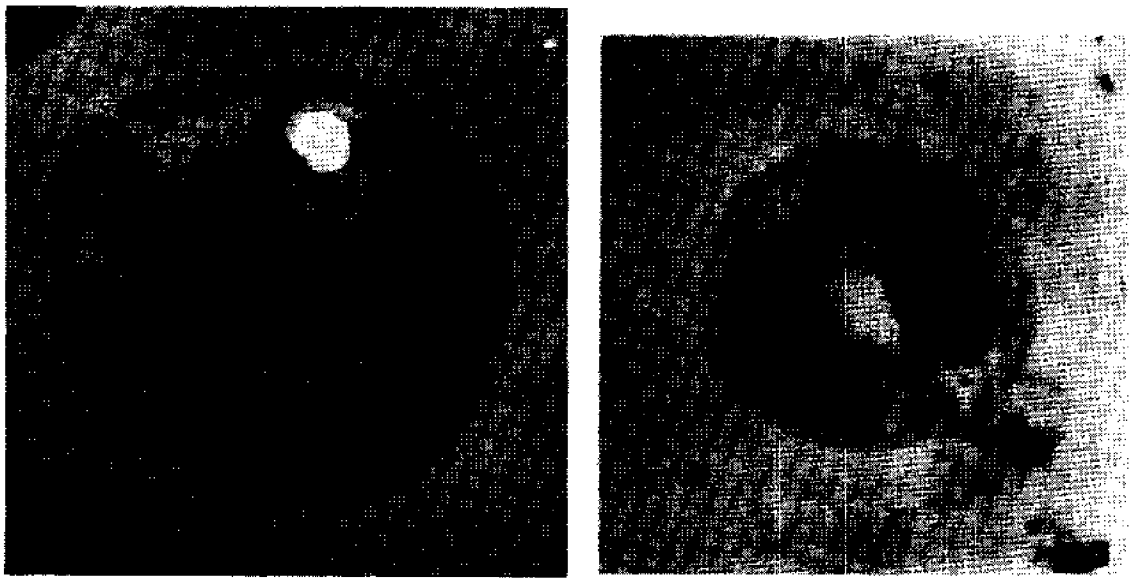
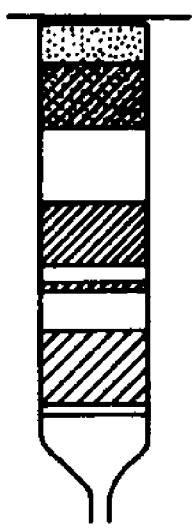


Fig. 7. Cross section showing plastid lamellae ending within the chloroplast without thylakoid cross-over; vacuole with lamellated vesicles.

Fig. 8. Cross section showing what may be a pyrenoid.



FILTER PAPER		RF
V	ORANGE YELLOW	0
IV	YELLOW	18
III	GREEN	52
II	ORANGE	70
I	YELLOW	98

Fig. 9. The chromatographic pattern of Monodus pigments.

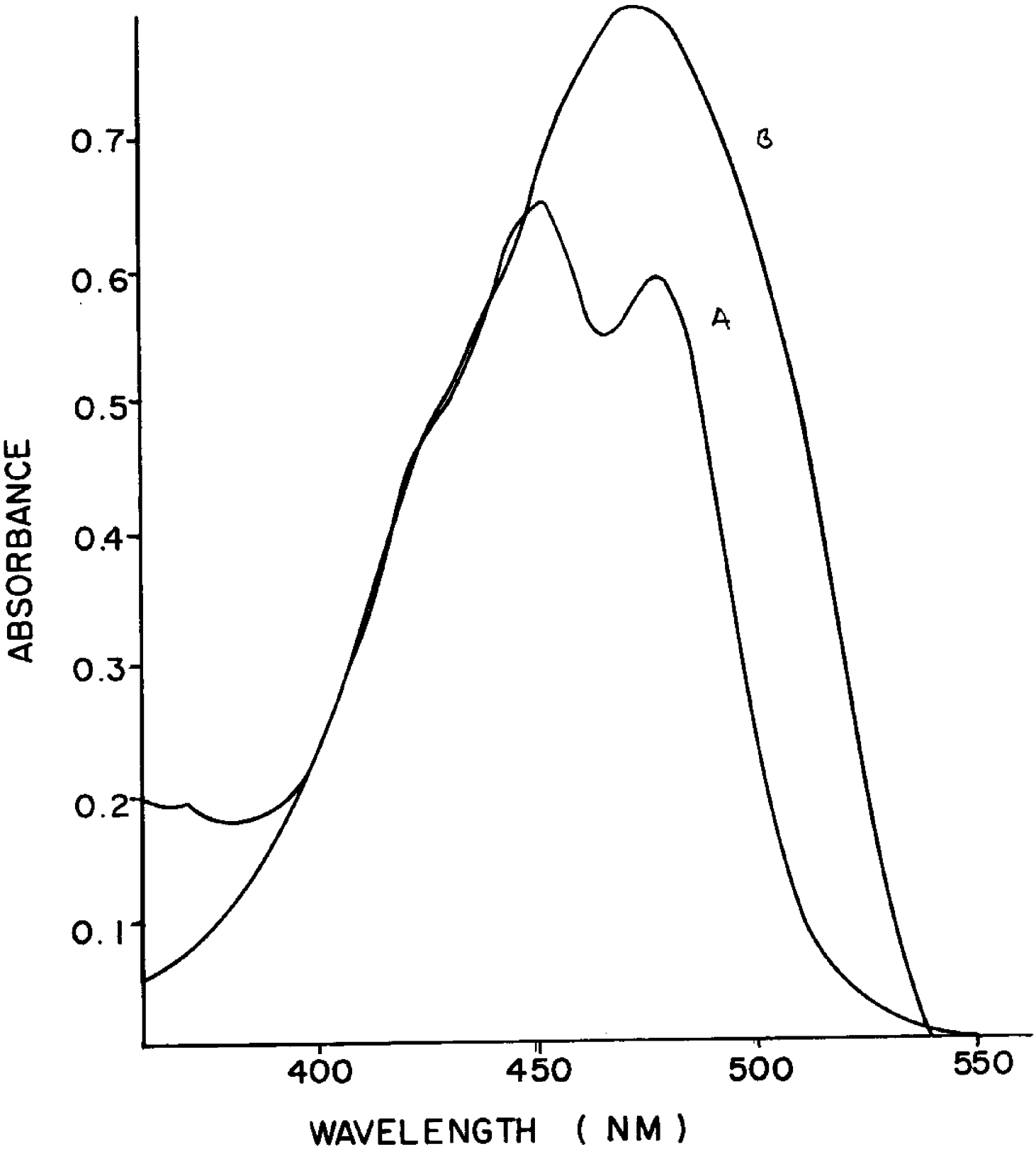


Fig. 10. Absorption spectrum of Fraction I (A = β-carotene) and Fraction II (B = canthaxanthin?) in ethanolic solution.

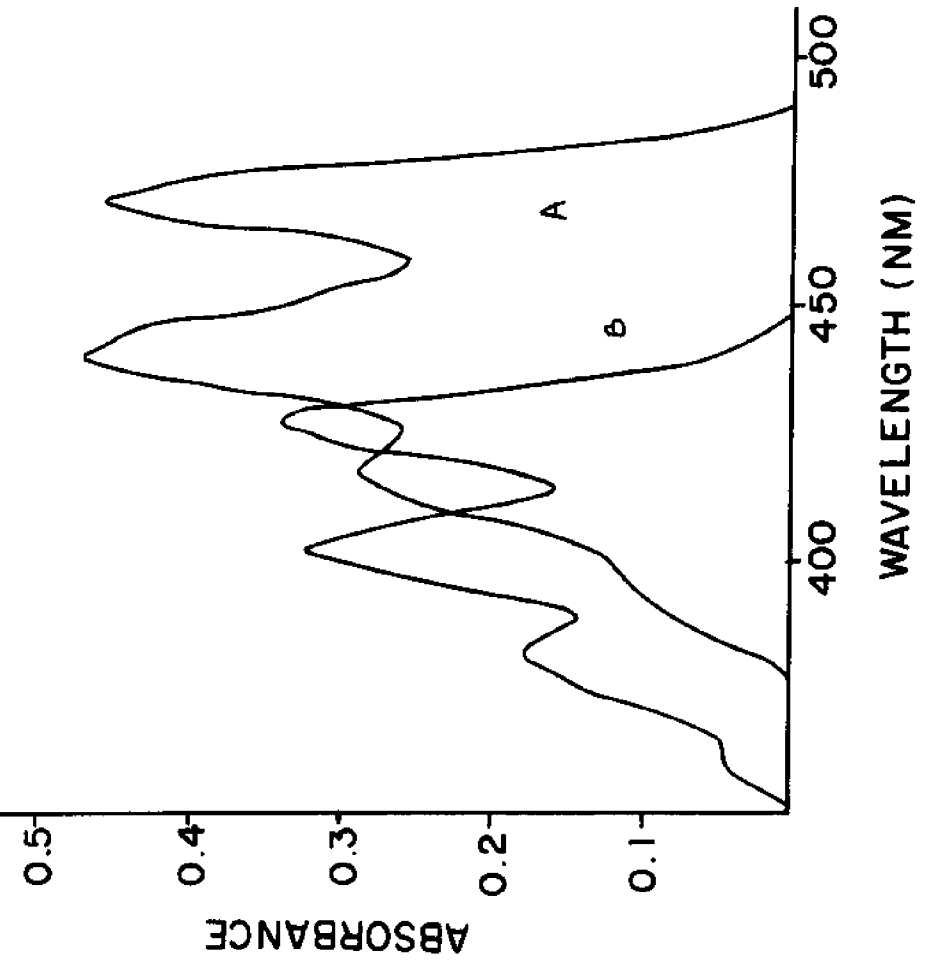


Fig. 11. Absorption spectrum of Fraction IV (=vaucheriaxanthin) in ethanolic solution, before (A) and after (B) treatment with HCl.

Fig. 12. Absorption spectrum of Fraction V (violaxanthin) in ethanolic solution before (A) and after (B) treatment with HCl.

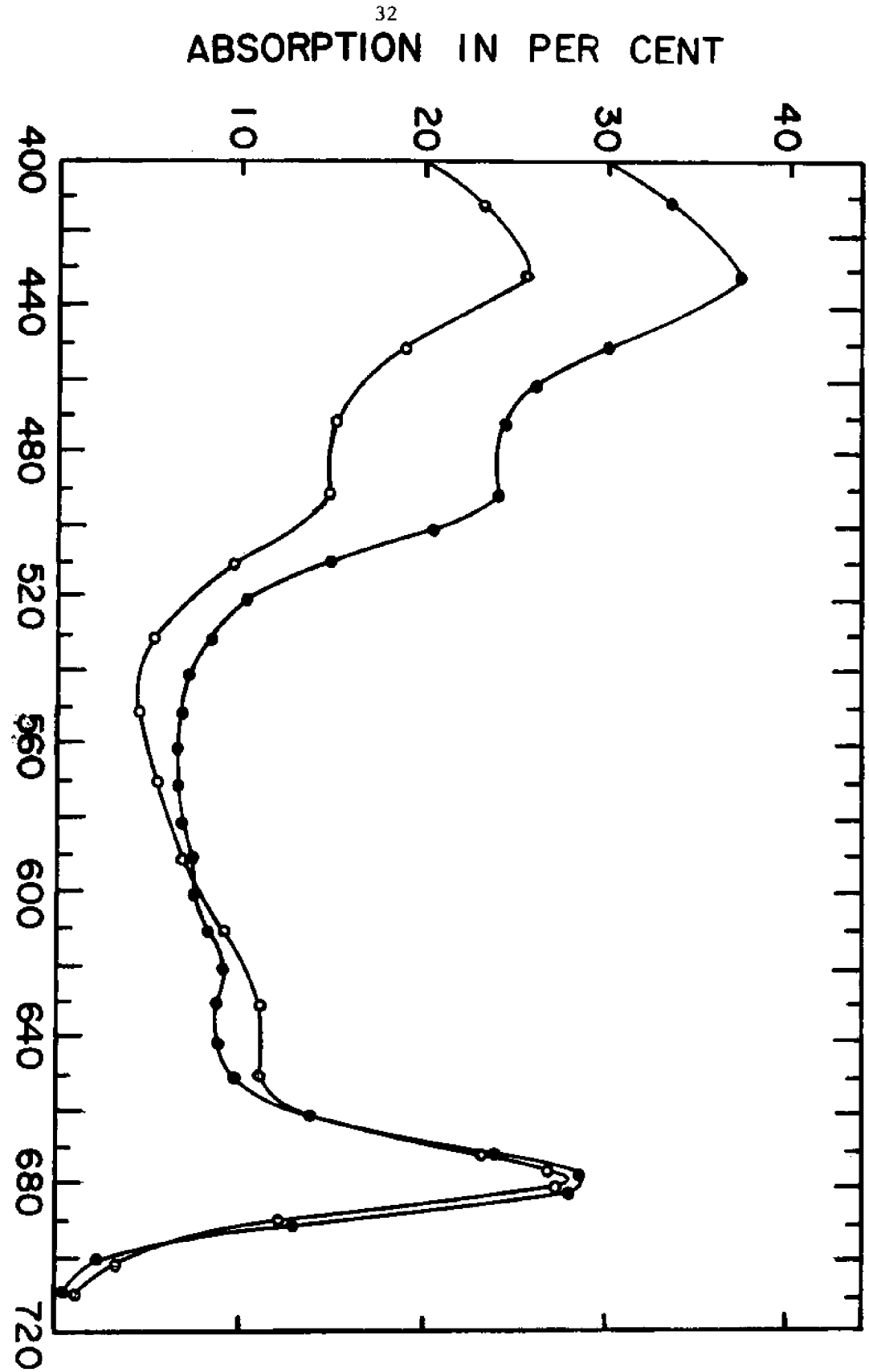


Fig. 13. Comparison of a photosynthetic action spectrum with an in-vivo absorption spectrum in Monodus.

A Study of Plankton Movement with Respect to Dissolved Oxygen in Ponds P-1 and C-1 in Summer, 1971

Daniel E. Leeper and William J. Woods

INTRODUCTION

The factors controlling vertical plankton movement in natural marine systems are well known. The primary factor involved is both the intensity and the duration of light. Due to the thorough mixing of natural systems, the dissolved oxygen content of the water remains relatively constant. Consequently, the oxygen concentration of the water has little, if any, effect upon vertical plankton movement.

However, in an artificial system such as the control ponds (C-ponds) and the polluted ponds (P-ponds) the potential for the dissolved oxygen concentration of the water to become an important factor in vertical plankton migration is greatly increased. The dissolved oxygen in the P-ponds drops down to near zero on a nightly basis and an early morning observer at the ponds will notice the presence of fishes and shrimp at the surface -- escaping the oxygen deprivation in the deeper waters. The effect of very low (and often zero) levels of dissolved oxygen must have some effect upon the plankton within the ponds and one would expect that plankton capable of movement would also escape the very low oxygen conditions of the bottom. The purpose of this study is to determine the effect of fluctuations in dissolved oxygen upon the vertical movement of both phytoplankton and zooplankton within these artificial systems.

PROCEDURE

Glass tubes 5 cm, 30 cm, 60 cm, and 80 cm in length were attached to stakes and then the stakes were driven into ponds C-1 and P-1 and left for the duration of the study. Water from the ponds was drawn through these glass tubes and used for oxygen determinations and pigment analysis at the various levels. Dissolved oxygen levels were determined by the Winkler method as outlined by Strickland and Parsons (2). Pigment analysis consisted of extraction and determination of the amount of chlorophyll a, b, and c and Astacin type carotenoids. Chlorophyll-a was plotted as an indication of the amount of phytoplankton present at the various levels. The Astacin type carotenoids are pigments indicative of the various levels of zooplankton (1). The Astacin type carotenoid level was plotted on the graphs as indicative of the zooplankton concentration at the respective levels.

Water samples were drawn at the four depths indicated above and were millipore filtered immediately. Fifty to one hundred milliliters were filtered depending upon the density of the plankton population. The millipore filters were then placed in a dark dessicator for one to four hours then ground using 90% acetone in a tissue grinder for one to two minutes. The grinder contents were then placed in a 15 cm centrifuge

tube, capped and shaken vigorously. The material was then allowed to extract in a refrigerator for one to eight hours. After extraction, the extract was again vigorously shaken and then centrifuged for thirty minutes. After centrifuging, the supernatant liquid was poured into a 1 cm curvette and absorbancies read on a Beckman DU Spectrophotometer at the following wavelengths: 750 mu (for turbidity of sample), 665 mu (for chlorophyll-a), 645 mu (chlorophyll-B), 630 mu (chlorophyll-c), 510 mu (Astacin type carotenoids), and 480 mu (non-Astacin type carotenoids).

The following formulas for chlorophyll concentrations as reported by Strickland and Parsons (2) were used:

$$Chl_a \text{ (ug/sample)} = 11.6(E_{665}) - 1.31(E_{645}) - .14(E_{630})$$
$$Chl_b \text{ (ug/sample)} = 20.7(E_{645}) - 4.34(E_{665}) - 4.42(E_{630})$$
$$Chl_c \text{ (ug/sample)} = 55(E_{630}) - 4.64(E_{665}) - 16.3(E_{645})$$

where E equals the absorbancy at the wavelength indicated. These values are also calculated for a 10 cm cuvette so the appropriate correction must be made.

Determinations of the Astacin type carotenoids were made from residual absorbancies at 510 mu and 480 mu as given by Francis Richards (1):

$$D_{res}510 = E_{510} - .0026(Chl_a) - .0035(Chl_b) - .0021(Chl_c)$$
$$D_{res}480 = E_{480} - .0019(Chl_a) - .0136(Chl_b) - .0054(Chl_c)$$

where D_{res} is residual absorbancy and $Chl_{a,b,c}$ are entered in mg/liter.

Astacin type carotenoids (mspu/1) = $2(4.45\ D_{res}510 - D_{res}480)$ where mspu equals milli-specified pigment units (1).

Two diel studies were conducted on both P-1 and C-1, the first diel having readings made every four hours and the second diel having readings made every six hours. In addition to the diel studies, a series of late evening — early morning samples were taken from both ponds (a total of six were done but two were discarded due to unacceptably high turbidity). The late evening — early morning samples had the advantage of showing movement in the absence of sunlight when the effect of low levels of oxygen would be at a maximum and without the discomforts of a dial study. The diel studies gave a more complete and accurate picture of what was going on over a twenty-four hour period.

RESULTS

Figures 1-4; 7-10 are comparisons of the sample taken in the evening and the sample taken before sunrise the following morning. The same scales have been used on the graphs so that comparisons can be made easily. The scales on the diels are also consistant through each diel facilitating comparison between graphs.

P-1 The most apparent relationship between oxygen, chlorophyll$_a$ and A-type carotenoids is the evening-to-morning decline in all three (except for occasional levels of Chlorophyll-a and A type) (Fig. 1-4). For each sample, as oxygen decreases toward the bottom, chlorophyll-a increases so that oxygen is always at a minimum at the bottom and chlorophyll-a is usually at a maximum at 80 cm. Minima for the chlorophyll-a concentration usually occurred at the 5 cm level. The A-type carotenoids (zooplankton) apparently are more sensitive to the lower levels of oxygen at the 80 cm level and would normally reach a maximum concentration at the 60 cm level. Minimum concentration for the A-type carotenoids occurred at the surface for the evening sample - probably reflecting their negative phototaxis - and either at the 5 cm or 80 cm level for the morning samples - reflecting the early morning light at the surface and the low oxygen concentration at the bottom. The two diel studies run upon the P-pond, (Fig. 5-6) reflect the effect of the sun on the various pigments and consequently upon the oxygen concentrations. Chlorophyll-a concentration increases from the 6 a.m. sample through the day. The 6 p.m. sample shows a decline in chlorophyll-a and this decline continues until the 6 a.m. sample the following morning.

The A-type carotenoids are the reverse of the chlorophyll-a in that daylight results in a decline in concentration, with increasing concentrations coming at the 6 p.m. samples and continuing until the 6 a.m. sample the next morning shows a decline.

C-1 The C-pond, while having lower levels of pigments, follows the same general trend as the P-pond. There is an inverse relationship between oxygen and chlorophyll-a with chlorophyll-a reaching its maximum concentration at the 80 cm level and oxygen being always at a minimum at this level. The C-pond evening and morning samples (Fig. 7-10) consistantly had increases in chlorophyll-a concentration at almost every level whereas the P-pond samples show a consistant decline for the same evening to morning period (Fig.1-4).

The A-type carotenoids show the same kind of evening to morning decline in concentration as in the P-pond, however, they normally reached a maximum at the 80 cm level as opposed to the P-pond maximum at 60 cm. Also this maximum at 80 cm was true of both morning and evening samples - perhaps reflecting less of an effect of low oxygen concentrations.

Minimum concentrations of A-type carotenoids were always at the 5 cm level in the C-pond regardless of the time of the sample. This also differs from the P-ponds which had minima at either the surface (5 cm) or bottom (80 cm). The reason for this pond to pond difference is probably the fact that 1) the zooplankton population as reflected in the concentration of A-type carotenoids is less in the P-pond and 2) there is more dissolved oxygen at the 80 cm level in the C-pond at the early morning minimum than at the same time and level in the P-pond. These two factors result in oxygen concentrations being of little significance in vertical migration in the C-pond.

The diels of the C-pond, Fig. 11-12, both show gains in the concentration of chlorophyll-a, A-type carotenoids, and oxygen until sometime in the afternoon (apparently around 2:00 p.m.). Chlorophyll-a concentration then continues to drop until 6 a.m. the next morning in diel I and in the case of diel II increases slightly at midnight then drops again at 6 a.m. (The rise at midnight was coupled with a complete lack of oxygen stratification due to wind).

The A-type carotenoid movement is essentially the same as that for chlorophyll-a in diel I and in diel II the concentration of A-type carotenoid increases steadily from 6 p.m. to 6 a.m. the following morning.

CONCLUSIONS

Oxygen plays a very minor role in the causation of vertical movement of plankton. This is especially true in the C-pond where the oxygen minimum rarely approaches zero even at the 80 cm level. Also the plankton population is smaller in the C-pond and thus oxygen consumption is less.

In the P-pond, oxygen concentration has more of an effect on plankton movement as it goes from supersaturation conditions during the day to almost zero during the early morning hours. There are apparently some species of zooplankton in the P-ponds that are capable of withstanding extremely low levels of dissolved oxygen for even when the oxygen level dropped to zero there were still zooplankton present in the samples. However, to the more oxygen sensitive plankton the low oxygen levels were a factor in their movement. The exceedingly low oxygen levels in the P-pond had less effect upon phytoplankton than on zooplankton. This may be a result of unflagellated and unciliated phytoplankton species being "trapped" at the lower levels and unable to move to a region of higher oxygen concentration.

The depth of the ponds plays an important factor in movement in that a steady wind mixes the ponds and destroys any oxygen stratification and therefore any plankton stratification that may have resulted from it.

In summation, oxygen plays a very minor role in the causation of plankton movement especially in the C-pond. However, in the P-pond where there is a much more drastic fluctuation in oxygen levels within a 24-hour period there is apparently some migration out of the oxygen deficient water into regions of higher oxygen concentration.

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(1) Richards, Francis A., and Thompson, Thomas G. 1952. The Estimation and Characterization of Plankton Populations by Pigment Analysis (II. A Spectrophotometric Method for the Estimation of Plankton Pigments). Journal of Marine Research. 12: 156-172.

(2) Strickland, J.D.H., and T. R. Parsons. 1968. A Practical Handbook of Seawater Analysis. Fisheries Research Board of Canada. Bull. No. 167.

FIGURE LEGENDS

Fig. 1-4. Oxygen, chlorophyll-a, and astacin-type carotenoid concentrations in P-1 at dawn and dusk.

Fig. 5. Diel changes in oxygen, chlorophyll-a, and astacin-type carotenoid concentrations in P-1.

Fig. 6. Diel changes in oxygen, chlorophyll-a, and astacin-type carotenoid concentrations in P-1.

Fig. 7-10. Oxygen, chlorophyll-a, and astacin-type carotenoid concentrations in C-1 at dawn and dusk.

Fig. 11. Diel changes in oxygen, chlorophyll-a, and astacin-type carotenoid concentrations in C-1.

Fig. 12. Diel changes in oxygen, chlorophyll-a, and astacin-type carotenoid concentrations in C-1.

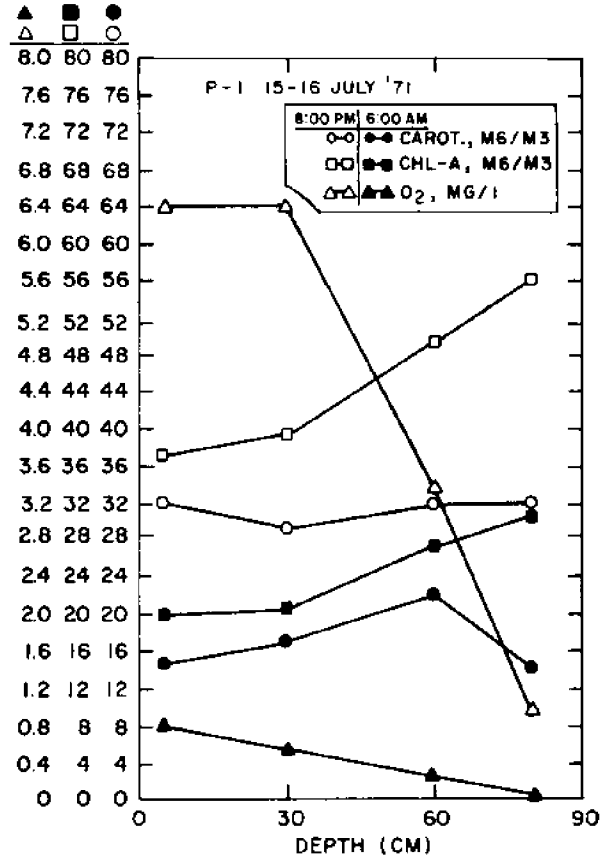

Fig. 1. Oxygen, chlorophyll-a, and astacin-type caroteniod concentrations in P-1 at dawn and at dusk, July 15-16.

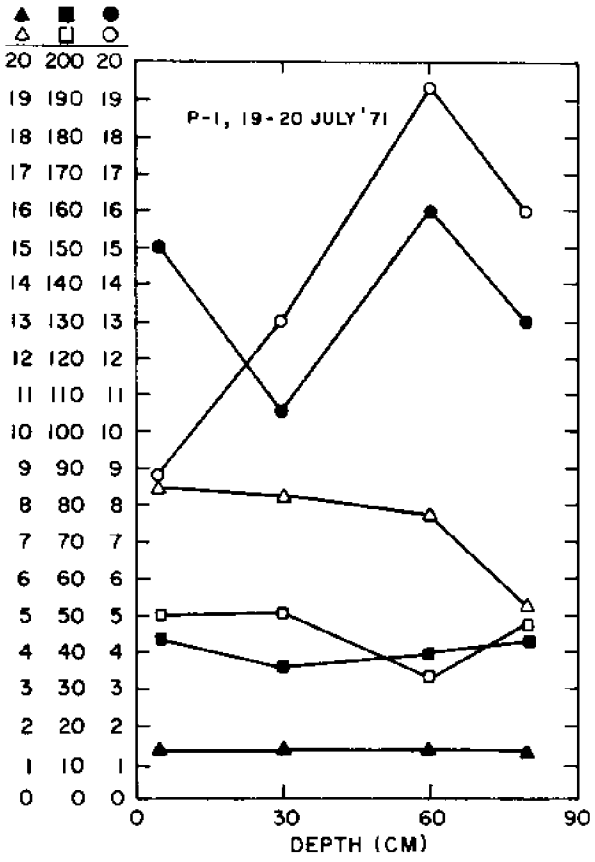

Fig. 2. Oxygen, chlorophyll-a, and astacin-type caroteniod concentrations in P-1 at dawn and at dusk, July 19-20.

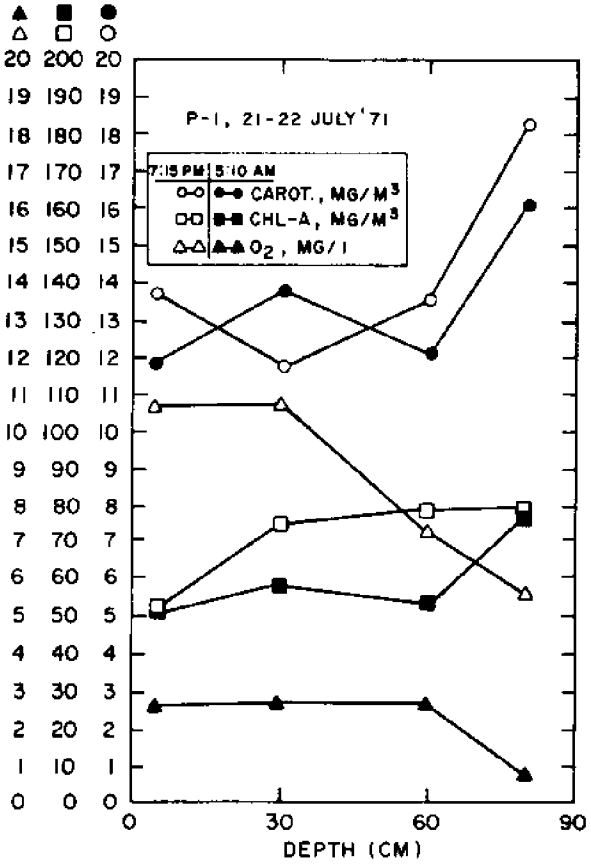

Fig. 3. Oxygen, chlorophyll-a, and astacin-type caroteniod concentrations in P-1 at dawn and at dusk, July 21-22.

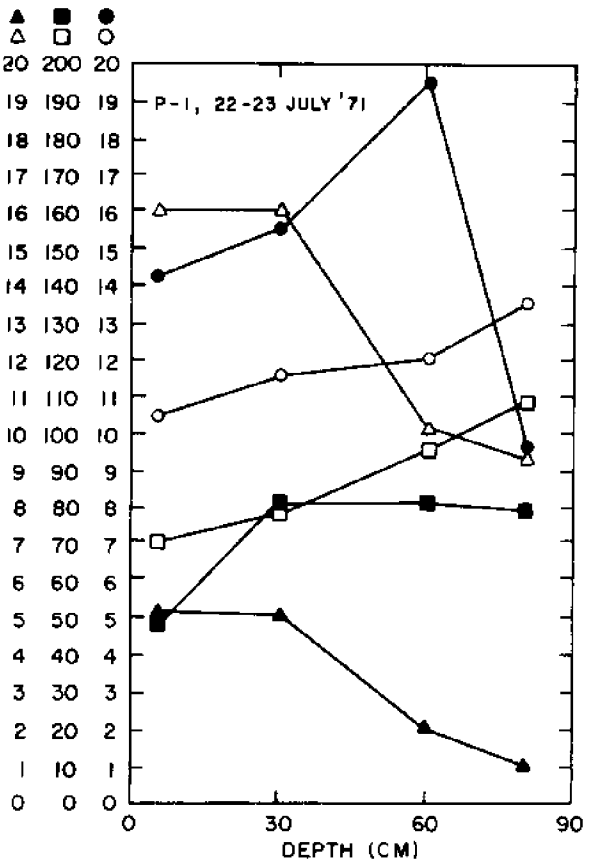

Fig. 4. Oxygen, chlorophyll-a, and astacin-type caroteniod concentrations in P-1 at dawn and at dusk, July 22-23.

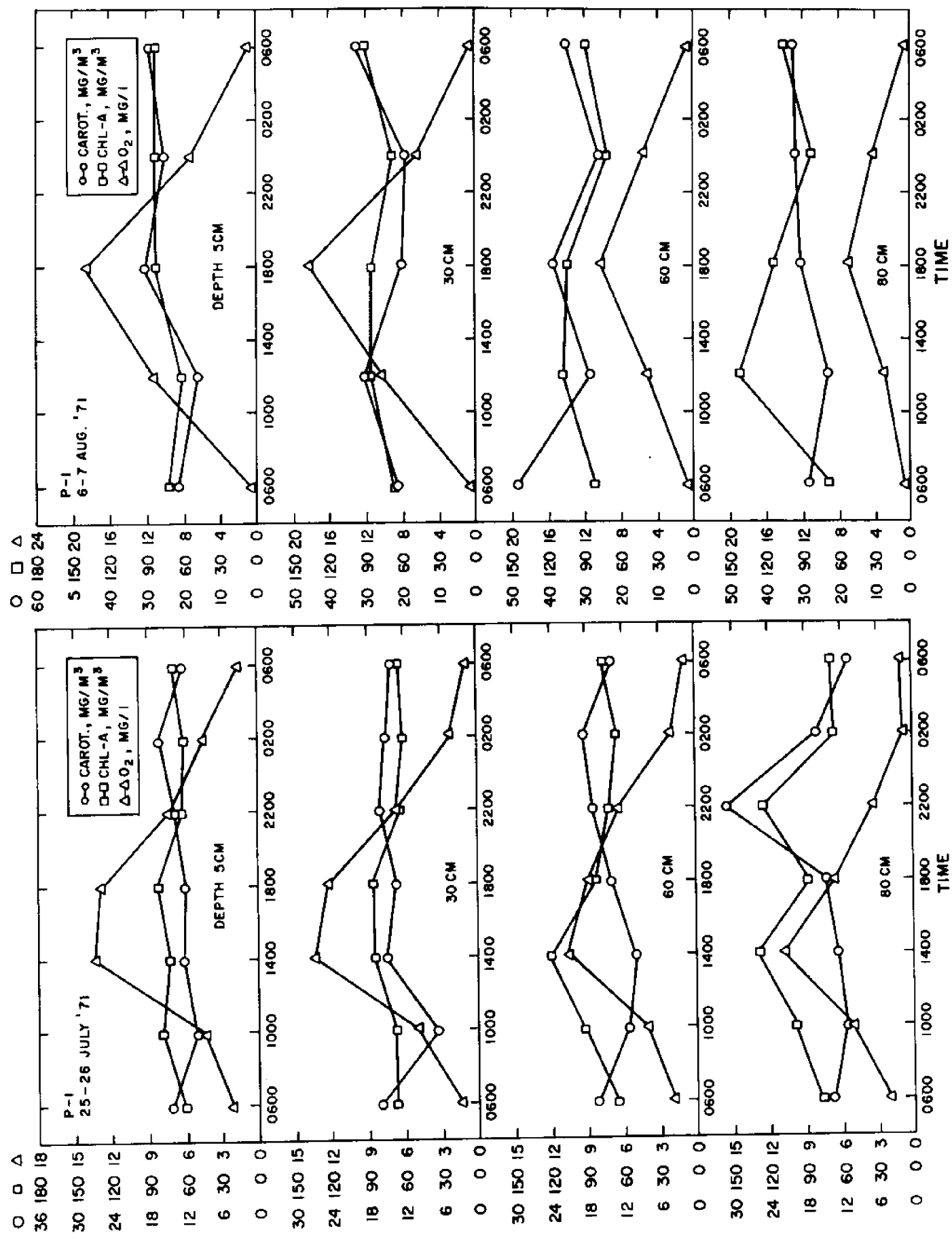

Fig. 5. Diel changes in oxygen, chlorophyll-a, and astacin-type carotenoid concentrations in P-1.

Fig. 6. Diel changes in oxygen, chlorophyll-a, and astacin-type carotenoid concentrations in P-1.

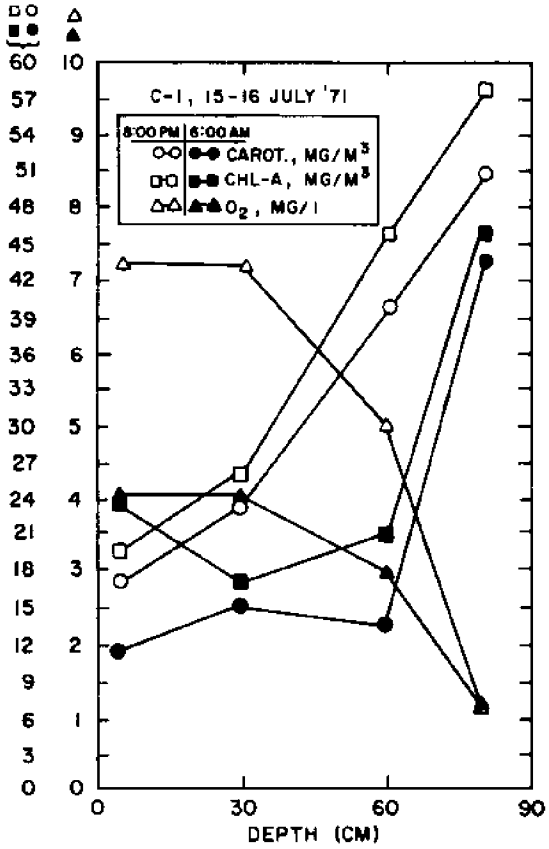

Fig. 7. Oxygen, chlorophyll-a, and astacin-type carotenoid concentrations in C-1 at dawn and at dusk, July 15-16.

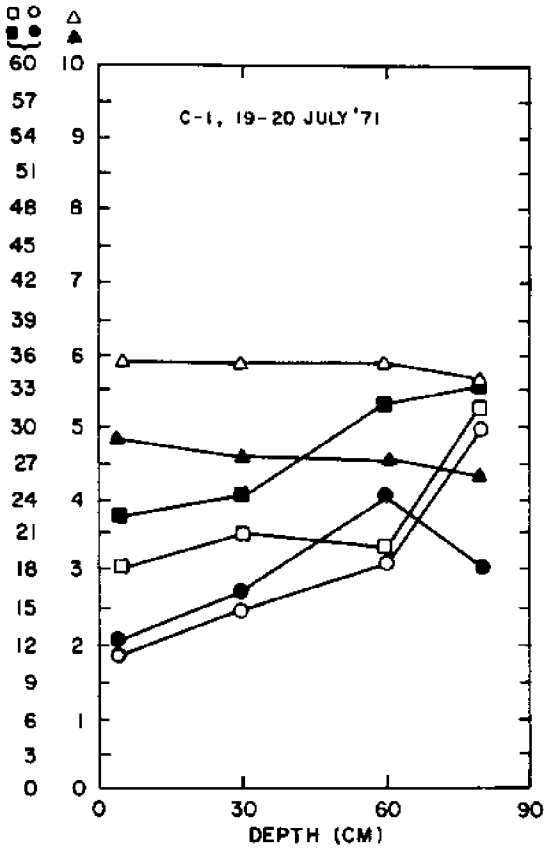

Fig. 8. Oxygen, chlorophyll-a, and astacin-type carotenoid concentrations in C-1 at dawn and at dusk, July 19-20.

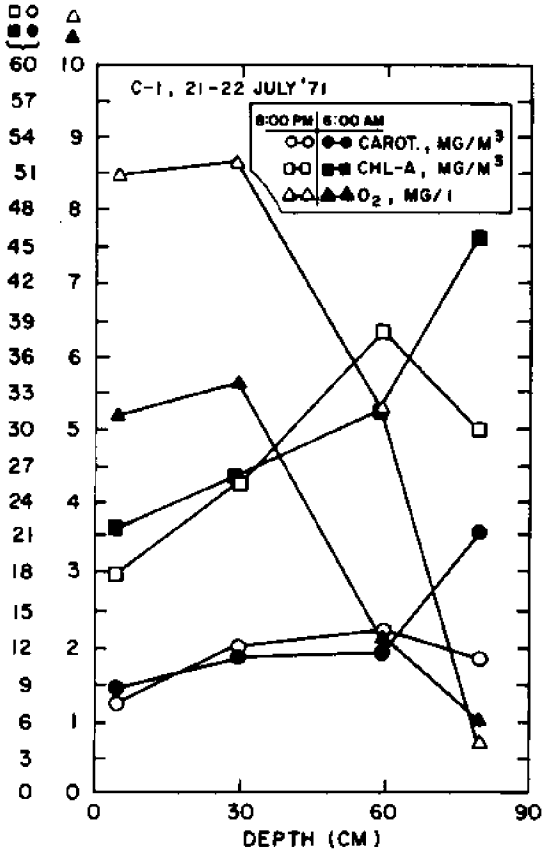

Fig. 9. Oxygen, chlorophyll-a, and astacin-type carotenoid concentrations in C-1 at dawn and at dusk, July 21-22.

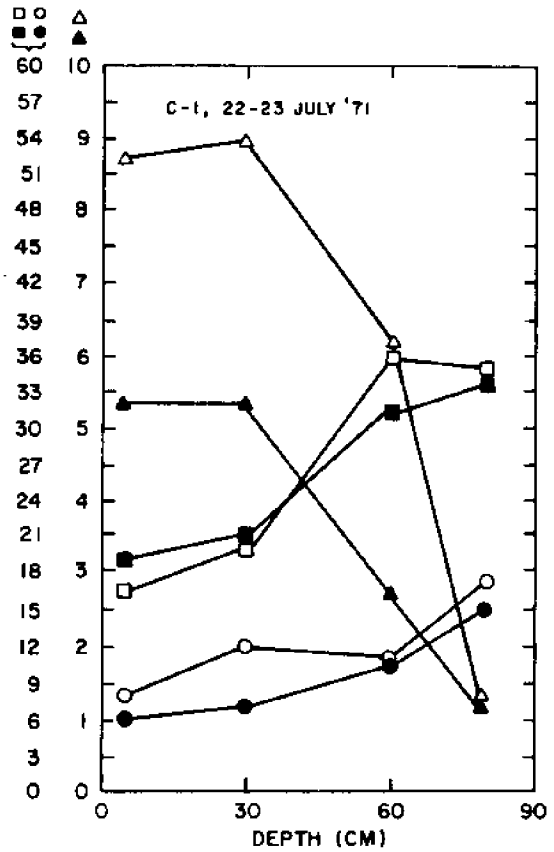

Fig. 10. Oxygen, chlorophyll-a, and astacin-type carotenoid concentrations in C-1 at dawn and at dusk, July 22-23.

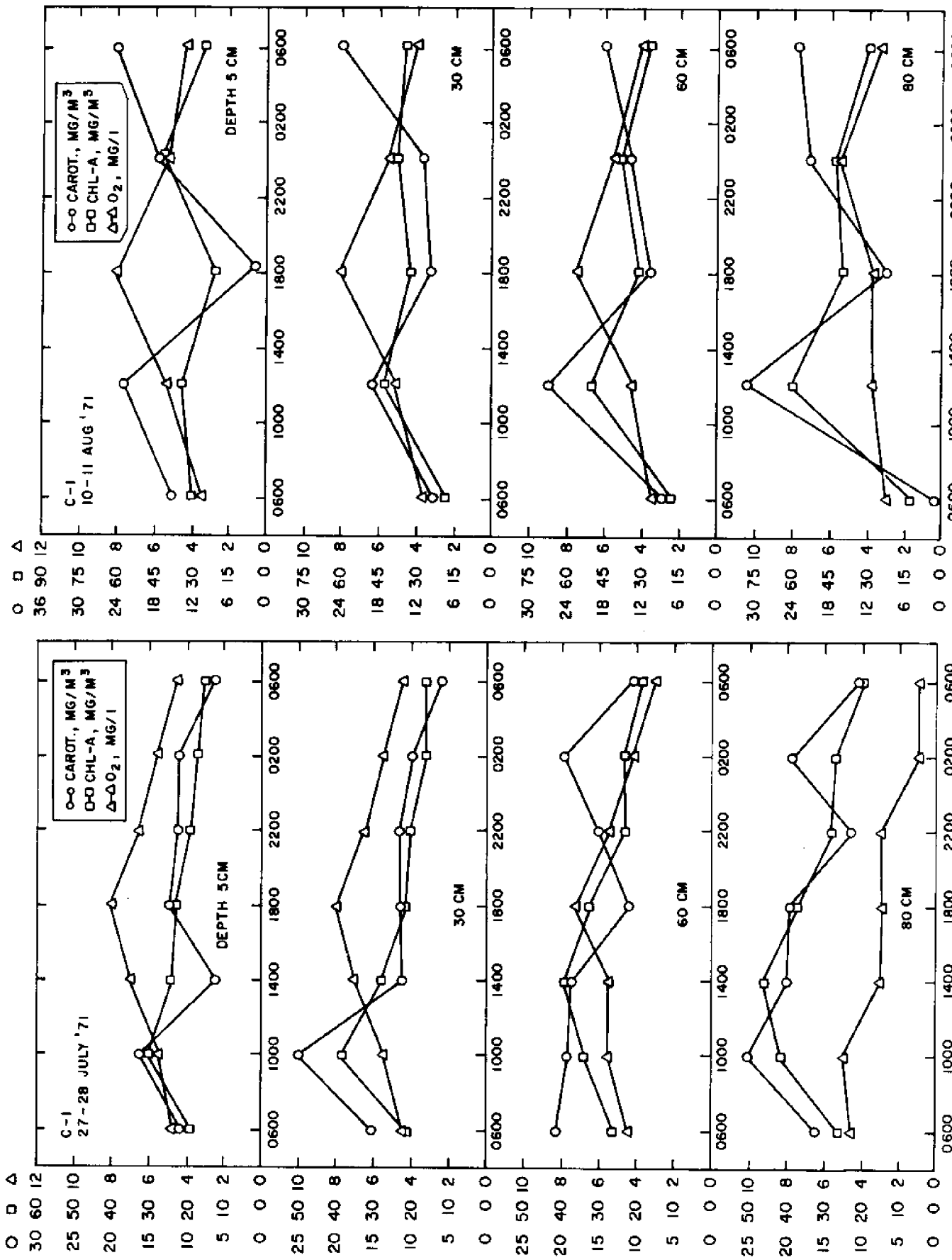

Fig. 11. Diel changes in oxygen, chlorophyll-a, and astacin-type carotenoid concentrations in C-1.

Fig. 12. Diel changes in oxygen, chlorophyll-a, and astacin-type carotenoid concentrations in C-1.

BACTERIA IDENTIFICATION

Teri Lynn Herbert

A short-term project related to the bacteria-shellfish study was done in the months of February, March and April. Douglas Halliday, a West Carteret High School senior assisted in the analysis of the water of P-pond #1 in an attempt to differentiate and identify the various genera of bacteria inhabiting the water.

Identification of the bacteria was based on physical and chemical characterization: colony size and appearance, ability to grow on specially-prepared differential media, and utilization of selected ions and chemical compounds. For a description and usage of each test applied to the bacteria, refer to the Difco Manual (Difco Laboratories, Inc. 1953), or any general bacteriology lab manual. The identifications were made using Bergey's Manual of Determinative Bacteriology (6th ed. 1948).

A tabular summary of the description and test results for each isolated bacterium follows this report.

Conclusions

Three species of Escherichia were isolated: E. coli, E. freundii, and an unspecified strain of E. coli, all typically found in polluted systems. Streptococcus liquefaciens and Proteus mirabilis were positively identified, and representatives of the Salmonella and Alcaligenes genera were described. Further identifications were not possible, due to inconclusive test results and/or inaccessible media materials and reagents.

References

1. American Society for Microbiology, 1948. Bergey's Manual of Determinative Bacteriology. Baltimore. Williams and Wilkins Co.

2. Difco Manual of Dehydrated Culture Media and Reagents for Microbiological and Clinical Laboratory Procedures, 1953. 9th ed. Detroit. Difco Laboratories, Inc.

Table 1. Bacteria characteristics and test results.

TEST	Unknown Bacteria #: 1	2	3	4	5	6	7	8	9
S S Agar	±	clear	-	-	-	-	pink	red	-
Citrate, Simmons	-	+	-	-	-	+	-	+	-
Starch	-	-	-	-	+	-	-	-	+
Methyl Red	+	-	+	-	+	-	+	-	+
Vogues Proskauer	-	-	-	+	+	+	+	-	-
Gelatin	+	-	-	-	-	-	+	-	-
Urea	-	-	-	-	-	-	-	-	-
Endo Agar	+	+	-	-	-	-	-	+	-
EMB Agar	-	-	-	-	-	-	-	+	+
Brilliant Green Bile Agar	-	-	+	-	+	-	-	+	-
3-Sugar 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.
Kliglers Iron Agar									
Gram Stain	-	-	-	-	-	-	+	-	-
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 chains

KEY: (-) 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 liquefacions
#8. Alcaligenes sp.
#9. Escherichia coli strain ?

COLIFORM INCIDENCE IN THE WATER AND SHELLFISH OF A MARINE OXIDATION POND

Teri Lynn Herbert
Marine Sciences Curriculum

Introduction

Shellfish cultivation for human consumption requires a survey of the levels of pathogenic organisms in the shellfish-growing area, and more importantly, in the shellfish tissues. These analyses are essential for waters suspected of fecal pollution, such as near a treated-sewage waste disposal, or near domestic livestock pasturage. Liu, et al (1967) showed that bacteria and viruses were found in shellfish (oysters and quahogs) several miles downstream from a sewage treatment plant. In the ponds located on Calico Creek at the Morehead City sewage treatment plant outfall, determination of enteric bacterial levels is needed before consideration of the ponds for mariculture purposes.

Many pathogenic bacteria are very sensitive to changes in their environment, and methods for detection of their presence are tedious and complicated, often with indefinite results (Pelczar and Reid, 1965). Consequently, coliform bacteria have been designated as indicator organisms: indicating that conditions are suitable for survival of pathogens, or simply that there is a local source of fecal contamination (Beard and Meadowcroft, 1935; Kabler and Clark, 1960).

This report presents the results of a study of the fecal coliform (enteric pathogen indicators) in the creek water mixing tank, in the mid-pond water, and in resident oysters of the Sea Grant ponds over the period September 1971 through March, 1972.

Methods

A method for detection of coliforms has been standardized by the U. S. Public Health Service for both water and shellfish analyses (Recommended Procedures for the Examination of Sea Water and Shellfish, 4th ed. 1970, AHPA, Inc.). For this study, Millipore Filtration was chosen for the mixed creekwater and mid-pond water samples, using the elevated temperature (44.5°C), mFC Broth technique for fecal coliform detection. For the oyster analyses, the presumptive and confirmed most probable number fermentation tube procedure was used. To prevent changes in bacterial numbers due to long storage or refrigeration, all samples of water and shellfish were cultured within two hours of collection. (Symons and Simpson showed in 1941 that even briefly stored samples gave erroneous bacterial counts.) Media was obtained in dry, premeasured form from Difco Laboratories and sterilized before use in the tests. All analyses were run in aseptic conditions, and glassware was also heat sterilized. Sterilized uninnoculated tubes of broth served as media controls during incubation periods.

Weekly fecal coliform analyses on water and shellfish were run from September 1971 through March 1972, for six months of counts on waste pond #1 and control pond #1. Duplicates of one hundred milliliters were filtered for each water analysis and at least twelve oysters (> 100 g) were collected per shellfish sample. All oysters originally came from lower Calico Creek and were washed before placing in the ponds, then allowed to remain there for at least two weeks before sampling. After washing, a preliminary test was done of these shellfish to establish a baseline of bacterial numbers for later comparison.

A record of weather conditions and treatment plant operations were kept throughout the six month study, including water temperature, rain and storm conditions and sewage plant shutdown (bypassing of sewage). The clearance rate or depuration time (how long it takes for the oyster to "clean" itself of bacteria when placed in non-contaminated water) was also measured to determine if this means of "cleaning" the shellfish was feasible for commercial purposes.

Results and Discussion

The bacterial levels in the creek water mixing tank samples ran consistantly higher than those of the mid pond. (See Table 1: each point graphed is an average of two duplicate samples.) Mixing tank counts ranged from 33.5 to 912 fecal colonies per 100 ml, while mid pond counts had a range of 1 to 173 fecal colonies per 100 ml. The percent reduction of bacteria from mixing tank to pond averaged 78.8% with a range from 21% to 98%.

Fecal coliform counts in the oysters showed two peaks during the period, one in September with 150 colonies per 100g sample and the other in November with 187.8 colonies per 100 g of shellfish. The September sample was taken the day Hurricane Ginger passed through the area; high winds and rain possibly mixed the bottom water and mud up, circulating more bacteria for the oysters to pick up. Salinity was down to 10‰ as a result of Ginger. In November, the creek pump bringing saline water to the ponds failed and was out of commission three days prior to sampling; salinity was again down to 10‰ and the stagnated water's temperature rose to 24°C, a few degrees higher than on previous days. All of these factors could allow for bacterial increase. The P-pond and mixing tank water also reflected high coliform counts, the stagnant tank water peaking over 900 colonies per 100 ml. sample.

All other counts remained below 43 colonies per 100 g and even decreased to zero colonies per sample for two consecutive weeks in January, 1972. These low counts occurred in cold weather: ice was formed on the pond over the oysters on January 18, and the oysters remaining alive probably did not open to feed again until the water warmed considerably a few weeks later.

During the six-month sampling period, no treated sewage was pumped directly into the P-ponds mixing tank. Any fecal pollution observed

in the ponds would have originated in the creek water which was used to fill the ponds (sewage plant treated effluent is dumped into the creek about ten yards from the experimental ponds.) Thus, bacterial populations were already diluted by the creek water before entering the mixing tank for further dilution. Resulting coliform counts reflected this to some degree: USPHS Pub. #33 (1964) sets a limit of 230 fecal colonies per 100 g shellfish sample allowed for safe consumption, and all of the samples in the six-month period were below this standard. A standard of 30 Millipore Filter fecal colonies per 100 ml of water (USPHS Pub. #33, 1964) is considered the limit safe for shellfish growing areas, and the coliform counts in the ponds and mixing tank exceeded this value in 59% of the time (pond) and 94% of the time (mixing tank).

From September to March, water temperatures fell as the seasons changed, with one sharp reduction to 2°C in January during freezing weather. On January 18, samples of water and shellfish were taken through a layer of ice on the waste pond. As noted above, bacteria counts dropped to zero for two weeks after the freeze; possibly due to the resulting inactivity of the shellfish as temperatures fell (Galtsoff, 1964).

Throughout the study, there appeared to be a correlation between lower water temperature, higher salinity, and less bacteria, with the temperature factor dominating (Figures 1 & 2). Galtsoff (1964) writes that oysters will survive in waters of salinities 5 to 32‰, and since the salinity range in the sea grant ponds always remained in the 9 to 21‰ range, the temperature differences would have the greater effect on the shellfish by regulating feeding schedules (uptake of bacteria). Thus, the lower temperatures slowed or stopped the oysters' feeding rate and bacterial counts were consequently lowered.

Depuration tests in February and April resulted in an estimated 12.5 days for the oyster to completely clear fecal bacteria out of its system. A large number of shellfish, all collected from one site at one time, were placed in P-pond #1 for 2.5 weeks prior to sampling, to allow time for bacteria uptake by the shellfish. The first test samples were taken from these P-pond oysters, then all shellfish were transferred to the control pond: an uncontaminated holding pond suitable for this test; and thereafter daily analyses were done until fecal counts stabilized at zero. To "clean" the shellfish required ten days in February and 15 days in April, averaging at 12.5 days, as previously stated. The depuration time is dependant on the feeding rate and schedule of the shellfish itself. These times could therefore increase during periods when the shellfish are not feeding, or are on a brief feeding schedule. Feeding schedules are influenced by temperature, salinities, and overall water conditions.

To determine the feasibility of growing shellfish in these sewage-fed ponds, a further study should be initiated to establish methods of reducing bacterial numbers in the pond water, consequently reducing shellfish bacteria to a safe level for public use. During this study when no treated sewage had been dumped directly into the ponds, oysters

were safely edible, but with resumption of sewage feeding, the shellfish will require a separate non-contaminated pond or tank for depuration before allowing their consumption.

Summary

1. Fecal coliform bacteria levels in the water and resident shellfish of the Sea Grant waste pond #1 were studied from September 1971 through March, 1972. The water was analyzed using Millipore Filtration, and the oysters examined using the Most Probable Number fermentation tube method. Resulting data were correlated with water temperature, weather conditions (wind, rain, storm conditions) and treatment plant operations.

2. Creek water in the P-pond mixing tank supported a greater population of fecal coliform bacteria than the mid-pond near-surface water.

3. During the six-month study, fecal counts in the resident shellfish (oysters) of P-pond #1 were below the USPHS safe limit of contamination. After treated-sewage feeding was again resumed (March 26, 1972) the shellfish bacterial count increased over the acceptible pollution level.

4. Low water temperature and higher salinities correlated with lower bacteria numbers, with water temperature the dominant factor. Local storm conditions appeared to have an effect of mixing the ponds, circulating bottom water and bacteria up to the surface.

5. Depuration time averaged 12.5 days.

6. Future use of the treated sewage fed ponds for the purpose of growing safely edible shellfish will require a depuration system to reduce bacterial contamination.

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Table 1. Fecal coliform counts in oysters.

Date	per 100 ml of Water				per 100g Oysters	
	C In	C	P In	P	C	P
9-3-71	9	0	241	6	1.2	17.9
9-10-71	1	5.5	430	104		
9-17-71	0	5	324	129.5	0	3.4
9-22-71	0	6	440	19.5	0	3.1
9-29-71	5	11	325	173	0	1[illegible]0
10-6-71	4	1.5	549.5	30.5	1.9	42.9
10-13-71	4	1.5	729	76.5	1.9	21.9
10-20-71	0	0	136	102	1.6	11.5
10-27-71	0	0	833	18.5	0	10.8
11-3-71	0	0	912	122	5.4	187.8
11-10-71	0	0		33	2.1	19.4
11-17-71	0	0	134	15	0	11.7
12-3-71	0	1	59.5	47	9.6	11.5
12-15-71	0	0	115.5	2.5	4.5	5.6
1-3-72	2	0	121	58	0	8.6
1-18-72	0	0	93.5	1	1.8	0
1-24-72	0	0	33.5	2	0	0
2-14-72	9	1	430	35.5	0	0.9
2-23-72	6	0	201	33	0	23
3-8-72	3	0	289	49	1.5	32
3-26-72	0	0	98	21	0	248

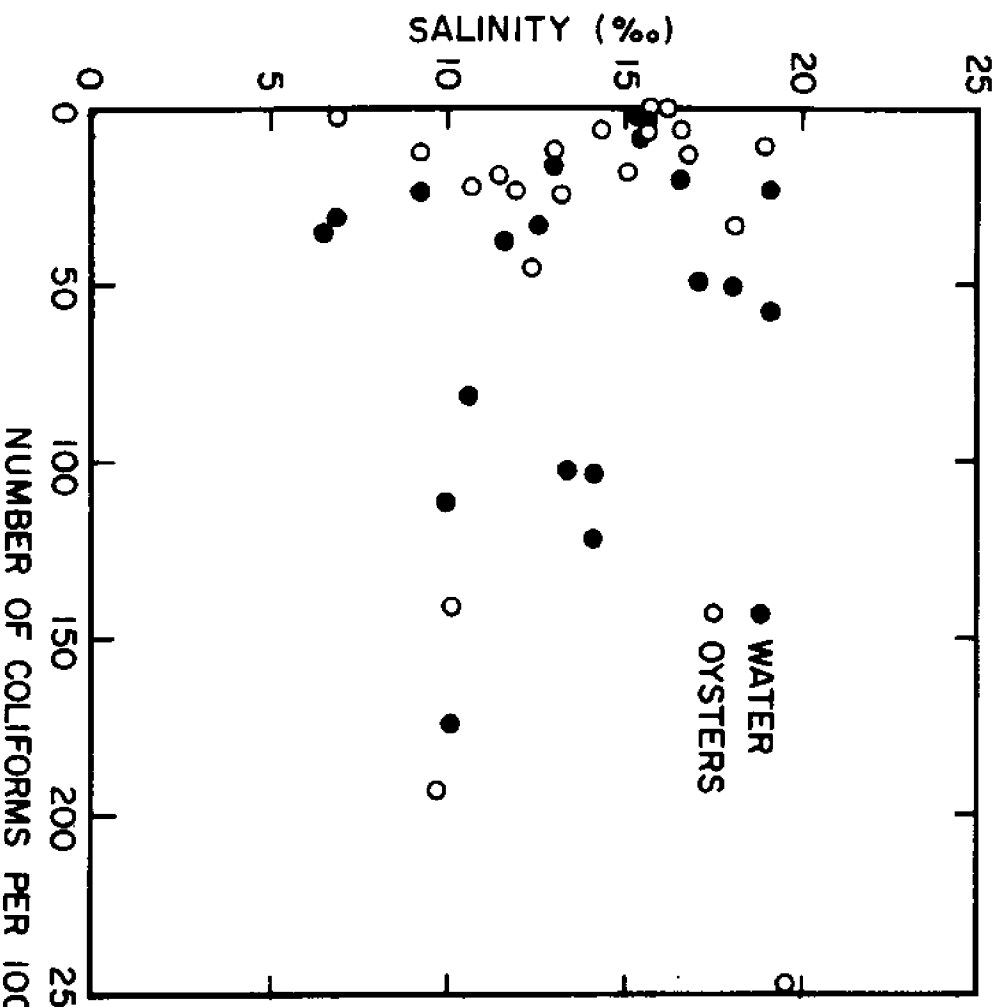

Fig. 1. Relationship of salinity to number of coliforms in waste ponds.

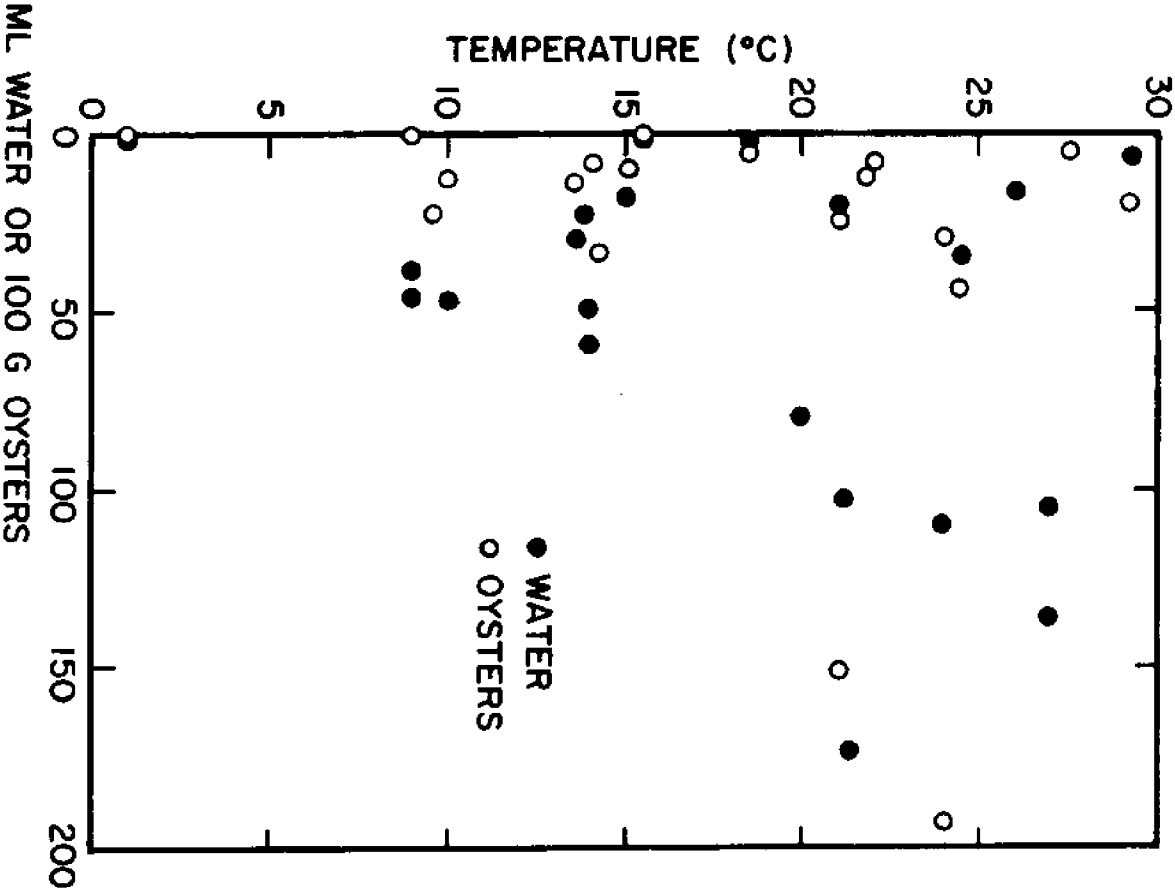

Fig. 2. Relationship of salinity to number of coliforms in waste ponds.

FORAMINIFERA IN ESTUARINE PONDS DESIGNED FOR WASTE CONTROL AND AQUACULTURE

Ann LeFurgey and Joseph St. Jean, Jr.

INTRODUCTION

Fossil foraminifera have long been used by geologists as diagnostic tools for classifying ancient strata and identifying oil-rich deposits. The usefulness of this protozoan extends also to modern sedimentary environments where living benthonic foraminifera cycle organic matter and accumulate energy as protoplasm and as skeletal material. Because different foraminiferal species occur in all ranges of salinity, form characteristic faunas in various estuarine environments, and are sensitive to the timing of energy sources such as fresh water inflow or organic nutrient input, foraminifera can also be used as aids to the energy classification of estuaries (Nichols, 1969). The purpose of the present study was to investigate cahracteristics of foraminiferal faunas in six similar estuarine ponds, three of which received additional energy input in the form of treated sewage effluent. Although the effluent ponds (P-1, P-2, P-3) were built on Calico Creek, Morehead City, N.C., and the control ponds (C-1, C-2, C-3) were constructed about one mile south on Bogue Sound, the primary factors governing foraminiferal distribution--salinity, temperature, and depth--were relatively equal in all six ponds. Therefore, faunal featues such as species composition, standing crops, productivities, diversities, and similarities, reflected the direct or indirect effects of an additional energy inflow, sewage effluent, upon the sedimentary microenvironments of the living foraminifera.

METHODS

To examine naturally occurring living populations, monthly sediment samples were collected using a small skiff or the access pier in each pond. Approximate locations of bottom sediment samples and samples from aquatic grasses and from pilings appear in Figs. 1 and 2. Bottom samples were taken either with a plastic coring tube, sampling area 14.4 cm^2; a small Petersen dredge, sampling area 176.0 cm^2; or a modified aquarium vacuum cleaner, sampling area 6.6 cm^2. Approximately the upper two centimeters of sediment was extracted from these samples. Samples were also scraped from deposits on the pilings of the access piers. Ruppia growing in the water and roots and lower leaves of Spartina growing at the water's edge were examined for possible epiphytic specimens.

To distinguish living specimens, all samples were preserved immediately in a mixture of isopropyl alcohol and rose bengal (Walton, 1952:60). Subsamples of approximately 5 ml were weighed and measured volumetrically, wet seived (Tyler 250 mesh, 63 μ openings), and dried at 40° C. Fifty subsamples were examined in entirety before and after drying to determine if drying had any adverse effects on delicate arenaceous and chitinous specimens. Total population counts, including living, dead, and numbers of specimens per species, were made for each subsample. Sediment sample and species data appear in Tables 1-7. After identification under a binocular stereoscope at 10 to 30X, specimens of the majority of species were re-examined for detailed morphological characters and photographed in the JEOL Scanning Electron Miscroscope, JSM II. Before insertion in the microscope, all foraminifera tests were coated under vacuum with gold or carbon/platinum. Operating voltages were 5, 15, or 25KV.

Pond temperatures and salinities, monitored daily, were noted for each sampling date, in addition to secchi disc values and water color (Table 1).

ENVIRONMENTS

General Pond Description

Each 0.1 acre pond (20 x 30 m) averages one-half meter in depth and has a maximum depth at the center of less than one meter (Fig. 1,2). Water enters through a 7.6 cm (diameter) pipe located on the pond periphery and drains through a standpipe placed near the center of each pond. Structural features, such as the shallow sandy margin and the deeper central area, as well as natural and artifical substrates, including patches of Spartina marsh grass, a shell reef-bar, a block reef, and treated wood pilings, provide a variety of microenvironments within individual ponds (Figs. 1, 2).

Water Sources

Both creek and sound communicate via Beaufort Inlet with an oceanic region of the North Carolina coast which is a transition area for temperate-tropical foraminiferal faunas. Because Calico Creek is the source of water mixed with sewage effluent in P-1, P-2, and P-3 and because Bogue Sound is the source of water mixed with city water in C-1, C-2, and C-3, the composition and nature of their waters have direct bearing on the ecosystems, and particularly on the foraminiferal populations, developing in the ponds.

Calico Creek is a narrow, shallow tributary of the Newport River complex which has tidal flushing and sporadic fresh water inflow. Spartina alterniflora marshes living along the creek edge are evidence of regular tidal flooding. Salinities fluctuate from 3 to 33‰, varying with tidal flow, distance from Beaufort Inlet, depth, and season. Mean water depth recedes to less than one meter at low tide, exposing large expanses of mud flats. Very fine silt and clay compose the creek bed, and the water bears a large silt load. Approximately 2.8×10^3 m^3 of treated effluent is released daily into the creek by the Morehead City sewage plant (Kuenzler, Wyman, and McKellar, 1971:10).

Bogue Sound is a shallow lagoon formed inside the North Carolina Outer Banks (barrier islands). Strong tidal currents flow through Beaufort Inlet, Bogue Sound's eastern connection with the open ocean. Salinity, which is less variable than in Calico Creek, ranges annually from 31 to 33 ‰. Seasonally, freshwater runoff enters from inland.

Mean annual bottom temperatures vary from 18.5° to 20.3° C; extreme bottom temperatures vary from 5° to 30° (Brett, 1963: figs. 8, 9, 10). Sediment is fine to very fine sand with a measurable proportion of clay in the lagoon-proper sediments.

Input water-flow patterns from Bogue Sound into the control ponds and from Calico Creek into the effluent ponds are diagrammed in Figs. 1 and 2. Sound waters passed into a central tank where the waters were combined with fresh water to form a homogeneous mixture of constant salinity before being pumped to each of the control ponds. A similar tank system was used at the effluent ponds to mix water from Calico Creek with effluent water from the sewage plant. Each of the effluent ponds received water from the sewage plant. Each of the effluent ponds received water from the central source to insure that input to all of the ponds was identical.

Emphasis is placed on the water sources because sediments suspended in these waters contained foraminifera. Foraminifera were seeded into the ponds through the pumps which drew water from either creek or sound. The homogeneity of the water supplied to all ponds of a group implied foraminiferal faunas should be alike in each group of ponds.

Hydrography

Stratification was minimal in the ponds because of their shallow depth and limited stirring by daily coastal winds. Water temperature approached that of ambient air temperature; seasonal extremes ranged from 0° to 36° C. In winter, the slight surface freezing in the ponds was more extensive and prolonged than in Bogue Sound or Calico Creek.

Salinity was maintained theoretically at 15 ‰ average; in actuality the salinity varied from 12 to 22 ‰ .

Light penetration in the effluent ponds was highly attentuated, particularly from late fall to early spring, when phytoplankton blooms were densest. The control ponds which had overall deeper light penetration were subject to heavy blooms only during the warmer months.

Geology

The earth basins of the six ponds were sealed with red clay from a Newport, North Carolina, quarry and lined with black muds from the Calico Creek marsh. Megascopic examination of the upper centimeter of pond sediments revealed a slight difference in mean sediment size between effluent and control ponds (LeFurgey, 1971). Both pond groups had coarse sediments at the edges, with the finest sands and silts

predominant in the central areas. Many samples from both groups contained fecal pellets and diatom frustules. The effluent ponds had a characteristicly higher proportion of heavy minerals. Clay minerals typical of the sediment source areas were found in all ponds: kaolinite, illite, and 14A hydroxy-interlayer mineral. Both control and effluent ponds had an upper layer of oxidized light-color sediments with much darker, reduced sediments below. The upper oxidized layer was thinner in the effluent ponds. Quantitative chemical analyses of the organic content of the sediments indicated little difference between the control and effluent ponds (Day, 1971; Rhyne and Hommersand, 1970).

FORAMINIFERAL FAUNAS

Control Pond 1, C-1

Quantitative samples taken in December, 1970, February, April, and June, 1971, contained a total of 23 species of foraminifera, 20 of which had living representatives. Overall the populations were not dominated by any particular species; Miliammina fusca (Brady) was the single most abundant species. Approximately equal abundances of Protelphidium tisburyense (Butcher), Ammotium salsum (Cushman and Bronnimann), and Elphidium clavatum Cushman (Tables 2 and 8) occurred in C-1. The total standing crop was relatively constant through the sampling period; the living proportion increased in April and June (Fig. 3). Average standing crop was 8.5 living specimens/cc of wet sediment to 6.0 dead specimens/cc. The largest living population for any one sample occurred in June, 48.8 living specimens/cc. Other large living populations were sampled in December, 15.3/cc, and April, 16.7/cc. Both samples were taken near the water inlet pipe (Fig. 10).

Control Pond 2, C-2

November, December, 1970, February, April, and June, 1971, quantitative samples yield 25 species total with 22 living species. Elphidium clavatum Cushman and Ammonia beccarii (Linnaeus) together constituted over 60% of the populations (Tables 3 and 8). Living standing crop varied from 9.2/cc in April to 35.5/cc in December (Fig. 3). Total standing crop ranged from 16.2/cc to 10.1/cc. Average standing crop was 23.0 living specimens/cc and 17.7 dead specimens/cc. The highest population density, 68.0 living/cc, occurred in a December sample taken to the right of the inlet pipe (Fig. 10). Other high values occurred in June, 65.0 living/cc; 53.8 living/cc.

Control Pond 3, C-3

The water of C-3 was so clear that one could see bottom on every sampling date (Table 1). Most of the samples included Ruppia maritima, an aquatic angiosperm growing over 80% of the area of C-3. December, 1970, February, April, and June, 1971, samples possessed a total of 25 foraminiferal species with 22 living species. Monthly living standing crop was highest in December, 77.1/cc, and lowest in February, 8.0/cc. Average standing crop for the entire sampling period was 39.5 living/cc and 12.4 dead/cc (Fig. 3). Cribroelphidium cf. C. vadescens Cushman and Bronnimann was confined to C-3. It occurred in a "bloom" sample in December taken from the east end of the pond (Fig. 10). Containing 162.6 living/cc, the sample was in 0.3 meters of water in an area of dense Ruppia growth. Elphidium clavatum Cushman and Ammonia becarii (Linnaeus), 24.4% and 14.7%, respectively, of the total living fauna, predominated in other months (Tables 3 and 8).

Effluent Pond 1, P-1

Analysis of November, December, 1970, February, April, and June, 1971, samples found 21 species present, 17 of which had living representatives. Elphidium clavatum Cushman made up 31% of the populations (Tables 4 and 8). Two arenaceous species and one calcareous species were next abundant: Ammotium salsum (Cushman and Bronnimann), Trochammina inflata (Montagu) and Ammonia becarii (Linnaeus). Monthly samples indicated populations of living individuals varying from < 1/cc in February (Fig. 4) to 10.1/cc in April. Average standing crop was 4.1 living/cc and 2.1 dead/cc. The highest values for living standing crop occurred near the inlet pipe in April, 18.8/cc, and in June, 30.0/cc. Other samples taken at the same time from other areas of the pond had values of 6.5/cc, < 1/cc, 0/cc, 1.4/cc (Fig 11).

Effluent Pond 2, P-2

Twenty species of foraminifera composed the foraminiferal faunas, 16 species living. Populations were dominated by Elphidium tumidum Natland which occurred first in December and reappeared in large numbers in June. Elphidium clavatum Cushman and Ammotium salsum (Cushman and Bronnimann) occurred throughout the sampling period and also increased in numbers in June. Average population size was 3.8 living specimens/cc, with a monthly average size ranging from < 1/cc to 14.2/cc (Fig. 4). Dead specimen average was 6.0/cc. One high standing crop appeared at the inlet pipe in June, 40.2 living/cc. Samples taken in the same area in April had 1.0 living /cc and in November 0/cc. Other high values were 30.4/cc and 47.2/cc in June.

Effluent Pond 3, P-3

The number of species found in P-3 totaled 27 with 23 living species. Six species of Elphidium occurred: E. advenum (Cushman), E. tumidum Natland, E. galvestonense Kornfeld, E. poeyanum (d'Orbigny), E. sp., and the dominant species E. clavatum Cushman. Ammonia beccarii (Linnaeus) was the only other species composing more than 10% of the total fauna. Monthly standing crop varied from < 1 to 14.4 living/cc and from 0 to 8.5 dead/cc. The average standing crop was 5.5/cc living and 2.7/cc dead. The highest standing crop recorded for a single sample was 36.0 living/cc. Other high values occurred also in June, 35.3/cc, 9.6/cc respectively. In December 20.2 specimens/cc were counted in a sample from sediments near the end of the access pier (Fig. 11).

Bogue Sound

Ammonia beccarii (Linnaeus), Protelphidium tisburyense (Butcher), and Elphidium clavatum Cushman were abundant at the Bogue Sound water intake pipe. Ammotium salsum (Cushman and Bronnimann) was common, along with many Miliolidae and several species of Rosalina.

Calico Creek

Foraminifera found near the water intake pipe in Calico Creek included Ammobaculites dilatatus Cushman and Bronniman, Trochammina inflata (Montagu), Ammotium salsum (Cushman and Bronnimann) and Ammonia becarii (Linnaeus). Several hundred meters downstream below the sewage outlet pipe Protelphidium tisburyense (Butcher) and Miliammina fusca (Brady) occurred abundantly. Percentages of calcareous and porcelaneous species increased with distance downstream toward Beaufort Inlet. A comprehensive study of faunas in the Inlet and in Core Creek, also a tributary of the Newport complex, has been contributed by Akers (1971).

COMPARISON OF FORAMINIFERAL FAUNAS

Dominant Species

From the total living population sampled in each pond, the percentage attributable to each species was calculated, and a classification of dominant, common, and rare species was made on the basis of the percentages (Table 8). Dominant species (Figs. 5, 6) were those occurring in three or more ponds at greater than 5%; common (Fig. 7), those occurring in three or moreponds at less than 5%; rare (Fig. 8), those occurring in four or more ponds at less than 1%. Cribroelphidium cf. C. vadescens Cushman and Bronnimann was placed in the common rather than the rare group because it was abundant, 26.4%, in C-3.

Elphidium clavatum Cushman was the most commonly occurring species in the ponds. It constituted over 25% of the faunas in C-2, C-3, P-1, and P-3; over 10% in C-1 and P-2 (Table 8). Schafer (1970 a, b) reported the "Elphidium incertum/clavatum" group as being one of the most pollution tolerant forms living in the Restigouche Estuary, Canada, and in Long Island Sound. Schafer and Sen Gupta (1969) found Miliammina fusca (Brady) abundant in polluted estuaries of New Brunswick and Maine. The opposite situation occurred in the effluent and control ponds. M. fusca was present as 1.5% of the total living population in P-1, missing from P-2, and present at less than 1% in P-3. However, it was the most abundant species in C-1 at 22% of the total living population and occurred in C-2 and C-3 at 10.5% and 5.0% respectively.

E. clavatum and M. fusca, together with E. tumidum Natland, Ammonia becarii (Linnaeus), and Ammotium salsum (Cushman and Bronnimann) dominated the faunas of the ponds.

Similarity

In order to quantitatively evaluate similar trends in faunal composition, numerical percentage similarity was calculated for all possible pairs of ponds (Table 9). According to the methods of Sanders (1960:143) and Murray (1970:58), the percentage of the total living fauna attributable to each foraminiferal species was calculated for each pond. Percentages of all species which occurred in any two ponds were compared, and the lowest percentage noted for each species pair. The sum of these least percentages was equal to the percentage similarity between the two ponds.

C-1 was less than 36% similar to all ponds except P-1. The 49.2% value for C-1/P-1 indicated similarity between two ponds from different groups. Studies of diversity of phytoplankton, zooplankton, and microsessile organisms (May, 1971:25) also suggested P-1/C-1 similarity. C-1 and C-2, 61.3% similar, were each less than 34% similar to P-2. C-2 was approximately 60%, and C-3, approximately 50%, similar to both P-1 and P-3.

Effluent ponds P-2 and P-3 were least similar, 25.4%. Both P-2 and P-3 were in the 50% range of similarity to P-1. P-2 was less than 35% similar to all ponds except P-1.

Differences among the ponds of the control group and among those of the effluent group were surprising because theoretically the water source, i.e. the major foraminiferal source, was the same for each group of ponds, even though source waters were different for the two groups (see Water Sources section). Additionally the other parameters most often cited as controlling foraminiferal distribution - depth, salinity, and temperature - were not different from pond to pond. These differences pointed to the existence of distinct microenvironments within each pond, made favorable or unfavorable by such variables as accessibility, food supply, oxygen content, pH, and bacterial concentration.

Species Diversity

Monthly indices of diversity for living and dead foraminiferal populations in both pond groups are illustrated in Fig. 9. This index, $S-1/\log_e N$, weights equally the number of species (S) and the number of individuals (N). The index is calculated for each sample and an average monthly index is calculated from all samples taken from a particular pond in a particular month.

Lowest living species diversity occurring in any pond in any month was 0.51, recorded in November for P-3. P-2 also had species diversity less than one, 0.74, in February. Little weight should be placed on the 0 indices for November and February in P-1, November in P-2, and February in P-3; the total living population sampled had less than 10 individuals with S=1 or 0 diversity. C-3 and C-2 had their lowest diversities, 1.05 and 1.09 in February and December respectively. Lowest diversity occurred in P-1 in April, 1.25, and in December in C-1, 1.80.

Highest living species diversity occurring in any pond in any month was 2.60, the April average for P-3. C-1, C-2, and C-3 each had a peak diversity index greater than 2: C-1, 2.35, June; C-2, 2.25, November; C-3, 2.20, April. Highest values in P-1 appeared for December, 1.80, and in P-2 for April, 1.25.

Among the control group, C-1 proved to be most diverse, with C-2 and C-3 about equal to each other yet lower than C-1 in living diversity. Among the effluent group, diversity tended to be greatest in P-1, intermediate in P-3 and lowest in P-2. Between the two groups living diversity was generally higher in the control ponds. Higher diversity in the control ponds has also been reported for phytoplankton (Campbell, 1971:137), zooplankton (May, 1971:25), fish (Beeston, 1971:190), and crustaceans (Walton and Williams, 1971: 211).

Diversities for dead assemblages in the control ponds closely paralleled diversities for living in the majority of samples. P-1 and P-2 diversities for dead assemblages tended to be higher than those for the living. Either more dead than living species were introduced or fewer species survived than entered the ponds. Evidence from other facets of the study (Living/Total Ratios section) supported the latter case.

Standing Crops

Standing crops of living specimens were greater during the sampling period in C-2 and C-3 than in any other ponds (Figs. 3 and 4). Peak populations occurred in December and June in C-2 and also in December and June in C-3. C-1 had largest standing crops in April and June, but on the whole had much smaller numbers of foraminifera than the other two control ponds. Juveniles of different species appeared in the control ponds samples every month, and juveniles were observed in April and June in the effluent pond samples.

The standing crop of living foraminifera in the effluent ponds was less than 1/5 of that in the controls. P-1 had highest living standing crops in April and June; P-2 and P-3, in December and June. Crops for all three effluent ponds were less than 1/cc in November and February.

Areal Distribution

Standing crops of living foraminifera, plotted according to sample location, illustrate the relationship of population density to microhabitat (Figs. 10 and 11). Some of the highest foraminiferal densities occurred near the water inlet pipes in C-1, C-2 (Fig. 10) and in P-1 (Fig. 11). These pipes were the points of introduction of foraminifera, which entered the ponds through sediments suspended in the water pumped from their adjacent natural habitats, Bogue Sound, for the control ponds; Calico Creek, for the effluent. Areas surrounding the pipe were thus continually being repopulated with new specimens from sound or creek. Because the introduction points coincided with the shallow sandy margins where benthic algae and associated

epiphytes were abundant, an extremely favorable microenvironment was created. Foraminiferal populations must have migrated subsequently around the peripheral regions.

Movement of individual foraminifera is not rapid, and the ability of foraminifera to populate the entire sediment area also depends upon (1) water circulation and turbulence, (2) rates of settling of foraminifera from the water, and (3) accidental transport of foraminifera with sediments distributed and displaced by man or other animals. Success following the multiple means of distribution would then be contingent upon location in a favorable microenvironment.

Areal distribution in C-3 (Fig. 10) was widespread and densities were consistently higher than in any other pond. Ruppia maritima, an aquatic angiosperm, and its epiphytic communities, provided abundant food for the foraminifera in this pond.

The plankton-dominated central areas appeared especially unfavorable in the effluent group. The highest standing crops occurred in the peripheral zone of P-2 with the exception of one June sample. P-3 had a similar peripheral distribution. Areas of increased phytoplankton production and reduced photic zone had decreased productions of benthic flora. Therefore, one of the major food sources for bottom dwelling herbivores was in low supply.

The central portion of the ponds was also the area of greatest O_2-pH stress during peak phytoplankton production. Daily, during a phytoplankton bloom,high photosynthesis preceeded high respiration rates with subsequent reduction in oxygen concentration. After the bloom peaked and died, bacterial activity increased and oxygen concentrations were almost fully depleted (Smith, 1971; Dillon and Woods, 1971).

Foraminifera have low oxygen requirements, but how long they can survive at reduced levels and how well they adjust to rapid changes in oxygen concentrations is not clear. In a comparison of faunal assemblages of the Beaufort area, Akers (1971) found marsh foraminifera at depths to 35-40 cm below the surface and in areas of a Spartina marsh not covered continually by water. These environments, presumably low in O_2, were also low in pH and supported only arenaceous foraminifera. Although the pond sediments were not sampled to such depths, the predominance of calcareous foraminifera in the upper sediments and the high pH of the pond waters suggested that the pond pH-O_2 conditions were not analagous to the conditions of Akers' areas.

Bradshaw (1961), in experiments with Ammonia tepida Cushman, calculated a critical O_2 level of 1.72 mg O_2/1 at 26°C, 35 ‰. At a

comparable temperature, but a lower salinity, the bottom waters of the control ponds varied diurnally from 0.8 to 7.9 mg O_2/l (Table 1); in the effluent ponds dissolved O_2 varied from 0.12 to 10.2 mg/l . The lower values in both groups of ponds were considerably less than the critical survival level for the species tested by Bradshaw. Ammonia tepida Cushman occurred in smaller numbers in the effluent than in the control ponds, indicating a critical O_2 level in nature between 0.1 and 0.8 mg/l.

Productivity

Foraminiferal productivity is a function of (1) initial population size, (2) proportion of the population which reproduces, (3) rate of reproduction, and (4) number of offspring produced (Murray, 1967). Little data is available on individual reproduction, but it is apparent that reproduction varies both with species and environment. Haman (1969), in a review of rates of reproduction, cited occurrences of hourly, weekly, monthly, and biannual reproduction of various species occupying a wide range of environments.

Because of these multiple contributing factors, only the population size at the time and place of each sampling was known with accuracy, the calculated productivities (Table 10) were, at best, an estimate of the relative rates of productivity in the ponds. The attempt at estimation of productivity was made primarily to aid understanding of the contribution of foraminifera to energy flows within each pond system.

The following assumptions were made in calculating productivity according to Murray's method (1967), and they yield a minimum or most conservative estimate:

(1) initial standing crop was the average of all the standing crops calculated per pond for the sampling period;
(2) reproduction occurred in all living individuals;
(3) reproduction occurred once per year;
(4) reproduction yielded one offspring per indiviual;
(5) dry protoplasmic weight of 2800 foraminifera = 12.4 mg or 1.05×10^6 foraminifera = 4.65 grams (Boltovskoy and Lena, 1969).

Average annual production in all control ponds was approximately three times that of the effluent ponds. The estimated control value, 0.37 g/m^2/yr, and effluent value, 0.14 g/m^2/yr, were valid as relative rates of reproduction, but should not be considered accurate absolute values.

Living/Total Ratios

The ratio of living to total benthic foraminifera is used to approximate relative rates of deposition in marine sediments (Phleger, 1960:190). Assuming a stable rate of production over the time of sediment accumulation, abundant living specimens relative to the total population mean fairly rapid addition of sediments other than foraminifera. A low percentage of living specimens relative to the total indicates a slow rate of supply of other sediments.

Averages of living/total ratios were relatively constant from month to month for C-3 (Table 11). The other ponds had variable monthly rates. Overall, the living/total ratio was higher in the effluent ponds, as would be expected from their higher rates of primary production and from effluent inflow. The higher number of living tests as compared to dead might indicate dissolution of tests after death. These variations could also be attributed to different rates of reproduction in the foraminiferal populations.

Trigonal Plots

The smaller benthic foraminifera are divided into three major groups on the basis of skeletal composition: (1) arenaceous or agglutinated skeleton made of sedimentary particles cemented together by the indivual foraminifera; (2) imperforate, porcellaneous skeleton of calcium carbonate secreted by the organism; and (3) perforate, calcareous skeleton also secreted by the organism. These three groups are, respectively, the sub-orders Textulariina, Miliolina, and Rotaliina. By plotting on a trigonal graph percentages of the three groups present in samples from different environments, Murray (1968) found that hyposaline lagoons and estuaries are rich in Rotaliina or Textulariina, or both, and have a low content of Miliolina. Similar plots were evident for the control and effluent ponds (Fig. 12). All samples were on or near the Rotaliina-Textulariina base line in accord with Murray's model.

Bandy, Ingle, and Resig (1965a) also used the trigonal analysis to show differences between living and dead populations in offshore outfall areas of California. Although arenaceous specimens, Textulariina, dominated the dead population at the outfall, perforate calcareous specimens, Rotaliina, were eight times as abundant as all others in the living population. The sewage effluent from the outfall increased the organic content of sediments which contributed to reduced pH values.

Calcareous tests are more easily dissolved in acidic conditions than are arenaceous. Thus when the calcareous foraminifera dies and

the protective protoplasmic body decays, it is subject to more rapid dissolution than the arenaceous foraminifera. This selective dissolution explains Bandy's anomalous results.

Comparison of living/dead in the present study revealed little difference in the population distributions. P-1 and P-3 dead populations were shifted toward the arenaceous or Textulariina side. The shift, however, was not sharp enough to indicate definite solution of tests. The high alkaline pH's occurring in surface and bottom waters of the effluent ponds discounted the possibility of acid solution, but since pH at the sediment-water interface and within the sediments was not determined the possibility of solution cannot be completely excluded.

COMPARISON WITH OTHER FAUNAS

The foraminiferal faunas of the Morehead City effluent and control ponds could not be readily distinguished on the basis of species composition or species diversity, two common indicators in pollution diagnosis. Significant differences occurred, however, in the standing crops and estimated productivity of the foraminifera. During the seasonal cycle observed, from November to June, the living populations of the effluent ponds were much smaller than those of the controls. The effluent itself was not toxic to the foraminifera, but the trophic structure which had evolved in the presence of sewage effluent limited the number of microhabitats favorable to foraminiferal growth and reproduction.

Although the six ponds had major similarities, singly and as groups, each pond had developed into a separate entity characterized by microenvironmental differences in foraminiferal populations. These microenvironmental variations have been noted in other marine and estuarine areas by Buzas (1965, 1968), Lee, et al. (1969), and Bradshaw (1968).

The isolated large populations of foraminifera within individual ponds were comparable to "blooms" of foraminifera described by Lee, et al. (1969) for samples from epiphytic communities with more than fifty living foraminifera. Tracer studies (Lee, et al., 1966) revealed that feeding by foraminifera on certain species of diatoms, chlorophytes, and bacteria was concentration dependent. Optimum feeding occurred when the concentration of food organisms was between 10^3 to 10^6 cells/ml of water, under laboratory conditions. Lee concluded that their feeding mechanism allows foraminifera to grow and reproduce rapidly, i.e. "bloom", when the algal food supply is high, to eat and reproduce slowly when the food supply is low.

If foraminifera living in the ponds rejected blue-green algae and most bacterial species, as they did in laboratory feeding experiments by Lee, et al. (1966), then the foraminifera must have depended upon living epiphytes associated with the algae for nourishment. When light penetration below one centimeter was sharply attenuated by dense plankton blooms in the effluent ponds, growth of all benthic flora such as blue-green algae, was restricted. When this algal growth was dimished, concentrations of living epiphytes suitable for foraminiferal food would have been too low to stimulate productivity.

Phytoplankton cell concentrations averaged 1.8×10^7 cells/ml in the effluent ponds; 3.0×10^6 cells/ml in the control cponds. Death of entire plankton blooms did occur suddenly (Campbell, 1971) with the majority of dead plankton individuals settling to the bottom. If foraminifera did feed on dead organisms which settled out of the water column, concentrations of dead phytoplankton periodically exceeded the optimum suggested by Lee, et al. (1966) and were high enough to inhibit foraminiferal feeding.

Using a model of trophic structure which included benthic foraminifera, Lipps and Valentine (1970) postulated that shallow water species on muddy substrates were microherbivores feeding on bacteria and living epiphytic diatoms. On a muddy bottom at greater depths the foraminifera subsisted mainly on bacteria and organic detritus.

Christiansen (1971), observing shallow and deep water species, concluded that shallow water species were primarily herbivorous. Deep water forms, living at depths where algae did not survive, ingested organic particles, nauplii and other larvae, nematodes, copepods, and even other foraminifera.

Estuarine forms seeded into the ponds were not adapted to a light-limited environment; therefore, they might not have consumed those alternative foods ingested by deeper water species. An interesting future study should investigate adaptations of the pond foraminifera to a light-limited food supply.

No macrophytes such as Ulva or Enteromorpha survived in the plankton-dominated portions of the ponds. Pure stands of Enteromorpha grew in the peripheral areas of the ponds in 1969-1970, but this alga did not prolifically reproduce in either group of ponds. Epiphytic communities of these benthic algae do support large populations of foraminifera, and scattered stands of algae in the pond periphery may account for the patchy "blooms" of foraminifera found also in the marginal area. High standing crops of living foraminifera in control ponds reflected the abundance of diatoms, a preferred food, in the benthic flora. Epiphytic communities of Ruppia in C-2 and C-3 also augmented the food supply.

Both Mueller and Lee (1969) and Bradshaw (1961) noted that bacteria provided nutrients essential for rapid growth and reproduction in foraminifera. They also observed that foraminiferal functions were inhibited at bacterial concentrations greater than approximately 1×10^6 cells/ml. Bacterial counts in the effluent ponds were in the range of 10^8 to 10^7 cells/ml, an order of magnitude greater than in the control ponds. At the sediment-water interface, bacterial concentrations were probably higher. Therefore, lower standing crops might also be the result of growth and reproductive inhibition by bacteria.

Bandy, Ingle, and Resig (1964a, b; 1965a, b) defined a prominent dead zone of foraminifera under deep ocean sewage outfalls off the California coast. The effluent was borne by freshwater through the outfall pipe from which it was discharged into deep, full-salinity waters. Foraminiferal numbers under the outfall were less than one per cc, but several hundred meters from the outfall both standing crop and species diversity of planktonic and benthic foraminifera increased phenomenally. A parallel situation existed in shallow Calico Creek where freshwater effluent was discharged daily. Populations at the discharge pipe were less than one per cc and increased downstream to more than twenty per cc. Phytoplankton blooms occurred in both areas, but the blooms never reached self-destructive densities because tidal flushing in the creek and longshore currents in the coastal zone continually renewed the waters. Sewage effluent entered P-1, P-2, and P-3 at a salinity of 15 ‰; moderately large populations did survive at the inflow pipe and in other regions of the ponds. Therefore, one must conclude that low standing crops in the effluent ponds, and perhaps in Calico Creek and off the California coast, were not reflecting toxicity of effluent to foraminifera. Microenvironmental restriction were imposed on the pond foraminifera by sewage-thriving phytoplankton regimes. Major environmental restrictions were imposed on the creek and coast foraminifera by freshwater carrying pollutants and not by pollutants themselves.

SUMMARY

1. Elphidium clacatum Cushman was the most commonly occurring species in both effluent and control ponds. Other important species included Ammonia becarii (Linnaeus), Miliammina fusca (Brady), Ammontium salsum (Cushman and Bronnimann), and Elphidium tumidum Natland.

2. Neither the three control ponds nor the three effluent ponds were true replicates. Although C-2 and C-3 were highly similar, C-1 most closely resembled P-1 in species composition. Both C-2 and C-3 were moderately similar to P-1 and P-3. P-2 and P-3 were moderately similar to P-1, but distinctly different from each other.

3. Both living and dead species diversities in the control ponds were generally higher; values ranged from 1.05 to 2.35, living, and 0.65 to 2.5, dead. Diversity of living species in the effluent ponds was from 0 to 2.6, a much wider range of values; diversity of dead species was 0 to 1.90.

4. Standing crops in the effluent ponds averaged 4.5 living specimens/cc; in the control ponds, 23.7 living/cc. Estimated productivity was approximately three times greater in the control ponds.

5. Microenvironmental differences in O_2, pH_u, type and concentration of food supply made foraminiferal distribution erratic. These environmental parameters were controlled primarily by dense phytoplankton blooms in the effluent ponds.

6. Municipal sewage effluent after secondary treatment is in itself not toxic to foraminifera or deleterious to their sedimentary environment.

FAUNAL LIST

Generic identifications were based on Loeblich and Tappan (1964); species determinations, on Ellis and Messina (1940, et seq.). The only exception was Elphidium clavatum Cushman, identified from Buzas (1966) who made a thorough and definitive study of three widely confused and misinterpreted species of Elphidium, including E. clavatum Cushman.

Small numbers of porcelaneous species present precluded to identify the individuals beyond genus. The genera Quinqueloculina and Triloculina were subsequently grouped under the single family heading, Miliolidae.

Ammoastuta salsa Cushman and Bronniman, 1948

Ammobaculites carassus Warren, 1957

Ammobaculites dilatatus Cushman and Bronnimann, 1948

Ammobaculites exiguus Cushman and Bronnimann, 1948

Ammonia beccarii (Linnaeus), 1758

Ammonia tepida Cushman, 1926

Ammotium salsum (Cushman and Bronnimann), 1948

Angulogerina sp.

Arenoparella mexicana (Kornfeld) emend. Anderson, 1951

Bolivina sp.

Buliminella elegantissima (d'Orbigny), 1839

Cribroelphidium cf. Cribroelphidium vadescens Cushman and Bronnimann, 1948

Elphidium advenum (Cushman), 1922

Elphidium brooklynense Shupack, 1934

Elphidium clavatum Cushman, 1930

Elphidium galvestonense Kornfeld, 1931

Elphidium poeyanum (d'Orbigny), 1839

Elphidium tumidum Natland, 1938

Elphidium sp.

Globigerinidae

Haplophragoides mexicanus Kornfeld, 1931

Haplophragmoides wilberti Anderson, 1952

Miliolidae

Miliammina fusca (Brady), 1870

Nonionella atlantica Cushman, 1947

Protelphidium tisburyense (Butcher), 1948

Reophax nan Rhumbler, 1911

Rosalina sp.

Tiphotrocha comprimata (Cushman and Bronnimann), 1948

Trochammina inflata (Montagu), 1808

Trochammina lobata Cushman, 1944

Trochammina macrescens (Brady), 1870

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Table 1. Sampling Data

Sampling Date	Pond	Salinity °/₀₀	Temp. °C	Secchi Disk cm-depth	Color	Sampler	Sample Number
6 November 1970	C-1	16.6	13.9	90	green	Core	1-4
	C-2	16.7	13.7	95	green		5-8
	C-3	17.1	13.4	visible	clear		9, 10
	P-1	16.7	15.1	55	green		11-14
	P-2	16.9	13.9	40	brown		15-18
	P-3	19.0	13.8	79	muddy green		19-23
4 December 1970	C-1	18.1	18.7	65	green	Core	25-29
	C-2	16.9	17.8	82	green		30-33
	C-3	18.5	17.8	140	clear		34-36
	P-1	17.8	17.5	23	bright green		37-39
	P-2	18.0	17.3	33	dark brown		40-42
	P-3	19.4	16.3	36	brown		43-45
19 February 1971	C-1	19.9	13.9	140	light green	Core	51-53
	C-2	19.7	13.8	140	light green		54-56
	C-3	20.9	13.8	140	clear		57-59
	P-1	13.2	13.5	26	bright green		60,61
	P-2	13.3	12.6	29	bright green		62,63
	P-3	13.9	12.1	25	bright green		64,65
1 April 1971	C-1	No data		140	green	Petersen	73,74
	C-2			140	green	Dredge	75,76
	C-3			140	clear		77A,78A
	P-1			29	bright green		66,67
	P-2			33	bright green		68,69
	P-3			31	dark green		70,71
4 June 1971	C-1	25.0	30.0	60	green	Aquarium	77J,78J,79-87
	C-2	24.4	30.0	80	green	Vacuum	88-95
	C-3	24.7	29.6	140	clear	Cleaner	96-99
	P-1	22.2	30.0	50	grey-green		100-108
	P-2	19.9	27.3	28	bright green		109-118
	P-3	22.2	29.6	51	grey-green		119-127

Control Pond 1

STATION NO.	26	27	28	29	51	52	53	73	74	77	78	79	80	81	82	83	84	85	86	87
LIVING SPECIES	2	2	9	12	3	10	8	4	14	13	17	5	12	6	14	1	0	9	8	8
TOTAL SPECIES	6	10	14	12	16	12	8	4	14	15	17	5	12	9	15	1	0	13	8	9
No. LIVING/CC(WET)	4.0	0.2	2.0	15.3	3.8	5.7	1.8	16.7	10.9	7.8	23.2	3.6	12.2	9.6	48.8	<1	0	6.5	7.2	3.6
TOTAL No./CC(WET)	10.0	8.0	14.9	28.0	28.2	15.9	4.6	19.7	13.6	29.4	29.0	4.0	17.0	13.4	50.0	<1	0	16.2	10.2	6.0
No. Individuals Living/Sample																				
No. Individuals Dead/Sample																				
Ammoastuta salsa																				
Ammobaculites crassus					0				2	2	2		1		2					1
A. dilatatus	0	0	1	10	4	1	1		0	0	0		0		0					0
A. exiguus	1	7	3	0	0	0	1		2	12	6		3	0	7			1		
Ammonia beccarii	0	0	3	15	7				0	8	5		2	1	0			0		
A. tepida	5	1	5	0	0	2	1		5	4	5		3		2			0		1
Ammotium salsum			4	3	2	0	1		0	3	0		0		0			1		0
Angulogerina sp.			6	0	7	1	1	1	7	8	1		1		2			0		1
Arenoparella mexicana			1	0	0	1	1	0	1	3	0		0		0			1		
Bolivina sp.			0	0	7	0	1	0	3	1	0		0		1			0		
Buliminella elegantissima			5	5	0	5	2	0	0	0	0		0		0			0		
Cribroelphidium cf. *C. vadescens*			1	6	1	7	1	0	5	0	5		0		0			0		
Elphidium advenum			4	6	0	0	0	0	3	0	0		0		0			0		
E. brooklynense					4					1								4		
E. clavatum	2				0					0	1									
E. galvestonense	3				20	3	2		16	7	32	5		6	44			3	1	
E. poeyanum						8	2		4	18	14	5		6	0			2	9	
E. tumidum				9																
Elphidium sp.				0														0	2	
Globigerinidae																		1	0	
Haplophragmoides mexicanus		0	0	12	1	1	1		10	2	4	1								
H. wilberti		3	2	24	7	0	1		5	3	4	0								
Miliolidae	0	0	0	16	0	0	2	4	4	4	5		2	5	16			3	1	3
Miliammina fusca	3	15	9	4	7	3	0	2	1	5	3	1	0	2	0			2	0	0
Nonionella atlantica											0	0			1					
Protelphidium tisburyense		0	1	2	1	1		16	8	8	40		1	21	89			13	12	2
Reophax nana		5	0	0	2	1		6	5	5	2		0	2	2			27	6	1
Rosalina sp.	9		4	42	7	2	1		19	8	8		28	1	27			3	4	1
Tiphotrocha comprimata	18		16	3	25	8	0		4	24	3		14	1	1			2	1	1
Trochammina inflata			0					3	1		1									
T. lobata			2					0	0		0									
T. macrescens		1	1	4	0	1			1	2	2		2		2			1		1
		0	8	0	1	0			6	0	0		0	4	0			0	1	0
		0	0	1	0				0	3	0		0	0	1				1	0
		2	1	0	1															
		0	3	2	0	2	1			2	1	1	2	3	1				1	
		2	0	0	5	0	0			0	0	0	0	0	0				1	

Table 2. Individual sample data for control pond 1.

Control Pond 2

STATION NO.	7	30	31	32	33	54	55	56	75	76	88	89	90	91	92	93	94	95
LIVING SPECIES	12	6	8	4	1	9	8	9	8	8	2	0	8	6	15	13	12	10
TOTAL SPECIES	12	8	9	4	1	9	8	10	9	9	13	0	11	11	15	15	12	11
No. LIVING/CC(WET)	23.0	16.5	21.8	68.0	<1	12.5	27.4	6.8	12.0	6.4	1.2	0	49.2	16.8	65.0	53.8	25.4	41.8
TOTAL No./CC(WET)	46.4	43.8	44.7	84.2	<1	22.5	38.3	8.8	23.0	9.4	76.8	0	95.0	25.0	85.8	92.6	45.2	61.6
No. Individuals Living/Sample																		
No. Individuals Dead/Sample																		
Ammoastuta salsa																		
Ammobaculites crassus																		
A. dilatatus														0				
A. exiguus													0	2	1	3		
Ammonia beccarii	9	5	10	46		8	16	2	4	4	1		5	0	0	0	58	
A. tepida	14	7	23	20		10	10	4	6	2	58		18	4	8	18	15	
Ammotium salsum	1	0	5	10			2		2	2	0		3	9	5	5		6
Angulogerina sp.	5	4	20	10		2	0		2	1	5		7	0	3	2	5	4
Arenoparella mexicana	2							0	0	3	0		11	0	5	6	6	4
Bolivina sp.	0							1	1		11		24	2	5	13	56	
Buliminella elegantissima																		
Cribroelphidium cf. *C. vadescens*	3		25							1						14	4	5
Elphidium advenum	2		25							7						1	2	3
E. brooklynense																		
E. clavatum	18	19	18			42	72	24	33	13	1		88	30	123	69	63	100
E. galvestonense	8	9	43			44	19	0	41	0	57		94	21	38	26	46	26
E. poeyanum	4												2		1	4	1	2
E. tumidum	3												0		0	0	0	0
Elphidium sp.	33	18	36			4	48	2	18		0		7		3	3	15	5
Globigerinidae	38	17	51			23	20	0	10		4		3		4	2	14	2
Haplophragmoides mexicanus	5	5	2			3		1	9	2			6		0	8	9	5
H. wilberti	7	4	4	4		4		0	1	2			5		4	2	2	0
Miliolidae				2														0
Miliammina fusca	3	2	3	9		2	3	4	3	1	0		0	0	7	2	1	1
Nonionella atlantica	0	0	0	1		0	0	0	0	0	2		4	2	0	2	0	3
Protelphidium tisburyense	1		1			1	1		4	1	0		0	3	4	1	5	0
Reophax nana	0		0			0	0		0	0	2		4	8	0	1	0	3
Rosalina sp.	2	2	3	9		5	1	4	4	8	0		8	6	6	3	4	1
Tiphotrocha comprimata	0	0	0	1		0	0	0	2	5	2		2	3	0	2	2	9
Trochammina inflata			1								6		6		7	2	1	1
T. lobata			0								0		2		0	1	0	0
T. macrescens			2								2				4	3		
															0	0		
															1	1		
															0	0		

Table 3. Individual sample data for control pond 2.

Control Pond 3

STATION NO.	34	35	36	57	58	59	77A	78A	96	97	98	99
LIVING SPECIES	8	9	14	0	5	4	10	11	9	16	7	5
TOTAL SPECIES	11	12	17	0	5	6	13	13	10	16	7	5
No.LIVING/CC(WET)	162.6	0.4	68.3	0	8.9	7.0	11.8	51.3	44.0	37.0	24.0	61.5
TOTAL No./CC(WET)	211.4	9.3	81.1	0	10.1	9.4	17.6	68.6	53.6	44.2	45.0	73.5

No. Individuals Living/Sample
No. Individuals Dead/ Sample

Ammoastuta salsa		0 1										
Ammobaculites crassus	1 0		0 2									
A. dilatatus												
A. exiguus												
Ammonia beccarii	40	6	11		2	8	1	41	25	75	19	16
	5	9	16		2	7	4	13	8	11	31	6
A. tepida	13	5	5			0	0	31	21	8		
	3	7	18			2	4	33	2	15		
Ammotium salsum												
Angulogerina sp.												
Arenoparella mexicana	0 1	1 0	2 2									
Bolivina sp.												
Buliminella elegantissima												
Cribroelphidium cf. *C. vadescens*	376		2		7		1	43		4	2	
	68		7		1		0	4		0	3	
Elphidium advenum							1					
E. brooklynense							1	0				
E. clavatum	96	4	108		12	9	2	34	35	24	5	102
	45	60	75		2	9	5	61	13	11	2	14
E. galvestonense										2		
E. poeyanum							4	1	1	0		
							4	0	0	0		
E. tumidum			16									
			12									
Elphidium sp.							3					
							5					
Globligerinidae												
Haplophragmoides mexicanus		1	0					4				
		0	1					0				
H. wilberti	0	3	3			0	1		2	3		
	5	7	7			1	0		0	0		
Miliolidae	43	4	3			1	5	0	42	6	3	0
	1	0	2			1	0	8	2	3	4	2
Miliammina fusca	0		0			0	0	1	8	5		0
	2		3			0	2	6	11	2		3
Nonionella atlantica												
Protelphidium tisburyense	4	3	3				2	20	0	2	5	
	0	4	4				0	3	1	1	9	
Reophax nana												
Rosalina sp.												
Tiphotrocha comprimata			2									
			0									
Trochammina inflata		0	7					3	1	2		
		8	0					1	0	0		
T. lobata		1	2					1		1		
		2	0					0		0		
T. macrescens												

Table 4. Individual sample data for control pond 3.

Effluent Pond 1

STATION NO.	13	14	37	38	39	60	61	66	67	100	101	102	103	104	105	106	107	108
LIVING SPECIES	1	0	7	0	3	1	0	8	3	8	15	5	2	1	0	1	5	2
TOTAL SPECIES	5	0	7	6	4	6	2	9	6	8	18	10	3	4	2	4	12	6
No. LIVING/CC(WET)	0.2	0	3.2	0.4	0.8	-	0	18.8	1.4	30.0	26.1	16.2	0.3	0.6	0	0.3	6.5	0.6
TOTAL No./CC(WET)	1.6	0	4.8	1.3	2.8	-	0.3	22.8	3.4	43.5	33.3	22.6	1.3	0.8	<1	2.0	19.1	2.4
No. Individuals Living/Sample																		
No. Individuals Dead/Sample																		
Ammoastuta salsa																		
Ammobaculites crassus								2 0		1 0	1 0						1 0	
A. dilatatus											1 2 3						0 1 3	
A. exiguus			1 1 0								1	2 1 6					1 3 7	
Ammonia beccarii	0 1		0	0 1	1 2 3	0 2		6 0	6 5			2	5 5	2 1	0 1			
A. tepida										3 1	5 1 8							
Ammotium salsum	1 0		6 3	0 2				5 1 0	0 5		2	2 9 1 9				0 1	1 7 3 2	0 1
Angulogerina sp.																		
Arenoparella mexicana				0 1		0 1		7 0	0 1			2 2	0 4	0 1	0 1		0 1	
Bolivina sp.																		
Buliminella elegantissima																		
Cribroelphidium cf. *C. vadescens*																		
Elphidium advenum																		
E. brooklynense																		
E. clavatum				0 2	1 4	0 5			3 0	3 1 1	2 9 5 0 1	3 7 6	2 1	1 0		1 0	1 2 3 0	2 0
E. galvestonense																		
E. poeyanum																		
E. tumidum			1 0				0 1					2 0 5						
Elphidium sp.																		
Globigerinidae																	1 0 0	
Haplophragmoides mexicanus	0 3					0 3		6 7		4 1 3	7 2	0 3				0 5	3	1 2
H. wilberti			2 0	0 3		0 1	0 1	0 1	0 1		1 0	2 0 1	0 1			0 1	0 1	
Miliolidae						0 1						0 1 0						
Miliammina fusca			3 0					1 0				2 2	0 2					0 1
Nonionella atlantica																		
Protelphidium tisburyense																		
Reophax nana																		
Rosalina sp.																		
Tiphotrocha comprimata											2 0							
Trochammina inflata	0 1		1 0		0 1			1 6 0	1 0	4 0	1 2 1	2 3		0 1			0 1	0 4
T. lobata										2 0	1 0 0	0 3						0 1
T. macrescens	0 2			0 1		1 0		4 0										

Table 5. Individual sample data for effluent pond 1.

Effluent Pond 2

STATION NO.	16	17	18	40	41	42	62	63	68	69	109	110	111	112	113	114	115	116	117	118
LIVING SPECIES	1	1	0	6	3	2	1	3	5	2	11	5	1	1	0	3	0	5	0	0
TOTAL SPECIES	2	2	0	6	7	5	1	5	9	2	11	9	2	1	0	4	0	9	0	3
No. LIVING/CC(WET)	0.3	0.2	0	6.0	3.3	0.8	0.6	0.1	1.0	0.9	40.2	22.3	1.3	0.8	0	30.4	0	47.2	0	0
TOTAL No./CC(WET)	0.4	0.7	0	6.4	6.0	2.6	0.6	0.2	7.2	0.9	51.0	35.6	1.3	0.8	0	37.2	0	71.2	0	5.6
No. Individuals Living/Sample																				
No. Individuals Dead/Sample																				
Ammoastuta salsa																				
Ammobaculites crassus					0/1						1/0									
A. dilatatus											1/0									
A. exiguus					0/1				3/0		4/0	3/5				0/1		0/5		
Ammonia beccarii				1/0																
A. tepida																				
Ammotium salsum	2/0			5/1	1/5	0/1	3/1	2/0	2/0		12/2	7/23				2/2		36/12		0/2
Angulogerina sp.																				
Arenoparella mexicana									1/1											
Bolivina sp.																				
Buliminella elegantissima																				
Cribroelphidium cf.*C. vadescens*				2/0					0/1											0/2
Elphidium advenum																				
E. brooklynense												0/1						1/0		
E. clavatum				5/3	0/3	0/12			0/5	3/10	15/15	32/16	0/2			6/5		13/18		0/16
E. galvestonense																				
E. poeyanum																				
E. tumidum				4/1	8/4	3/1					60/22	36/12	2/0	4/0		55/16		56/20		
Elphidium sp.																				
Globigerinidae																				
Haplophragmoides mexicanus	0/1	1/2			1/3				0/1		5/1	2/14						8/3		
H. wilberti								0/1												
Miliolidae						0/1		0/1	1/0											
Miliammina fusca		0/1						0/1										0/1		
Nonionella atlantica																		0/3		
Protelphidium tisburyense																		0/1		
Reophax nana																				
Rosalina sp.																				
Tiphotrocha comprimata									1/0		1/0									
Trochammina inflata					0/1	1/0		1/0	0/7		0/0	0/4								
T. lobata																				
T. macrescens				1/0						1/0	1/0	0/1								

Table 6. Individual sample data for effluent pond 2.

Effluent Pond 3

STATION No.	19	20	21	22	23	43	44	45	64	65	70	71	119	120	121	122	123	124	125	126	127
LIVING SPECIES	1	0	0	0	2	1	2	6	2	0	9	9	9	2	0	4	7	1	9	9	7
TOTAL SPECIES	4	0	0	1	2	2	3	6	2	0.2	9	13	12	2	5	12	12	7	11	14	10
No. LIVING/CC(WET)	1.7	0	0	0	1.4	0.2	20.2	0.2	0.3	0	7.2	2.9	9.6	0	0	36.0	6.4	0	55.0	35.3	2.4
TOTAL No./CC(WET)	7.7	0	0	0.3	1.4	0.4	23.6	2.6	0.3	0.2	8.8	3.7	14.0	0.8	2.0	53.6	9.4	5.8	74.0	59.2	6.3
No. Individuals Living/Sample																					
No. Individuals Dead / Sample																					
Ammoastuta salsa																	0				
Ammobaculites crassus								1									1		1	0	
A. dilatatus								0			4	1	2				2		1	2	
A. exiguus											0	0	0				1		0	0	
Ammonia beccarii	0				2		7	1			4	4	3		0	11	6	0	21	33	0
A. tepida	4				0		0	1			0	0	0		1	10	1	2	6	16	3
Ammotium salsum												2	5			3	1		7	9	0
Angulogerina sp.								2			2	0	2			7	1		8	5	5
Arenoparella mexicana								2			3	1	6			4	4	1	3	5	3
Bolivina sp.								0					0			0	0	0		0	
Buliminella elegantissima								0			1	0	1			1	1	1		5	
Cribroelphidium cf. *C. vadescens*	0																			1	
Elphidium advenum	1										5		0							0	
E. brooklynense											1		1								
E. clavatum	6						35		1		0		6		0		15	0	19	14	1
E. galvestonense	3						6		0				1		1		1	2	3	8	2
E. poeyanum	0						1				2				0		0	0	6	4	1
E. tumidum	2										0	2							1	3	0
Elphidium sp.												0		2						0	
Globigerinidae													6	0						4	
Haplophragmoides mexicanus													1	1						0	
H. wilberti											1	0		0	0	0		0	0	2	1
Miliolidae											0	2	2	0	1	2		1	1	4	0
Miliammina fusca												0	0			1	1		1	0	0
Nonionella atlantica												2	1				0		0	0	2
Protelphidium tisburyense					5	1		1	1		4	1			0		0	0	1	1	1
Reophax nana					0	0		0	0		0	0			1		2	3	1	6	0
Rosalina sp.												1	1								
Tiphotrocha comprimata												0	0								
Trochammina inflata				0		0		2		0	1					1	0	1	4	1	0
T. lobata				2		1		0		1	0	1	1			3	1	3	2	1	2
T. macrescens												0	0			0					
																2					
																0					
																1					

Table 7. Individual sample data for effluent pond 3.

Table 8. Percentages of dominant, common, and rare living species occurring in the total living foraminiferal populations of the control and effluent ponds in November and December, 1970, and February, April, and June, 1971.

Living Species	Percentage (%) Occurring in Total Living Population					
	C-1	C-2	C-3	P-1	P-2	P-3
Dominant						
Ammonia beccarii	4.9	25.8	14.9	7.6	1.1	11.2
Ammotium salsum	10.5	1.4	5.0	17.3	16.6	4.6
Elphidium clavatum	12.6	37.0	26.4	37.0	17.0	66.5
Elphidium tumidum	1.1	10.0	3.1	5.5	54.0	<1
Miliammina fusca	22.4	10.6	5.0	1.6	-	<1
Common						
Ammobaculites exiguus	4.2	<1	<1	3.4	2.5	0
Ammonia tepida	<1	3.0	4.2	2.6	<1	4.5
Cribroelphidium cf. *C. vadescens*	0	2.7	26.4	0	<1	0
Haplophragmoides mexicanus	3.3	0	<1	4.7	3.9	0
H. wilberti	6.8	1.6	<1	1.3	<1	<1
Protelphidium tisburyense	17.1	<1	2.4	0	0	1.8
Trochammina inflata	4.2	<1	<1	9.4	<1	1.3
Rare						
Ammobaculites crassus	1.0	0	<1	1.3	<1	<1
A. dilatatus	4.6	0	<1	<1	<1	1.1
Arenoparella mexicana	1.5	<1	<1	2.4	<1	<1
Bolivina sp.	0	0	0	0	0	<1
Buliminella elegantissima	0	0	0	0	0	<1
Elphidium advenum	0	0	<1	0	0	0
E. brooklynense	<1	0	0	0	<1	0
E. galvestonense	0	<1	<1	0	0	1.2
E. poeyanum	0	0	<1	0	0	<1
E. sp.	0	3.1	<1	0	0	<1
Globigerinidae	0	<1	0	<1	0	0
Miliolidae	<1	<1	6.5	<1	<1	2.2
Nonionella atlantica	0	<1	0	0	0	0
Reophax nana	<1	<1	0	0	0	0
Rosalina sp.	<1	<1	0	0	0	<1
Tiphotrocha comprimata	1.5	<1	<1	<1	<1	0
Trochammina lobata	<1	0	<1	0	0	<1
T. macrescens	2.0	<1	<1	4.5	<1	0

Table 9. Percentage (%) similarity calculated for all combinations of ponds.

	C-1	C-2	C-3	P-1	P-2	P-3
C-1	-	35.7	35.9	49.2	34.6	29.7
C-2	35.7	-	61.3	58.7	33.8	57.7
C-3	35.9	61.3	-	50.1	30.4	54.1
P-1	49.2	58.7	50.1	-	49.9	55.5
P-2	34.6	33.8	30.4	49.9	-	25.4
P-3	29.7	57.7	54.1	55.5	25.4	-

Table 10. Estimated annual productivity of foraminifera in control and effluent ponds, expressed as grams (dry protoplasmic weight) per pond.

	Standing Crop		Annual Productivity			
	No. Living per cc	No. Living per cm^2	No. per m^2 ($x10^4$)	grams (dry weight) per m^2	Pond Area m^2	grams per pond
C-1	8.5	4.16	4.16	0.19	500	95.
C-2	23.0	8.06	8.06	0.37	559	206.8
C-3	39.5	11.63	11.63	0.51	525	267.8
E-1	4.1	2.56	2.56	0.09	394	35.5
E-2	3.8	2.43	2.43	0.09	438	39.4
E-3	5.5	3.10	3.10	0.14	382	53.5
Average						
C	23.7	8.24	8.24	0.37	528	195.4
E	4.5	2.72	2.72	0.14	404	56.6

Table 11. Ratio of living to total number of foraminifera found in each pond per month, used as an index to relative rates of sediment deposition.

	C-1 %	C-2 %	C-3 %	E-1 %	E-2 %	E-3 %
Nov.	-	50	-	12.5	43	30
Dec.	30	58	77	48	63	76
Feb.	23	67	82	0	67	67
April	83	56	73	74	24	82
June	63	57	79	62	69	62
Average	54	58	77	62	66	63

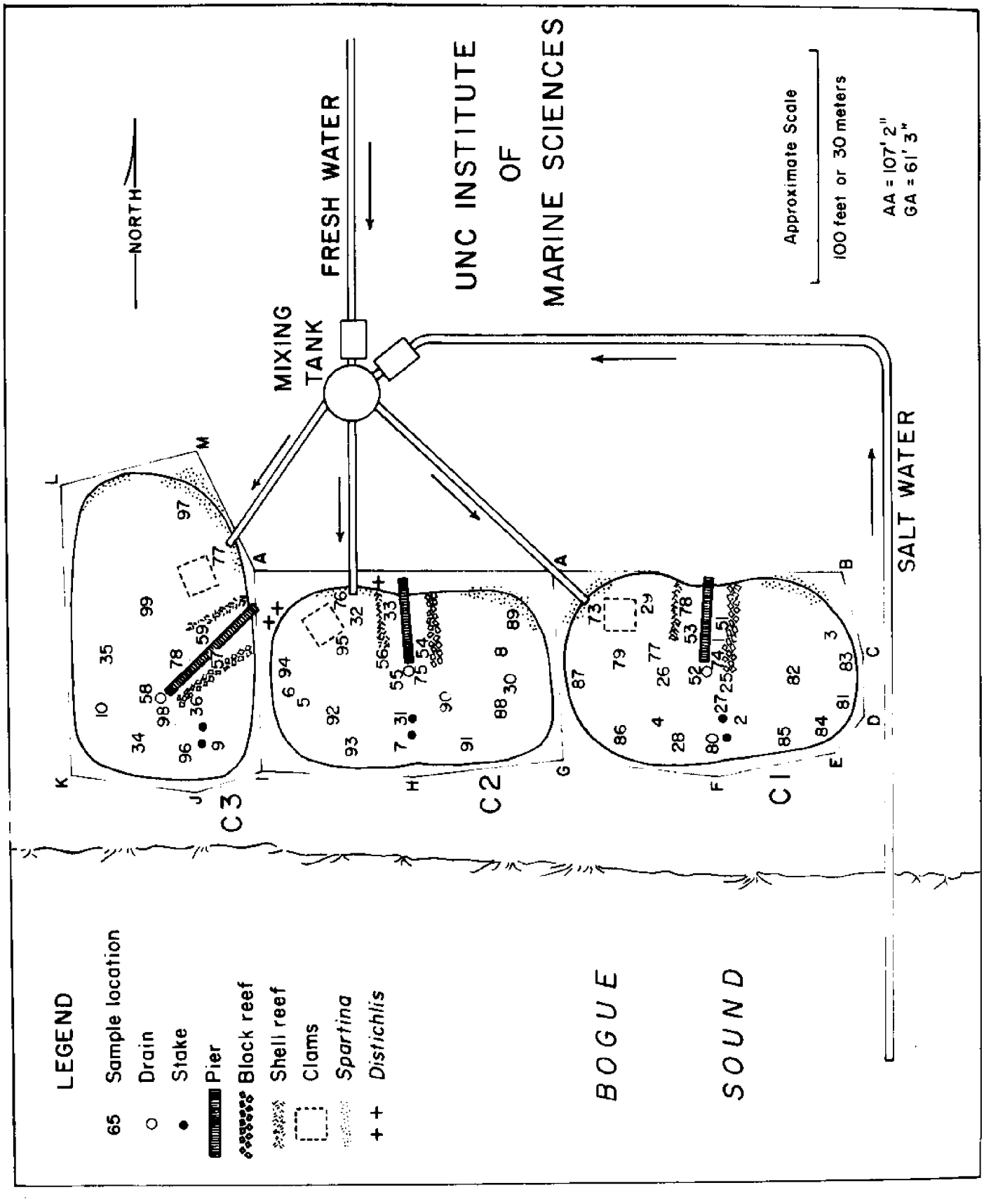

Fig. 1. Sketch of control ponds illustrating water flows and approximate locations of sampling stations.

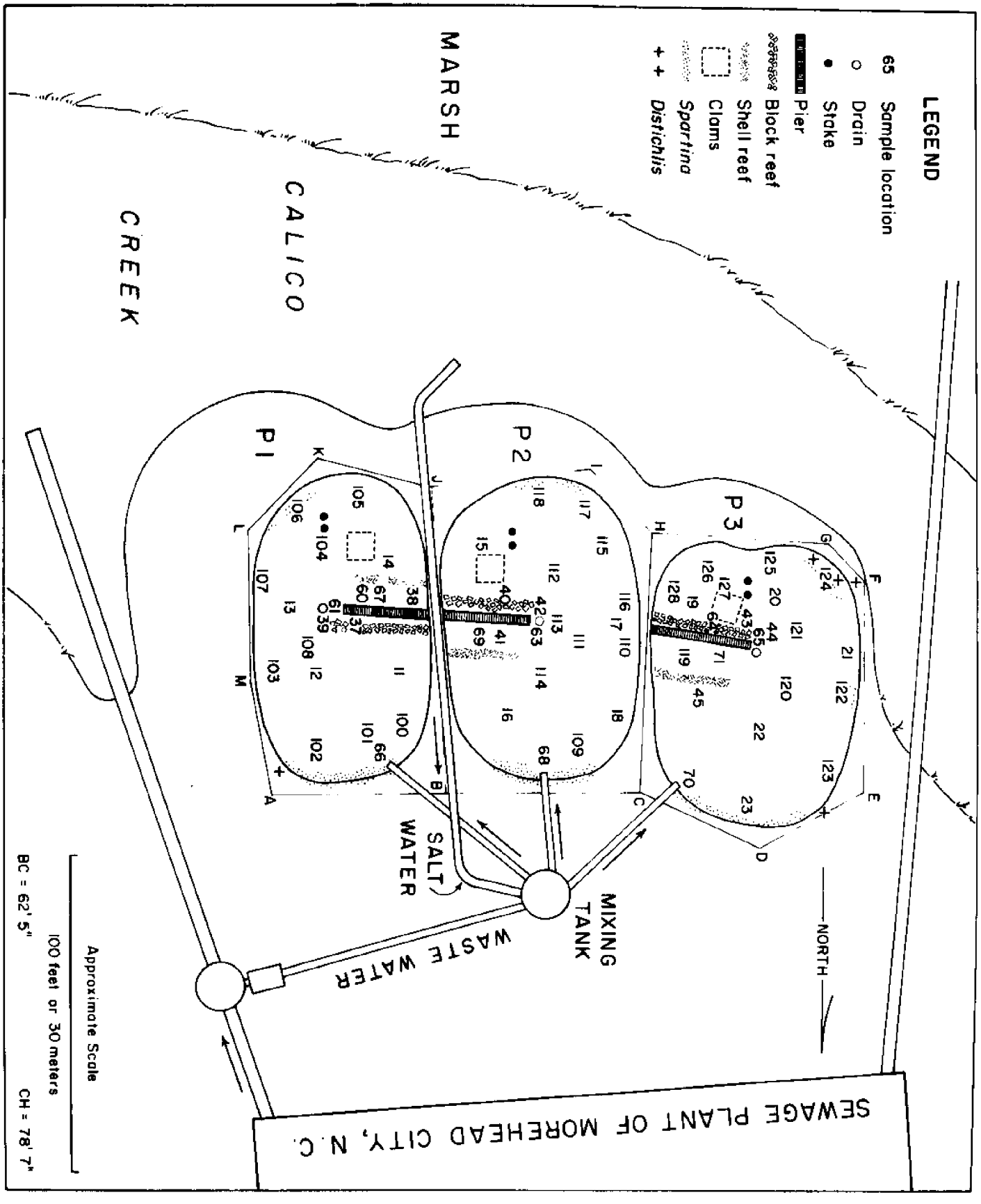

Fig. 2. Sketch of effluent ponds illustrating water flows and approximate locations of sampling stations.

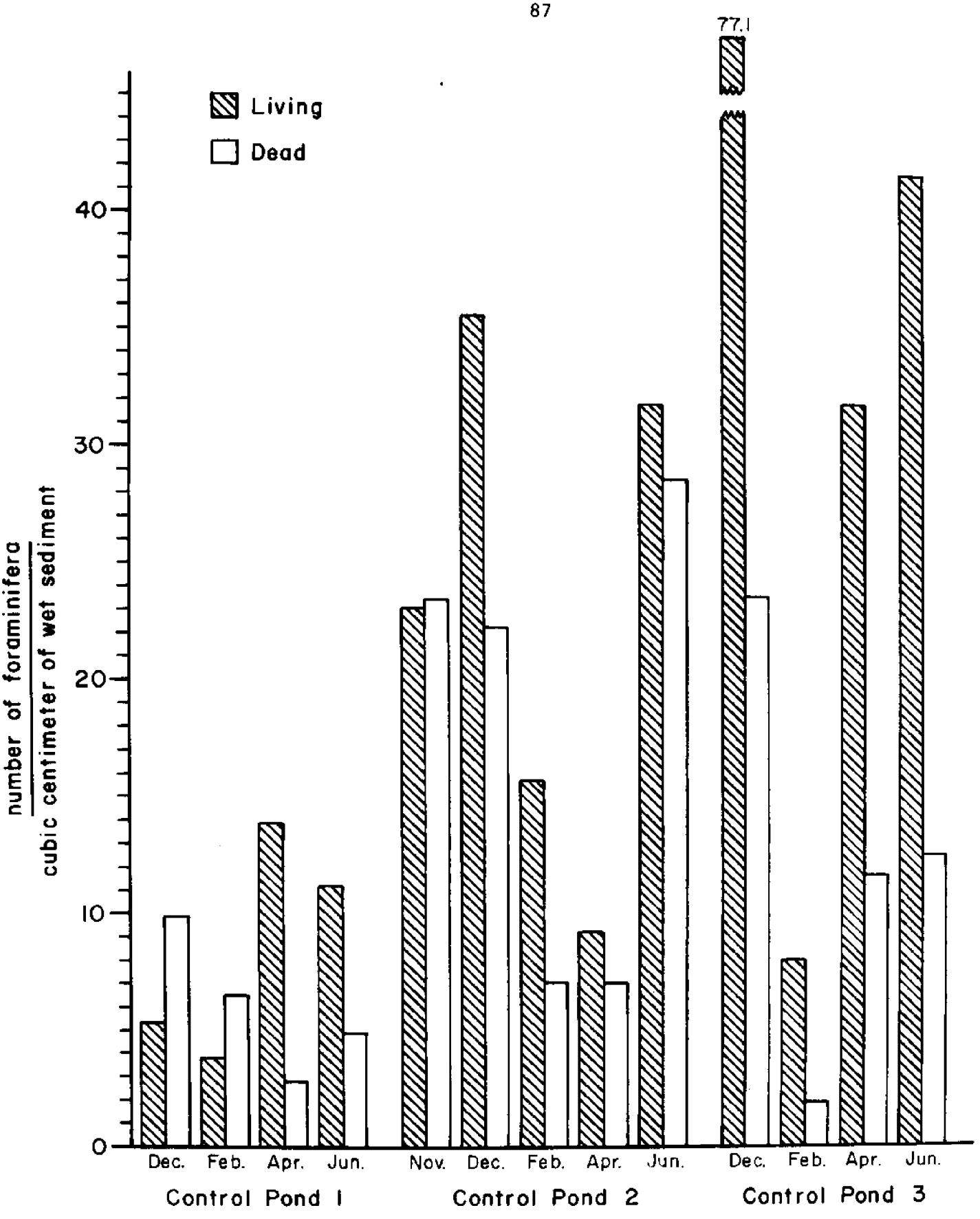

Fig. 3. Average monthly standing crops of living and dead foraminifera in the control ponds, expressed as number of foraminifera per cubic centimeter of wet sediment and calculated from standing crops of individual samples taken in each pond in November, December, 1970, and February, April, and June, 1971.

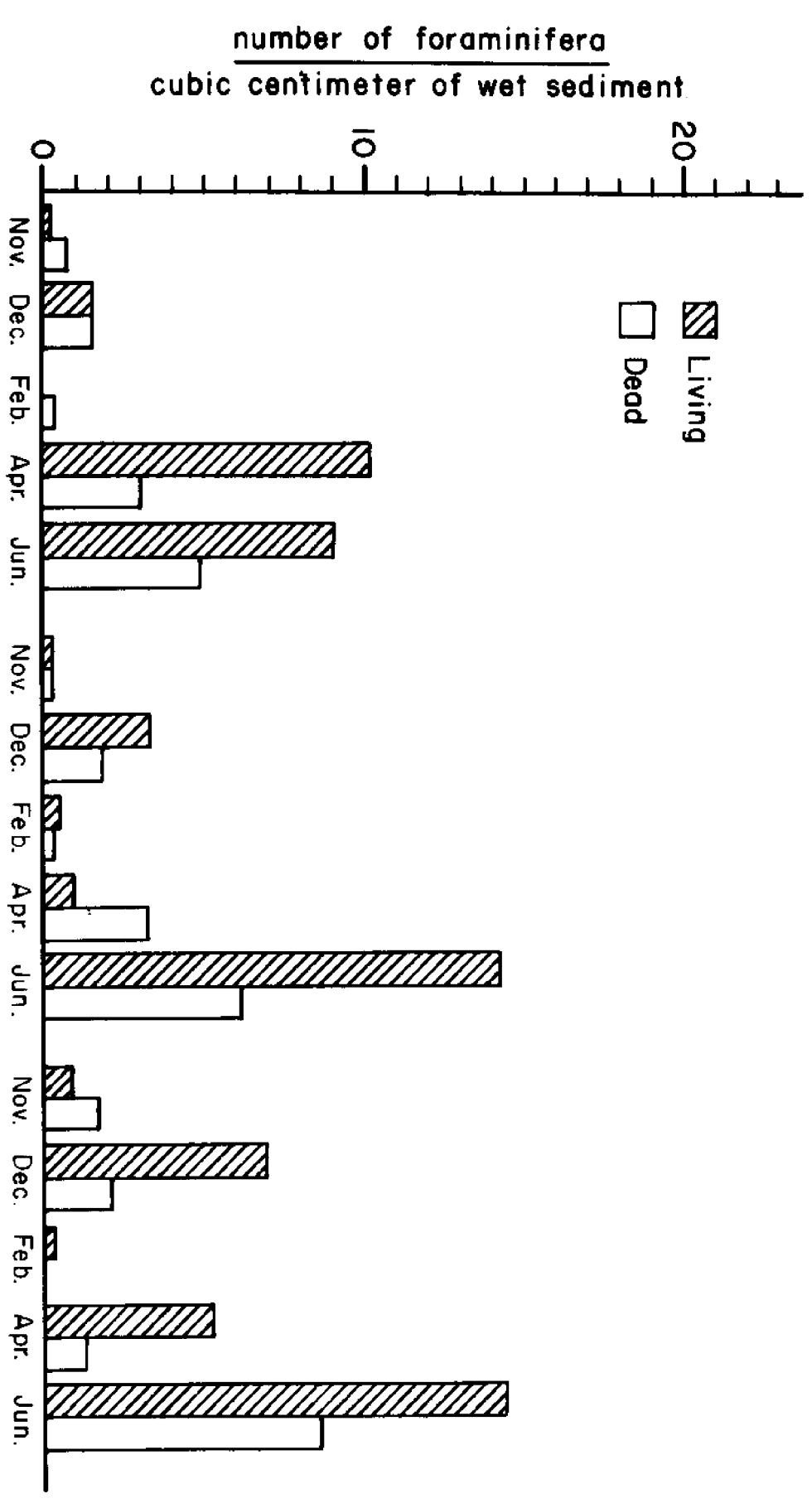

Fig. 4. Average monthly standing crops of living and dead foraminifera in the effluent ponds, expressed as number of foraminifera per cubic centimeter of wet sediment and calculated from the standing crops of individual samples taken in each pond in November, December, 1970, and February, April, and June, 1971.

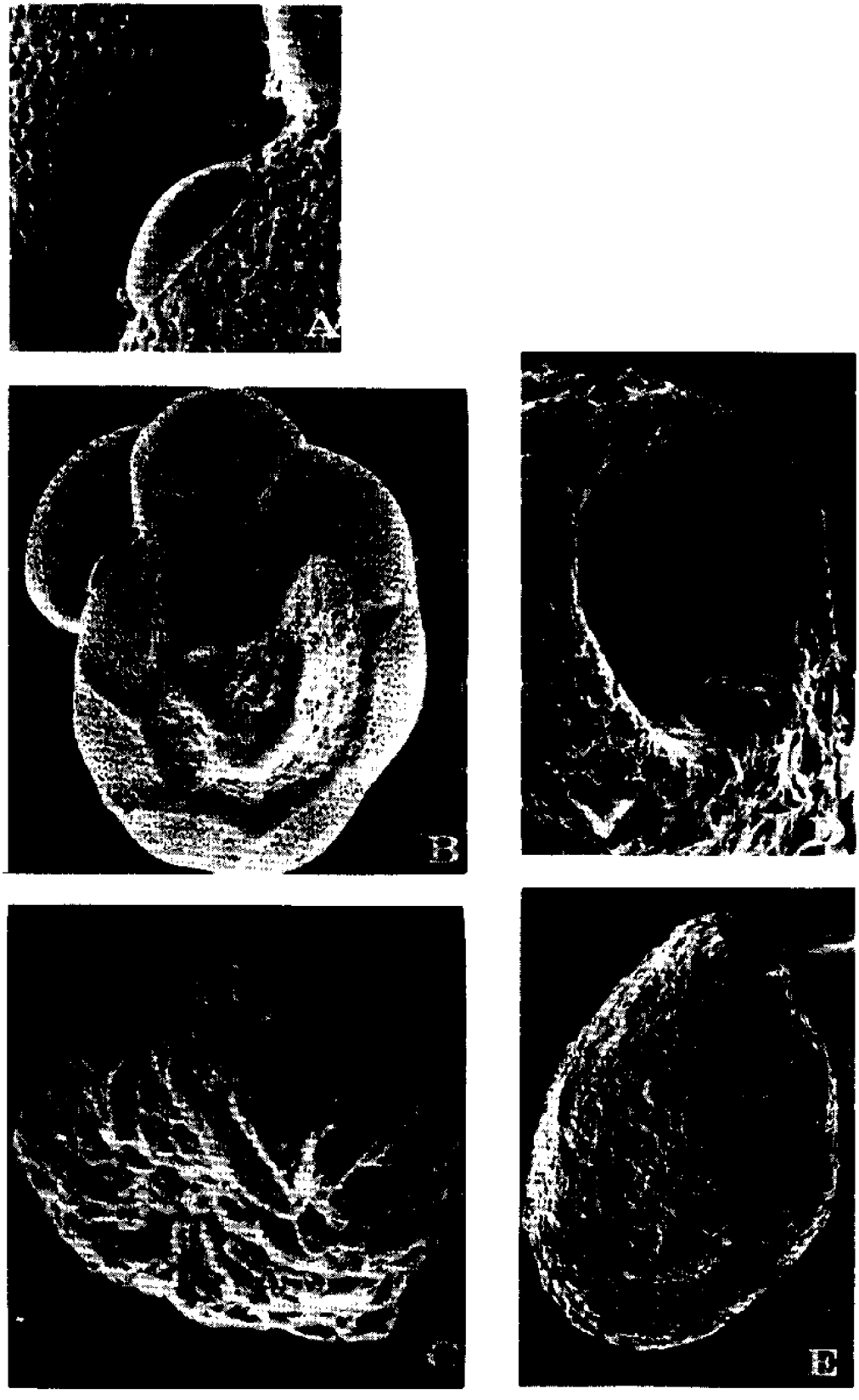

Fig. 5. Scanning electron micrographs of dominant species. A, B. Ammonia becarii (Linnaeus). A. Ostracod valve on last chamber x 280, B. x 90. C. Ammotium salsum (Cushman and Bronnimann) x 315. Note single large crystal below aperture. D, E. Miliammina fusca (Brady). D. aperture x 800, E. x 220.

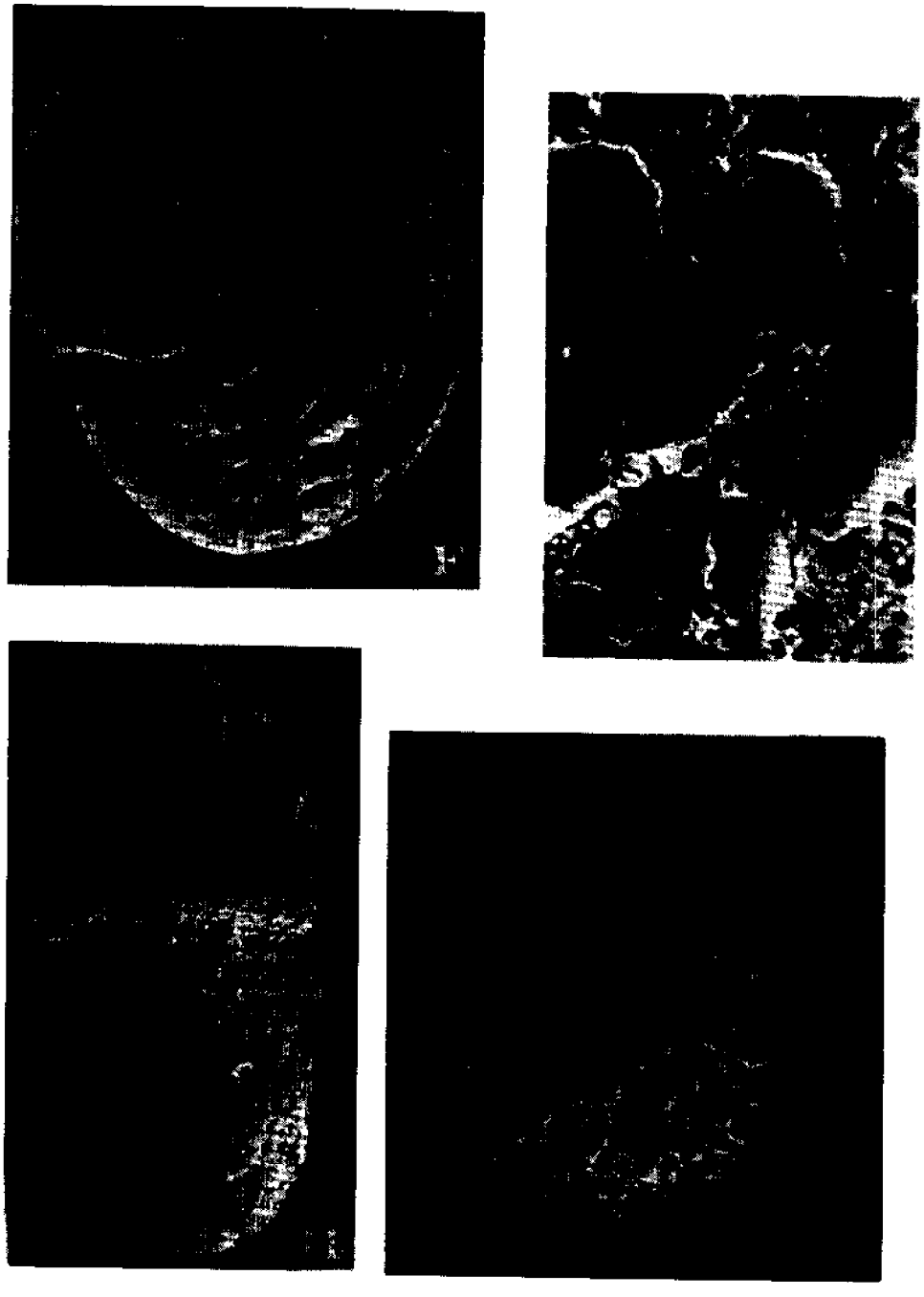

Fig. 6. Dominant species. F, G. Elphidium clavatum Cushman. F. x 260, G. etched and pitted umbilical area x 675. H, I. Elphidium tumidum Natland. H. apertural face x 340, I. side view illustrating prominent retral processes, depressed and etched umbilical area x 310.

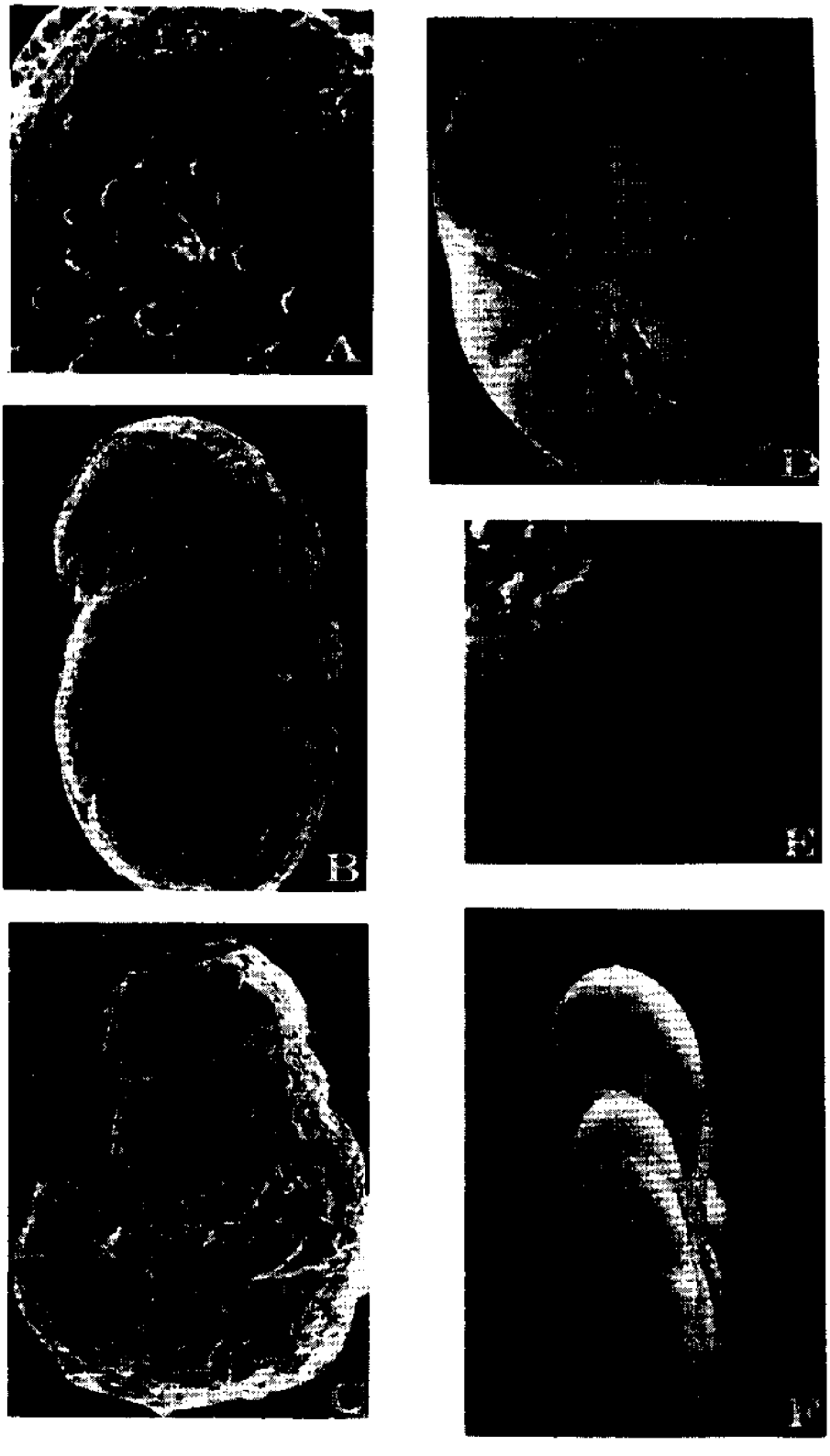

Fig. 7. Scanning electron micrographs of common species. A B. Cribroelphidium cf. C. vadescens Cushman and Bronnimann. A. aperture with ornamentation x 1075, B. x 750. C. Ammobacu - lites exiguus Cushman and Bronnimann x 290. D, E, F. Protelphidium tisburyense Butcher. D. x 180, E. tubercles at apertural face and suture lines x 695, F. edge view emphasizing depressed suture lines and umbilical area x 150.

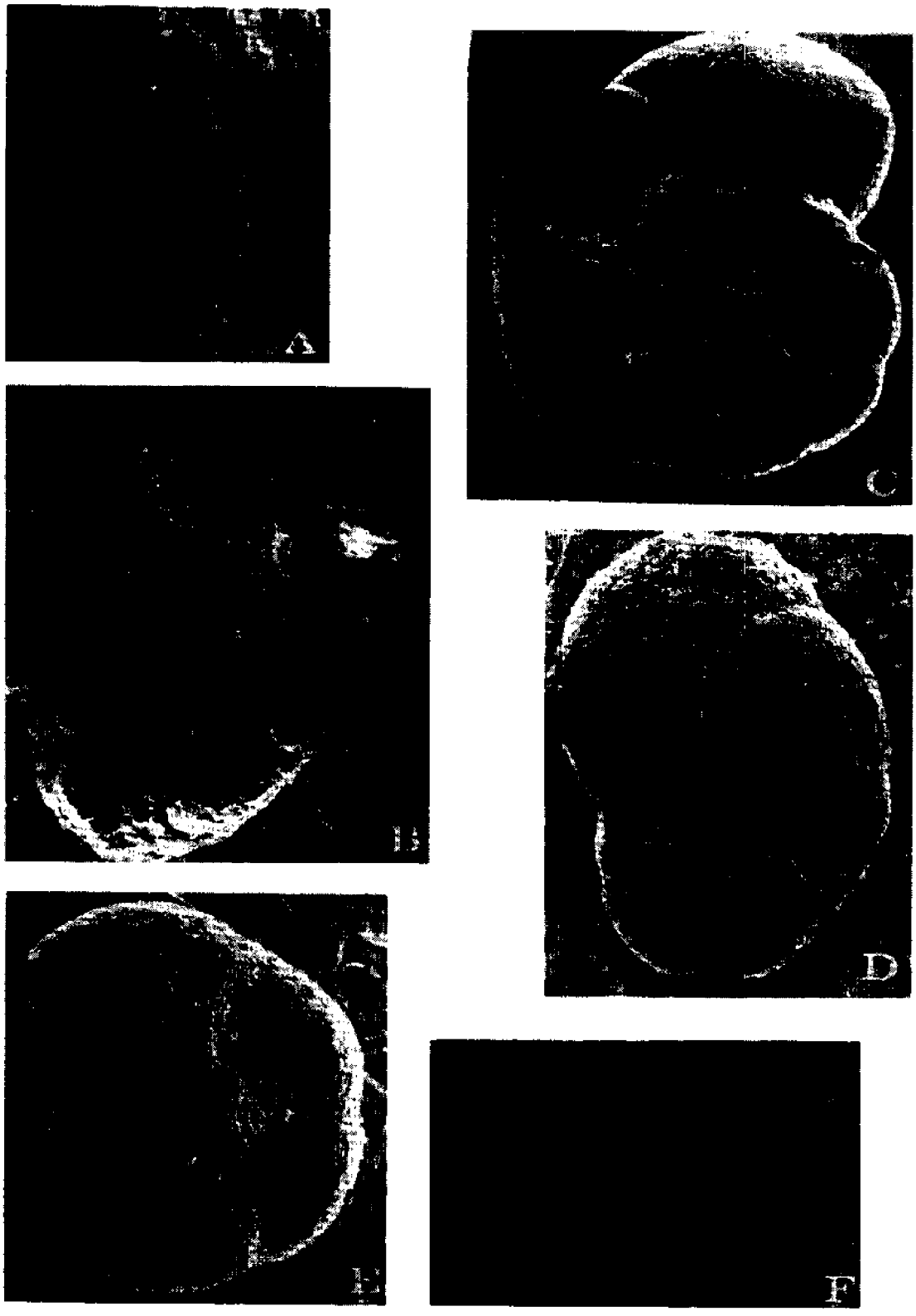

Fig. 8. Scanning electrom micrographs of rare species. A, B. Ammobaculites dilatatus Cushman and Bronnimann. A. spicules with orientation perpendicular to test x 590, B. x 195. C. Trochammina macrescens Cushman x 270. E F. Arenoparella mexicana (Kornfeld). E. x 115, F. closely cemented arenaceous wall x 290.

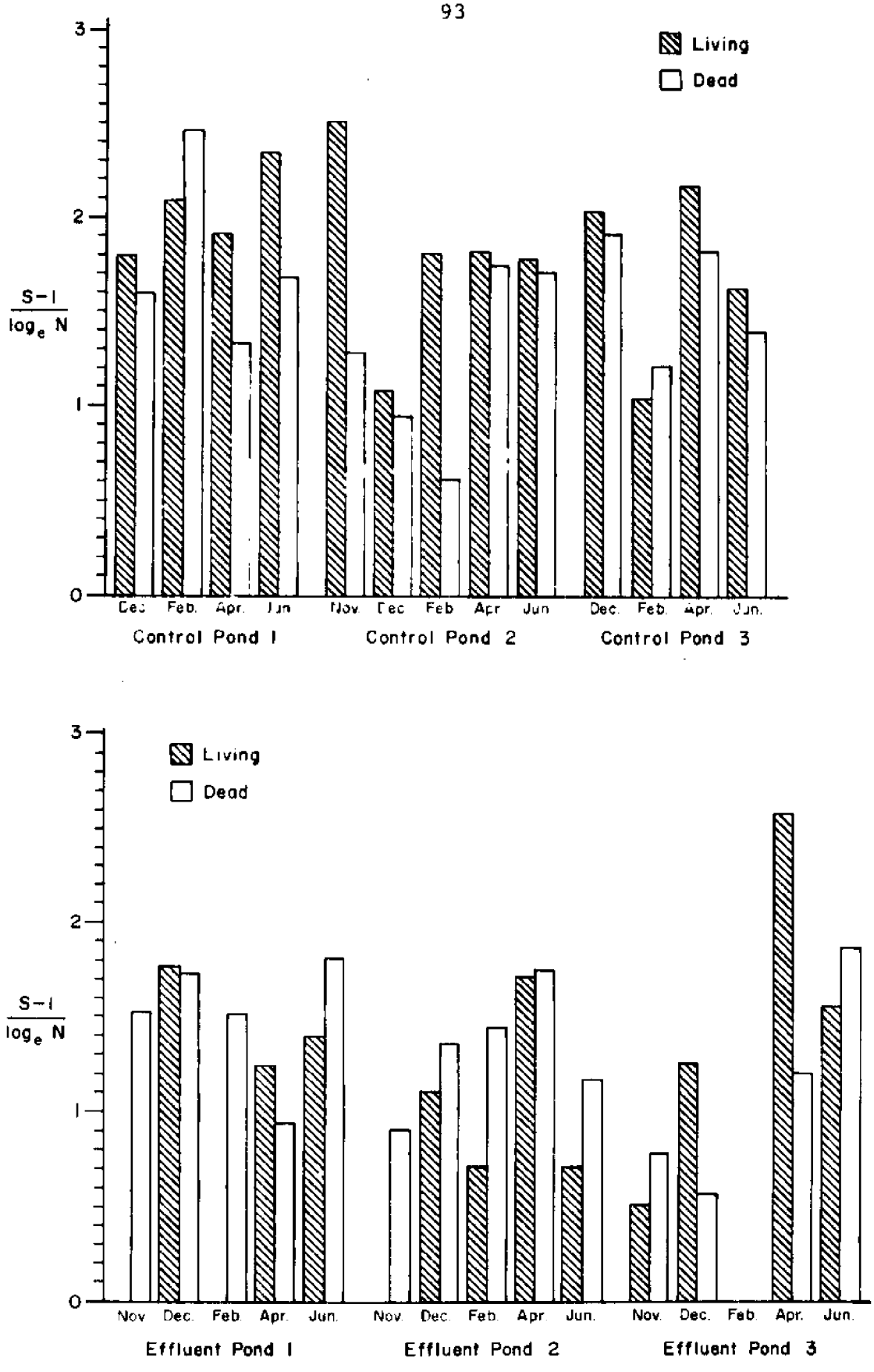

Fig. 9. Average monthly species diversity of living and dead foraminifera in the control and the effluent ponds. Species diversity = S-1 $\log_e N$, where S = number of species present, $\log_e N$ = natural logarithm of the number of individuals present per sample.

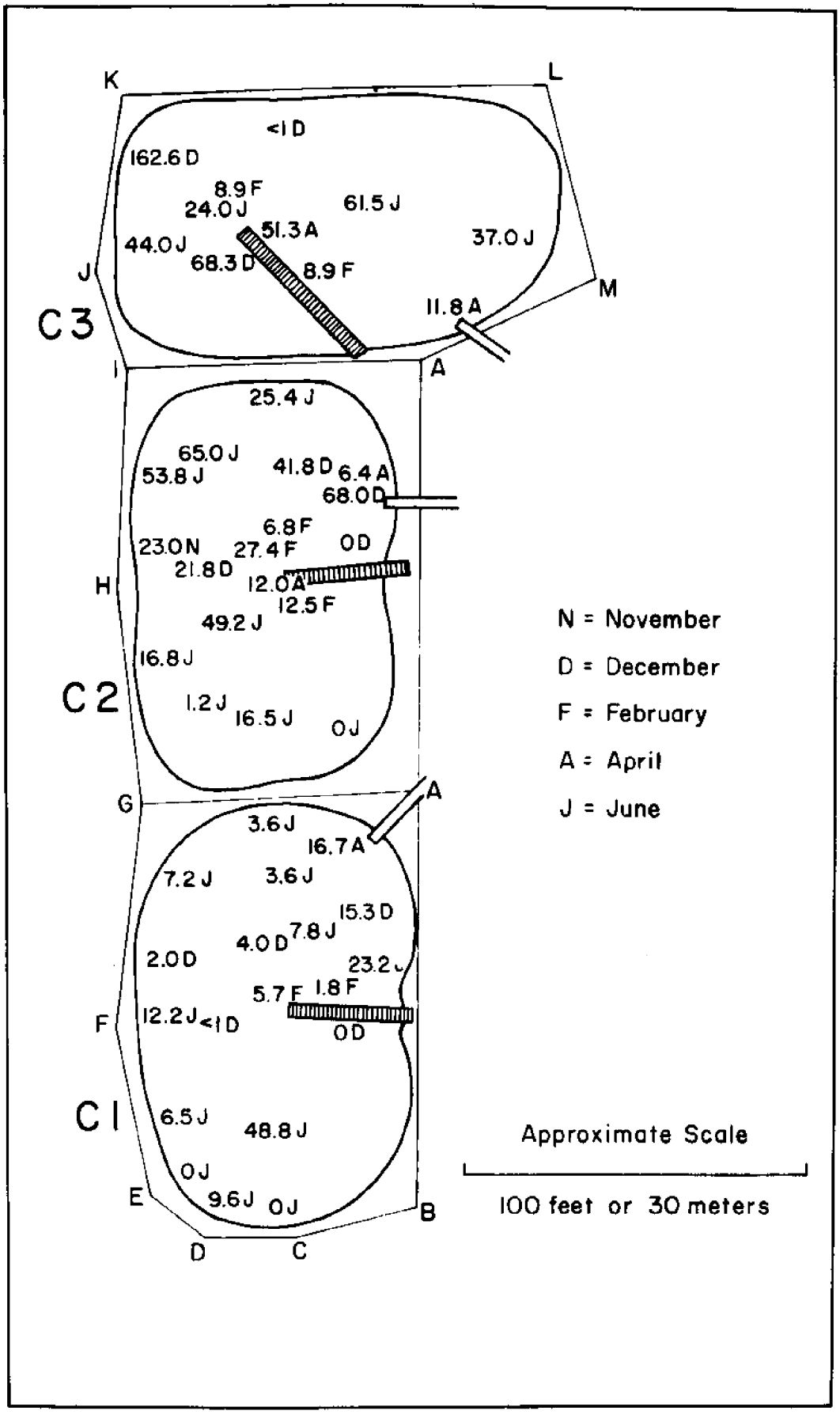

Fig. 10. Areal distribution of standing crops of living foraminifera in individual monthly samples in the control ponds expressed as number of living foraminifera per cubic centimeter of wet sediment.

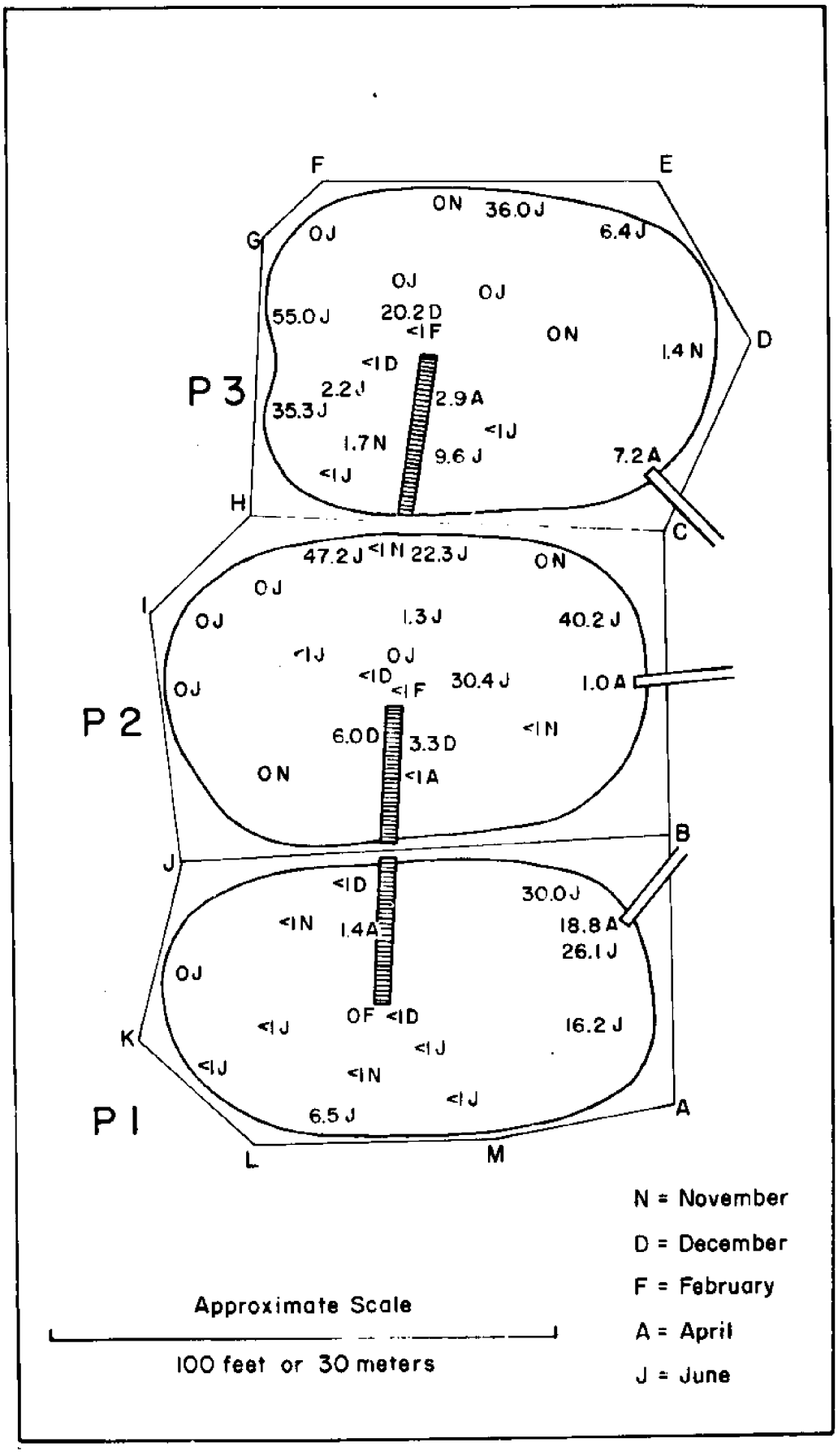

Fig. 11. Areal distribution of standing crop of living foraminifera in individual monthly samples in the effluent ponds expressed as number of living foraminifera per cubic centimeter of wet sediment.

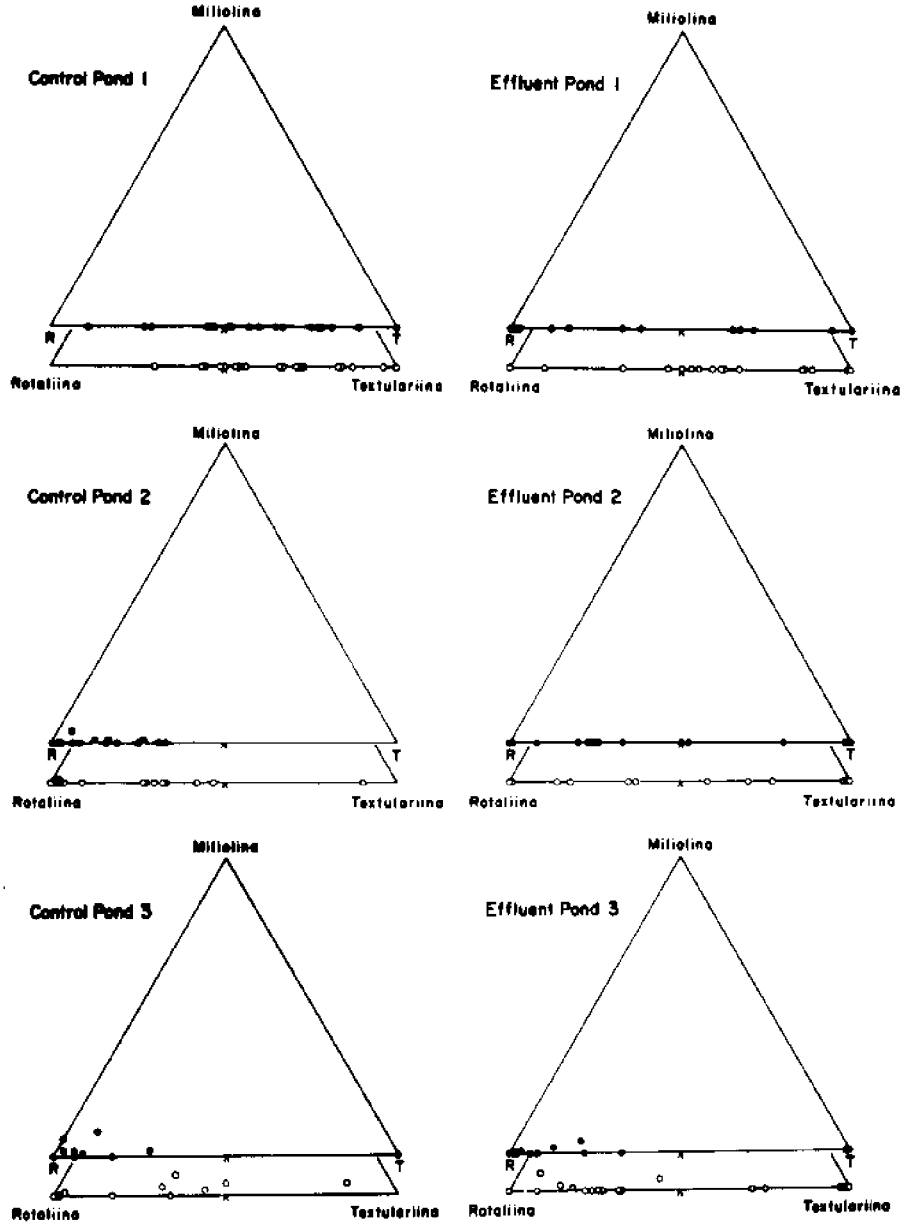

Fig. 12. Trigonal graphs of the percentages of living and dead Textulariina, Rotaliina, and Miliolina found in individual samples from control and effluent ponds. Upper triangle living, lower, dead.

Niche Partition among *Corophium acutum*, *C. lacustre* and *C. acherusicum* (Crustacea:Amphipoda). Effects of Salinity and Turbidity.

Richard S. Fox and Austin B. Williams

This is a recently initiated study and no results are available. Permanent sampling stations have been established at 16 localities in the Newport and North Rivers while 2 additional stations are located in the "P" and "C" ponds (Fig. 1). These stations were established to provide standard collections of corophiids from a regular range of salinities and turbidities. These stations were arranged along 2 transects beginning in Beaufort Inlet at Ft. Macon and in one case extending up the Newport River to the "narrows" and in the second case extending up the North River to State Road 1300. A branch of the Newport River transect extends up Calico Creek to the Morehead City sewage treatment plant. The North River transect will serve as a control against which the Newport transect will be compared to determine the effects of turbidity and organic particulates. Both transects will yield independent data concerning the effects of salinity.

The animals under consideration are fouling organisms which construct detritus tubes on submerged objects. To assure regularly available uniform samples it was decided to use artificial substrates at each station. This avoided the necessity of relying on natural substrates which would not always be present or uniform. The artificial substrates were designed to allow monthly replacement and will be used to provide data on population size and structure, fecundity and reproductive patterns for each of the 3 species.

The artificial substrate sampler consists of a 3.8 x 13.7 cm strip of pine lathing attached to a 3.8 x 3.8 x 25 cm pine block which is suspended about 30 cm below the water surface (Fig. 2). The base is bouyed up by a 2 l plastic jug and is held vertical by a 1 kg weight. The entire apparatus is held in poisition by a 4.5 kg anchor. When possible the samplers were positioned in about 2 m of water at high tide. Stations 1, 2, 10, and 16, however, could not be placed at this depth because of physical characteristics of the river. Each sampling device was equipped with a second replaceable plate to provide replication.

In addition to the standard samplers described above, 7 stations (1, 4, 6, 8, 12, 14 and 16) were equipped with a second artificial substrate designed to provide information on successional changes in the population characteristics of the 3 amphipod species. These samplers are similar to the standard samplers but are provided with 4 replaceable plates. One plate will be removed each quarter for a year.

Each station will be visited monthly by boat or car. The plates will be replaced and the old ones returned to the laboratory for analysis. Salinity and turbidity samples will be taken and returned to the laboratory for determination. Temperature will be measured in the field. Future work will include determination of physiological salinity optima and salinity and turbidity preferences of the 3 species.

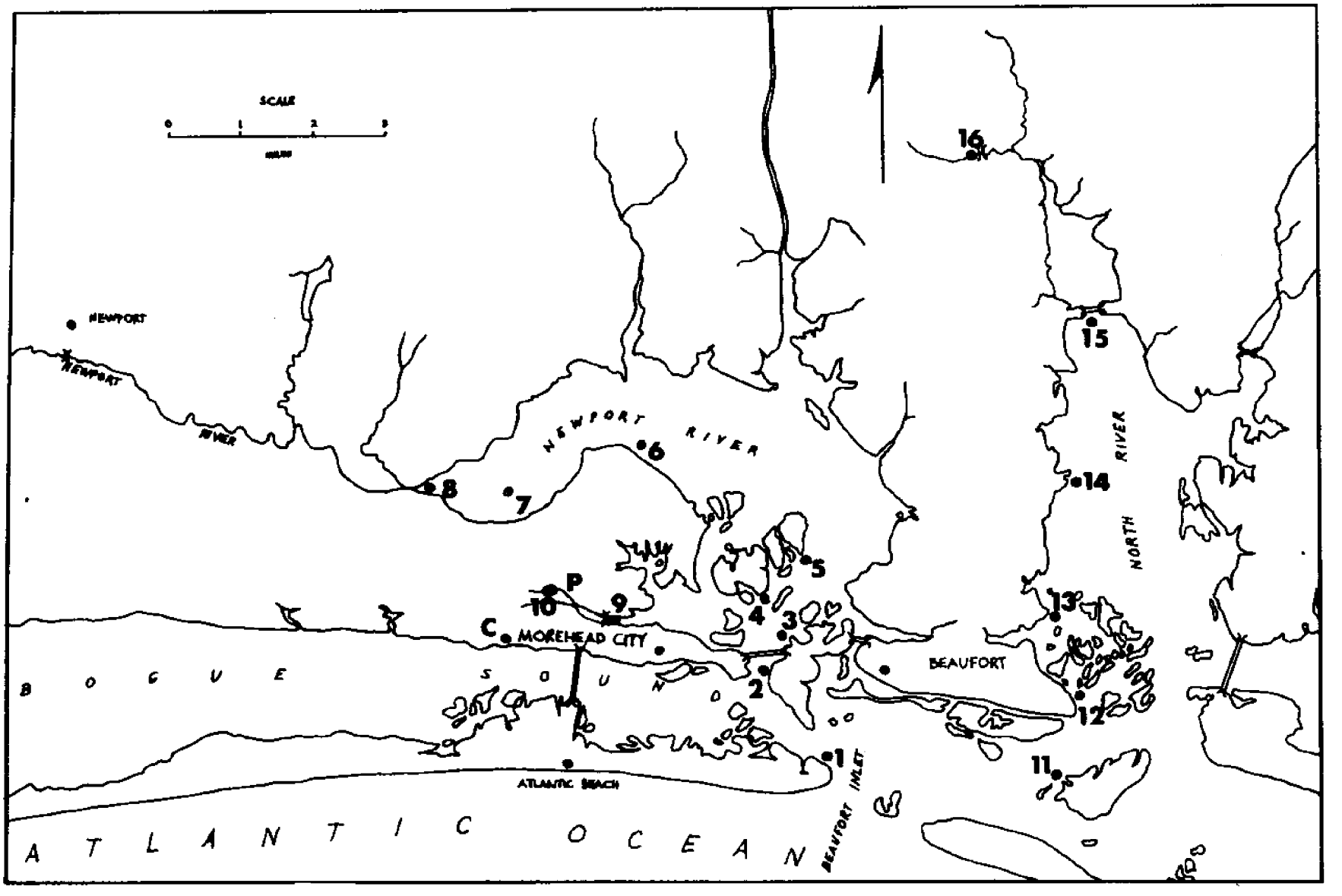

Figure 1. Newport-North River area, Carteret County, North Carolina showing sampling stations. (After North Carolina State Highway Commission map 031)

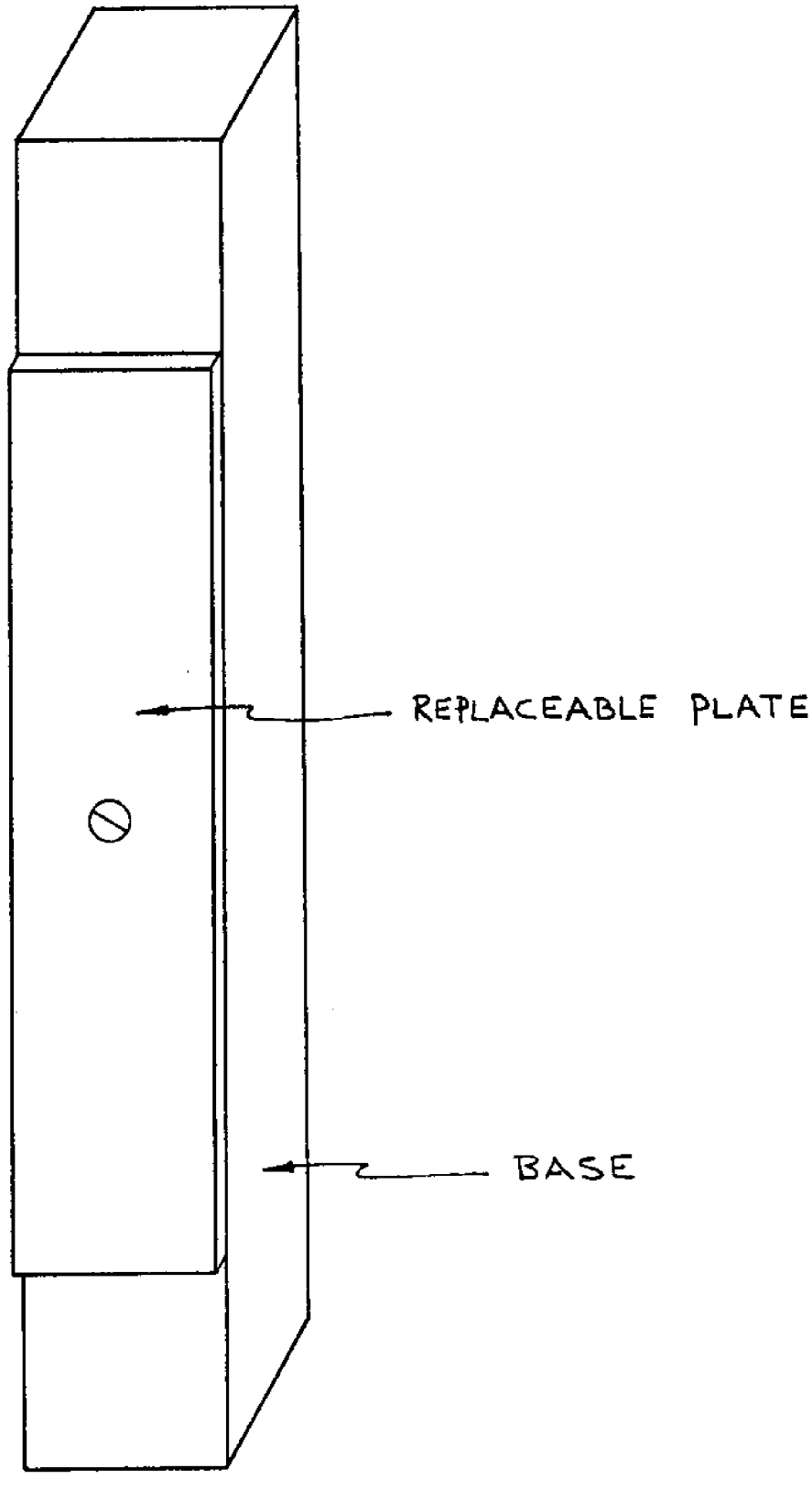

Figure 2. Artificial substrate sampler.

Nutrient Inputs and the Responses of the Salt Marsh Gastropod Littorina irrorata, Say.

Progress Report, September 1, 1972

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Department of Zoology
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Chapel Hill

Introduction

Due to the importance of estuaries as nursery grounds for many commercial and sport fisheries, studies of the most productive and, therefore, most important part of the estuary, the intertidal and shallow water areas can be of immeasurable value. Spartina salt marshes provide a very large portion of the nutrients available in the nursery grounds through the production and subsequent decomposition of the Spartina grass. Numerous animals take part in the pathway from the grass to the estuary and the gastropod Littorina irrorata, a detritus-Aufwuchs feeder is an especially conspicuous member.

Preliminary sampling of the salt marshes and Littorina populations in the Morehead City, Beaufort, and Beaufort Inlet area have demonstrated differences among several marshes in both Littorina populations and the structure and biomass of marsh grasses. These differences are especially pronounced in those marshes which were "polluted" by increased nutrient inputs from treated sewage effluents. This study was initiated to investigate the reasons behind the Littorina population differences and to define more clearly the role of the marsh snail in the salt marsh system.

Study Area and Methods

Three Spartina salt marshes were chosen which represented different levels of nutrient input. Calico Creek marsh represents the high nutrient input marsh using the Morehead City sewage treatment plant secondarily treated effluent as the increased input. Ft. Macon marsh represents the low nutrient marsh. It is located approximately one and one-half miles west of Beaufort Inlet and is flushed with largely undiluted sea water from the inlet to a greater degree than the other marshes. Russell's Creek is the marsh representing an intermediate nutrient input. It is located north of the Beaufort Inlet about five miles up the Newport River.

Sampling has thus far indicated that in terms of Littorina density, individual size, biomass per unit area, Spartina grass density and grass height, the three marshes remain in the same order as their nutrient inputs rank them (Table 1). In each of the three marshes three cages have been established and stocked with a predetermined number of measured, marked individuals. The snails were marked with a numbered tag affixed to the shell with clear epoxy glue and were measured for length and width. In

each marsh one cage contains the same density of individuals as is found in the surrounding marsh. The second cage contains only one-half the density found in the surrounding marsh, and in the third cage, twice the density of the surrounding marsh. It is assumed that one-half as many individuals as is normally found will have twice the resources available to each individual and that twice as many individuals as is normally found will have only one-half the resources available to each individual, when compared to the resources available to normal density populations. In addition, two extra cages were set up in Calico Creek and stocked with snails from Russell's Creek and from Ft. Macon, in the density normally found in these marshes (Russell's Creek $120/m^2$, Ft. Macon $80/m^2$). Approximately once a month since July, 1971, the marked snails have been removed from the cages and measured to follow growth and mortality.

Results

If as is expected either quantity and/or quality of the nutrients available to the population in Calico Creek is greater than that of either of the other two marshes, and the snail populations are resource-limited, then the greatest growth should be evidenced by the animals in Calico Creek. At this time, very little of the approximately 38,000 data points have been completely correlated or verified statistically. Certain trends have been developing, however, indicating that the Calico Creek snails are growing faster. This is particularly noticeable in the young individuals which exhibit the greatest growth, as well as in the older adult individuals. It is not unusual for adults in Ft. Macon marsh to exhibit virtually no measurable increase in size for periods of up to six months, while adults in the other marshes increase at a more frequent regular rate. In addition, within each marsh, the snails in the low density cages appear to be growing faster than the snails in the normal density cages. These, in turn, exhibit a faster rate than the snails in the high density cages. Also, the snails in Calico Creek from Russell's Creek and Ft. Macon appear to be growing faster than the snails in their own respective marshes, across all size classes.

Nutrient analysis of samples of water and marsh bed taken in 1971 are proceeding slowly. The analysis indicates that Calico Creek is an enriched marsh with higher quantity and/or quality of available resources.

Several unusual events have occurred in the marshes which will require special explanation. In recent months the Ft. Macon population has experienced an unusually high death rate, both in the cages and in the surrounding marsh. There are several possible explanations for this. In June and July of this year, severe storms kept the tide level very high in the Ft. Macon marsh, completely submerging the Spartina for periods up to three to four days. One of the behavioral patterns of Littorina is to climb the stalks of grass (at high tide) and remain out of the water. With the grass completely submerged, it is possible that these prosobranch snails were simply unable to acquire necessary oxygen. Experimentation is currently underway to determine if it is possible to drown Littorina by keeping them submerged for long periods of time.

A second event that has been observed is the elimination of grass in the high density cages in both Calico Creek and Russell's Creek. The only known difference between these cages and the others in each marsh is the density of snails. An explanation for this phenomenon is difficult since the snails were thought not to consume the living Spartina grass. Additional cages are being set up with high densities of snails to determine whether or not the denuding is repeatable. (Also further chemical analyses of the cage bed are being done to find out why no new grass has sprouted.)

Table 1

Population parameters of *Littorina irrorata* in three marhses, Morehead City, N. C. Values are given as the average ± (S. D.); numbers in brackets indicate sample size.

Marsh	*Littorina*/m^2 Juv.	Adult	Mean Adult Size (mm) w	l	Mean Adult Mass (mg)	Mean Juv. Mass (mg)	*Littorina* Biomass (mg/m^2)	Grass Height
Calico Creek	2	158	18.7±1.0(50)	23.7±1.5(50)	173.3±42.9(41)	50.4±25.9(20)	27513.8±(50)	73±9
Russell's Creek	4	116	17.5± .7(50)	22.1± .9(50)	115.6±22.6(30)	38.4±18.3(20)	13331.2±(50)	56±6
Ft. Macon	9	71	16.8± .8(50)	21.2±1.1(50)	112.7±22.7(30)	36.0±15.1(20)	8325.7±(50)	43±4

PRELIMINARY INVESTIGATION OF MACRO-INFAUNAL POPULATIONS WITHIN THE C AND P PONDS

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University of North Carolina

INTRODUCTION

The purpose of this study was to determine qualitatively what species of larger infaunal invertebrates have become established in the substrate of the ponds. Later investigations would include studies on population densities, biomass determinations and measurement of various ecological factors. Since quantitative measurements were not the primary objective, most of the data presented here are from direct observations. Samples were taken during the months of November, December, January, May and August, 1970-1971, with emphasis placed on devising appropriate methods for sampling in this environment.

METHODS

A modified suction-corer (Riedl and Ott, 1970) was used to extract substrate from each of the three enriched and controlled ponds. The core sampler consisted of a piece of plastic tubing 69 cm long and 7 cm in diameter. The penetrating end was slightly sharpened on the inside edge. Two holes were drilled 6 cm from the top to receive a wooden dowel 20 cm in length which acted as a handle. A rubber stopper perforated by a 1 cm hole containing a 10 cm length of glass tubing was placed in the proximal end of the corer. To this was attached a length of rubber tubing.

In use the apparatus was simply inserted into the substrate while air being sucked out (by mouth) facilitated penetration of plastic tube. A standard pinch clamp on the rubber tubing maintained a vacuum within the sampler during extraction from the substrate. A sample was freed for removal by releasing the clamp. This method will not only extract samples of coarser sediments, it will also maintain layering within sediment samples. The use of a core sampler instead of an Ekman grab has been shown to be far more practical for this type of investigation (Paterson and Fernando. 1970). Quantitative samples from pre-determined depths are easy to obtain.

Once removed, a sample was placed in a bucket of water and mixed by hand. The suspension was then poured, in part, through a series of standard sieves with mesh openings ranging from 4.00 mm to 0.5 mm. A larger 0.5 mm sieve was constructed from stainless steel screening to ease the sieving process (Spooner and Moore, 1970). Organisms were periodically removed from the screens with fine forceps and deposited in a dish containing isotonic magnesium chloride. Identifying characteristics and colors were noted with aid of a dissecting microscope before preserving the specimens in 5% formalin.

RESULTS

Core samples were taken at 1, 4 and 8 m from shore (the latter constituting the approximate center of the pond and therefore the deepest region). Substrates in the C and P ponds appeared similar toward the center and deeper areas. Both were highly reduced, consisting of large amounts of decaying organic matter and composed predominantly of very fine sand. In the P ponds, this sand substrate covered most of the bottom except for the periphery (1 m from the water's edge). However, substrate in the C ponds became obviously more oxidized and increased in grain size with decrease in depth.

The dominant populations consisted of Capitella capitata (Fm. Capitellidae) and Laeonereis culveri (Fm. Nereidae), both polychaete worms. Two species of oligochaetes were also found in considerably smaller numbers (Dr. Elizabeth A. McMahan, personal communication). The latter were often lost in the mesh of a 0.5 mm screen and could best be picked up with a 0.25 mm sieve. In both the C and P ponds, C. capitata outnumbered L. culveri; however, in the enriched ponds the ratio was considerably higher (Table 1).

Except for samples taken in May, populations within the substrate of both series of ponds seemed will established. Examination of the May samples from the P ponds failed to show that a phytoplankton bloom and subsequently an oxygen crash occurred at this time in the P ponds (Table 2). In the C ponds where there was no record of a bloom, populations of L. culveri and C. capitata were still found.

DISCUSSION

Core samples were generally limited to the upper 10 cm of substrate. From aquaria set up in the laboratory containing substrate form the C and P ponds, and from an occasional deep core sample (using the same sampling device but merely inserting it further into the substrate), it was noted that C. capitata were often found in sediment deeper than 10 cm. For an accurate analysis of the macro-infaunal organisms, samples deeper than 10 cm need to be taken and sieves with a mesh 0.5 mm need to be used.

The study further showed that population densities within this environment are not static. The organisms sampled appeared to vary seasonally showing their lowest densities following phytoplankton blooms which resulted in sever oxygen depletion. Whether or not this is the critical factor has yet to be tested. The worms can, however, reestablish themselves quickly. There is no doubt that the organisms living in the substrate constitute a major portion of the biomass of the ponds.

TABLE 1

SMALL BOTTOM ANIMALS IN CORES JULY 4, 1971 (data compiled by H. T. Odum and E. A. McMahan). Values are per 38 cm^2 core.

	C Ponds		P Ponds	
Species	Number	Wet wt. g.	Number	Wet wt. g.
Capitella capitata	145	0.54	456	2.11
Laeonereis culveri	57	0.39	113	0.49
Oligochaeta sp. (A)	49	0.16	32	0.09
Oligochaeta sp. (B)	6	0.03	1	0.04

TABLE 2

OXYGEN CONCENTRATIONS IN Mg/l (from records kept at the Institute of Marine Sciences, Morehead City, N.C.)

Date of Specimen Samples	Date of ∂_2 Determination	A.M. P-1	A.M. P-2	A.M. P-3	P. M. P-1	P. M. P-2	P. M. P-3	A. M. C-1	A. M. C-2	A. M. C-3	P.M. C-1	P.M. C-2	P.M. C-3
	11-14-70	14.32	10.26	13.86	30.18	22.02	26.59	8.34	8.23	9.75	13.34	9.80	10.74
Nov. 11, 1970	11-15-70	10.20	7.32	9.48	19.70	15.28	18.37	7.74	8.26	7.99	8.82	8.49	9.01
	11-16-70	8.48	7.27	9.11	24.22	21.69	20.70	7.44	7.82	7.63	8.56	10.51	11.02
													))
	12-13-70	9.29	8.68	8.90	22.51	16.87	16.37	7.88	8.12	7.67	8.17		9.23
(Dec. 14, 1970	12-14-70	10.41	11.11	10.44	17.72	15.25	14.74	7.66	7.54	7.42	8.14	9.00	9.43
Dec. 15, 1970	12-15-70	12.09	10.70	11.70	18.92	17.68	15.61	7.58	8.62	8.06	8.87	9.75	10.73
	12-16-70					NOT AVAILABLE							
	1-25-71					NOT AVAILABLE							
Jan. 26, 1971	1=26-71					NOT AVAILABLE							
Jan. 27, 1971	1-27-71	8.48	10.13	10.85	12.96	11.49	13.20	10.61	9.22	9.54	11.46	12.58	11.39
	1-28-71	12.49	11.38	11.70	14.78	13.90	14.71	10.86	11.28	11.79	10.70	13.09	11.76

Date of Specimen Samples	Date of O_2 Determination	A. M.			P.M.			A.M.			P. M.		
		P-1	P-2	P-3	P-1	P-2	P-3	C-1	C-2	C-3	C-1	C-2	C-3
	5-20-71				NOT AVAILABLE								
May 21, 1971	5-21-71	0.91	0.86	0.79	4.92	2.92	5.86	7.35	6.27	5.72	8.64	8.88	7.13
May 22, 1971	5-22-71				NOT AVAILABLE								
May 23, 1971	5-23-71				NOT AVAILABLE								
	5-24-71	0.43	0.87	0.83	5.48	10.60	8.97	6.20	7.14	5.73	7.68	10.18	9.88
Aug. 14, 1971	8-14-71				NOT AVAILABLE								

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FISHES OF EXPERIMENTAL MARINE PONDS RECEIVING TREATED SEWAGE WASTE AND OF NEARBY WATERS OF THE NEWPORT RIVER

by R. Hyle

INTRODUCTION

A study of the fishes in the Sea Grant ponds was initiated in June 1970. The main objective of the study was to compare the species composition, numbers, and biomass of the fish populations in the control and waste ponds. The study was completed with the final harvest of the ponds in the summer of 1971.

After the summer harvest of June 1971, the ponds were prepared for the culture of penaeid shrimp and bait-fish, Fundulus heteroclitus. The pond fish populations derive from several sources, (1) recruitment stock remaining in the ponds after harvest, (2) eggs and larvae introduced by the creek pump, (3) eggs, larvae, and juveniles introduced by back flooding through the standpipe (noted only in P-3 and (4) adults introduced into the ponds as seed stock.

Fundulus heteroclitus, the common mummichog, is used during the spring, summer and fall by fishermen to catch "flatfish", Paralichthys sp. Local bait distributors market the mummichog at $1.00/dozen (W.R. Bradley, Sportman's Fishing Pier, Morehead City, N.C.). Most distributors capture their supply in the local creeks in which the fishes are quite abundant at times. The culturing of bait-fish in ponds such as the experimental Sea Grant ponds, however, has advantages over the taking of bait-fish in natural creeks: (1) the pond harvest is an easier task, (2) the bait-fish are available in the ponds when not available in the natural creeks. Gordon (1968) relates that the bait-fish industry in the Great Lakes cannot catch enough minnows in the summer when the demand is high.

The main objectives of this latter study were to investigate the feasibility of raising bait-fish, the common mummichog, in experimental marine ponds, and compare their production in ponds receiving treated sewage waste and in non-enriched control ponds.

A study of the fish fauna of the Calico Creek basin and the entire Newport River Estuarine Complex was also begun in the summer of 1970 as a dissertation research project.

Increasing awareness of the quality of the environment has brought the estuaries into national concern. Their importance as fin and shell-fish production areas and wildlife sanctuaries is well known. Recently, for example, the Sport Fisheries Institute Bulletin (April, 1970) noted that in the state of Maine, "the average annual crop of shellfish and bait worm harvest from each acre of estuary was worth $33,503 in the market place... by comparison the best market garden farms in Maine yield $2,000 in crops per acre per year". Further, it was emphasized that "general overall reduction of productivity of the estuaries by pollution or other factors, say 20, 40, 60 or 80 percent, etc., would cause corresponding reduction in yield on the continental shelf".

Classifications exist of emerging new systems associated with man, such as estuarine ecosystems that receive sewage wastes, systems resulting from dredging, systems with pesticides, etc. These are recognized as new types of ecological systems where man's particular influence is great (Odum, Copeland and McMahan, 1968).

The Newport River Estuary, North Carolina, lies in the South Atlantic Estuarine Zone, and according to the National Estuary Study (U.S. Fish. Wildl. Serv.,1970) has been classified as a relatively unmodified estuary. Nevertheless, the Newport River estuarine complex. is receiving treated sewage wastes and is undergoing modifications. The Calico Creek-Crab Point Bay area receives treated sewage wastes from the Morehead City sewage treatment plant, and the Newport River proper receives treated sewage wastes from the Newport treatment plant and the West Carteret High School treatment plant.

Another potential stress on the estuary is periodic dredging of the channels in the area. Recent harbor depth dredging has pumped vast spoil on the area just north-east of the U.S. 70 bridge east of Morehead City, inundating and destroying several marsh habitats and adjacent waterways.

In summary, the Newport River estuarine complex is subject to environmental alterations through treated sewage input and dredging soil deposition. The objectives of this study were to assess the value of the Newport River estuarine complex as a fish nursery ground and fish production area and to study the fish fauna seasonal structure and composition and their utilization of such a modified estuarine system.

Partial support was received from Sea Grant for the fish fauna survey completed in Calico Creek. The data presented here includes only a comparison of the fish fauna of Calico Creek and that of similar stations in the Newport River. Sample locations are shown in Fig. 1.

METHODS

Ponds

The mummichog populations were monitored by trapping. The sunken fish traps were 2'x4' constructed of aluminum angle and 1/8" hardware cloth; the traps were unbaited and fished for 24 hour periods. Fish catches were sorted and lengths and weights were taken. Mark-recapture and catch per unit effort techniques were employed for population estimates (Lagler, 1966). A harvest of the P-ponds was completed in August 1972 using a 60' sein with a 1/4" mesh and the sunken traps. The C-ponds were not harvested. Pond C-1 exhibited a sparse fish population, and pond C-3 exhibited an engulfing growth of Rupia maritima, which made harvesting impossible.

Calico Creek and Nearby Waters of the Newport River Estuary

Physical Characteristics

The Newport River Estuary Complex was mapped (using depth recording devices and aerial survey) to establish depth contours and volumes and to locate sampling stations. Depth contours of the Newport River Estuary are shown in Fig. 1. The contours represent soundings taken at high water between June 1970 and March 1971.

During 1972-73, water mass movement was investigated. Seabed drifters (McCullock, Peterson, Carlson, and Conomos, 1970; Folger, 1971) were released seasonally from designated points. The drifters were released just prior to trawl sampling to possibly increase returns within the system.

Concurrently with monthly sampling, temperature, salinity and oxygen were measured at surface and bottom at designated stations. Temperature was taken with a hand thermometer, salinity with a refractometer, and oxygen analysis was by the Winkler method currently used at the Institute of Marine Sciences, Morehead City, N.C.

Fish Fauna

Adult and juvenile fishes were collected at 20 stations thoughout the system using various gear. During 1971, the shallow waters of the estuarine complex were sampled monthly by skiff using a 20' otter trawl and a surface 1/2 meter plankton net. The sampling interval was 10 minutes. The deeper waters were sampled on a monthly basis by the R/V Machapunga using a 40' trawl and a surface 1 m plankton net. Again, the sampling interval was 10 minutes.

Small sunken traps (modified fyke nets with wing extensions) were also employed in the shallow waters from September 1971 to December 1971. The traps were discontinued in December because of the continued fouling and time-consuming maintenance.

During 1972, trawl sampling was continued monthly in the deeper channels and quarterly in the shallows. On the quarterly sample, all stations (deep and shallow) were sampled twice, once at night and once the following day, approximately 12 hours apart.

All fish catches were sorted, identified to species, weighed, and measured. All associated organisms were noted in the catch. When large samples were taken, it was necessary to subsample the catch. Large samples were only taken in deep water with the large net.

Also during 1972, selected fish species (dominant resident or regulars) were examined for food contents and analyzed for age and growth. These selected species were also tagged to note their resident or transient use of the estuary.

RESULTS AND DISCUSSION

Ponds

Population estimates of the mummichog by mark-recapture studies are shown in Table 1. Mark-recapture results were incomplete for July in C-3 and P-3; population estimates were not possible. No fish were trapped in C-1, however, juvenile mummichogs were dipped from the pond. The population estimate from trap catches during the harvest of P-3 is about 1600 fish. (Fig. 2)

The 1972 sein harvest results of the P-ponds are given in Table 2. Harvestable fish (45 to 100mm standard length) were also taken by trapping prior to the seining. Length frequencies of a subsample from P-1 are presented in Figure 3. The total harvest results are presented in Table 3. Extrapolation of these results of the 1/8 acre ponds gives a dollar value of $511/acre to the harvest.

During the past year, after the 1971 harvest, a shift occurred in the proportions of sheepshead minnows, Cyprinodon variegatus, and the mummichogs in P-3. The sheepshead minnow was absent in P-3 in the 1971 harvest, and the mummichog made up 92% of the harvest by weight. However, in the 1972 harvest, the sheepshead minnow comprised 28% by weight of the harvest compared to 39% for the mummichog. The structure of P-1 remained relatively unchanged. The sheepshead minnow made up 89% by weight of the 1971 harvest and the mummichog 10%. Similar proportions were present in the 1972 harvest in which the sheepshead minnow made up 84% of the harvest by weight and the mummichog 10%.

In order to acquire maximum yield of the mummichogs, efforts would have to be made to control competing fish species such as the sheepshead minnow and the mullet, Mugil cephalus. Bearden (1967) cites the disadvantage of many undesirable species introduced during flooding into salt ponds used for game fish culture. The non-resident mullet (incapable of breeding in ponds) whose young migrate into the estuaries could be controlled with screens on the intake pump and standpipe. The resident sheepshead minnow would have to be controlled through trapping and seining, which would possibly give the mummichogs a competitive advantage.

Salt ponds are naturally highly productive (Hiatt, 1947; Bearden, 1967) as are enriched sewage ponds; however the marine ponds receiving treated sewage waste appear to be more suitable for the culture of the bait fish, F. heteroclitus than natural salt ponds (control ponds). The sewage ponds have shown (Hyle, 1971; Beeston, 1970) a selectivity for non-commerical species such as the resident mummichog, which shows a wide tolerance to extreme flucuations in temperature, salinity and oxygen. Also, often it is necessary to remove rooted vegetation from fish culture ponds (Bryan and Allen, 1969). The problem of rooted vegetation developed in pond C-3; however, rooted vegetation is excluded from the soft bottom of the turbid sewage ponds.

In summary, the high productivity and selective nature of salt ponds receiving sewage waste increases their feasibility for efficient production of the bait fish, F. heteroclitus.

Calico Creek and Nearby Waters of the Newport River Estuary

Physical Characteristics

The entire Newport River Estuary has an average depth of approximately 1 meter at high water. Over 88% of the complex lies below the 2 meter contour. The upper part of the estuary is a broad shallow basin, and the lower part is cut by man-made channels maintained to depths of 3 to 4 meters. Calico Creek is one of several shallow creeks in the complex; depths average less than 2 meters at high water. A narrow channel extends from the marsh headwaters into the Calico Creek basin where very little relief is encountered. Upper Calico Creek is characterized by a mud bottom. Sample locations were chosen in the lower basin, since trawl and traps used in the upper creek became clogged with mud and debris rendering them inoperable.

Temperature, salinity and oxygen data are presented in Tables 4-10. During 1971 and 1972, the lowest temperatures occurred at surface and bottom during February. Temperatures rose during the spring and summer to reach a maximum at the surface and bottom during August.

The salinities varied with each tidal cycle and were influenced by runoff and river input. Coffee colored water was often observed after heavy rains and was found to be lower in salinity than surrounding waters. Salinity minima occurred during October 1971 and February 1972. A salinity maximum occurred with decreasing river input during August both in 1971 and 1972.

Oxygen values decreased toward summer and increased during the winter months. Oxygen lows occurred in July 1971 and August 1972. Oxygen maxima occurred during November 1971 and February 1972. Low oxygen values did occur during the warm summer months, and low slack tides did not reveal oxygen depletion in the Calico Creek basin or in the Newport River. There is apparently enough tidal and wind mixing in this shallow system to prevent oxygen depletion.

Fish Fauna

The discussion here will be limited to the fish fauna survey in the Calico Creek basin and a comparison of the data with that of river stations similar in hydrography and physiography.

Fish species and numbers are presented in Tables 4-10. During the two year period, 56 species representing 33 families were taken by trawling and trapping at the Calico Creek and Newport River sites; 37 species were taken in the Calico Creek basin and 45 species in the Newport River. The trawl survey revealed similar species numbers in the creek and river systems. During the two year period, monthly trawl sampling collected 30 species in Calico Creek and 33 species at the Newport River Station.

The seasonal structure in the Calico Creek basin remained

uniform over the two year sampling period, however, some variation occurred at the river station. During 1971, the species number was highest during the fall-winter period and lowest in the summer at the Calico Creek station. The river station in 1971 showed the highest species number during the fall period and the lowest during the winter. During 1972, the species number was again highest during the fall-winter period and lowest during the summer in Calico Creek. At the river station in 1972, the seasonal structure shifted slightly; the highest species number was during the winter-spring period and the lowest during the summer-fall period. Dahlberg and Odum (1970), conducting a trawl survey of the Georgia coastal fishes, found the highest species number in the spring, summer and autumn periods and the lowest in the winter period.

The species composition was dominated by the resident or regular forms (occurring practically every month in fair numbers), Leiostomus xanthurus and Lagodon rhomboides, throughout the sampling period. Much of the species assemblage is made up of seasonal and occasional forms. This supports the thesis of Tyler (1971) that "at latitudes where there is relatively greater annual temperature fluctuation, there are proportionately more species in the temporal components and fewer in the regular component".

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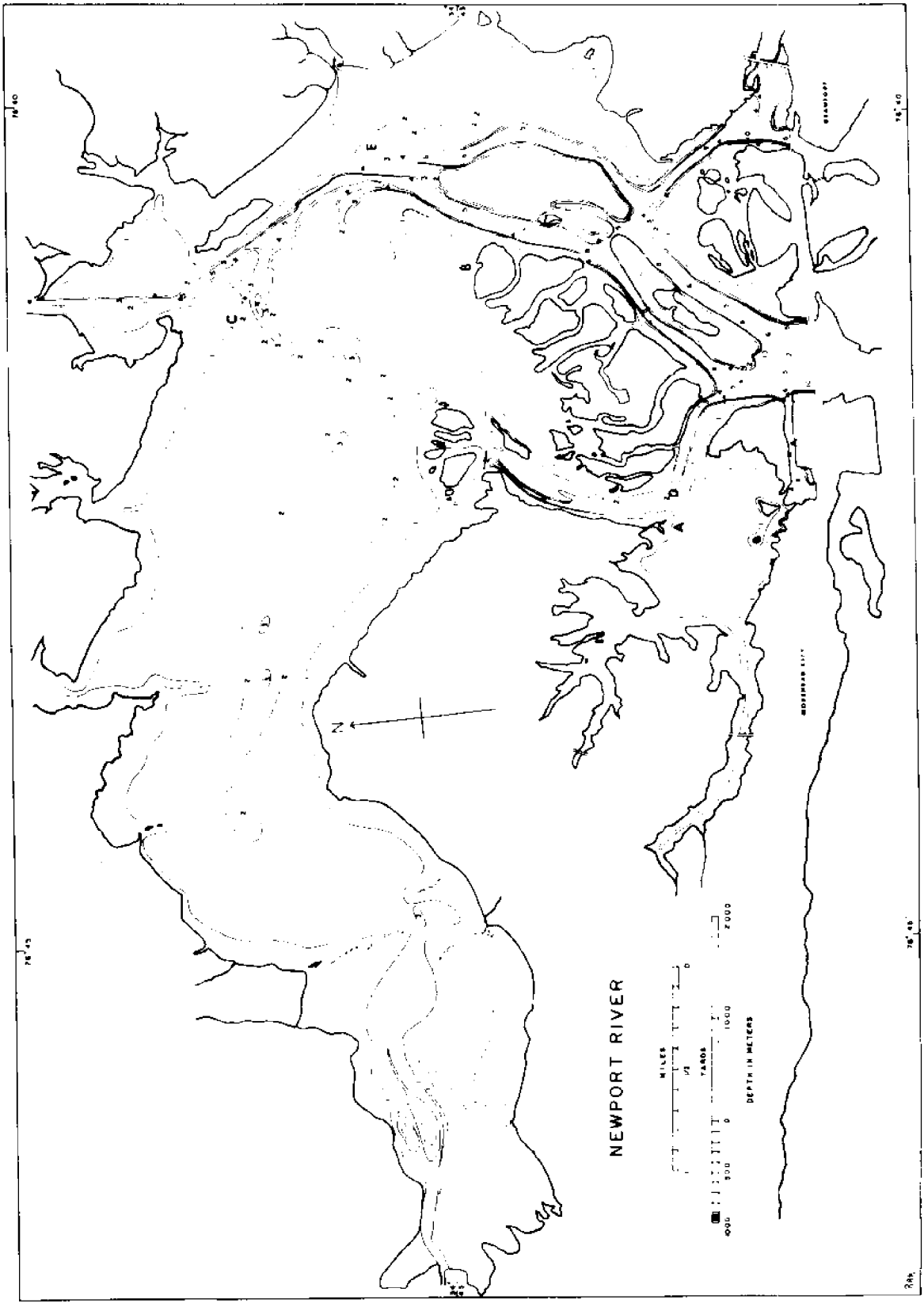

Fig. 1. Sample locations, A-Calico traps, B-Spoil traps, C-Range traps, D-Calico trawl and E-Newport River trawl. Depths (m) are from soundings at high water.

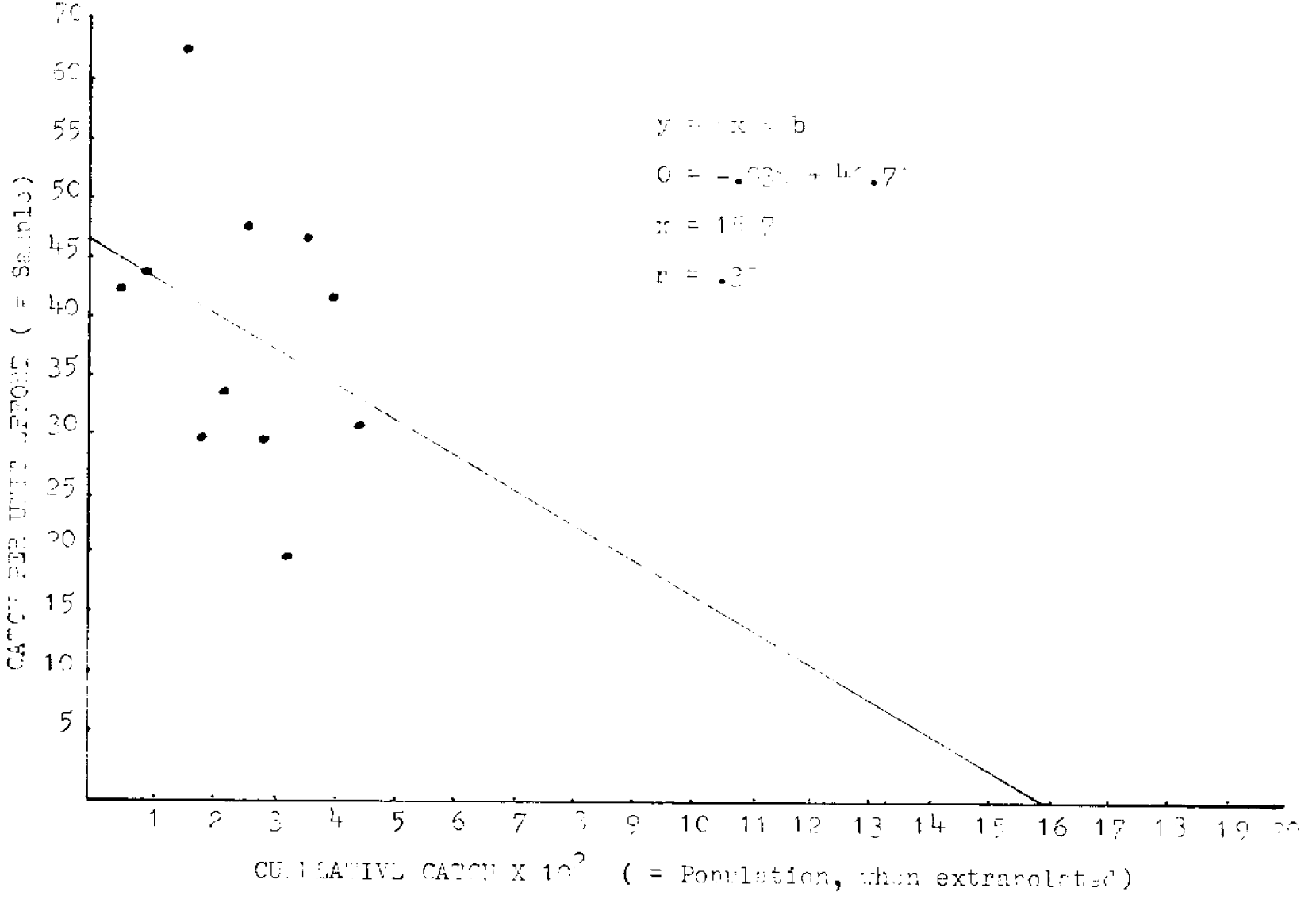

Fig. 2. Population estimate of *F. heteroclitus* in P-3 .

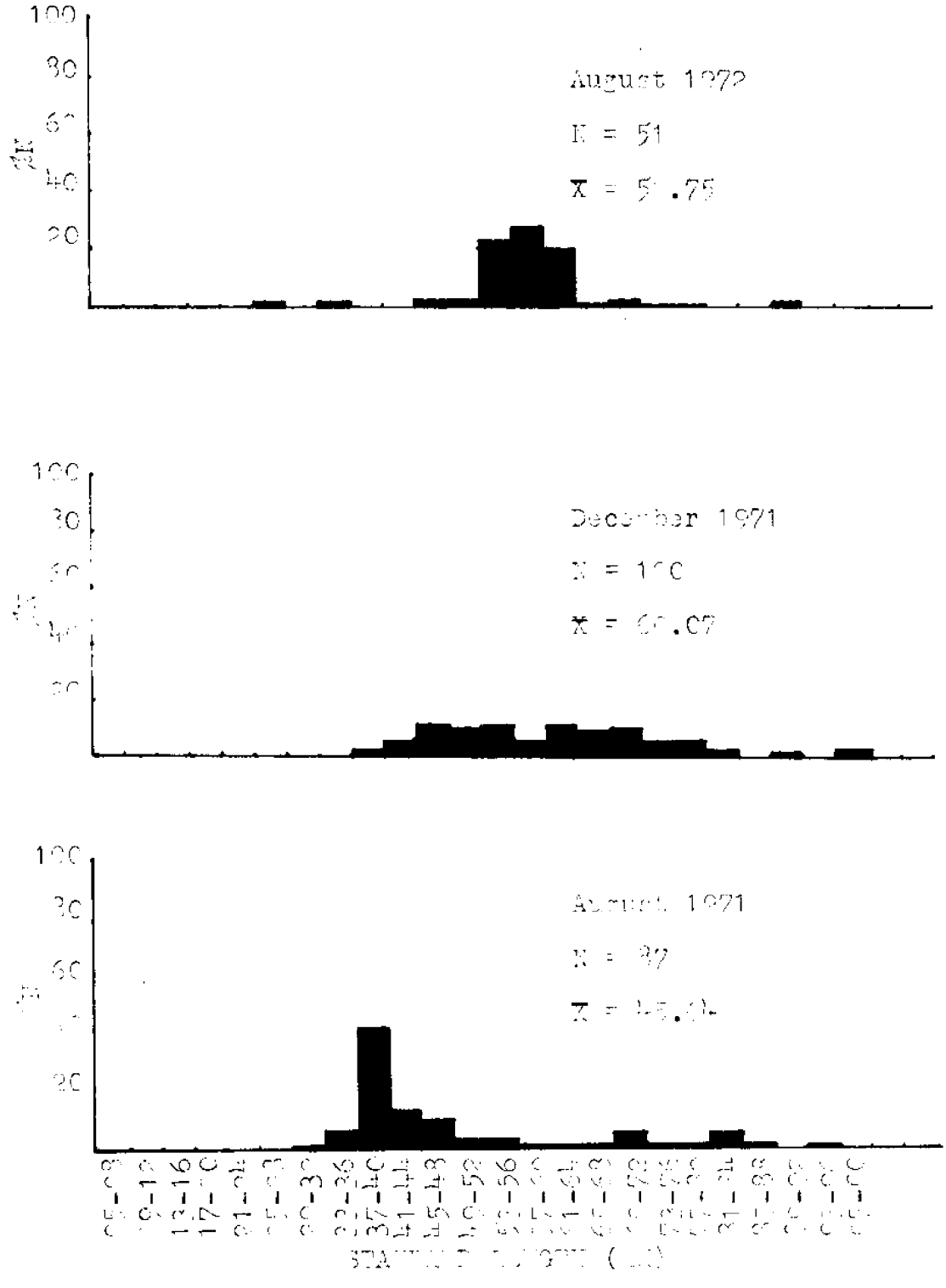

Fig. 3. Length frequencies of *A. heterosetifera* in [illegible]-1.

Table 1. Population estimates of Fundulus heteroclitus based on mark-recapture studies.

Pond	August 71	December 71	July 72
C-3	5,123	3,296	-
P-1	468	302	869
P-3	909	5,321	-

Table 2. Weights and numbers of fishes collected by seining during 1972 harvest.

Species	P-1			P-3		
	Wt.*	No.	%Wt.	Wt.*	No.	%Wt.
C. variegatus	5,550	4,753	84	1,440	1,233	28
F. heteroclitus	710	100	10	2,040	283	39
A. rostrata				800	2	16
M. cephalus	470	2	6	934	10	17

*wet weight in grams

Table 3. Trapping results and total harvest of F. heteroclitus.

P-1			P-3	
Wt.*	No.		Wt.*	No.
		Trap		
3,864	624		2,576	431
		Total Harvest**		
4,574	724		4,616	714

*wet weight in grams
**sein and trap

Table 4. Species and numbers collected in Calico trap - 1971 and surface temperatures (°C) and salinities (°/oo).

Species	September							October								November-December				
	8	10	15	17	22	24	Total	4	8	13	18	24	27	29	Total	3	10	24	1	Total
Opsanus tau	1				1	1	3	1						1	2					
Bairdiella chrysura	2	2	10	1	1	2	18	4	2	3	8	19	1		37	1	4		3	8
Lagodon rhomboides	2	7	2	23	13	10	57	8	20	4	20			11	63	7	12		19	38
Orthopristes chrysopterus	4	3	2		1		10		6	1	2		6	2	17					
Trinectes maculatus		1					1													
Leiostomous xanthurus			6	10	13	1	30	10	227	22	68	11	18	58	414	17	14		6	37
Lutjanus griseus					1		1				1				1		1			1
Micropogen undulatus					1		1			1					1					
Paralichthys albigutta					1		1	1							1					
Centropristes philadelphicus									1		1				2					
Symphurus plaguisa											1	1			2				1	1
Cynoscion nebulosus													1*	8*	9					
Selene vomer													1		1					
Eucinostomus gula																1				1
Monacanthus hispidus																1				1
Spheroides maculatus																	1			1
Dorosoma cepedianum																		1		1
Total # species			9								10							8		

Table 4. Continued.

	September							October						November-December			
	8	10	15	17	22	24	4	8	13	18	24	27	29	3	10	24	1
Salinity	31.8	32.9	25.8	28.5	32.9	25.2	24.7	22.5	21.7	21.5	9.9	14.9	11.5	20.8	16.4	28.5	30.7
Temp.	27.6	27.5	28.0	27.2	28.2	26.3	26.0	23.0	21.4	21.4	22.4	24.9	22.8	24.0	15.2	11.8	13.3

* caught on station by hook and line.

Table 5. Species and numbers collected in spoil bank trap - 1971 and surface temperatures (°C) and salinities (°/oo).

Species	September						October							November-December				
	10	15	17	22	24	Total	4	8	13	24	27	29	Total	3	17	24	1	Total
Lagodon rhomboides	1	1	3	8	3	16	30	7	4	1	1	1	44	2			17	19
Orthopristes chrysopterus	2	2	2	2	1	9	16	5	2	3	5	2	33	1				1
Trinectes maculatus	1	1				2	1		1	1			3					
Bardiella chrysura	3	5		1		9		2	5	3	6		16	1			1	2
Opsanus tau		2		1	1	4		1	2				3	1				1
Chaetodipterus faber			1			1												
Leiostomus xanthurus							4		1		1		6	2			1	3
Micropogon undulatus							1		1				2					
Centropristes striatus							1						1					
Paralichthys albigutta							1						1					
Lutjanus griseus								1					1				1	1
Cynoscion nebulosus								2*					2					
Prionotus evolans									1				1					
Paralichthys dentatus										1			1	1				1
Strongylura marinus														1				1
Sphyrna barracuda																1		1
Fundulus majalis																1		1
Total # species			6						13						10			
Salinity	28.5	25.2		29.6	25.8		20.8	20.8	9.7	8.8	8.1	10.4		21.9		26.3	22.5	
Temperature	27.5	27.0		27.2	27.0		26.0	22.2	20.9	22.6	25.3	22.0		25.5		11.4	11.2	

Table 6. Species and numbers collected in range trap - 1971 and surface temperatures (°C) and salinities (°/oo).

Species	September							October						November-December				
	8	10	15	17	22	24	Total	4	8	13	27	29	Total	10	17	24	1	Total
Lagodon rhomboides	2	9	12	14	6	10	53	14	34	38	9	4	99		67	11	11	99
Bairdiella chrysura	4	35	15	1	5	22	82	20	69	67	18	4	178	11	2			13
Leiostomus xanthurus			29	1	5	1	36	11	8	79	31	2	131	6	6	1		13
Chaetodipterus faber			3	1	1	2	7											
Orthopristes chrysopterus			1	3	4	2	10	6	4				10					
Micropogon undulatus				1			1		1	1			2	1			1	2
Lutjanus synagris				1			1											
Trinectes maculutus								1		1			2	1				1
Paralichthys lethostigma								1					1					
Centropristes striatus								1					1					
Lutjanus griseus								2	2	1			5					
Caranx sp.										1			1					
Opsanus tau											1	1	2			1		1
Anguilla rostrata												1	1					
Conger oceanicus														1				1
Symphurus plaguisa															2			2
Fundulus heteroclitus														1		2		3
Total # Species				7						12					9			
Salinity	32.9	29.9	26.3	25.2	25.2	20.8		19.7	19.7	10.8	12.8	7.1		16.4	25.0	24.1		21.9
Temperature	27.4	27.5	27.5	27.9	28.0	27.5		26.2	23.2	21.0	23.5	22.5		15.0	15.5	11.0		11.3

Table 7. Species and numbers collected in Calico Creek monthly trawl samples - 1971 and surface and bottom temperatures (°C) and salinities (°/oo).

Species	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.
Lagodon rhomboides	5	1	1	4	6	1		4	9	
Urophycis regius	1									
Menidia menidia	4									
Archosargus probatocephalus	2									
Scophthalmus aquosus	1									
Peprilus triacanthus			1	2						
Orthopristes chrysopterus				1		1			1	
Dasyatis sayi						1				
Prionotus carolinus						1				
Anchoa hepsetus								18	1	
Paralichtys dentatus									1	
Synodus foetens									2	1
Spheroides maculatus									2	
Centropristes philadelphics									1	
Menticirrhus saxatilis									1	
Micropogon undulatus									3	
Leiostomus xanthurus									1	
Bairdiella chrysura									1	
# species	5	1	2	3	1	4	0	2	11	1

Table 7. Continued.

		Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.
Salinity Range	S	28.0-30.1	29.1-29.1	32.3-34.5	34.5-33.4	31.6-30.5	33.4-33.4	35.1-35.1	31.8-32.9	13.2-17.5	20.8-20.8
	B	28.0-30.1	29.1-27.1	32.9-35.5	35.1-33.9	29.4-31.6	34.0-33.4	35.1-35.1	31.8-31.8	17.5-19.7	20.8-20.8
Temp. Range	S	7.3 7.4	12.3	15.4-15.4	23.0-23.0	24.9-25.1	25.6-28.7	29.7-28.8	27.5-27.2	20.5-20.5	15.0-15.0
	B	7.3 7.4		15.4-15.1	22.4-22.5	25.3-25.1	25.7-25.7	29.7-28.8	27.0-28.0	20.0-20.5	15.0-15.0
Oxygen mg/L	S	5.82	5.78	6.4	7.62	4.44	4.55	5.50	5.50	6.82	8.16
	B			8.1	6.51	4.44	4.93	6.64	6.64	7.05	10.67

Table 8. Species and numbers collected in Newport River - Old Black Channel monthly trawl samples - 1971 and surface and bottom temperatures (°C) and salinities (°/oo).

Species	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.
Urophycis regius			1							
Lagodon rhomboides			13	99	20	13	18	19	29	149
Anchoa mitchilli			12	1	1				24	1
Leiostomus xanthurus				11	1				9	9
Orthopristes chrysopterus				2	1	1	1			
Ancylopsetta quadrocellata				1				1		
Paralichthys dentatus				1						
Symphurus plaguisa				1						
Anchoa hepsetus							52			
Trinectes maculatus								2		
Eucinostomus gula								1		3
Micropogon undulatus									2	
Synodus foetens										1
Etropus crossotus										4
Mentricirrhus saxatilis										1
Peprilus triacanthus										2
Eucinostomus argenteus										9
# Species	0	0	3	7	4	2	3	4	4	9

Table 8. Continued.

		Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.
Salinity Range	S	23.7-23.7		32.9-30.0	31.9-31.9	31.6-32.2	32.9-32.9	35.1-35.1	29.6-25.8	14.2-13.1	16.8-16.7
	B	23.7-23.7		32.9-30.0	32.9-32.9	31.6-32.2	34.0-34.0	36.1-35.1	29.6-27.4	17.8-16.4	19.9-17.3
Temp. Range	S	7.4- 7.4		15.8-16.9	23.0-23.4	25.4-25.7	25.7-25.8	29.3-29.4	27.0-27.5	21.7-21.5	15.2-18.1
	B	7.4- 7.4		15.7-16.9	25.5-22.8	25.4-25.6	25.7-25.8	29.2-29.2	27.0-27.5	20.5-20.3	15.3-15.1
Oxygen mg/L	S	5.68		6.95	7.13	5.98	3.79	6.14	6.14	6.73	7.71
	B			7.59	7.28	5.86	3.53	6.87	6.87	6.43	8.20

Table 9. Species and numbers collected in Calico Creek quarterly trawl samples - 1972 and surface and bottom temperatures (°C) and salinities (°/oo).

	11 Feb 10			12 May 11			18 Aug 17			21 Nov 20		
	Day	Night	Total	Day	Night	Total	Day	Night	Total	Day	Night	Total
Symphurus plaguisa		1	1		2	2				1	10	11
Urophycis regius	1	2	3								1	1
Prionotus scitulus		1	1									
Brevoortia tyrannus	1	7	8							1	1	2
Anchoa mitchilli	8	10	18	1	1	2	1		1			
Paralichthys dentatus		1	1									
Micropogon undulatus		2	2									
Leiostomus xanthurus	1	10	11	5	1	6				16	1	17
Lagodon rhomboides		6	6	5	1	6		4	4	10	6	16
Syngnathus fuscus		1	1									
Alosa aestivalis	1		1									
Menidia menidia	10		10									
Peprilus triacanthus	3	3										
Prionotus evolans	1	1										
Syngnathus louisianae					1	1						
Ophidion welshi					3	3					1	1
Orthopristes chrysopterus				1	2	3						
Prionotus carolinus					2	2						
Pomatomus saltatrix				1		1				1		1

Table 9. Continued.

		11 Feb 10 Day	11 Feb 10 Night	11 Feb 10 Total	12 May 11 Day	12 May 11 Night	12 May 11 Total	18 Aug 17 Day	18 Aug 17 Night	18 Aug 17 Total	21 Nov 20 Day	21 Nov 20 Night	21 Nov 20 Total
Chilomycterus schoepfi					1		1						
Monacanthus hispidis									1	1			
Synodus foetens								1		1		1	1
Eucinostomus argenteus											1		1
Bairdiella chrysura											3	2	5
Opsanus tau												1	1
Menticirrhus americanus												1	1
Etropus crossotus												2	2
Paralichthys albigutta												1	1
# Species		8	10	14	6	8	10	2	2	4	7	12	14
Salinity Range	S	9.91-1.0	7.2-4.4		32.9-32.3	33.4		33.9-32.8	28.5-33.4		19.7-23.0	20.8-21.9	
	B	11.0-13.7	7.2-4.9		32.9-32.9	32.9		33.9-32.8	29.5-33.4		21.9-25.2	22.5-23.0	
Temperature Range	S	5.1 5.5	5.5 5.0		17.5-19.0	20.5		27.0	26.0		13.5-14.0	14.8-14.7	
	B	5.5 5.8	5.0 4.5		18.0-19.0	19.8		27.0	25.5		13.1-13.0	14.5-14.5	
Oxygen mg/L	S	11.61	11.38		8.13	8.30		4.90	6.26		8.16	8.00	
	B	12.06	10.69		7.58	7.02		5.33	7.89		8.16	7.75	

Table 10. Species and numbers collected in Newport River - Old Black Channel quarterly trawl samples - 1972 and surface and bottom temperatures (°C) and salinities (°/oo).

Species	11 Feb 10 Day	11 Feb 10 Night	Total	12 May 11 Day	12 May 11 Night	Total	18 Aug 17 Day	18 Aug 17 Night	Total	21 Nov. 20 Day	21 Nov. 20 Night	Total
Peprilus triacan thus	10	1	11									
Mugil cephalus	1	1	2									
Etropus crossotus		1	1		3	3					1	1
Symphurus plaguisa		5	5	1	3	4		1	1		4	4
Anchoa mitchilli	4	10	14		1	1				1		1
Prionotus evolans	2	2	4									
Paralichthys dentatus		3	3	1	1	1						
Brevoortia tyrannus	4	2	6									
Micropogon undulatus		1	1					1	1			
Lagodon rhomboides	15	4	19	11	28	39	3	1	4	7	16	23
Leiostomus xanthurus		24	24	12	38	50		3	3		9	9
Synodus foetens	1		1									
Urophycis regius	5		5									
Menidia menidia	4		4									
Prionotus carolinus	1		1									
Chilomycterus, schoepfi					2	2						
Spheroides maculatus				2	1	3						
Ancylopsetta guadrocellata					4	4						
Trinectes maculatus					1	1	3	4	7			

Table 10. Continued.

Species	11 Feb 10 Day	Night	Total	12 May 11 Day	Night	Total	18 Aug 17 Day	Night	Total	21 Nov 20 Day	Night	Total
Ophidion welshi					3	3						
Orthopristes chrysopterus				3	22	25		7	7			
Prionotus tribulus					1	1						
Paralichthys lethostigma				1		1						
Paralichthys albigutta				1		1						
Anchoa hepsetus							6		6			
Opsanus tau								1	1			
Prionotus scitulus								2	2			
Hypsoblennius hentzi								1	1			
Eucinostomus argenteus										7	3	10
Eucinostomus gula										2	7	9
Membrus martinica											2	2
Centropristes striatus											1	1
# Species	10	11	15	8	13	15	3	9	10	4	8	9
Salinity Range S	8.3-10.5	2.8 2.8		33.9-33.4	30.7-31.8		27.4-28.5	27.9-30.2		21.4-26.3	22.5-24.7	
B	13.2-12.1	3.3 2.8		22.9-25.1	32.9-31.8		27.4-24.6	30.9-32.9		24.10-26.3	23.0-24.7	
Temp. Range S	5.5 5.0	6.0 6.0		20.5-21.5	20.0-20.0		26.0-26.5	27.0-26.8		13.8-14.5	14.5-14.2	
B	5.3 6.0	4.8 6.0		19.5-20.0	18.7-18.5		26.5-25.8	26.0-26.0		13.0-13.5	14.5-14.2	

Table 10. Continued.

		11 Feb 10 m			12 May 11			18 Aug 17			21 Nov 20		
		Day	Night	Total	Day	Night	Total	Day	Night	Total	Day	Night	Total
Oxygen mg/L	S	11.21	11.68		7.11	7.89		5.79	6.83		8.84	8.57	
	B	10.67	11.45		6.87	7.89		5.79	7.62		8.30	8.16	

OBSERVATIONS ON BIRD ACTIVITY AROUND PONDS

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INTRODUCTION

Casual observation of bird activity by almost every investigator involved in the self-designing brackish-water ecosystems study indicated that birds were both a drain on and a contributor to energy webs in the two sets of experimental ponds. Initially no one was charged with responsibility for recording bird activity, but when it became evident that this should be done to help complete the accounting of energy flow, a part-time observer was engaged to collaborate on the project. One of the authors, C. J. S., teacher in the Carteret County Public School System, did the field work, and prepared this report in conjunction with A. B. W.

Seventeen species of birds were found closely associated with the ponds. All observations recorded from early September, 1970 to late August, 1971 are summarized below.

ANNOTATED CHECKLIST

1) Duck, Black (Anas rubripes). Summer. Two in P-pond area, one adult female and one immature; probably nested nearby; in all P ponds.

2) Duck, Mallard (Anas platyrhynchos-mixed species). Spring, summer. Most frequently dabbling and washing in P-1, or around P-1 where nests were located in grass; numbered about 45.

3) Egret, American (Caserodius albus egretta). Fall, winter, spring. Wading in P ponds capturing small fish; mostly in P-1, occasionally in P-2. During middle of day these birds flew across creek to roost in pines.

4) Egret, Snowy (Egretta thula thula). Summer, fall, winter. Mostly on banks of P-1, sometimes P-2, also perched on stakes in ponds capturing fish. During middle of day these birds flew across creek to roost in pines.

5) Grackle, Boat-tailed (Cassidix mexicanus). Summer. Two immature birds observed feeding around P-1.

6) Heron, Eastern Green (Butorides virescens virescens). Year round. Capturing fish from piers, stakes and banks of P ponds; occasionally wading in shallows, most frequently in P-1 and P-2.

7) Heron, Great Blue (Ardea herodias). Winter. Wading in P ponds catching fish, most frequently in P-1.

8) Heron, Little Blue (Florida caerula). Fall. Wading and catching fish, mostly in P-1.

9) Heron, Louisiana (Hydranassa tricolor ruficollis). Spring, fall. Perching on piers and pond banks, and feeding in shallow water, especially P-1. Not so abundant as Snowy Egrets.

10) Heron, Black Crowned Night (Nycticorax nycticorax hoactli). Summer. Seen near but not in P ponds in June.

11) Kildeer (Charadrius vociferus). Winter. Banks of P ponds feeding on small invertebrates.

12) Kingfisher, Eastern Belted (Megaceryle alcyon alcyon). Fall. Probably diving in all P ponds in effort to catch small fish.

13) Plover, Semipalmated (Charadrius semipalmatus). Fall. Visiting insect feeder around P ponds.

14) Rail, Northern Clapper (Rallus longirostris crepitans). Summer, fall. Found around P ponds during high tides. In Spartina growth near edges of all P ponds.

15) Sandpiper, Spotted (Actitis macularia). Spring, fall. Probably visiting area during migration; feeding on insects and on invertebrates around edge of ponds.

16) Skimmer, Black (Rynchops nigra). Summer. Skimming surface of all P ponds but most feeding occurred in Calico Creek.

17) Tern, Least (Sterna albifrons). Spring, summer. Flying over and diving into all P ponds.

DISCUSSION

Observation indicates that birds visiting the ponds were feeding there. The major predation appeared to be on fishes; herons, egrets and terns were frequent visitors. Most activity occurred in the P ponds and was concentrated there in P-1 and P-2. On days when no birds were observed, tracks and droppings around the edges and in shallows often gave evidence of visits by the predators. Windy days may have had a damping influence on bird visits because the pond waters were stirred by wind action. Most of the wading birds seek quiet water where they are better able to locate prey.

In contrast to the P-ponds, almost no bird activity was observed in the C-ponds, none during winter. This relative absence may have been caused by greater human activity in the area around the C-ponds as well as absence of adjacent marshes in contrast to the relatively isolated P-ponds largely surrounded by marshes.

Observation began during fall migration. An almost immediate decline in density can be seen from the records (Tables 1 and 2). Low levels of few species that remained resident during winter (herons, egrets) were partially suppressed by presence of hunters who frequented the P-pond area during November-January. During spring migration increased activity began in April. The increase levelled off during early summer when nestlings began to move about in search of food from mid June onward.

There are no quantitative data to support observations by A. B. W. and others that bird activity around the P ponds was greater during the summers of 1969 and 1970 than in summer of 1971, but the impression is a clear one. A reason for this may be that during the latter part of June and early July, 1971, all large fish, mollusks, crustaceans and rooted plants were harvested from the ponds. The human activity plus resulting scarcity in food supply for avian predators left in the ponds seemed to have a direct effect resulting in reduction of visits by birds to the ponds.

A log of observations follows in Tables 1, 2, and 3. Observation times were limited to hours when the observer, C. J. S., was free to be in the field. However, early morning or late afternoon-evening were judged to be prime times for observation, and except for transient records such as one occurrence of a juvenile grebe in C-3 during fall, and some others unnoted, the record is thought to be reliable.

TABLE 1

LIST OF BIRD SPECIES OBSERVED IN EXPERIMENTAL (P) PONDS FROM SEPT. 1970 TO AUG., 1971

Date	Species	Number	Ponds	Observation Time
9/9/70	Spotted Sandpiper	1	P-1	6:45 - 7:30 P.M.
	Kingfisher	1	"	
	Semipalmated Plover	2	P-1 & 2	
9/10/70	None		P	6:45 - 7:45 P.M.
9/11/70	American Egret	4	P-1, 2, 3	5:00 - 6:00 P.M.
9/13/70	Snowy Egret	10	P-2	6:45 - 7:30 A.M.
	Louisiana Heron	1	"	
	American Egret	1	"	
	Clapper Rail	1	"	
	Least Sandpiper	2	"	
9/22/70	None		P	4:00 - 4:30 P.M.
9/23/70	Sandpiper (?)	1	P-1	5:00 - 5:45 P.M.
9/28/70	None		P	3:00 - 3:15 P.M.
10/5/70	None		P	4:20 - 4:45 P.M.
10/10/70	American Egret	1	P-3	8:00 - 8:30 A.M.
10/11/70	None		P	7:45 - 8:15 A.M.
10/27/70	Snowy Egret	2	P-1	10:10 -10:45 A.M.
10/28/70	None		P	4:15 - 4:30 P.M.
11/6/70	None		P	4:00 - 4:10 P.M.
11/12/70	American Egret	2	P-1	2:10 - 2:15 P.M.
11/17/70	American Egret	1	P-1	3:55 - 4:15 P.M.
	Snowy Egret	1	P-1	
11/18/70	None		P	4:00 - 4:15 P.M.
11/23/70	None		P	4:30 - 4:45 P.M.

TABLE 1, CONTINUED

Date	Species	Number	Ponds	Observation Time
11/26/70	None		P	9:15 - 9:30 A.M.
11/27/70	None		P	9:10 - 9:20 A.M.
11/29/70	Snowy Egret	1	P-1	10:15 -10:30 A.M.
	Little Blue Heron	1	P-1	
11/29/70	None		P	3:00 - 3:30 P.M.
12/1/70	None		P	5:15 - 5:30 P.M.
12/6/70	None		P	4:00 - 4:10 P.M.
12/30/70	Great Blue Heron	1	P	9:00 - 9:10 A.M.
1/3/71	None		P	9:00 - 9:10 A.M.
1/8/71	None		P	4:00 - 4:10 P.M.
1/10/71	None		P	10:00 -10:10 A.M.
1/16/71	None		P	8:00 - 8:15 A.M.
1/20/71	Killdeer	4	P-1	4:00 - 4:15 P.M.
1/22/71	None		P	4:30 - 5:30 P.M.
2/2/71	American Egret	1	P-1	3:55 - 4:15 P.M.
	Great Blue Heron	1	P-2	
2/7/71	None		P	4:15 - 4:30 P.M.
2/12/71	Great Blue Heron		P	4:15 - 4:45 P.M.
2/21/71	None		P	10:00 -10:15 A.M.
3/1/71	None		P	3:45 - 4:00 P.M.
3/7/71	None		P	12:30 -12:45 P.M.
3/9/71	None		P	4:00 - 4:15 P.M.
3/20/71	None		P	11:00 -11:10 A.M.
3/20/71	None		P	4:30 - 4:35 P.M.
3/27/71	None		P	10:45 -11:00 A.M.

TABLE 1, CONTINUED

Date	Species	Number	Ponds	Observation Time
3/31/71	Great Blue Heron	1	P-2	5:55 - 6:15 P.M.
4/1/71	Green Heron	1	P-2 & P-3	4:50 - 6:10 P.M.
	Mallard Duck	2	P-1	
4/4/71	Mallard Duck	5	P-1	10:30 -11:30 A.M.
	Green Heron	1	P-1	
4/19/71	None		P	4:45 - 5:00 P.M.
4/24/71	Green Heron	1	P-1	11:45 -12:10 P.M.
	Spotted Sandpiper	1	P-3	
4/25/71	Green Heron	1	P-1	6:30 - 6:45 P.M.
4/27/71	Louisiana Heron	1	P-1	5:45 - 6:15 P.M.
5/3/71	American Egret	1	P-1	5:30 - 5:45 P.M.
5/7/71	None		P	5:30 - 5:45 P.M.
5/11/71	None		P	4:30 - 4:45 P.M.
5/17/71	None		P	5:30 - 5:45 P.M.
5/24/71	Mallard Duck	10	P-1	4:50 - 5:30 P.M.
	Least Tern	1	P-1 & P-2	
6/11/71	Least Tern	1	P-1	6:30 - 7:00 P.M.
6/12/71	Green Heron	1	P-1	8:00 - 8:30 P.M.
6/14/71	Mallard (young)	12	P-1	6:30 - 7:30 P.M.
	Green Heron	1	P-1	
6/21/71	Green Heron	1	P-1	12:15 - 1:15 P.M.
	Mallards (mixed)	8	P-1	
6/22/71	Clapper Rail	4	P-1, 2,3	7:45 - 9:45 A.M.
	Mixed Ducks (Mallards)	8	P-1	
6/22/71	None		P	11:00 -12:00 A.M.
6/27/71	None		P	8:30 - 8:35 P.M.

(NOTE)* Restocking program beginning

TABLE 1, CONTINUED

Date	Species	Number	Ponds	Observation Time
6/28/71	None		P	8:25 - 8:30 A.M.
6/28/71	None		P	6:00 - 7:15 P.M.
6/30/71	None		P	8:25 - 8:35 A.M.
7/2/71	None		P	8:25 - 8:40 A.M.
7/7/71	Mallards	2	P-1	2:30 - 3:00 P.M.
7/15/71	Boattailed Grackle Black Duck	2 2	P-1 P-3	10:30 -11:35 A.M.
7/15/71	Least Tern	1	P-1, 2	3:30 - 5:00 P.M.
7/16/71	Black Skimmer	1	P-1, 2,3	6:00 - 7:00 A.M.
7/19/71	None		P	2:30 - 3:00 P.M.
7/20/71	Least Tern	3	P-1, 2,3	5:30 - 6:10 P.M.
7/21/71	None		P	12:45 - 1:00 P.M.
7/26/71	None		P	8:00 - 8:30 P.M.
8/2/71	None		P	11:45 -12:10 P.M.
8/6/71	None		P	1:55 - 2:15 P.M.
8/6/71	None		P	5:50 - 6:15 P.M.
8/10/71	None		P	9:25 -10:00 A.M.
8/17/71	None		P	3:00 - 3:15 P.M.
8/19/71	Snowy Egret	1	P-2	11:00 A.M.

TABLE 2

LIST OF BIRD SPECIES OBSERVED IN CONTROL (C) PONDS
FROM SEPT., 1970 TO AUG., 1971

Date	Species	Number	Ponds	Observation Time
9/15/70	None		C - Ponds	4:30 - 5:00 P.M.
10/19/70	Green Heron	1	C -3	4:10 - 4:20 P.M.
10/10/70	None		C - Ponds	8:45 - 9:10 A.M.
10/11/70	"		"	8:20 - 8:30 A.M.
10/28/70	"		"	4:00 - 4:10 P.M.
11/23/70	"		"	4:00 - 4:15 P.M.
11/26/70	"		"	9:35 - 9:50 A.M.
11/29/70	"		"	10:40 -10:50 A.M.
12/8/70	"		"	3:45 - 4:00 P.M.
2/7/71	"		"	3:45 - 4:00 P.M.
2/21/71	"		"	4:45 - 5:00 P.M.
3/14/71	"		"	11:30 -11:45 A.M.
5/12/71	"		"	4:00 - 4:05 P.M.
7/9/71	"		"	10:30 -10:35 A.M.

AN ANNOTATED BIBLIOGRAPHY ON ECOLOGICAL REQUIREMENTS AND CULTURE OF PENAEID SHRIMPS, 1960-June, 1971

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INTRODUCTION

Profitable culture of penaeid shrimps for commercial harvest in countries of the western hemisphere has been a dream of scientists, experiment station technologists, and commercial fishermen for a long time. Cultural practices established long ago in rice paddies and similar impoundments from the Philippines through southeast Asia to southwestern India depended on flooding with waters known to contain developing young of the species, and retention of the water until reasonable growth was attained, whereupon the maturing shrimp were captured in weirs as they migrated back toward the sea on ebbing tides. The Japanese carried cultural practices forward considerably when they perfected methods of inducing captive Penaeus japonicus to spawn and produce viable larvae which were then reared to marketable size. Success of this venture, which depends in large part on marketing live shrimp for the high priced tempura and suki dishes, encouraged attempts to rear other species for profit both in Japan and elsewhere. Results of these attempts are gradually being published mostly in circulars, trade and news items, reports, or patent descriptions, while a few are contained in the primary scientific literature.

The tertiary treatment ponds in the present experiments seemed to offer a possibility for production of penaeid shrimp during warm months if management could produce conditions suitable for rapid growth, e.g. maintenance of satisfactory oxygen levels, since other ecological requirements seemed adequate. As background for conducting an experiment in such pond culture of penaeids, experience of others was sought in literature. Lists of publications dealing with penaeid shrimps have already been published, as have books and articles on aquacultural practices. Here, however, is an annotated bibliography of 76 references covering the period 1960-June, 1971, and containing only these references which have direct bearing on culture of penaeid shrimps in impoundments. Bibliographies of these papers contain references to the older literature.

SELECTED ANNOTATED BIBLIOGRAPHY

Aldrich, D. V. 1963a. Physiology and behavior program, p.55-56. In: Biological Laboratory, Galveston, Tex., fishery research for the year ending June 30, 1962. U. S. Fish Wildl. Serv., Circ. 161.

Postlarval and juvenile brown shrimp salinity tolerance ranges are noted. In addition, survival at temperature-salinity combinations is briefly discussed. Comparative survival experiments between juveniles and postlarvae in relation to temperature ranges are noted.

_____. 1963b. Tolerances to environmental factors, p. 57-60. In: Biological Laboratory, Galveston, Tex., fishery research for the year ending June 30, 1962. U. S. Fish Wildl. Serv., Circ. 161.

Study to determine shrimp behavior in different salinity gradients. Also studied was postlarval brown shrimp survival in various temperature-salinity combinations. Similar studies on brown whrimp juveniles were undertaken, and tolerances of postlarvae and juveniles were noted.

_____. 1964. Behavior and tolerances, p.61-64. In: Biological Laboratory, Galveston, Tex., fishery research for the year ending June 30, 1963. U. S. Fish Wildl. Serv., Circ. 183.

Results of two experiments are given: behavior of postlarval grooved shrimp in a given salinity to determine the influence of previous salinity history and the influence of temperature on distribution in a salinity gradient; tolerance of postlarval grooved shrimp subjected to various temperature-salinity combinations.

_____. 1965. Experimental biology program, p.76. In: Biological Laboratory, Galveston, Tex., fishery research for the year ending June 30, 1964. U. S. Fish Wildl. Serv., Circ. 230.

A comparison between brown and white shrimp survival at different termeratures is noted. In addition, the influence of temperature on growth of postlarval brown shrimp is discussed.

_____, C. E. Wood, and K. N. Baxter. 1968. An ecological interpretation of low temperature responses in Penaeus aztecus and P. setiferus postlarvae. Bull. Mar. Sci. 18: 61-71.

From author's abstract: Postlarvae of Penaeus aztecus and P. setiferus were exposed to varying temperatures under controlled conditions. P. aztecus regularly burrowed into silty clay substrate as temp. fell to 12-17°C, and emerged as temp. rose to 18-21.5°C. Under identical conditions, P. setiferus showed neither type of activity. Burrowing at low temp. is interpreted as a behavioral mechanism having survival value in northwestern Gulf of Mexico for P. aztecus, which usually arrives during summer. Temperatures which stimulated burrowing of postlarvae of P. aztecus in lab. are comparable with natural water temps. off Tex. and La. coasts during winter. Furthermore, temp. range which stimulated emergence of this organism from substrate in lab. is similar to that associated with hypothesis that most postlarvae of this shrimp species hibernate in burrowed condition for at least a portion of winter in northwest Gulf of Mexico.

Allen, D. M., and T. J. Costello. 1969. Additional references on the biology of shrimp, family penaeidae. U. S. Fish Wildl. Serv., Fish. Bull. 68(1): 101-134.

Updated the general bibliography first compiled by Chin and Allen.

Bang, K. S. and Y. K. Ro. 1969. Studies on the artificial hatching of the Korean shrimp (in Japanese, English abstract). Bull. Fish. Res. and Dev. Agency (Pusan, Korea) No. 4: 93-102.

By studying artificial hatching of Penaeus orientalis the following was observed: at temp. of 23°C and over, spawning was speedy but quanity and health bad; whereas at 18-19°C spawning was slower but quanity and health were good; development rate speedier at 21°C by 4 hours from that in 18°C experiment.

Baxter, K. N. 1963 Abundance of juvenile shrimp, p.31-32. In: Biological Laboratory, Galveston, Tex., fishery research for the year ending June 30, 1962. U. S. Fish Wildl. Serv., Circ. 161.

Shrimp postlarval abundance in relation to temperature fluctuations was briefly discussed.

Broad, A. C. 1965. Environmental requirements of shrimp, p.86-91. In: Clarence M. Tarzwell (ed.), Biological problems in water pollution. U. S. Div. Water Supply Pollut. Contr., 3rd Seminar 1962.

A review of other workers' findings. Discussion includes: salinity and distribution; salinity ans survival of juveniles; temperatures in relation to growth, mortality, spawning, and migrations.

Broom, J. G. 1969. Pond culture of shrimp on Grand Terre Island, La., 1962-1968. Proc. Gulf Carib. Fish. Inst., 21st Sess., p.137-151.

Mortalities due to O_2 deficiencies are briefly discussed. Included are average temperature and salinity of ponds for 19 months.

Chambers, G. V. and A. K. Sparks. 1959. An ecological survey of the Houston ship channel and adjacent bays. Publ. Inst. Mar. Sci., Univ. Tex. 6: 213-250.

From the author's abstract: This report includes comparison of data on temp., chlorinity, dissolved oxygen, and related factors with data on fish, shrimp, and crabs caught in trawls.

Chapman, C. R., J. C. Parker, C. R. Mock. R. D. Ringo, E. Pullen, A. Inglis, and C. H. Koski. 1964. Ecology of western Gulf estuaries, p. 50-54. In: Biological Laboratory, Galveston Tes., fishery research for the year ending June 30, 1963. U. S. Fish Wildl. Serv., Circ. 183.

Temperature and salinity in relation to sample catch of white shrimp are d discussed. Temperatures affecting general physiology and lethal temperatures of white shrimp are suggested.

Chin, E. and D. M. Allen. 1959. A list of references on the biology of shrimp, family Penaeidae. U. S. Fish Wildl. Serv., Spec. Sci Rep. Fish. 276: 1-143.

A valuable list of literature to date.

Christmas, J. Y., G. Gunter, and Patricia Musgrave. 1966. Studies of annual abundance of postlarval penaeid shrimp in the estuarine waters of Mississippi, as related to subsequent commercial catches. Gulf Res. Rep. 2(1): 177-212.

Salinity and temperature data are given for sampling stations. A brief analysis of occurrence and abundance of postlarval shrimp in relation to these two factors is given.

Cook, H. L. 1969. A method of rearing penaeid shrimp larvae for experimental studies, p.709-715. In: Proc. World Sci. Conf. Biol. and Culture FAO Fish. Rept. No.57, Vol.3.
The effects of temperature and salinity are given.

_____. 1969. Larval Culture, p.5-6. In: Rept. of Bur. Comm. Fish. Biol Lab., Galveston Texas, fiscal year 1968. U. S. Fish and Wildlife Serv., Circ. 325.
Experimental data on the effects of different temperatures on survival of brown shrimp larvae.

_____, and M. J. Linder. 1969. Synopsis of biological data on the brown shrimp Penaeus aztecus aztecus Ives, 189, p.1471-1498. In: Proc. World Sci. Conf. Biol. and Culture of Shrimps and Prawns, Mexico City, Mexico, 12-21 June 1967. FAO Fish. Rept. No.57, Vol.4. Synopsis.

_____, and M. Alice Murphy. 1966. Rearing penaeid shrimp from eggs to postlarvae. Proc. 19th Ann. Conf., Southeast. Assoc. Game Fish Comm., 1965: 283-288.
Description is given of physical facilities in which mass cultures of penaeids have been reared from eggs to postlarvae. The metal chelator EDTA was added to water in which shrimp were grown to stimulate production of Skeletonema for protozoeal stages. Naupliar stages required no food; Artemia were supplied for postlarvae. Larvae of Penaeus aztecus developed more rapidly at 30°C than at lower temperatures. Salinity varied from 20.5 to 36.0 ppt during rearing trials in which P. aztecus larvae were reared to postlarvae. Addition of mixed algal cultures as food gave better survival than additions of thier individual components. Autoclaved sea water was the best medium. Details of filtration chambers and handling characteristics of the larval stages are included.

_____, and M. Alice Murphy. 1969. The culture of larval penaeid shrimp. Trans. Amer. Fish. Soc. 98(4): 751-754.
Data on larval development, hatching and larval survival in relation to temperature. Effects of slainity on larval survival.

Costello, T. J. and D. M. Allen. 1969. Synopsis of biological data on the pink shrimp Penaeus duorarum duorarum Burkenroad, 1939, p.1499-1538. In: Proc. World Sci. Conf. Biol. and Culture of Shrimps and Prawns, Mexico City, Mexico, 12-21 June 1967. FAO Fish. Rept. No.57, Vol.4.
Synopsis.

Cummings, W. C. 1961. Maturation and spawning of the pink shrimp, Penaeus duorarum Burkenroad. Trans. Amer. Fish. Soc. 90(4): 462-468.
Data show a correlation between occurrence of early ripe and ripe pink shrimp on Tortugas Grounds. Suggested cautiously that temp. may be a triggering mechanism for spawning, however, temp. was only envir. factor cpnsidered.

Eldred,Bonnie, R. M. Ingle, K. D. Woodburn, R. F. Hutton, and Hazel Jones. 1961. Biological observations on the commercial shrimp, Penaeus duorarum Burkenroad in Florida waters. Fla. State Ed. Conserv. Mar. Lab., Prof. Pap. Ser. 3. 139p.
Exhaustive study with an exhaustive literature cited section. Discussed are temp. and salinity data for all stations, migrations in relation to low temp., relation of temp. to distribution, spawning and population densities, and growth in relation to temp.

_____, Jean Williams, G. T. Martin, and E. A. Joyce, Jr. 1965. Seasonal distribution of penaeid larvae and postlarvae of the Tampa Bay area, Florida. Fla. State Bd. Conserv. Mar. Lab., Tech. Ser. 44. 47p.

Spawning in relation to temp. for P. duorarum, spawning in relation to temp. for larger specimens, optimum spawning temp., and distribution of post-larvae in relation to temp.

Ewald, J. J. 1965. The laboratory rearing of pink shrimp, Penaeus duorarum Burkenroad. Bull. Mar. Sci. 15: 436-449.

Water temp. compared optimum temp. of lab and natural environment and found a contradiction. Gave data for growth in relation to temp. Discussed variation in larval development along with temp. Discussed shrimp mortality using waters from Biscayne Bay as against Gulf Stream waters.

Fujinaga (Hudinaga) M. 1969. Kuruma shrimp (Penaeus japonicus) cultivation in Japan, p.8110832, In: Proc. World Sci. Conf. Biol. and Cult. of shrimps and Prawns, Mexico City, Mexico, 12-21 June 1967. FAO Fish. Rept. No.57, Vol.3.

The effects of environmental conditions on hatching and development of larvae is discussed. Also included are data for O_2 and temp. in experiments to determine maximum cultivation density of Kuruma Shrimp.

_____, 1969. Method of cultivation of penaeid shrimp. U. S. Patent Office, Pat. No.3477, 406 14p. Pat'd Nov.11, 1969.

Covered the same area as Miyajura, M. 1969.

Fuss, C. M., Jr., and L. H. Ogren. 1966. Factors affecting activity and burrowing habits of the pink shrimp, Penaeus duorarum Burkenroad. Biol. Bull. (Woods Hole) 130: 170-191.

From author's abstract. The purpose of the study was to determine the effects of light, temp., salinity, fatigue, persistend daily rhythms, availability of food, bottom types, and bottom water currents on the daily activity and burrowing habits of adult pink shrimp (Penaeus duorarum), and to attempt an estimate of their relative importance. Results of modified in situ and controlled observations were combined to present a better assessment of behavior in the natural habitat. An extensive literature cited is included.

Griffith, G. W. 1966. Growth and survival of shrimp, p.43-45. In: Annu. rept. of Bur. Comm/ Fish. Biol. Lab., Galveston, Texas, fiscal year 1965. U. S. Fish. Wildl. Serv., Circ. 246.

Short-term experiments to determine survival rates of juvenile and subadult white shrimp under various combinations of temperature and salinity.

_____. 1967. Growth and survival, p. 32-33. In: Rept. Bur. Comm. Fish. Biol. Lab., Galveston, Texas, 1966. U. S. Fish Wildl. Serv., Circ. 268.

The effects of temperature on growth of postlarval shrimp are discussed.

Gunter, G. 1961. Habitat of juvenile shrimp (family Penaeidae). Ecology 42: 598-599.

A brief account of the importance of salinity in the distribution of Penaeus setiferus, P. aztecus, and P. duorarum.

_____. 1961. Some relations of estuarine organisms to slainity. Limnol Oceanogr. 6: 182-190.

Abundance of Penaeus setiferus in low salinities and correlations of the size of shrimp with a given salinity are discussed.

_____, J. Y. Christmas, and Rosamond Killebrew. 1964. Some relations of salinity to population distributions of motile estuarine organisms, with special references to penaeid shrimp. Ecology 45: 181-185.

Discussed salinity as an environmental limit and as a factor affecting the distribution of important penaeids. Included is a discussion on salinity limits and optimum salinities.

_____, and J. C. Edwards. 1969. The relation of rainfall and fresh-water drainage to the production of the penaeid shrimps (Penaeus fluviatilis Say and Penaeus aztecus Ives) in Texas and Louisiana waters, p.875-892. In: Proc. World Sci. Conf. Biol. and Culture of Shrimps and Prawns Mexico City, Mexico, 12-21 June 1967. FAO Fish. Rept. No.57, Vol.3.

Analyzed total rainfall from 1927-1964. Attempted to correlate the catch of penaeids with rainfall.

Hedgepeth, J. W. 1966. Aspects of the estuarine ecosystem p.3-11. In: R. F. Smith, A. H. Swartz, and W. H. Massman (ed.) A Symposium on estuarine fisheries. Amer. Fish. Soc., Spec. Publ. No.3

Brief mention of penaeid shrimp. Discussed the seasonal differential abundance of shrimp. Related it to salinity and nutrient cycles rather than temperature.

Hoese, H. D. 1960. Juvenile penaeid shrimp in the shallow Gulf of Mexico. Ecology 41: 592-593.

Investigation to determine the reasons for almost exclusive occurence of juveniles in low salinity environments. Author considered that other factors besides salinity may be the cause of distribution and that salinities in broad ranges are inconsequential to young shrimp.

Hoestlandt, H. 1969. Recherches sur le cycle biologique di la crevette Penaeus duorarum Burkenroad 1939 Au Dahomey (English abstract), p.687-707. In: Proc. World Sci. Conf. Biol. and Culture of Shrimps and Prawns, Mexico City, Mexico, 12-21 June 1967. FAO Fish. Rept. No.57, Vol.3.

The relationship between salinity and the density of four populations is examined.

Hudinaga, M., and J. Kittaka. 1966. Studies on food and growth of larval stage of a prawn, Penaeus japonicus, with reference to the application to practical mass culture (in Japanese, English summary). Information Bulletin on Planktology in Japan 13: 83-94. Nihon Purankuton Kenkyu Renrakukai Ho.

This journal is listed in indices under both English and Japanese titles. The second entry is transliteration of Japanese title & the second part of title means that articles are published in both Japanese and English text.

The author's abstract includes the same material as listed for these authors on the card for the 1967 publication. The journal is the same in both cases.

_____, and J. Kittaka. 1967. The large scale production (Sic) of the young Kuruma prawn, Penaeus japonicus Bate. Information Bulletin on Planktology in Japan, Commemoration No. of Dr. Y. Matsue, p.35-46.

From authors summary. Raising of larvae and propogation of plankton foods done simultaneously in culture tanks 10 x 10 x 2m built outdoors, receiving direct sunlight, filled with raw sea water filtered through 80-100 mesh net. 30-100 mature female shrimp placed in each tank for spawning. Nutrient salts added in water beginning prior to day hatched nauplii passed into zoeal stage (200g KNO_3 and 20g K_2HPO_4 /tank/day). Water in culture tank held without changing until 1 week after larvae passed into postlarval stage, then 1/5 of total water exchanged with fresh sea water daily. Simultaneous propogation of plankton food and raising of young shrimp done while aeration was applied. With this method it is possible to maintain balance of larval shrimp and food for long periods. It made possible a drastic reduction of foods such as cultured diatoms, Artemia and little neck clams which were necessary in the conventional method. Survival rate from nauplius stage to young shrimp stage was 24% and it was possible to produce an average of 1000×10^3 young shrimp per tank.

_____, and Mitsutake Miyamura. 1962. Breeding of "Kuruma" prawn (Penaeus japonicus Bate). (Transl. by Yosuke Hirono). Oceanogr. Soc. Jap., 20th Anniversary Volume, p.1-20.

Temperatures for rearing are given. Amount of oxygen demand is calculated and discussed.

Hughes, D. A. 1969. On the mechanisms underlying tide-associated movements of Penaeus duorarum Burkenroad, p.867-874. In: Proc. World Sci. Conf. Biol. and Culture of Shrimps and Prawns. Mexico City, Mexico, 12-21 June 1967. FAO Fish. Rept. No.57, Vol.3.

Response of postlarvae and juveniles to salinity changes, tide associated movements as a consequence of responses to salinity changes, and evidence for rhythm controlling receptivity to slainity decreases in juveniles are given.

_____. 1969. Responses to salinity change as a tidal transport mechanism of pink shrimp Penaeus duorarum. Biol. Bull. 136(1): 43-53.

Investigated the supposition that response to salinity changes may contribute to the tidal transport mechanism. By selective use of tidal currents as transporting media the movements of animals into and out of inshore waters are facilitated and the position of certain species with such areas maintained.

Joyce, E. A., Jr. 1965. The commercial shrimps of the northeast coast of Florida. Fla. Bd. Conserv. Mar. Lab., Prof. Pap. Ser. 6. 224p.

The importance of temperature and salinity on growth, spawning and distribution is discussed (p.177-182).

Kim, K. D. 1967. Studies on the artificial culture of Penaeus orientalis Kishinouye. Proc. Indo-Pacific Fish. Comm. 12(2): 253-257.

Development, breeding and rate of growth studies were carried out. Temp. data in relation to development, food uptake, movement and growth are given.

Kinne, O. 1963. The effects of temp. and salinity on marine and brackish water animals. I. Temp., p.301-340. In: Harold Barnes (ed.) Oceangr. Mar. Biol. Ann. Rev. 1.

An exhaustive treatment of the present knowledge of the effects of temp. on marine and brackish animals.

_____. 1964. The effects of temp. and salinity on marine and brackish water animals. II. Salinity and temp., salinity combinations, p.281-339. In: Harold Barnes (ed.) Oceangr. Mar. Biol. Ann. Rev. 2.

An exhaustive treatment of the present knowledge of the above.

KutKuhn, J. H. 1966. The role of estuaries in the development and perpetuation of commercial shrimp resources, p.16-36. In: R. F. Smith, A. H. Swartz, and W. H. Massman (ed.) A symposium on estuarine fisheries. Amer. Fish Soc., Spec. Publ. No.3.

Summarized knowledge concerning environmental factors and commercial shrimp populations. Included temp. and salinity factors in estuaries and response to environmental changes in estuaries. Also included: natural adversities, modifications by man and extensive literature cited.

Kutty, M. N. 1969. Oxygen consumption of the prawn, Penaeus indicus H. Milne Edwards and Penaeus semisuleatus DeHaan, p.957-969. In: Proc. World Sci. Conf. on the biol. and Culture of Shrimps and Prawns, Mexico City, Mexico, 12-21 June 1967. FAO Fish. Rept. No.57, Vol.3.

Discussed the influence of starvation on oxygen consumption, the influence of weight on oxygen consumption, and the oxygen consumption in relation to ambient oxygen.

Linder, M. J. and H. L. Cook. 1969. Synopsis of biological data on the white shrimp Penaeus setiferus (Linnaeus) 1767, p.1439-1470. In: Proc. World Sci. Conf. Biol. and Culture of Shrimp and Prawns, Mexico City, Mexico, 12-21 June 1967. FAO Fish. Rept. No.57, Vol. 4.

Synopsis.

Lorio, W. J. 1967. The biology of the brown shrimp, Penaeus aztecus, in impoundments. Proc. 18th Ann. Conf., Southeast. Assoc. Game Fish Comm., Oct. 18, 19, 20, and 21, 1964 Clearwater, Florida, p.539-549.

Six 1/3 acre ponds in Rockefeller Wild Life Refuge, Cameron, La., Av. 3 ft. deep, each with one or two 6 foot trenches, and filled with water ranging from 7-20ppt. salinity. Ruppia maritima covered up to 85% of pond area at times.

Filled with water before April and partly cleared of fish with rotenone. Beam trawl captures from canal used to stock ponds at 400 to 600 lbs. shrimp per acre. Fed Purina Fish Chow, mixed fish population, & 100 striped mullet. Shrimp allowed to live & grow for 6 mos. Samples taken periodically. Harvested by draining, trawling & seining. Growth rates were initially high, but tapered off (lengths). Statistical analysis of stocking rates & diets showed no significant differences among treatments, standard defiations becoming smaller with each successive sample moving toward an average size with time. Uniform size approached.

Production compared with high rate of 240 lbs. per acre of P. setiferus produced in another experiment cited. Here only 10 lbs. per acre harvested.

Rapid decrease in number of shrimp after 3rd week in August. Dietary change to more invertebrates in sotmach contents as shrimp mature militate against continued growth?

Dissolved Oxygen range, 1-14 ppm.
Dissolved CO_2 range 0-9 ppm.
pH 7-9.6
Temp. 30-36 C.
Sulfides, negative.

Mahmudul, K. and D. V. Aldrich. 1970. Influence of diet on the feeding behavior, growth, temp., tolerance. Diets influenced survival, growth and thermal resistence of postlarval Penaeus aztecus and P. setiferus. Depart. Wildl. Sci., Galveston, Mar. Lab. Texas A&M Univ. TAMU-SG-70-226. 80p.

From author's abstracts: Laboratory study on food preference, survival growth, temp., tolerance. Diets influenced survival, growth and temp. resistance of the shrimps. Results indicated initial diet preference, survival, growth and resistance to high temp. are independent qualities of foods as indicated by these 2 species of shrimps.

McFarland, W. N. and B. D. Lee, 1963. Osmotic and ionic concentrations of penaeidean shrimps of the Texas coast. Bull. Mar. Sci. Gulf Carib. 13: 319-417.

From author's abstract. The serum and muscle ions, Na, K, Ca, Mg and Cl and serum osmotic concentration of two euryhaline forms, Penaeus setiferus and P. aztecus serum is hyposmotic to sea water (ca. 89%_ and the serum ions account for 94-96 percent of the osmotic concentration. In the stenohaline penaeids, Trachypeneus similis and Sicyonia dorsalis the serum is slightly hyposmotic to sea water (ca. 97-98%). Serum ions account for 95% of the osmotic concentration in T. similis, but only 84% in S. dorsalis. High Mg levels occur in S. dorsalis, but not the other penaeids and this relates to its more sluggish activity. Muscle K concentrations are highest in the euryhaline penaeids, intermediate in T. similis and lowest in S. dorsalis. Thus, T. similis in its serum ions, has affinities to the euryhaline species to which it is closely related, and, with respect to muscle K and serum osmotic concentration, affinities to S. dorsalis with which it shares some ecological similarities. Differences in serum regulation of ions and osmotic concentration in P. setiferus and P. aztecus under hyposaline and hypersaline conditions, are shown to coincide with the different salinity distribution of the species in nature.

Miyamura, M. 1969. Method for the artificial culture of Shrimp. U. S. Pat. 3, 473, 509, Oct. 21, 1969. U. S. Patent Office.

Temperature and oxygen data are presented for the artificial rearing of shrimp from the egg stage through stages of spawning, hatching, development of nauplius, through the zoea stage, the mysis stage, postlarvae stage and on through full development to the adult stage.

Munro, J. L., A. C. Jones, and D. Dimitrius. 1968. Abundance and distribution of larvae of the pink shrimp (Penaeus duorarum) on the Tortugas Shelf of Florida, Aug. 1962-Oct. 1964. U. S. Fish Wildl. Serv., Fish. Bull. 67(1): 165-181.

From author's summary. Spawning in each year usually reached a peak during the month of the highest bottom-water temp. The month in which the bottom water was warmest varied from year to year, and the months of maximum spawning activity varied accordingly.

The center of spawning tended to move toward deeper water as the season progressed, and the last heavy spawning was in depths of more than 30 m. (16.7 fathoms). This movement may be correlated with temp. decrease in shallow water, but the movement of adult shrimp into deeper water is the factor which is most directly responsible.

Naylor, E. 1965. Effects of heated effluents upon marine and estuarine organisms. Adv. Mar. Biol. 3: 63-103.
No mention of penaeids, however, his section on "Utilization of Heated Effluents" could be useful in aquaculture investigations.

Parker, J. C., H. W. Holcomb, Jr., W. G. Klussman and J. C. McNeill. 1971. Distribution of aquatic macro-fauna in a marsh on West Galveston Bay, Texas and possible effect thereon. TAMU-SG-71-208 (Texas A & M Univ. -Sea Grant). 32p.
Lethal effects of temp. on Penaeus setiferus.

_____, C. R. Mock, E. J. Pullen and R. D. Ringo. 1964. Ecology of western Gulf estuaries, p.63-67. In: Biological Laboratory, Galveston, Tex., fishery research for the year ending June 30, 1964. U. S. Fish. Wildl. Serv., Circ. 230.
Hydrological data is presented along with seasonal abundance of shrimp. Growth and concentration of postlarval shrimp in areas of low salinities is discussed briefly.

Ringo, R. D. 1965. Dispersion and growth of young brown shrimp, p.68-70. In: Biological Laboratory, Galveston, Tex., fishery research for the year ending June 30, 1964. U. S. Fish. Wildl. Serv., Circ. 230.
Growth of brown shrimp in relation to water temp. is discussed.

Roessler, M. A., A. C. Jones, and J. L. Munro. 1969. Larval and postlarval pink shrimp Penaeus duorarum in south Florida, p. 859-866. In: Proc. World Sci. Conf. Biol. and Culture of Shrimps and Prawns, Mexico City, Mexico, 12-21 June 1967. FAO Fish. Rept. No.57, Vol.3.
The following are given: Optimum temp. for larvae; temp. in relation to mysid stages; importance of bottom temp. to spawning; max. spawning temp.; and temp. decline and larval response.

Sick, L. V. 1970. Larval distribution of commercially important Penaeidae in North Carolina. Jour. Elisha Mitchell Sci. Soc. 86(3): 118-127.
Discussed the temp. and salinith requirements of adult (spawning) and larval shrimp. Reviewed other workers' findings on salinith & temp. effects on shrimp spawning and growth, and presented sample densith of larval stages in offshore plankton.

St. Amant, L. S., J. G. Broom, and T. B. Ford. 1966. Studies of the brown shrimp, Penaeus aztecus in Barataria Bay, Louisiana, 1962-1965. Proc. Gulf Carib. Fish. Inst., 18th Annu. Sess., p.1-17.
Correlation between environmental parameters (temp. & salinity) and shrimp production. Investigation includes: postlarval recruitment, movements and temp., growth and temp.-salinity; and juvenile growth rates, occurrence and movements in relation to temp.-salinity.

_____, K. C. Corkum, and J. G. Broom. 1963. Studies on growth dynamics of the brown shrimp, Penaeus aztecus, in Louisiana waters. Proc. Gulf Carib. Fish. Inst., 15th Annu. Sess., p.14-26.

Pattern of movements, density and growth rates of brown shrimp in nursery areas. Factors controlling above are discussed--temp. and salinity.

Temple, R. F., and C. C. Fischer. 1967. Seasonal distribution and relative abundance of planktonic-stage shrimp (Penaeus spp.) in the northwestern Gulf of Mexico, 1961. U. S. Fish. and Wildl. Serv., Fish. Bull. 66(2): 323-334.

The following was considered: Average catches of planktonic-stage Penaeus spp. during different temp. conditions; seasonal abundance trends correlated with temp.; spawning in relation to bottom temp.; overwintering.

Wheeler, R. S. 1969. Culture of juvenile and adult shrimp, p. 6-7. In: Dept. of Bur. Comm. Fish. Biol. Lab., Galveston, Tex., Fiscal Year 1968. U. S. Fish. Wildl. Serv., Circ. 325.

The following information was given: low survival of shrimp in ponds in relation to O_2 depletion; ability of shrimp to overwinter in ponds; growth in relation to temp.

Wiesepape, L. M. and D. V. Aldrich. 1970. Effects of temp. and salinity on thermal death in postlarval brown shrimp, Penaeus aztecus. TAMU-SG-71-201 (Texas A & M Univ.-Sea Grant). 70p.

From author's abstract: Laboratory studies on the effects of temp. and salinity of resistance of postlarval Penaeus aztecus to high temp. Acclimation to increased temp. with concurrent changes in salinity. Resistance time increased with increasing acclimation temp. and decreased with increasing lethal temp. Evidence was found of a new salinity-temp. tolerance relationship for an estuarine organism.

Author also included Literature Cited giving reference to temp. and salinity effects on animals other than penaeids. Literature Cited includes references on physiological implications.

Williams, A. B. 1960. The influence of temp. on osmotic regulation in two species of estuarine shrimps (Penaeus) Biol. Bull. (Woods Hole) 119: 560-571.

Study to determine the ability of P. setiferus and P. duorarum to regulate their internal salinities in a variety of salinity and temp. combinations.

_____ and E. E. Deubler. 1968. A ten-year study of meroplankton in North Carolina estuaries: Assessment of environmental factors and sampling success among bothid flounders and penaeid shrimps. Chesapeake Sci. 9(1): 27-41.

From author's abstract: Assessment of effects of environmental factors, including temperature and slainity, on sampling success for postlarvae shrimp is discussed. Aside from seasonal variations, light had greatest effect on sampling success.

Zein-Eldin, Z. P. 1960. Nutrition and respiration, p.34-35. In: Galveston Biological laboratory fishery research for the year ending June 30, 1960. U. S. Fish Wildl. Serv., Circ. 92.

Attempts to find a satisfactory artificial nuturent medium are discussed. Also, a study of oxygen uptake was made for white and brown shrimp. Oxygen consumption per unit weight is discussed.

_____. 1963. Effect of slainity on growth of postlarval penaeid shrimp. Biol. Bull. 125(1): 188-196.

From author's abstract. Experiments designed to evaluate the effects of salinity upon the growth of postlarvae of the white shrimp, Penaeus setiferus, and either the brown shrimp, P. aztecus, or the pink shrimp P. duorarum. .

_____. .964. Growth and metabolism, 65-67. In: Biological Laboratory, Galveston, Tex., fishery research for the year ending June 30, 1963. U. S. Fish. Wildl. Serv., Circ. 183.

The following experiments were conducted: Influence of salinity on growth; influence of temp-salinity combinations on growth; amount of food required at various temp.; survival rates in relation to temp. and salinity; the effects of density on growth.

_____. 1966. Shrimp metabolism, p.41-43. In: Annual report of the Bureau of Commercial Fisheries Biological Laboratory, Galveston, Texas, fiscal year 1965. U. S. Fish Wildl. Serv. Circ. 246.

Discussed temp. tolerances of postlarvae brown and white shrimp; effect of temp. and salinity of growth; survival of shrimp exposed to variou combinations of temp. and salinity.

_____. 1967. Metabolism of estuarine organisms, p.31-32. In: Rept. Bur. Comm. Fish. Biol. Lab., Galveston, Tex., 1966. U. S. Fish Wildl. Serv., Circ. 268.

Investigation of the effects of salinity and temp. on oxygen requirements of postlarval shrimp.

_____ and D. V. Aldrich. 1964. Laboratory studies of shrimp tolerances to salinity and temp. (Abstract). Proc. Gulf Carib. Fish. Inst., 16th Annu. Sess., p.121.

Only an abstract, but data are given.

_____ and D. V. Aldrich. 1965. Growth and survival of postlarval Penaeus aztecus under controlled conditions of tmep. and salinity. Biol. Bull. (Woods Hole) 129: 199-216.

From author's introduction: Studies designed to test the combined effects of temp. and salinith on the survival and growth of postlarval brown shrimp.

_____ and G. W. Griffith. 1965. Growth, tolerances, and metabolism of estuarine-marine organisms, p.77-81. In: Biological Laboratory, Galveston, Tex., fishery research for the year ending June 30, 1964. U. S. Fish Wildl. Serv. , Circ. 230.

Study to determine enviornmental factors influencing growth of Penaeus aztecus and P. setiferus. The following studies were carried out: Effects of various temp-salinity combinations on young white shrimp and a comparison of tolerances of the two species; a comparison in reference to molting frequence and temp. between the two speciew; effect of temp. on growth of postlarval brown shrimp; effect of changing temp upon growth of young brown shrimp; and salinity and temp. tolerances of juveniles and subadult brown shrimp.

_____ and G. W. Griffith. 1966. The effect of temp. upon the growth of laboratory-held postlarval Penaeus aztecus. Biol. Bull. (Woods Hole) 131: 186-196.

A follow-up of previous studies. Includes an exhaustive study of the effects of temp. in the range (15^{o} - 35^{o}C) commonly encountered by the postlarvae.

_____ and G. W. Griffith. 1969. An appraisal of the effects of salinity and temp. on growth and survival of postlarval Penaeids, p.1015-1026, In: Proc. World Sci. Conf. Biol. and Culture of Shrimps and Prawns, Mexico City, Mexico, 12-21 June 1967. FAO Fish. Rept. No.57, Vol.3.

From author's abstract. Growth and survival experiments conducted in the laboratory on postlarval Penaeus aztecus Ives and P. setiferus (Linnaeus) showed that, in general, both species tolerated a broad range of temp. and salinity, but some differences between species existed. At specific temp. which ranged from 11°C, P. aztecus was more tolerant than P. setiferus of temp. of 15°C or lower. In contrast, P. setiferus was more tolerant than P. aztecus at 30°C to 35°C. Test salinities between 2 and 40 °/oo showed that P. aztecus tolerated higher salinities than did P. setiferus.

Specific combinations of salinity and temp. were tested to determine whether these factors interacted to affect postlarval shrimp. Combinations of low temp. and low salinity were detrimental. The effects of salinity and temp. on growth, survival, and tolerances of postlarvae as observed in the laboratory are discussed as related to overwintering of postlarval P. aztecus; rate of growth as observed in nature; time of entry of the two species into the nursery areas; and the effects of simultaneous decrease of salinity and temperature.

A ^{14}C TRACER STUDY OF A SIMPLE MARINE FOOD CHAIN WITH POSSIBLE APPLICATION TO POND AQUACULTURE

Keith Gates*

INTRODUCTION

The Institute of Marine Sciences maintains three Sea Grant salt water ponds that receive effluent from the secondary sewage treatment plant serving Morehead City, North Carolina (Odum and Chestnut 1970). The input of high concentrations of organic material and inorganic nutrients coupled with a relatively long flushing time enables these ponds to support high rates of primary production (Kuenzler and Chestnut 1971). Sewage effluents stimulate productivity, but the increased fixed energy is not necessarily channeled into products beneficial to man (Jeffries 1962; Barlow et al 1963; Odum and Chestnut 1970; Kuenzler and Chestnut 1971). Ryther and Mathiessen (1969) suggested that these highly productive systems could be utilized for intensive aquaculture. Such systems would serve as tertiary treatment oxidation ponds to reduce the problems of sewage treatment and produce a marketable product.

The following is the first progress report of a preliminary investigation to determine the applicability of a simple marine food chain consisting of phytoplankton, the American oyster, Crassostrea virginica, and commercial Penaeid shrimp (i.e., white, brown, or pink) to aquaculture in polluted salt water ponds. The American oyster, a filter feeder, can remove suspended particles from sea water ranging from approximately 0.9 to 12 μ in size (Haven and Morales-Alamo 1970). These particles are trapped on the gills and then sorted. Some particles are bound in mucous packets and rejected as pseudofeces while others are carried to the mouth, ingested, and eventually rejected as true feces (Chipman 1959). The disposition of filtered particles depends in part on the type and number of particles involved. Loosanoff and Engle (1947) fed varying concentrations of the alga Chlorella to oysters and found that densities between 1.2 and 5.4 X 10^6 cells/ml. produced a large fraction of pseudofeces and a relatively small fraction of true feces. As the cell concentrations were reduced the production of pseudofeces decreased and the production of true feces increased.

During 1970 the ponds under consideration maintained an average phytoplankton density of 1.8 X 10^6 cells/ml. with peak blooms reaching 10^7 cells/ml. (Campbell 1971). This average phytoplankton density falls within the range of maximum pseudofece production for Loosanoff and Engle's Chlorella feeding experiment in 1947. It follows that a large portion of the suspended phytoplankton normally available only to filter feeders would be tied up in compact packets of oyster feces and pseudofeces. This

* Under the direction of Dr. W. J. Woods.

contention is supported by Muse (1971) who found high concentrations of chlorophyll in the feces and pseudofeces of oysters grown in the ponds.

The purpose of this study is to determine through the use of radioactive carbon (^{14}C) tracer techniques if young adult commercial Penaeid shrimp can eat and assimilate the compacted organic material from the feces and pseudofeces of American oysters grown in polluted salt water ponds. To obtain an overall view of the carbon cycle in this system the amount of fixed ^{14}C in oyster tissue, shrimp tissue, phytoplankton (before and after feeding), dissolved organic carbon (before and after feeding) and in feces and pseudofeces will be determined for each of the following conditions:

1. Oysters + shrimp + phytoplankton tagged with ^{14}C
2. Shrimp + phytoplankton tagged with ^{14}C
3. Shrimp + phytoplankton filtrate tagged with ^{14}C
4. Shrimp + millipore filtered sea water + $H^{14}CO_3^-$

Naturally a duplicate control receiving no radioactive material will be run simultaneously for each trial.

METHODS

The initial concern of this study was the development and standardization of ^{14}C tracer techniques to be used for the determination of the amount of radioactive material fixed in phytoplankton samples, tissue samples, and in the dissolved organic carbon fraction of water samples using a Nuclear Chicago Unilux III liquid scintillation counter. This phase is completed and the actual feeding experiments are now underway. The following is a general outline of the methods used for a typical experimental trial.

Water samples are taken from the polluted ponds and filtered through a number 10 plankton net to remove any zooplankton. Thirty-two liters of this water are placed in each of two aerated aquaria housed in an environmental chamber maintained at approximately pond temperature. The chamber maintains a continuous light intensity of 220 foot-candles supplied by fluorescent bulbs. One aquarium is tagged with $H^{14}CO_3^-$. The other is the control. The plankton is allowed to grow and assimilate the $H^{14}CO_3^-$ for four days (W.J. Woods, pers. comm.). At the end of four days another plankton count is made for each aquarium. Small aliquots from each are filtered through a 0.45u Millipore filter. The filters are placed in a dessicator for drying. The filtrate is collected and frozen. Four oysters and four to six Penaeid shrimp that have been acclimating in Millipore filtered sea water of proper salinity for 24 hours are placed in each aquarium. At the end of four days another series of samples and counts are taken. Then 16 liters of pond water from each of two carboys maintained in

natural light for four days (one tagged and one control) are passed through two plankton centrifuges. The plankton is collected and diluted to 500 ml with Millipore filtered sea water. A plankton count is made and aliquots are taken for filtering. The phytoplankton is then added to each aquarium. The process is repeated four days later. At the end of four days of feeding samples are again taken and counts are made. The oysters and shrimp from each aquarium are collected and placed in Millipore filtered sea water for twenty four hours to remove all radioactive material from their digestive tracts. They are then frozen. Feces and pseudofeces from each aquarium are collected and frozen.

The oyster, shrimp, phytoplankton, and feces and pseudofece samples are prepared for oxygen combustion in 500 ml filtering flasks (Davidson and Oliverio 1967; Davidson et al. 1970). All frozen oysters from each aquarium are ground together in a blender. The shrimp are treated in the same manner. These samples are then weighed and dried in an oven along with the feces and pseudofeces at 80°C. Small samples (less than 100 mg) of dried oyster tissue, shrimp tissue, feces and pseudofeces, or a Millipore filter are ignited in the oxygen flasks. The evolved CO_2 is passed through a recirculating gas train (maintained at Y2 atmospheric pressure) consisting of a water cooled condenser and Drierite to remove any water vapor and stannous chloride to remove any interfering halogens (Weyman et al. 1967; Moore, et al. 1968). The evolved CO_2 is collected in 15 ml of 2:1 V/V methanol-phenethylamine. After collection this solution is diluted to 25 ml with methanol. A 5 ml aliquot is withdrawn and placed in a scintillation vial containing 10ml of scintillation fluid (300 mg. POPOP and 5g PPO in one liter of toluene - Wolfe and Shelske 1967). The samples are kept in the dark for 24 hours to reduce the effect of any chemoluminescence and then counted in the scintillation counter.

A Van Slyke wet digestion is used to determine the activity of any ^{14}C tied up in the dissolved organic carbon portion of the system (Van Slyke and Folch 1940; Van Slyke et al. 1951; Kuyper et al. 1964; Weyman et al. 1967; Moore et al. 1968). A 5 ml sample of filtrate is placed in a 125 ml filtering flask and acidified with concentrated H_2SO_4 to release any inorganic ^{14}C in the system. Another 5 ml sample of filtrate is placed in a 125 ml filtering flask and digested according to the Van Slyke procedure. The evolved CO_2 in each case is passed through the same gas train used in the oxygen flask combustion.

RESULTS AND DISCUSSION

No conclusive results have been obtained from the feeding experiments at this time. However, the work is continuing.

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Shrimp culture feasibility study

William L. Rickards and Austin B. Williams

INTRODUCTION

The long-range goals of the overall sewage study program include the development of methods of aquaculture which will utilize the effluent of sewage treatment plants as well as providing a screening device for organisms with the ability to withstand the environmental fluctuations which occur in ponds receiving the effluent.

During the two previous years of the pond project, penaeid shrimp were stocked in both the control ponds and the sewage ponds. The results of these stocking attempts were described by Beeston (1970, 1971). In both cases, the shrimp survived and grew in the control ponds but failed to survive in the sewage ponds. At the time, it was the opinion of the project personnel that failure of the shrimp to survive in the "P" ponds was due to the very low concentrations of dissolved oxygen in the water at night (Smith, 1971) as well as rather wide diurnal pH fluctuations (Laughinghouse and Kuenzler, 1971).

Despite the failure of penaeid shrimp to survive, aquacultural interest in them because of their economic value prompted the study describe below. The objectives of this study were to modify the oxygen and pH regimes in one of the "P" ponds and to subsequently stock the pond with penaeid shrimp to determine whether or not factors in addition to oxygen and/or pH had been responsible for the previous shrimp mortalities.

I. HABITAT IMPROVEMENT STUDY

In an attempt to alter the "P" pond habitat so that penaeid shrimp would be able to survive, P-2 was selected for a study in which the pond was aerated. It was hoped that a relatively small amount of aeration would alleviate the extremely low dissolved oxygen encountered in the early morning hours by other investigators (Smith, 1971). Since penaeid shrimp generally require dissolved oxygen levels of at least 2.0 ppm, we considered 3.0 ppm as a desirable level to attempt to maintain in P-2.

Methods: Prior to aeration, pond maintenance procedures involved routine daily measurement of dissolved oxygen, temperature, and pH in the evening and early morning. In addition, a diurnal dissolved

oxygen curve was determined on 20-21 June, 1971. On 14 July 1971, aeration of the pond was begun by means of a 3/4 horsepower oil-less compressor and 200 feet of perforated plastic air hose. The hose was arranged in a four-leaf clover pattern so that all areas of the pond received aeration. During the period 14-22 July, diurnal oxygen, pH, and temperature fluctuations were monitored. Temperature and pH were measured by recording instrumentation at the pond. Dissolved oxygen was determined by Winkler titration of samples fixed at the pond and returned to the laboratory.

Results and Discussion: Prior to aeration, the dissolved oxygen varied diurnally from 0.0 mg/l to about 13.5 mg/l as shown in Figure 1. Values plotted in Figure 1 are averages of triplicate measurements taken at different depths and locations in the pond.

Following aeration, fluctuations in the diurnal curve were moderated (Figure 1). Neither the daytime peak nor the early morning low point reached those experienced without the aeration. Of greatest interest was the maintenance of an average minimum dissolved oxygen value of 3.4 mg/l over the seven day period of measurement.

In addition, the pH which had at times varied from 7.5 to nearly 10.0 on a diurnal basis was now being maintained within a much narrower range, 7.6 to 8.3. Water temperatures did not differ noticeably between the two periods being compared.

Use of the compressor and perforated air line did not stir up the bottom sediments. This could have been a problem since increased turbidity in the system would have resulted in lower rates of photosynthesis by the phytoplankton possibly pushing the system past the point of compensation where production balances respiration. This would not be desirable since phytoplankton production is to be the basis for food chains to be investigated as sources of aquacultural products.

Conclusions: Aeration of pond P-2 by the means employed maintained dissolved oxygen well above 2.0 mg/l which had been accepted as the desirable level.

Aeration had no detectable effect on temperature, but diurnal fluctuations in pH were moderated as had been desired at the beginning of the study period.

As a result of the modification of the pond environment into one which was more favorable for penaeid shrimp, it was decided to undertake studies to determine whether or not shrimp could now survive and grow in the "P" ponds.

II. SHRIMP SURVIVAL

The objective of the shrimp study was to determine whether or not penaeid shrimp would survive in the modified environment of pond P-2. If survivors were found, growth would then be measured.

Methods: on 20 August 1971, juvenile white shrimp (Penaeus setiferus) were seined from Hoop Hole Creek, a tidal slough on the sound side of Bogue Banks near Atlantic Beach. The shrimp were transported to the laboratory and transferred to a holding pen through which water from Bogue Sound circulated. The shrimp were held overnight and were released into ponds C-2 and P-2 the next morning. Thus, on 21 August, C-2 received 221 juveniles and P-2 received 211 juveniles.

Additional juvenile P. setiferus were added to the same ponds on 13 September bringing the total numbers of shrimp to 321 in C-2 and 287 in P-2. No attempt was made to stock the ponds to carrying capacity. A sample of 35 of the juveniles were preserved in 10% formalin for weight and length determinations.

On 10 November seine hauls were made in each pond stocked. We recovered 60 shrimp from C-2 and 33 shrimp from P-2. Weight and length were determined for each shrimp, and they were preserved in formalin for subsequent analysis of gut contents.

Because shrimp were released into the ponds on two occasions, 21 August and 13 September, those shrimp recovered on 10 November were in the ponds for either 82 or 59 days. Data discussed in the following sections are based on an average stocking date of 1 September giving an average duration in the ponds of 70 days.

Results and Discussion: Figure 2 shows the average initial and final weights of the shrimp recovered from P-2 and C-2. Whereas shrimp in both ponds were stocked at the same average weight (1.4 grams) those in P-2 grew to a slightly greater average weight (14.8 grams) than did those in C-2 (13.45 grams). Average shrimp lengths were also somewhat larger for shrimp from P-2 than for those from C-2 (Table 1), 121.5 mm as compared to 113.3 mm.

The increase in weight of shrimp in P-2 was 13.4 grams in approximately 70 days or 0.97 mm in length per day. Such growth compares favorably to that of shrimp in natural populations in North Carolina (McCoy and Brown, 1967; McCoy, 1968; Williams, 1955). Thus, it would appear that the "P" pond environment may be made suitable for growing penaeid shrimp simply by aeration of the water.

Differences in the numbers of shrimp recovered from P-2 and C-2 may have been due to several reasons. However, the most likely reason would be the difference in bottom type between the two ponds. C-2 has a fairly firm bottom in which seining is much easier than in P-2 which has a very soft, muddy bottom into which the shrimp can burrow and which makes seining very difficult.

In addition, mortality of the stocked shrimp may have been greater in P-2 than in C-2. This could not be verified since it was impossible to recover all of the shrimp in either of the ponds. The presence of numerous blue crabs in P-2 could have resulted in greater losses of shrimp in that pond than in C-2 in which fewer blue crabs were seen.

Also shown in Figure 2 are the changes in heads-on count (number of shrimp per pound) which occurred during the study. When stocked, the juveniles were approximately 300 count heads-on, and they were approximately 30 count in P-2 and 50 count in C-2 on 10 November. Shrimp of 30 count weight are well within the marketable size range. Thus, shrimp of commercial size were produced in the pond receiving treated sewage effluent.

Supplementary feeding was not employed during the study, and it is assumed that the shrimp were feeding on the numerous small organisms and accumulated detritus in the ponds. Verification of this will be possible when the gut contents of the preserved shrimp are examined.

Conclusions: Penaeid shrimp were able to survive and grow in the aerated "P" pond. It is now possible to proceed with studies of penaeid shrimp food chains utilizing treated domestic effluent in aquacultural applications.

Shrimp growth in the "P" ponds approximated that found in natural populations of penaeid shrimp in North Carolina. Thus, there appeared to be no detrimental effects on growth resulting from the extremely eutrophic environment employed.

It appears that such eutrophic environments may be beneficially employed as grow-out ponds in shrimp culture operations since shrimp of marketable size were produced. In addition, it might be possible to obtain at least three crops from such grow-out facilities in North Carolina depending on the duration of favorable water temperatures.

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Aquaculture in brackish water ponds receiving treated domestic sewage: preliminary studies. Presented to Atlantic Estuarine Research Society, Myrtle Beach, South Carolina. April 13-15, 1972.

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Table 1. Data obtained from shrimp stocked in ponds P-2 and C-2. Shrimp lenths were measured from the tip of the telson to the base of the rostrum.

	number stocked	average # of days in pond	number recovered	initial avg. weight (gms)	initial avg. length (mm)	final avg. weight (gms)	final avg. length (mm)
Shrimp in Pond P-2	287	70	33	1.40	57	14.77	121.5
Shrimp in Pond C-2	321	70	60	1.40	57	13.45	113.3

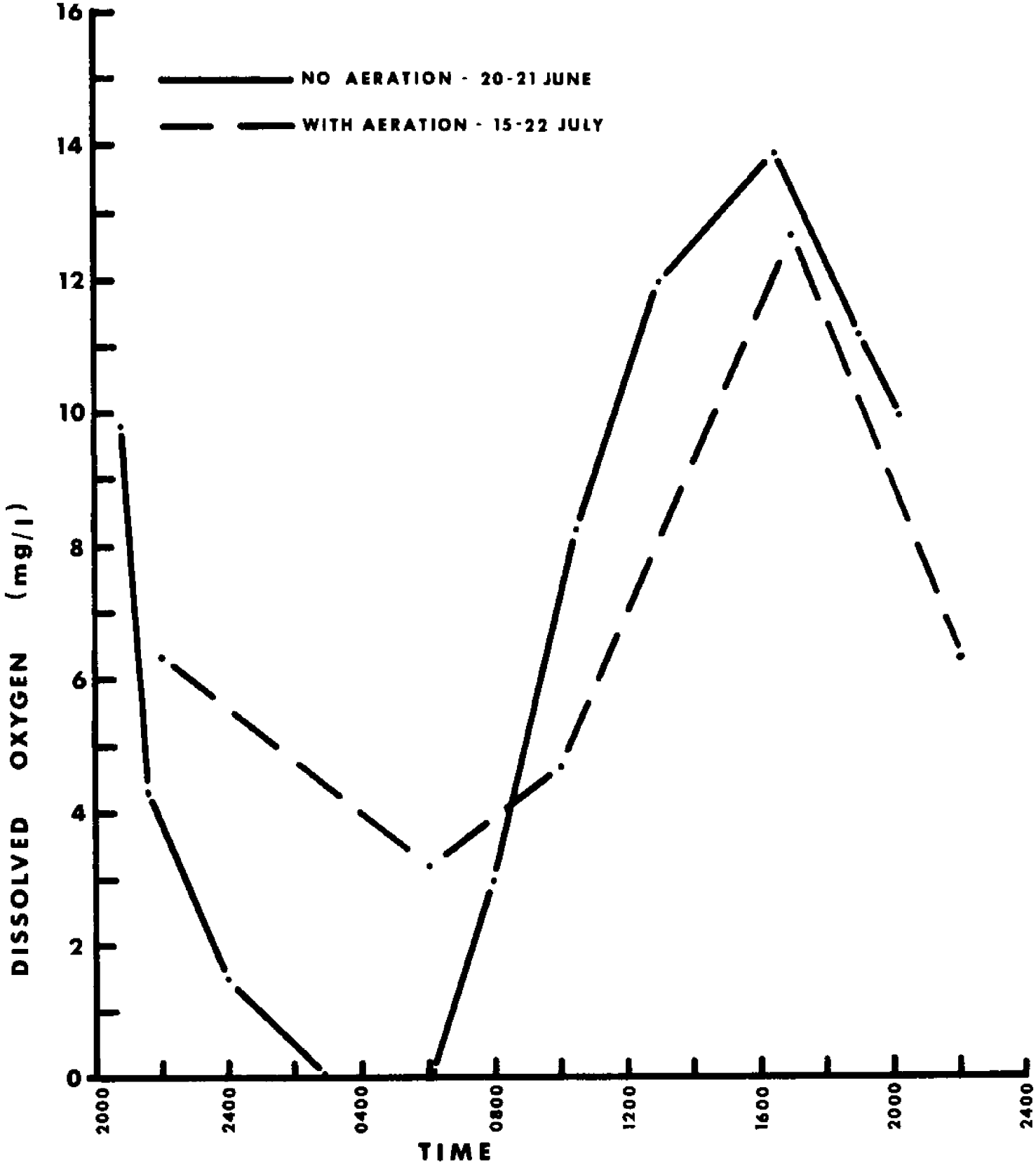

Figure 1. The average dissolved oxygen in milligrams per liter on a diurnal basis for Pond P-2 before and during aeration.

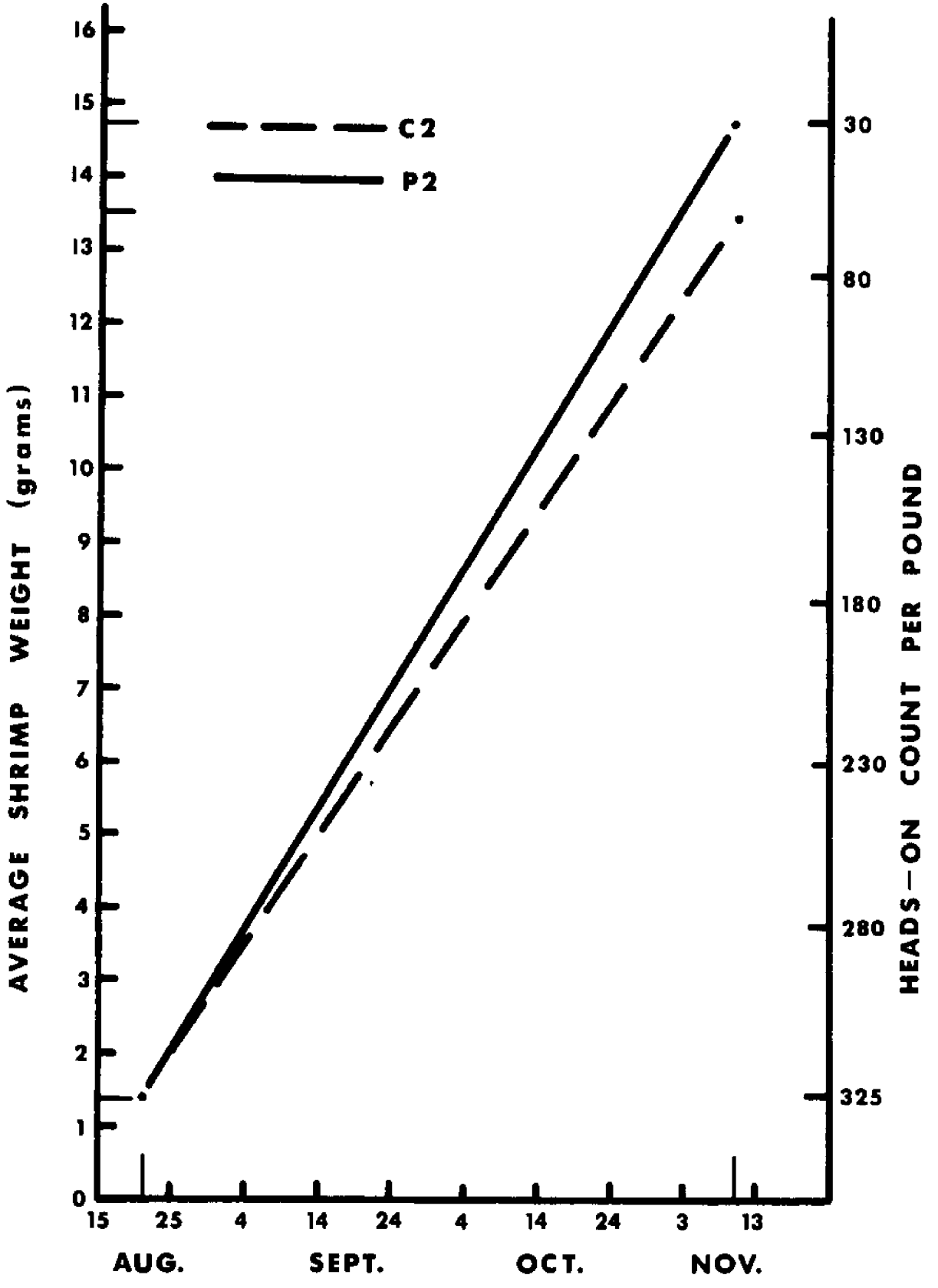

Figure 2. Changes in the average weight and heads-on count (number of shrimp per pound) during the study period for shrimp stocked in ponds P-2 and C-2.

VARIATIONS IN CONDITION OF OYSTERS FROM EXPERIMENTAL PONDS

Barbara Muse and A. F. Chestnut
Marine Sciences Curriculum

INTRODUCTION

A wide range of quality in oysters is evident in processing for market. The term "fatness" used in general descriptions of condition of oysters can be misleading for it refers to an index of condition which is attributed to the abundance of glycogen and not to lipids. Among the methods used for determining condition are glycogen measurements and calculations of percentage of dry weight. Previous workers have shown marked fluctuations in glycogen content, usually related to the reproductive cycle (Walne, 1970; Galtsoff, et al, 1947; Engle 1950, 1957; Haven, 1960; Menzel and Hopkins, 1952; Hopkins et al, 1954). The seasonal pattern of change in oysters from geographical localities varies with local climatic conditions, abundance and kinds of food available, and intensity of feeding (Galtsoff, 1964). There are no reported studies of oyster condition from North Carolina estuaries, but patterns of fluctuation are probably similar to those reported from Chesapeake Bay and South Carolina.

These studies were confined to oysters from experimental and control ponds constructed in 1968 in Morehead City, N.C. for a Sea Grant project to study self design of an estuarine ecosystem (Odum and Chestnut, 1970; Kuenzler and Chestnut, 1971). One phase of the project was directed toward possibilities of aquaculture in ponds enriched with treated sewage affluent. The enriched ponds typically have high plankton concentrations, as high as 10^7 cells/ml, with over 151 species of phytoplankton (Campbell, 1971). Utilization of phytoplankton by filter feeders, such as oysters, would be reflected in glycogen and dry weight percentage values.

METHODS

Wire baskets containing adult oysters from Bogue Sound or Calico Creek were placed in the ponds and tested after about two weeks to determine their condition. Oysters from Calico Creek were placed in control pond one to determine how long it took them to become acclimated after being moved to the pond environment. Additional samples were also taken from the Newport River, which is an area of commercial production. The oysters were collected at first only from control pond one and polluted pond one, and only single oysters were analyzed. Starting with May 16, 1971, five to 12 oysters were collected from the ponds each time.

There are many methods used by laboratories for determining the condition of oysters. Three methods mentioned by Shaw, Tubiash, and Barker (1967) and Engle (1950) are index of condition, percentage of solids, and glycogen determination. The most common procedure is a determination of the index of condition, which is generally calculated by dividing the dry weight of the meat (in grams) times 100 by the volume of the shell cavity (in cubic centimeters). The second most common method is the percentage of solids, which is determined from the dry weight of the oyster meats. The most precise measure of oyster quality is the determination of the glycogen of the oyster tissue. Percentage of solids and glycogen analyses were the two methods used in these studies to determine condition of oysters.

Dry Weight

Shaw, Tubiash, and Barker (1967) state that there are two methods for obtaining dry weights of oysters: freeze drying and oven drying. The percentage of solids is lower for oven-dried oysters than for freeze-dried oysters, indicating that volatile substances other than water are being removed by oven drying or that a small quantity of water is retained in the freeze-dried meats. The oven drying method was used in these studies.

The oysters were ground up either in a Waring blender or a tissue grinder. Generally, between five and 10 grams of each sample of ground oyster tissue were poured into a preweighed 10 cc. beaker and weighed. The oyster samples were then dried in a drying oven at about 85°C. The oysters were reweighed after several days in the oven. From these wet weights and dry weights percentage dry weight was determined by dividing the dry weight (in grams) times 100 by the wet weight (in grams).

Glycogen Analysis

Two colorimetric methods of analysis were used to determine glycogen in this study, the diphenylamine and anthrone methods. The general procedure is to extract the glycogen by alkaline digestion of small aliquots of the ground oyster meats (50 to 100 mg). Duplicate, or more often triplicate, samples of tissue were obtained. The glycogen is then isolated by precipitating out the glycogen with alcohol and centrifuging it down. The glycogen is heated with the reagent. The acid of the reagent hydrolyzes the glycogen to glucose, and the glucose reacts with the reagent to produce a green color. Glycogen analysis was started on May 30, 1971, using diphenylamine reagent. The diphenylamine method proved inconvenient in that only a limited number of samples could be determined since samples had to be read within an hour after removal from the hot water bath. Starting with August 21, 1971, the anthrone method of glycogen analysis was used.

In the anthrone reaction, according to Durham (1959), glycogen is hydrolyzed to glucose by heating it with the strong sulphuric acid of the reagent, and then the glucose is dehydrated to 5-hydroxymethylfurfural.

In the procedure the centrifuge tubes and tissue samples were weighed and 1 cc. of NaOH was added to each centrifuge tube. The tubes were heated in a boiling water bath for 30 minutes, removed from the bath, 5 cc. of hot distilled water added, allowed to cool to room temperature, then 7 cc. of 95% ethanol were added to each tube. At the end of 12 to 24 hours the tubes were centrifuged, the supernatant liquid decanted, and the glycogen dissolved in 10 cc. of distilled water. The glycogen solution was diluted with hot distilled water and 1 cc. of this final dilution added to 10 cc. of anthrone reagent. The samples and the standards were heated for seven to eight minutes in a boiling water bath, removed and cooled to room temperature and read in 1 cm. cuvettes at 620 mu. Up until February 2, 1972, oyster glycogen was used as a standard. Starting on February 2, 1972, D-glucose was used as a standard, and a formula was used for obtaining glycogen concentrations. The glucose standard reading was multiplied by 0.9, thus obtaining the equivalent glycogen reading. The formula is:

$$\frac{\text{concentration of glucose standard (=0.100mg.)}}{(0.9) \times \text{corrected absorbance of glucose standard}} \times \text{corrected absorbance of sample} \times \text{dilution factor} = \frac{\text{mg. of glycogen}}{\text{sample}}$$

The corrected absorbance is the absorbance minus the absorbance of the reagent blank. Glycogen was expressed as a percentage of the total solids.

RESULTS

Percentage of dry weight of oysters from enriched (P) ponds and control (C) ponds are contained in Table 1. The dry weight figures for the control pond oysters ranged from 11.1-26.9%. From September 29, 1970 to June 1971, the dry weight of C-1 oysters fluctuated between 20.4% and 24.8%. In mid July, 1971, C-1 dry weight dropped to 13.5%, then increased to 22.3%. The dry weight values during the months of September and October, 1971, for C-1 and C-2 oysters were 26.9% and 21%, but C-3 oysters showed a value of 14.7%. From November, 1971, to March, 1972, the dry weight values fluctuated up and down between 15.5% and 21.7%, with the C-1 oyster values consistently in the upper part of this range. At the end of March all three ponds showed a sharp drop down to 15% in C-1 and C-3. C-2 and C-3 oysters continued to have low values, 15.2% or lower, while C-1 rose again to a value of 21.2% on June 6, 1972, when the experiment was terminated.

The dry weight figures for the enriched ponds ranged from 13.1% to 27.3%. From September to December, 1970, the dry weight fluctuated between

TABLE 1. The Percentage Dry Weight of Oysters from Control (C) and Enriched (P) Ponds

Date	C-1	C-2	C-3	P-1	P-2	P-3
9/29/70	23.7			19.7		
11/6/70	21.5			21.3		
11/29/70	23.4			19.5		
12/22/70	22.6			21.9		
1/3/71	22.6			19.2		
1/28/71	21.9			15.6		
2/6/71	24.8			18.0		
2/27/71	24.0			17.1		
3/6/71	24.1			14.4		
3/18/71	21.4			17.5		
4/4/71	22.1			22.9		
4/10/71	21.4			19.8		
4/24/71	20.6			21.8		
5/16/71	20.5			21.5		
5/30/71	20.4			19.5		
6/20/71	20.7			17.9		
7/14/71	13.5			13.1		
8/3/71	22.3			22.3		
8/15/71	23.6			19.6		
8/21/71	21.5			19.5		
9/7/71	24.7			19.9		
9/21/71	21.0	11.1		18.5	19.5	
9/ 27-30/71	21.2	15.6	14.5	16.7	15.5	1617
10/4-6/71	20.2	16.0	13.8	21.4	18.8	19.0
10/11-14/71	19.4	15.9	12.4	17.1	17.1	17.1
10/18-20/71	26.9	21.0	14.7	19.2	18.2	19.3
10/25-27/71	18.8	15.6	13.2	17.6	17.1	19.0
11/1-3/71	20.4	17.4	19.4	21.4	20.1	23.0
11/15-17/71	21.7	19.9	21.5	21.5	23.9	24.3
12/2-3/71	21.0	20.6	18.5	19.4	21.2	19.1
12/7/71	18.8					
12/13-15/71	19.8	17.5	16.5	20.7	21.6	22.3
1/4-6/72	21.0	19.4	17.6	22.4		22.5
1/17-18/72	19.4	19.5	16.4	21.4	21.8	20.7
2/2-4/72	20.5	17.2	15.7	22.5	21.5	22.2
2/14-16/72	21.4	18.1	19.0	19.5	21.1	20.1
2/27-29/72	19.9	19.7	19.8	25.1	24.1	23.5
3/13-15/72	16.5	19.8	21.5	25.0	25.1	25.7
3/27-30/72	14.6	17.9	15.0	25.6	26.9	26.2
4/10-11/72		14.6	15.0	27.2	27.3	27.3
4/17/72	17.3					
4/24-26/72	17.9	13.0	14.5	25.4	25.8	26.9
5/8-9/72	18.7	14.6	15.2	26.0	25.9	25.1
5/23-24/72	18.8	13.5	14.3	23.2	24.5	20.8
6/6/72	21.2			23.5		

19.5% and 21.9% in P-1, dropped in January, 1971, and reached a low value of 14.4% in early March, 1971. A peak occurred in early April to 22.9%, then fell sharply in mid-July to 13.1% with a rise in early August to 22.3%. In contrast to the control ponds, the three enriched ponds usually had dry weight values that were fairly close together. In November all three ponds had high dry weight values, to 24.3% for P-3, and remained high from December, 1971 to February, 1972, ranging from about 19 to 22%. At the end of February a maximum of 27.2% was reached which continued into April, 1972.

Two methods of analysis were used in determining glycogen levels. Table 2 contains results of a comparative determination of the same sample by the time methods. The results obtained showed no significant difference in the two methods.

TABLE 2. Comparison of the Diphenylamine and Anthrone Methods with Oysters from C-1

Method	Sample	% glycogen
diphenylamine	C-1	34.0
anthrone	C-1	35.0

The percentage of glycogen in oysters from the two series of ponds are tabulated in Table 3. The percentage of glycogen in the control ponds ranged from 10 to 47%. Fluctuations continued through the summer, with sharp drops in mid July and mid August. In early September glycogen rose to 47% then fell during the latter part of September. In October, 1971, values ranged from 11% to 40%. The three control ponds had widely diverging values which were not in phase, for instance, when values were low in C-2 they were high in C-1 and C-3. In mid November the values in the three ponds were close, but they soon diverged again with C-1 maintaining higher values than the other two ponds.

The percentage glycogen in the enriched ponds ranged from 14 to 56%. On May 30, 1971, P-1 glycogen was 14%, but it rose on June 20, 1971 to 36%. It continued to fluctuate up and down during the summer and was similar to results of C-1 to the end of August. A decrease occurred in mid July and values of 39% were recorded in P-1 during the first part of October. Glycogen analysis on the other two P-ponds (P-2, P-3) in the month showed small peaks during late October and early November and continued to rise to a peak in April, 1972, with values of 51% to 56%. The glycogen peak was followed by a sharp drop in the latter part of May in all three ponds, to 34% in P-1 and P-2 and 21% in P-3.

DISCUSSION

The glycogen figures for the pond oysters were higher and covered

TABLE 3. The Percentage Glycogen Content of the Oysters Control (C) and Enriched (P) Ponds

Date	C-1	C-2	C-3	P-1	P-2	P-3
5/30/71	27.0			14.0		
6/20/71	42.0			36.0		
7/14/71	23.0			22.0		
8/3/71	36.0			29.0		
8/15/71	21.0			17.0		
8/21/71	35.0			25.0		
9/7/71	47.0			26.0		
9/21/71	29.0	11.0		27.0	27.0	
9/27-30/71	32.0	33.0	20.0	24.0	27.0	21.0
10/4-6/71	28.0	21.0	18.0	39.0	32.0	24.0
10/11-14/71	40.0	25.0	18.0	28.0	29.0	25.0
10/18-20/71	33.0	11.0	39.0	21.0	27.0	28.0
10/35-37/71	40.0	35.0	21.0	31.0	36.0	31.0
11/1-3/71	39.0	25.0	29.0	34.0	34.0	39.0
11/15-17/71	31.0	30.0	31.0	36.0	32.0	33.0
12/2-3/71	42.0	31.0	26.0	36.0	33.0	33.0
12/7/71	38.0					
12/13-15/71	37.0	32.0	32.0	32.0	34.0	36.0
1/4-6/72	35.0	26.0	21.0	38.0		40.0
1/17-18/72	28.0	29.0	23.0	37.0	38.0	35.0
2/2-4/72	44.0	28.0	22.0	44.0	47.0	46.0
2/14-16/72	39.0	35.0	18.0	42.0	48.0	47.0
2/27-29/72	46.0	24.0	10.0	48.0	49.0	51.0
3/13-15/72	21.0	27.0	17.0	39.0	46.0	47.0
3/27-30/72	26.0	27.0	28.0	43.0	52.0	49.0
4/10-11/72		22.0	29.0	51.0	56.0	53.0
4/17/72	28.0					
4/24-26/72	29.0	15.0	20.0	42.0	50.0	55.0
5/8-9/72	28.0	11.0	12.0	44.0	42.0	42.0
5/23-24/72	33.0	22.0	12.0	34.0	34.0	21.0
6/6/72	31.0			34.0		

a wider range than values reported for Chesapeake Bay oysters. The glycogen values were intermediate between the high and low values of results from Long Island Sound. The pond oysters had higher glycogen values than the average carbohydrate values of southern oysters, except for four instances in the C ponds. The glycogen content (based on dry weight) of the oysters in the ponds ranged from 10% to 47% for the C-pond oysters and from 14% to 56% for the P-pond oysters.

The dry weight figures for the pond oysters ranged from 11.1% to 26.9% for the C-pond oysters and from 13.1% to 27.3% for the P-pond oysters. The dry weight figures for the oysters in both sets of ponds were similar to those for Chesapeake Bay, except they never got quite as low.

There are three papers from Chesapeake Bay which give glycogen and dry weight figures. Galtsoff, Chipman, Engle, and Calderwood (1947) give glycogen as a percentage of the wet weight and the percentage of water in the oysters. These have been converted to the glycogen as a percentage of the dry weight and the percentage of solids for this discussion. In the York River and Piankatank River glycogen ranged from 3.06% to a high of 32.5%. The dry weight for these two rivers was from 8.4% to 29.7%. Engle (1950) had a glycogen range of from 3% to 35%, based on dry weights, in his studies of oysters in Chesapeake Bay (Maryland). Over a ten year period Engle (1958) states that the average monthly low was 13-14% solids and the average monthly high was 19-20% solids. The lowest solids content was 6.1%, taken during a period of abnormally low salinity and the highest solids content was 26.5%. In Long Island Sound (Galtsoff, 1964) the glycogen content based on dry weight ranged from 4% to 75+% during 1933. The following year, 1934, the minimum after spawning was around 15%, and the accumulation of glycogen was more gradual and increased about 27%. Lee and Pepper (1956), in their graph of the average carbohydrate content in monthly samples of southern oysters (based on dry weight), had a range of about 11% to 38% (cited from Galtsoff, 1964).

Neither set of ponds showed a well marked seasonal cycle. This is probably because the ponds are shallow and well mixed throughout the year so that the nutrients and algae which are continuously pumped in do not settle out. As a result nutrients are always available to the algae, and algae are always available to the oysters. Temperatures were generally favorable for feeding, except on a few occasions when it was so low that feeding would cease. The oysters are capable of feeding at all ranges of salinity found in the ponds. Although they do not feed as well at the low salinity values, it was rare that the salinity ever dropped to this level. In other words, there were very few days of the year when the oysters were not feeding on the algae to some extent. For these reasons, the pond oysters did not have as marked a seasonal cycle as would be found in an estuary. Similar results were obtained in the laboratory by Sayce and Tufts (1968). It was found that supplemental feeding improves oyster condition at all times of the year, but that optimum feeding rate varies by season or as a result of changes in

physical parameters. It follows that oysters cultured in ponds should be available over much more of the year than wild oysters and it is possible that oysters can be fattened up better in enriched waters.

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THE EFFECTS OF TREATED SEWAGE EFFLUENTS ON NITROGEN FIXATION AND AMMONIA DIFFUSION

by Martin E. Raps*

INTRODUCTION

As a part of the "Sea Grant Pond Project" the purpose of this investigation was to study the effects of treated sewage effluents on the nitrogen cycle of experimental ponds. Nitrogen in its many forms is a major constituent of these effluents. Accordingly, certain, if not all, processes within the nitrogen cycle of these model estuaries should reflect these inputs. Of particular interest in this study were the effects of these wastes on the processes of nitrogen fixation and ammonia diffusion.

Because nitrogen fixers are not dependent upon an external source of combined nitrogen, it is possible that they would have a selective advantage in the C-ponds where combined inorganic nitrogen levels appear to be low. It is also possible that, because nitrogen fixation is an expensive process in terms of energy requirements, heterotrophic N-fixers would prosper better in the P-ponds where energy-rich organics are plentiful. Autotrophic N-fixers might also prosper in the P-ponds if, owing to the high levels of phosphorus and organics, nitrogen was found to be the limiting nutrient.

With regards to ammonia diffusion, waters enriched with sewage waste effluents would be expected to raise ammonia concentration and establish conditions for increased ammonia diffusion. Therefore, determinations of the diel and seasonal rates of nitrogen fixation for the water and underlying sediments, and the rates of seasonal ammonia diffusion for both sets of ponds constitute the major objective of this study.

Also of interest in this study were the effects of sewage waste effluents on primary productivity. Thus, as an additional objective, the nitrogen budgets of the two sets of ponds were compiled and the data used to develop analog models representing the salient trophic levels in the nitrogen cycle for the two pond types.

METHODS AND MATERIAL

Nitrogen Fixation

Sample Preparation and Analysis.

A modification of the acetylene reduction technique described by Stewart et al. (1967) was employed to determine nitrogen fixation rates. Purified grade acetylene gas (0.5 cc at 0.1 atm) was injected into 8 cc rubber stoppered serum bottles containing either 2.0 cc of

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pond water concentrated 50x (see below) or approximately 2 cc of pond sediment. Silicon grease was applied around the stopper as a sealant. In addition, some samples were covered with aluminum foil. Except as noted each sample was incubated for 2 hours, either in situ or in the laboratory after which 0.5 cc of 50 per cent trichloroacetic acid (TCAA) was added to stop the reaction. Preliminary studies determined that ethylene was not generated by TCAA in contact with the rubber stoppers as previously reported by others. Equivalent volume evacuations were made prior to each addition in order to maintain atmospheric pressure. Ethylene production was measured with the use of a Varian Aerograph model 600D gas chromatograph outfitted with a H-flame ionization detector and a 9 foot long, 1/8 inch diameter teflon column packed with Porapak R, 50-80 mesh. The analyses were run at room temperature and the output of the detector was traced on a Sargent model 250-T recorder. High purity N_2 at a flow rate of about 25 cc/min served as the carrier gas. High purity ethylene was used for making standards. Samples with no added acetylene and with acetylene and TCAA added before incubation, served as controls.

Sampling Techniques

Except as noted, water samples were taken from the 30 cm depth within 1 m of the pond standpipe, and particulate matter was concentrated 50x by using a Foerst centrifugal pump. The mean of duplicate samples was used to represent "pond water" N-fixation rate. In all cases, sampling sites were chosen to minimize the influence of the pier's shadow during incubation (Fig. 1).

Preliminary studies showed that the pond sediments consisted of two distinct sections (top sediments and lower sediments) which could be identified visually. The "top sediments" of the P-ponds were between 1 and 2 cm deep, had a light green to brownish-green appearance with a powdery consistency (reflecting the presence of algae and detritus), and could readily be mixed by the actions of crabs, worms and water movement. The C-pond top sediments were about 1 cm deep, had a dark green to brownish-green appearance, were less powdery and coincidentally were not mixed as often or as thoroughly as those of the P-ponds.

The "lower sediments" of the P-ponds extended from the top sediments down to the clay bottom (which begins approximately at the 20 cm depth), had a grey-black to black appearance, were very much consolidated on the order of thick mud, embodied a slight odor of hydrogen sulfide, and the gaseous phase rendered a characteristic methane peak on the gas chromatograph. The C-pond lower sediments also extended from the top sediments down to the clay bottom (at about the 20 cm depth), had a grey-black to black appearance, were consolidated but slightly more granular than those of the P-ponds, and the gas characteristics were less prominant.

In both pond types, the first 2-4 cm of the lower sediments were slightly lighter in appearance compared to the rest of the core.

Top sediment samples were taken from each pond at the 20, 30, and 50 cm depth within 1 m of the pier. Sample cores about 1 1/4 cm long were taken by using a 5 cc capacity, 1.5 cm outside diameter plastic syringe with needle adapter end removed. The modified syringe, which resembled a cylinder and piston, could be inserted into the wide mouth vials to deposit the sample, insuring minimal disturbance of the sediment core. The mean of the three rates obtained was used to represent the "top sediment" rate of N-fixation.

For lower sediments, a large sediment core was made by inserting a 50 cm long, 4.4 cm inside diameter plexi-glass tube about 20 cm into the pond bottom at the 50 cm depth. The submerged tube was tightly plugged with a number 10 rubber stopper enabling the tube and enclosed core to be withdrawn from the pond bottom. Except as noted, small samples cores about 1 1/4 cm long were taken from the large core at the 5, 10 and 15 cm interval (i.e. sediment depth) using the modified syringe. The mean of the data obtained was used to represent the "lower sediment" rate of N-fixation.

Incubating Procedures

After acetylene addition, each *in situ* sample was fastened to the string of a weight system and incubated at the depth and position from which it was originally taken, with the exception that lower sediment samples were covered with foil and incubated on the pond bottom along with top sediment samples. All samples were incubated with vials in the upright position. In some cases, samples were covered with aluminum foil.

Samples used for studying seasonal variations were collected between 1000 and 1100 hours (EST) and incubated from 1200 to 1400 hours (EST). For diel studies, the 2-hour incubations were run every 3 hours (for sediment samples) and every 6 hours (for water samples) starting at 2300 hours (EST) the day before.

Samples selected for laboratory studies were incubated at 22 ± 2°C under a light intensity of 400 f-c. Enrichment and isolation techniques used were described by Staley (1970), and media used for isolation were based on those of Stephenson (1948) and Staley (1970).

AMMONIA DIFFUSION

A modification of the technique developed by Stratton (1969) was employed to estimate the rate of ammonia diffusion from the pond surface to the atmosphere. The recirculating, essentially closed system consisted of a plastic dome, a flow meter, an air pump, a flask containing hydrochloric acid, and interconnecting plastic tubing (Fig. 2a). A fully closed loop, consisting of the foregoing without the clear plastic dome provided a system blank (Fig. 2b). And an open system, consisting of a flow meter, pump, and trap provided an "atmospheric blank" (Fig. 2c).

In theory, ammonia diffusing from the water surface under the plastic dome is bubbled into the flask and trapped in the 0.1 M HCL solution. After each run, the trapped ammonia was analyzed in the laboratory using the method described by Solorzano (1969).

Except as noted, experiments were run for 24 hours starting at 2100 hours on the evening before the day for which the data was recorded.

NITROGEN ANALYSES AND ANALOG MODELING

Field Procedures

Samples for studying seasonal variation in nitrogen forms were taken at 1800 hours on the same day selected for N-fixation and/or ammonia diffusion experiments. Samples representing pond water were taken at the 30 cm depth within 1 m of the standpipe. Samples representing incoming pond water were taken from the mixing tanks at the 20 cm depth. The latter were taken during the high tide closest to 1800 hours, when the mixing tanks were full and in the process of supplying the ponds with mixed water. The samples were preserved with mercuric chloride in accordance with the procedures described by Jenkins (1967) and kept frozen until analyses were made.

Laboratory Analyses

Sample analyses for nitrogen species were conducted at the Limnological Laboratory of the Department of Environmental Sciences and Engineering, University of North Carolina, Chapel Hill. Analyses were carried out using Technicon Controls (TM) equipment, and the automated procedures outlined in the FWPCA Methods for Chemical Analysis of Water and Wastes (1969).

NO_3+NO_2-N was determined on filtered samples by reducing nitrates to nitrates with either cadmium or hydrazine, then reacting with acidic sulfanilamide and N-1 naphthethylenediamine dihydrochloride to form the azo dye. NO_2-N was determined by the same procedure without initial reduction, and NO_3-N was obtained by subtraction. The color was read at 520 nm.

Total and filtered (Kjeldahl) nitrogen was determined by digesting with acid at 360°C, then reacting with alkaline phenol and sodium hypochlorite to form indophenol blue. The color was intensified by adding sodium nitroprusside and read at 630 nm. NH_3-N was determined by running filtered samples through the same procedure without digesting. Millipore (TM) type HAWP 02500 0.45u membrane filters were used for analyses of dissolved nitrogen species.

Chlorophyll *a* levels were determined at the Institute of Marine Sciences, Morehead City, using the method described by Lorenzen (1967).

Analog Modeling.

Idealized models of the nitrogen flow through the ponds were based on the work of Dugdale and Goering (1967), Dugdale (1967) and Odum (1969, 1971). Analog networks representing the models were constructed using integrators as salient trophic levels and nutrient sources with multipliers to depict biological pathways. Temperature inputs were those recorded during ammonia diffusion experiments; salinity and insolation data were obtained from the sea pond log book.

RESULTS

General.

The obvious disparity between the P-pond and C-pond chlorophyll a levels on any given day (Fig. 3) appears to support the generally accepted conclusion that inflowing nutrient and energy-rich sewage effluents are directly responsible for the increased eutrophic conditions frequently observed in receiving waters (Weiss and Wilkes, 1969).

In this investigation pond samples were analyzed for the common forms of nitrogen (Fig. 4-7). Although the major source of nitrogen in the P-ponds originated from treated sewage effluents, an extrapolation from nitrogen to sewage for the purpose of establishing causality cannot be made owing, in particular, to the varying ratios of the sewage constituents. Furthermore, the coincidence of certain nitrogen curves with the curves for either chlorophyll a, N-fixation, or ammonia diffusion does not necessarily imply that a definitive causal relationship exists. As in all in situ studies of this nature (where elucidation of the most influencing environmental factor is not assured), only relationships of association are able to be drawn.

The effects of human and meteorological events

A cursory examination of Figures 4 and 5, depicting total nitrogen levels for P-pond, C-pond and mixing tank waters, reveals a striking similarity among the curves. This similarity, which is best exemplified by Figure 4, is a drastic reduction in nitrogen concentration which begins in late August, lasts the entire month of September, and ends by October 2, 1971. During this "September decline", a similar drop occurred in pond water salinities and, as shown in Figure 8. this drop in salinity appears to be directly related to meteorological events. For example, on August 24, just prior to the major tropical storm (which dumped more than three inches of rain in 12 hours and caused backflooding of all ponds), the average salinity in the P-ponds was 19.2 ppt and the total nitrogen was 2.5 g N/m^3; on October 2, after the tropical storm, hurricane, and high tides (which caused additional back-flooding), the average salinity was 10.2 ppt and the average total nitrogen was 0.75 g N/m^3 (Figures 4 and 8. Thus the P-pond waters experienced a 47 per cent decrease in salinity and a 70 per cent decrease in total nitrogen. It is therefore possible that a major cause for the decrease in P-pond total nitrogen duringthis "September decline" was the result of meteorological events.

However another factor which accounts not only for the September decline, but also explains why the P-pond mixing tank total nitrogen remained low throughout the rest of the investigation, is that the sewage effluent pump was turned off on August 28th and inadvertantly left off by the pond attendant until the spring of 1972. As a result, sewage effluents were supplied ot the P-ponds only after mixing with, and being diluted by, Calico Creek water. However, even after the sewage pump was turned off, the P-ponds were supplied with total nitrogen at nearly twice the rate supplied to the C-ponds (Fig. 4).

A third factor which could have an effect on the rate of nitrogen supplied to the P-ponds is the seasonal change in the number of vacationers in Morehead City; that is, part of the September decline might by the result of vacationers leaving the area near the end of summer, thus reducing the amount of raw sewage generated. Such a seasonal pattern in the inflowing organic carbon of the sewage effluent was observed in 1970 during which time all pumps were operating properly (Day, 1971).

The drop observed in the total nitrogen of ponds C-2 and C-3 during the same period can also be explained as a result of meteorological events. For example, on August 24, the average total nitrogen for C-2 and C-3 was 0.75 g N/m^3, whereas on October 2, the average was 0.32 g N/m^3. This represents a decrease of 57 per cent. In the same period, the average salinity decreased from 25.6 ppt to 13.4 ppt representing a decrease of 48 per cent. Note, however, that pond C-1, operating during the summer with a higher standing crop (Fig. 3) and higher levels of total nitrogen, experienced a greater decline in total nitrogen than did C-2 and C-3 which by October 2, resulted in a level comparable to the other two C-ponds (Fig. 4). The fact that C-2 and C-3 each supported an abundant standing crop of the seagrass, Ruppia maritma, and C-1 did not, probably accounted for the observed differences in the C-pond water nitrogen levels (Odum et al., 1972).

In contrast to the nitrogen drop observed in the P-pond mixing tank, the C-pond mixing tank experienced a more gradual drop during September (Fig. 4) which reflected the seasonal pattern in Bogue Sound water total nitrogen.

NITROGEN FIXATION

Seasonal N-Fixation

Similar patterns observed in the seasonal N-fixation rates of the pond water and top sediments of both pond types suggest the importance of autotrophic N-fixing organisms. Rates in June were low, with peaks developing through August and September, followed by decreases to very low rates by January (Fig. 9 and 10). Granhall and Lundgren (1971) also observed highest seasonal rates in August and September while studying N-fixation in Lake Erken, Sweden. They measured a rate of 6 x 10^{-3}

ng N/cc-hr compared to average peak rates of 8×10^{-2} ng N/cc-hr in the P-ponds and C-ponds respectively. During a study on Lake Mize, a subtropical floridean lake, Keirn and Brezonik (1971) observed a maximum rate of 3.26 ng N/cc-hr at the 3 m depth; a rate which is comparable to the average C-pond maximum. Their observed peak, however, occurred in mid June when solar radiation was at or near its maximum for the area. Thus, waters higher in latitude, such as Lake Erken and the experimental ponds, experienced peak N-fixation later in the summer.

For pond water, the average P-pond N-fixing rate for any given sampling date was 2-3 times greater than the average C-pond rate, with the only exceptions being two inordinately high P-1 readings in early September which were over 4 times greater than the average water rates for C-ponds (Fig. 9). The drop between September and October observed in C-pond N-fixing rates appears to be associated with the meteorological events discussed earlier (Fig. 8). A similar drop in P-pond N-fixing rates was probably the result of sewage pump shut-down as well as the result of storm effects. The drastic drop observed in the P-pond water rates between November and December appears to be related to a coincident temperature drop (Fig. 11) although the steady drop in insolation undoubtedly was a contributing factor (Fig. 12). The C-pond water rates were extremely low from November on; therefore, detection of a similar drop was not possible.

Through October, the P-pond top sediments were fixing at the rate of 6-9 ng N/cc sediment-hr, which is approximately 7-10 times greater than the N-fixing rate of the C-pond top sediments. After the beginning of November, the sediment rates of both pond types decreased until by early December the average P-pond top sediment rate was about .4 ng N/cc sediment-hr, or about 4 times greater than the N-fixing rate of the C-pond top sediments (Figure 10).

For the top sediments of both pond types, the storm period appeared to have little or no effect on N-fixing rates. Similarly, the sewage pump shut-down caused no immediate effect on P-pond top sediments; however, the effect may be gradual and therefore difficult to distinguish from light and temperature effects.

From mid-November on, the relatively low rates of N-fixation measured in the top sediments of both pond types may have been the result of a drastic temperature drop which occurred in the first week of November (Fig. 11). However, the precipitous drop in P-pond top sediment rates which preceeded that temperature drop was apparently man-induced (Fig. 10). On October 21 and 27, aluminum paint covered 25 per cent of the water surface of P-2 and P-3, and 100 per cent of the water surface of P-1. As noted in the Sea Pond log book, the incident was the result of spray painting the wire fences surrounding the waste treatment facility. For each of the two dates cited, visible evidence of paint disappeared from the water surface in a couple of days. It is therefore possible that the P-pond top sediments felt the impact of the paint more than the water column and the drastic reduction in N-fixation was just one more manifestion of this incident.

In contrast to the seasonal fluctuations in N-fixing rates of pond waters and top sediments, a relatively constant rate was measured in the lower sediments throughout the extent of the investigation; however, a small decrease in both pond types was evident from June to January (Fig. 13). On the whole, an average P-pond

lower sediment rate of 1.0 ng N/cc sediment-hr was about 1.3 times greater than the average C-pond lower sediment N-fixing rate.

Relative Ecological Significance

During the 8-month period under investigation, P-pond top sediments fixed about 4 times more nitrogen per unit volume than did the lower sediments; but because there were 15-20 times more lower sediments than top sediments per pond, the ecological significance of the former with respect to fixing molecular nitrogen was actually four times as great. However, assuming a lower sediment denitrification rate equal to half the rate of N-fixation (Keeny 1972), the net contribution of new fixed nitrogen by the lower sediments is about twice as much as that of the top sediments.

In addition, P-pond water turned out to be the least significant of the three regimes. Although there was 40-60 times more water than top sediments, the latter fixed 100 times more nitrogen per unit volume than did pond water. As a direct consequence of the ponds' relatively shallow depth, which in itself lessens the relative significance of pond water, the penetration of solar radiation made possible the substantial development of phototrophic N-fixers in the top sediments. Water of significantly greater depth obviates the development of such algal mats.

Thus for the P-ponds during the 8-month investigation, top sediments fixed about twice as much nitrogen as did pond water, and lower sediments fixed about twice as much nitrogen as top sediments (Fig. 9, 10, 13). Using the same approach for the C-ponds, we find that the contribution of pond water and top sediments is about equal, and that the lower sediments fix about 20 times more nitrogen than either the pond water or top sediments (Fig. 9, 10, 13).

Taking into consideration the above pond physiography and allowing for 50 per cent denitrification in the lower sediments, the relative seasonal contribution of fixed nitrogen by the phototrophic zone (pond water and top sediments) and non-phototrophic anaerobic zone (lower sediments) is illustrated in Fig. 14. For the P-ponds the contribution of the lower sediments is shown to be slightly more than that of the phototrophic zone up to October, after which the significance of the lower sediments contribution becomes quite apparent owing primarily to the decline of phototrophic N-fixation. However, for the C-ponds, the greater contribution of the anaerobic heterotrophs (lower sediments) is apparent throughout the year.

There is a remote possibility that the rate of N_2 diffusion into the lower sediments might be the limiting factor for N-fixation. If this were the case, then the N-fixing rates for all ponds as determined by acetylene reduction would be higher than the actual rates, and the significance of the lower sediments as a producer of fixed nitrogen would be diminished.

In general, the N-fixing organisms functioning in the lower sediments were limited to anaerobic heterotrophs, whereas those of the phototrophic

zone included anaerobic phototrophs (photosynthetic bacteria) as shown by the results of the following studies.

Of particular ecological significance is the fact that, although each regime of the P-ponds fixed more nitrogen per unit volume than did its respective regime in the C-ponds, the average total P-ponds N-fixation of 1.7 N/pond-day amounted to only 4.8 per cent of the total nitrogen flowing into the P-ponds, whereas the average total C-pond N-fixation of 1.0 N/pond-day amounted to about 5.0 per cent of the total nitrogen flowing into the C-ponds. Thus, on the basis of nitrogen inputs, the ratio of vertical inputs to horizontal inputs appears to be about equal for both pond types.

Levels of Significance Between C and P-ponds.

Statistical support for the conclusion that the rate of N-fixation is greater in the P-pond than in the C-pond during the summer is furnished by the results of the expanded study conducted on August 15 (Table 1).

For pond water, total N-fixation (light vials) was found to be greater at the 90 per cent level of significance in P-pond water than in C-pond water. However, the difference in heterotrophic activity was not found to be significant (i.e. $\alpha < 90$). In addition, the results showed that for 1200 to 1400 hour incubation period, 90 per cent of the N-fixing activity in the P-pond water (and about 70 per cent in C-pond water) was attributable to phototrophs, and in view of the high dissolved oxygen levels regularly present in the water column, these phototrophs were algae. These results can be compared with the findings of Kusnetzov (1968) who concluded that, with respect to algae, heterotrophs played a minor role in the N-fixing activity in lakes.

For the top sediments, the results of the expanded study (Table 1) indicated that, with respect to the C-ponds), P-pond total N-fixation was greater at the 99.9 per cent level of significance, and P-pond heterotrophic N-fixation was greater at the 99.5 per cent level of significance. In the lower sediments, P-pond heterotrophic N-fixation was greater at the 90 per cent level of significance. Ligh vials for lower sediments actually registered a lower value than their respective dark vials and, because of this, were discarded. This was probably due to light inhibition, as it known some anaerobes are sensitive to light as well as oxygen (Brooks et al., 1971).

Therefore, the results in all three regimes support the association between high organic matter and high N-fixation, especially phototrophic. Horne (1972) found the same association between dissolved organics and algal N-fixation existing in tropical waters.

The results also show that for the 1200 to 1400 hour incubation period about 95 per cent of the N-fixing activity in the top sediments (70 per cent in C-pond top sediments) was attributable to phototrophic organisms.

On the basis of their appearance, and the presence of H_2S and methane in the lower sediments, it was assumed that on the whole the top sediments of both pond types functioned aerobically, and the lower sediments of both pond types functioned anaerobically. Although enrichment techniques were not entirely successful, some identifications were made from samples taken August 15 which could be interpreted to support this assumption. A blue-green alga of the family Nostocaceae (Anabaena) was isolated from top sediment samples of P-1, P-2, and C-1, and Azotobacter sp. was isolated from top sediments of all ponds except C-3. Both of these N-fixers function aerobically. In addition, microscopic examination following partial enrichment also revealed the presence of one or more species of purple sulfur bacteria in the top sediments of P-1 and P-2. It should be noted that the presence of an organism does not necessarily imply that it is functionally operating as a N-fixer. For example, it is difficult to imagine the presence of a functional (N-fixing) niche for the purple sulfur bacteria in the P-pond top sediments, because solar energy among other things would have to be made available to an anaerobic environment. Although light reaches the pond bottom most of the time (except during the dense blooms), the isolation of Anabena tends to substantiate the aerobic nature (however weak it may be) of the P-pond top sediments. However, the continual oxidation of the high energy detritus which exists in the P-pond top sediments as a result of the inflowing effluents, could maintain a very low oxygen environment, low enough for the proliferation of anaerobic microorganisms. In addition, the overall lower oxygen environment within the P-pond top sediments could partially account for the higher N-fixing rates with respect to the C-pond top sediments,because the efficiency of the N-fixing mechanism in both aerobic heterotrophs and autotrophs is increased as oxygen tension is decreased, reaching a maximum at about 4 per cent standard atmosphere (Parker, 1954; Dilworth and Parker, 1961; Stewart and Pearson, 1970,) The high organic loads would also provide the necessary energy for N-fixation required by the inefficient aerobic heterotrophs, such as Azotobacter (Stewart, 1969). Similarly the high amounts of energy-rich organics filtering down to the lower sediments could support a larger community of anaerobic heterotrophic N-fixers, such as the ubiquitous Clostridium (Stewart, 1969).

Therefore, a rationale exists for the observance of higher rates of N-fixation in the sediments of waters receiving treated sewage effluents.

Diel N-Fixation

With respect to the diel studies conducted on July 30 and November 4, the highest rate of N-fixation for both pond water and top sediments occurred during the 1200 hour incubation period (Fig. 15, 16). Because similar results were obtained in preliminary studies, mid-day was chosen to incubate samples for seasonal N-fixation studies. Preliminary studies also determined that no measurable change occurred in lower sediment rates as a function of time of day).

During diel studies on Lake Erken, Granhall and Lundgren (1971) found that peak rates occurred at mid-day; in fact 25 percent of the daily

N-fixation was measured between 1100 and 1300 hours. Stewart, Fitzgerald, and Burris (1967) found highest rates occurring just after mid-day (1300 hours) in a study on Lake Mendota.

Compared to the diel profiles of July 30, the lower amplitudes of the November profiles were probably a result of seasonal decrease in solar energy and, to a lesser extent, temperature. Also, by comparing the overall shape of the July 30 P-pond profiles with those of November 4, the effect of the "shorter day" becomes evident. This is particularly true for P-1 and P-2 pond water rates as shown by the relatively sharper mid-day spikes obtained for November 4 (Fig. 15).

Assuming that the data obtained from the 0000 and 2400 hour incubation represent purely heterotrophic activity, and assuming that heterotrophic N-fixation does not change drastically with respect to time of day (as implied by the expanded study of August 15th), one can calculate the contribution of heterotrophs to total N-fixation by dividing the nearly rectangular area formed by the 0000 and 2400 hour ordinates (representing heterotrophic activity) by the total area under the diel curve(Fig. 15, 16).

Using this method on pond water data (Fig. 15), the daily contribution of heterotrophs to total N-fixation for July 30 ranged from 27-45 per cent for the P-ponds and 21-37 per cent for the C-ponds; for November 4 the heterotrophic contribution ranged from 38-46 per cent for the P-ponds and 45-63 per cent for the C-ponds. That is to say, in pond water of both pond types, the role of phototrophs in N-fixation decreased as winter approached.

In the top sediments, the daily heterotrophic contribution for July 30 ranged from 33-43 per cent for the P-ponds and 53-66 per cent for the C-ponds; for November 4 the heterotrophic contribution ranged from 39-52 per cent for the P-ponds and 24-32 per cent for the C-ponds (Figure 16). For the P-pond top sediments, the role of the heterotrophs increased as winter approached just as in the case of all pond waters. However, heterotrophic contribution in the C-pond top sediments, was substantially curtailed.

Except for the C-pond top sediments, the results from this exercise support the conclusion that for these two regimes, solar radiation played a more important role than temperature. Otherwise, heterotrophic activity should have also declined in more or less proportion to the phototrophic activity.

N-fixation and Depth

Variations in the rate of N-fixation with respect to pond water appears to be limited to "surface effects" (Fig. 17a). For pond water, the rates were relatively constant from 20 cm to 60 cm in most cases. An apparent depression in N-fixation at the air/water surface, most evident in the P-ponds, might have been the result of excess solar radiation or temperature; however, such effects usually extend over a range of 3-5 m rather than 20 cm (Raymont, 1963). It is also doubtful that selective herbivorous feeding, or bouyancy phenomena on the part of the responsible phototrophs could account for such

depressions (Raymont, 1963). It is also possible that the effect was just "apparent" because all surface data, except for P-1, fall within the 90 per cent confidence interval (the rejection quotient).

The slight increase observed at the 80 cm depth, with respect to the rates at the 20-60 cm depths, although possibly apparent, could have been the result of occasional stirring of the top sediments causing a dispersion of N-fixing algal cells into the 30 cm region.

Wheras little or no variation in N-fixation was measured between the top and lower sediments of the C-ponds, top sediments of each P-pond fixed about 3 times more nitrogen on July 10 than did the sample taken from the 2 cm sediment depth (Fig. 17b).

In Waccasassa Estuary, an unpolluted embayment in northwest Florida, Brooks, et al. (1971) measured N-fixation rates of approximately 5 ng N/cc sediment/hr. in the 2-5 cm sediment depth and about 0.1 ng N/cc sediment/hr in the 5-20 cm sediment depth. They were unable to detect N-fixation in the flocculent top 2 cm of sediments. However, in order to obtain replicate samples, the authors blended together two samples from each station in order to achieve homogeneity. It is possible that such stirring may have upset the low aerobic and anaerobic microcosms by introducing more oxygen into them from the interstitial and overlying waters, thus, in effect, inhibiting or altogether poisoning the oxyphobic nitrogenase systems (Postgate, 1969; Drozd and Postgate, 1970). During studies preliminary to this investigation this author observed that stirring of pond sediments consistenly yielded lower results compared to controls, and thus stirring was eliminated from the procedures.

For similar reasons, water and sediment samples were not purged with nitrogen-free gas (20 per cent O_2, 0.04 per cent CO_2, balance Argon) as is often employed by other researchers (Stewart, Fitzgerald and Burris, 1967; Brooks et al., 1971). The rationale for using such a procedure is to increase the efficiency of acetylene reduction. However, because as little as 0.1 atm acetylene inhibits N-fixation by 95 per cent, its use is unwarranted for in situ studies in view of the possible errors incurred by disrupting (changing the oxygen environment of) the sample, be it water or sediment (Postgate, 1969; Granhall and Lundgren, 1971)

Limiting Nutrient and N-Fixation

It has been suggested that the macronutrient having the most control over phytoplankton growth (i.e. limiting nutrient) in the P-ponds was inorganic nitrogen (Woods, 1970; Hommersand and Talbert, 1971). Although a definitive statement regarding this issue was not the intended purpose of this investigation, a point can be made with respect to N-fixation which would tend to support the above suggestion.

As outlined in the introduction, it would appear logical that a selective advantage for N-fixing organisms should develop in environments limited by combined nitrogen. The results of this investigation confirm that such a niche had developed, and that it was stronger and broader in

the P-ponds than in the C-ponds even though nitrogen inputs to the former were greater. The rationale for this apparent paradox derives from the fact that, compared to the often quoted 15:1 N/P ratio (representing the relative proportion of those two macronutrients in the "average" algal cell), the inflowing sewage effluents exhibited a N/P ratio of about 1.5:1 (Hommersand and Talbert, 1971), and the P-pond water exhibited a N/P ratio of about 3:1 (Woods, 1970; Masarachia, 1971). Thus in order for the P-pond ecosystems to more fully utilize the incoming phosphorus and organic carbon (i.e. in accordance with the observed P-pond standing crops), a requisite source of combined nitrogen, namely ammonia, was provided through N-fixation.

Ammonia Diffusion

The rate of ammonia diffusion from the surface of each pond into the above atmosphere was measured about once every three weeks throughout the extent of the investigation (Fig. 26, 27). The average P-pond rate was found to be 2-3 times greater than the average C-pond rate. The factors most responsible for this were the higher pH in the P-ponds, and the higher ammonia concentration in the inflowing water (Table 2, Fig. 6). The higher pH in the P-ponds was the result of higher alkalinity in the inflowing water (Day, 1971), as well as the response to the photosynthetic activity of Monodus (Hommersand and Talbert, 1971); higher ammonia concentration in the mixing tank was, either directly or through Calico Creek, the result of inflowing treated sewage effluents.

Note, however, the difference between the ammonia concentrations occurring throughout the summer in the P-pond mixing tank and in the P-ponds (Fig. 6). This in itself alludes to the greater P-pond diffusion, and possibly to the greater metabolic uptake or nitrification of ammonia that might have occurred within the P-pond communities.

The highest diffusion rate (46 mg N/m^2-day) was recorded in P-2 on August 24 when the daily maximum pH was 9.9 and the ammonia concentration was 0.005 g N/m^3. The average P-pond rate was about 14 mg N/m^2-day during the summer, and about 3 mg N/m^2-day from October to December (Fig. 18). The C-pond average rate was approximately 6 mg N/m^2-day through September, and about 1 mg N/m^2-day to December. The efficiency of the 24 hour runs was about 80 per cent.

While studying alkaline water impoundments, Stratton (1969) recorded a rate of 35.5 mg N/m^2-day for Elfin Forest Lake, California, which had a pH of 9.8 and an ammonia concentration of 0.47 g N/m^3. For Santee holding pond (California) he recorded a rate of 97.9 mg N/m^2-day with a pH of 9.1 and an ammonia concentration of 1.75 g N/m^3 (Stratton, 1970).

pH and Ammonia Concentration.

Because the mid-afternoon P-pond pH consistently reached 9.5 or higher, a resultant shift in the equilibrium equation of the hydrolytic reaction (see Section II) favored the gaseous form of ammonia (NH_3) over the protonated form (NH_4^+). When the concentration of ammonia gas sufficiently surpassed the saturation level, diffusion occurred. The saturation level is a function of the partial pressure of the gas in the above atmosphere and the temperature and salinity of the water (Raymont, 1963). These factors, plus incoming ammonia ($NH_3 + NH_4^+$), pH, wind and water velocity, and other factors defining surface area and quality of the air/water interface, all influence the rate of ammonia diffusion. For the most part, it can be assumed that temperature, salinity and partial pressure were the same for both pond types, and therefore could not account for the differences observed between the rate of diffusion. However, because the C-ponds were openly exposed to Bogue Sound, they probably experienced higher velocity winds (increasing effective surface area) than did the P-ponds which were more secluded. Also, as a result of the inflowing treated sewage effluents, the P-pond surfaces were probably covered with surface active agents which would reduce the effects of winds as well as create a potential energy barrier to the diffusion process. But with both of these factors operating, they would if anything tend to decrease the difference and are therefore antithetic to the observed facts. Thus higher pH and greater ammonia input were the major factors responsible for the higher rates of ammonia diffusion observed in the P-ponds.

Relative Ecological Significance.

The P-ponds lost about 1.8 g N/pond-day through ammonia diffusion, or 5.1 per cent of the average total nitrogen flowing into the P-ponds. The C-ponds lost about 0.9 g N/pond-day, or 4.5 per cent of the average total nitrogen flowing into the C-ponds. Although the average P-pond loss was double the average C-pond loss, the per cent loss from each pond with respect to its total nitrogen inflow was relatively constant.

However, the comparison holding the greatest ecological significance is that made between N-fixation and ammonia diffusion. As mentioned before, the average total N-fixation was about 1.7 g N/pond-day for the P-ponds, and about 1.0 g N/pond-day for the C-ponds. Therefore, the contribution of vertical inputs (through N-fixation) was just about equal to the vertical losses (through ammonia diffusion) for each of the six ponds during the 8-month period investigated.

Although the finding is quite significant, it must be remembered that this applies only to shallow ponds. Therefore this would not apply to most lakes, especially those lacking a euphotic benthos. However, it should be applicable to coastal estuarine embayments.

Analog Modeling

Monodus Bloom.

For three consecutive winters, the P-ponds developed a dominant xanthophyte alga, Monodus guttula, which after a rapid die-off (crashing) was replaced each May by a more diversified plankton flora (Odum et al., 1972). It is believed that Monodus requires HCO_3^- as a source of photosynthetic carbon (Hommersand and Talbert, 1971), and as a result the pH began to rise from the onset of the early November bloom (Fig. 2).

Hommersand and Talbert (1971) suggested that the major Monodus bloom occurring in April was in response to temperature rising above 20° C, and that the May crash was the result of nitrogen limitation brought on by warmer temperatures and thus higher metabolic uptake of nitrogen. They were less clear on what caused the more gradual disappearance of the Summer flora and take-over by Monodus in November. However, because Monodus consistently dominated the P-ponds for the last three winters, this strongly suggests that the two events were also temperature controlled (Fig. 11).

Although less likely, there is the possibility that combined nitrogen concentrations which existed at the end of September were so low that the Summer flora were unable to maintain their high demands for nitrogen, even when their metabolic rates decreased with decreasing temperature. This assumes that the Summer flora, by their very nature of being warm temperature acclimated/warm temperature selected organisms, would metabolize relatively faster, and would possess relatively higher K_t and V_{max} values for combined nitrogen than would the winter acclimated/winter selected Monodus. The advantage of such an explanation is that it describes a mechanism for nitrogen to be limiting during the late summer through early winter period. Although this explanation might be applied successfully to the data of this investigation, it would not explain the high levels of combined nitrogen measured in the winters of 1968 and 1969 (Woods, 1970). Nevertheless, in all four years (1968 through 1971), Monodus consistently began its growth phase in late October/early November and crashed in May. Therefore, a simpler and perhaps more reliable explanation for the occurrence of these events is temperature.

Analog Model.

Utilizing P-pond temperature, chlorophyll a, and nitrogen data, a mathematical model was developed to describe P-pond phytoplankton growth in terms of nitrogen (N) and temperature (T) inputs (Fig. 19). The phytoplankton community was split into two parallel circuits, one representing the diversified summer phytoplankton (P_1), and the other representing the winter phytoplankton (P_2) dominated by Monodus.

Because the model comprises a set of differential equations, model solutions can be readily obtained by use of an analog computer (electronic differential analyzer). The analog circuitry (program) representing the model is illustrated in Fig. 20, and the nitrogen flow diagram, upon which the model is based, is shown in Fig. 21.

The prerequisite assumption made, when utilizing nitrogen data to develop a model on phytoplankton growth, is that nitrogen is the limiting nutrient. And similarly, because temperature is included, it should be considered as the limiting physical parameter. Therefore, the accuracy with which a model describes the observed data is a measure of the validity of the basic assumption.

Fundamental to the operations of the electronic differential analyzer are the assumptions that transport coefficients (K's) are constant with respect to input variables, and that mulitplication expresses the best relationship among the variables in each transport term. In truth, transport coefficients vary with temperature and with substrate concentration when the latter is below the level required for V_{max}.

In order to develop a model of manageable size which could fit the EAI-20 analog computer, further assumptions had to be made. The zooplanktonic grazers and decomposing bacteria were assumed to vary in direct proportion to phytoplankton levels, with the ability to recycle ammonia back to the nitrogen pool fast enough to allow their elimination as an integrating element. Although eliminated, their function as "fast" recyclers is still indicated by the pathways leading from the phytoplankton back to the nutrient pool (Fig. 21). There is no doubt that the assumption is untrue; however, the simplified model becomes manageable and the resulting solutions can be evaluated in light of their qualifying conditions.

As indicated in Fig. 20, two switches, a comparator, and a variable diode function generator (VDFG) give greater flexibility to the program. Switch (A) delivers two rates of nitrogen input. This approximates the conditions experienced before and after the September decline (Figures 4-7). Switch (B) provides two values for the P_1 growth transport coefficient, a higher value for the summer and a lower value for the winter. The comparator automatically selects between K_{13a} to K_{13b} depending on the difference between temperature and reference. That is, when temperatures drop below 18° C, the larger K (K_{13b}) is selected, thus in effect increasing the death rate. And lastly, the VDFG generates the temperature profile for the season, thus the influence of temperature on transport coefficients if provided by a multiplying function (Fig. 19).

Transport coefficients were based on those presented by Dugdale (1967), MacIsaac and Dugdale (1968), and Eppley, Rogers and McCarthy (1969).

The results of the trial-and-error procedure for finding the set of transfer coefficients that are reasonable, and result in the "best fit", are illustrated in Fig. 22. The effects of the 47 per cent dilution by the September storm period have been added "after-the-fact". This curve was achieved with K_{13a} = .15/wk, K_{13b} = 1.5/wk, K_{31a} = .8/wk and K_{31b} = 7.0/wk; thus this indicated the necessity for quick recycling of P_1 nitrogen back into the nitrogen pool (N) for subsequent uptake by Monodus. In order to prevent a total drain on the nitrogen pool it was necessary to use the data for total filtered nitrogen instead of combined nitrogen. Therefore, persistent bacterial action may be providing a slow but continuous source of ammonia for the growth of Monodus. In this case, the total (filtered) nitrogen inflowing rates were set back one week in an attempt to partially account for the time required by bacteria to break it down into readily usable forms.

Although the "fit" is not perfect, it does suggest that temperature is the most influential physical factor, and that nitrogen may be the most controlling macronutrient.

SUMMARY AND CONCLUSIONS

The effect of treated sewage effluents on the rates of N-fixation and ammonia diffusion of three estuarine ponds were determined for an 8-month period starting in June, 1971. N-fixation was measured using the acetylene reduction technique whereby ethylene is produced at a theoretical

rate equal to 1.5 times the rate that ammonia would be produced. A floating plastic dome and air pump system was employed for measuring the rate of ammonia diffusion from the pond surface into the above atmosphere. Temperature and nitrogen data was used to develop an analog computer program relating phytoplankton growth to the input variables. The following constitutes the major findings of this investigation:

(1) P-ponds fixed nearly twice as much nitrogen as did the C-ponds. However, the ratios of total N-fixation to total inflowing nitrogen were about the same for both pond types.

(2) Peak N-fixation rates occurred during mid summer in water and top sediments of both. On a diel basis, peak rates occurred at mid day, thus suggesting the importance of phototrophic N-fixation.

(3) For the P-ponds over the entire investigation, the top sediments fixed about twice as much nitrogen as did the pond water, and the lower sediments fixed about four times as much nitrogen as did the top sediments. However, assuming that 50 per cent was subsequently denitrified, the lower sediments contributed about twice as much fixed (combined) nitrogen to the pond system than did the top sediments.

(4) For the C-ponds over the entire investigation, the top sediments and pond water fixed about the same amount of nitrogen, but the lower sediments fixed about 20 times more nitrogen than either the top sediments or pond water.

(5) For pond water, the daily phototrophic contribution to N-fixation was 54-73 per cent for the P-ponds, and 37-79 per cent for the C-ponds. For top sediments, the daily phototrophic contribution was 48-67 per cent for the P-ponds, and 34-76 per cent for the C-ponds. In general, the rate of heterotrophic N-fixation increased with the approach of winter.

(6) For each pond, the total contribution through N-fixation was offset by nearly the same loss through ammonia diffusion.

(7) Peak ammonia diffusion rates occurred in mid summer for both pond types.

(8) From the solution of the analog computer program, temperature appeared to be the most controlling physical factor initiating the decrease in summer phytoplankton flora and the start-up of Monodus.

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Table 1. Mean ($\bar{x}$), variance (S^2), and t-values for light (L) and dark (D) *in situ* N-fixation conducted on August 15, 1971.

			N-Fixation P-ponds	N-Fixation C-ponds	t-value	P<
Pond Water (ng N/cc water-hr)10^2	(L)	$\bar{x}$	5.3	3.7	1.450	.10
		S^2	3.9	3.4		
	(D)	$\bar{x}$	0.6	1.0	-0.894	*
		S^2	0.4	0.8		
Top Sediments (ng N/cc sediment-hr)	(L)	$\bar{x}$	9.45	0.82	9.278	.001
		S^2	6.24	0.63		
	(D)	$\bar{x}$	.59	0.10	3.13	.005
		S^2	.16	0.06		
Lower Sediments (ng N/cc sediment-hr)	(D)	$\bar{x}$	1.72	0.96	1.672	.10
		S^2	1.12	0.74		

*No statistical significance

Table 2. Partial list of average pH measured at 1600 hours during ammonia diffusion experiments.

Date	C-Ponds	Date	P-Ponds
June 9	7.9	June 16	8.6
July 6	8.2	July 6	8.9
August 14	8.1	August 24	8.9
September 12	8.1	September 12	8.7
October 25	8.0	November 2	9.3
November 12	8.2	November 19	9.4
December 12	8.3	December 12	9.6

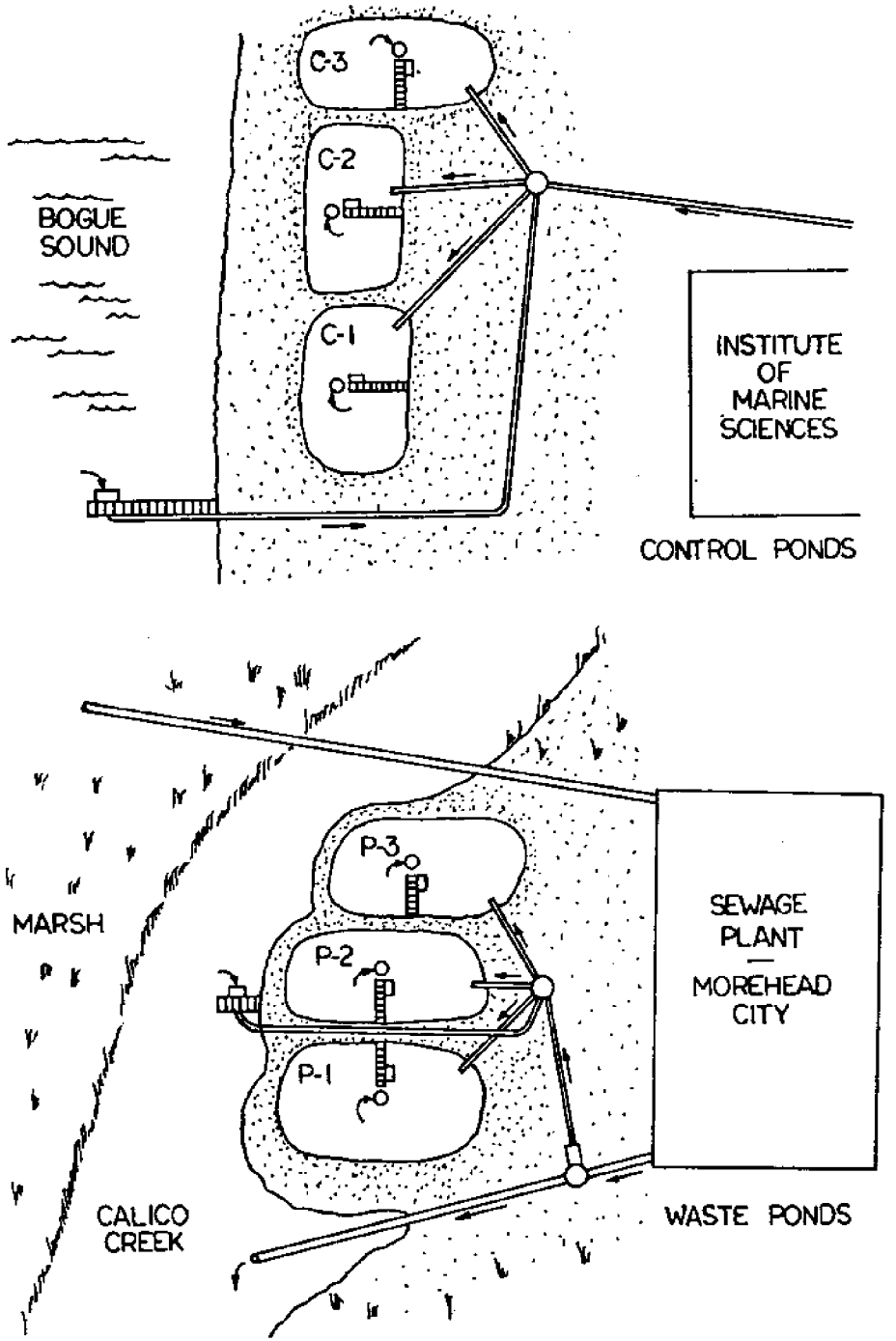


FIGURE 1. SITE PLAN OF P- AND C-PONDS

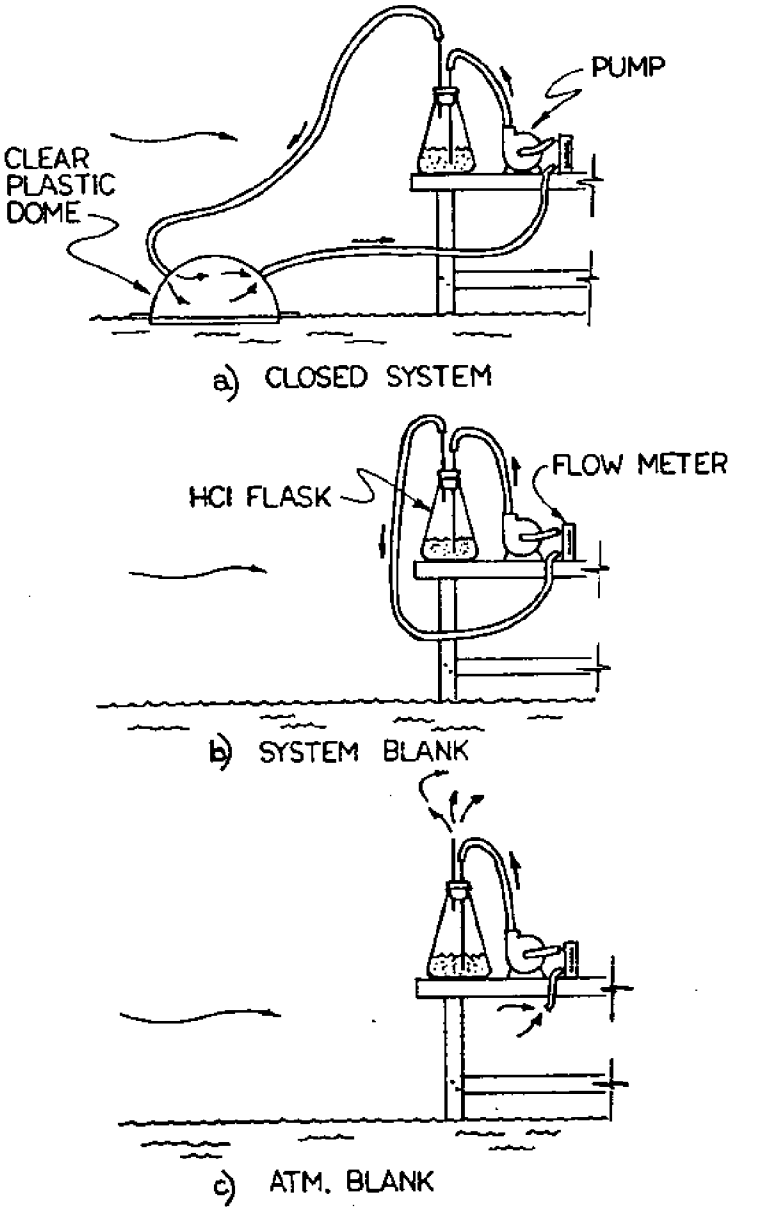


FIGURE 2. AMMONIA DIFFUSION APPARATUS

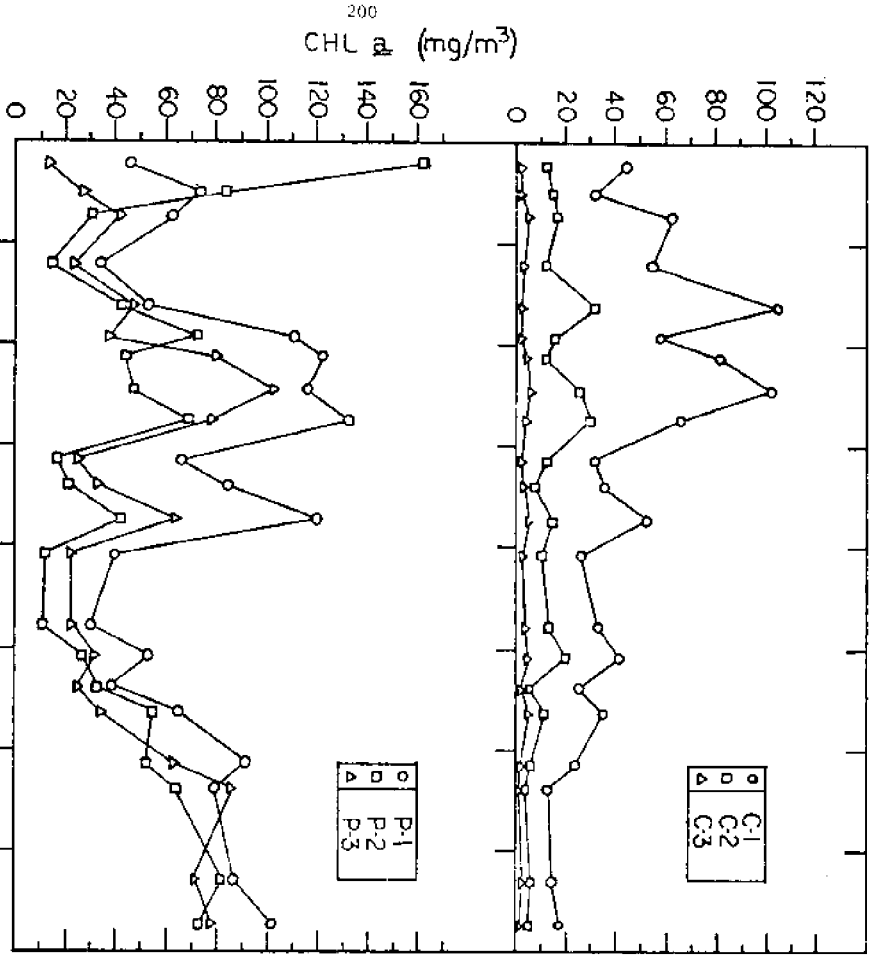


FIGURE 3. CHLOROPHYLL a

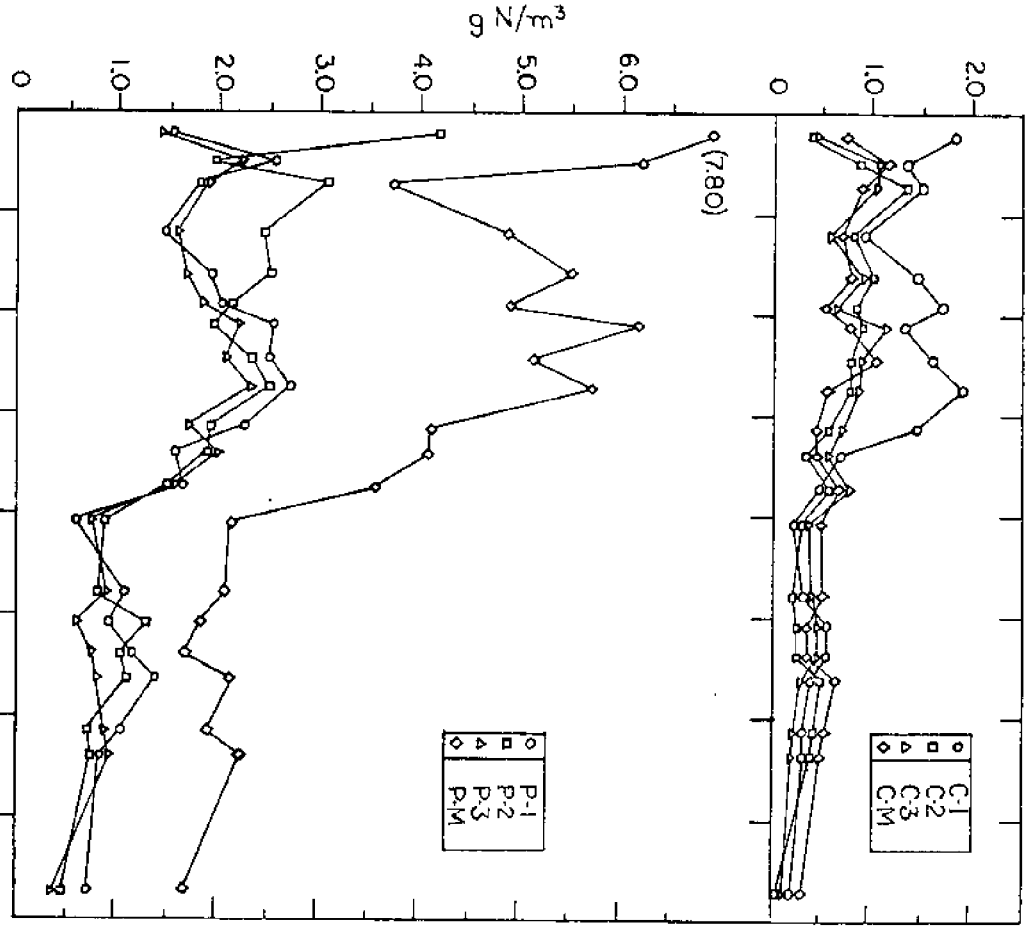


FIGURE 4. TOTAL N (RAW)

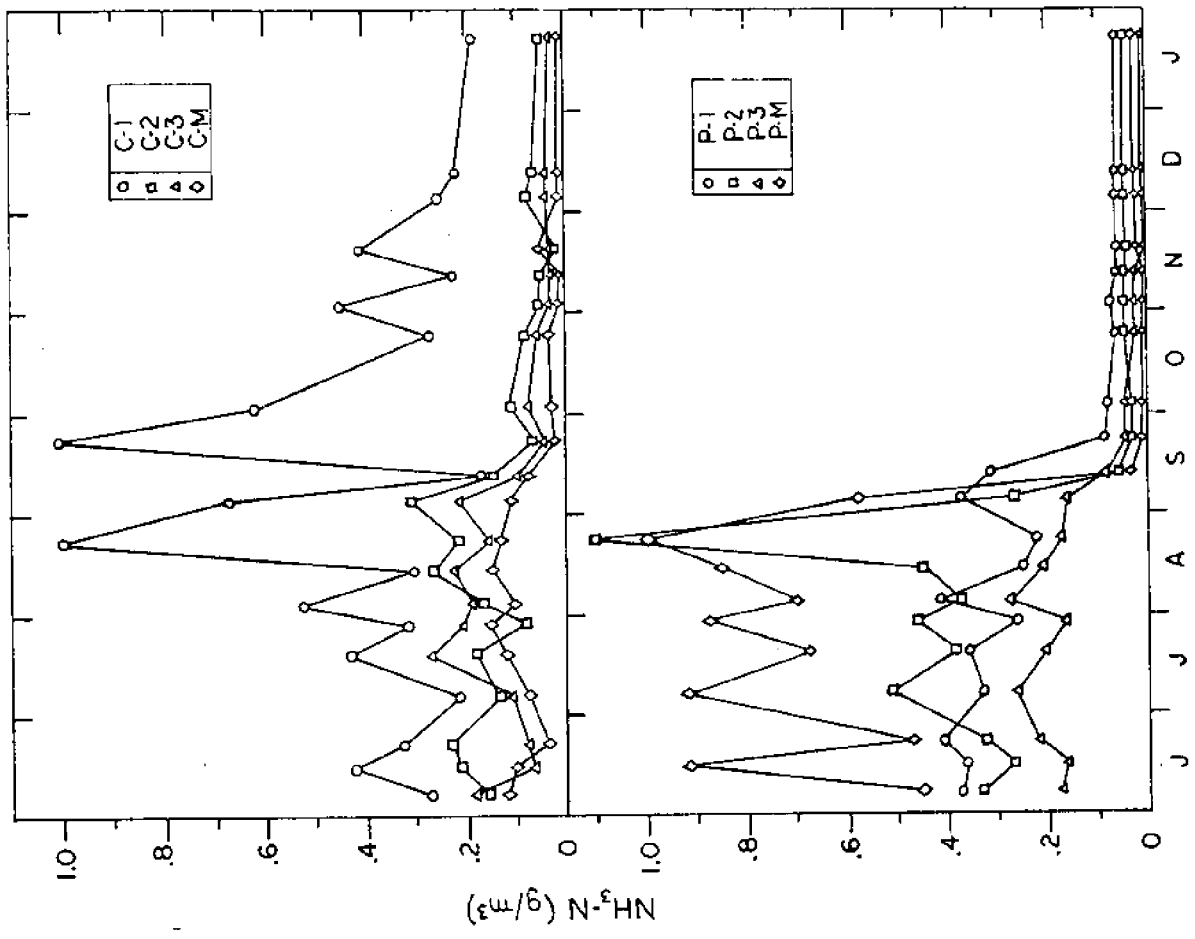


FIGURE 6. NH3-N

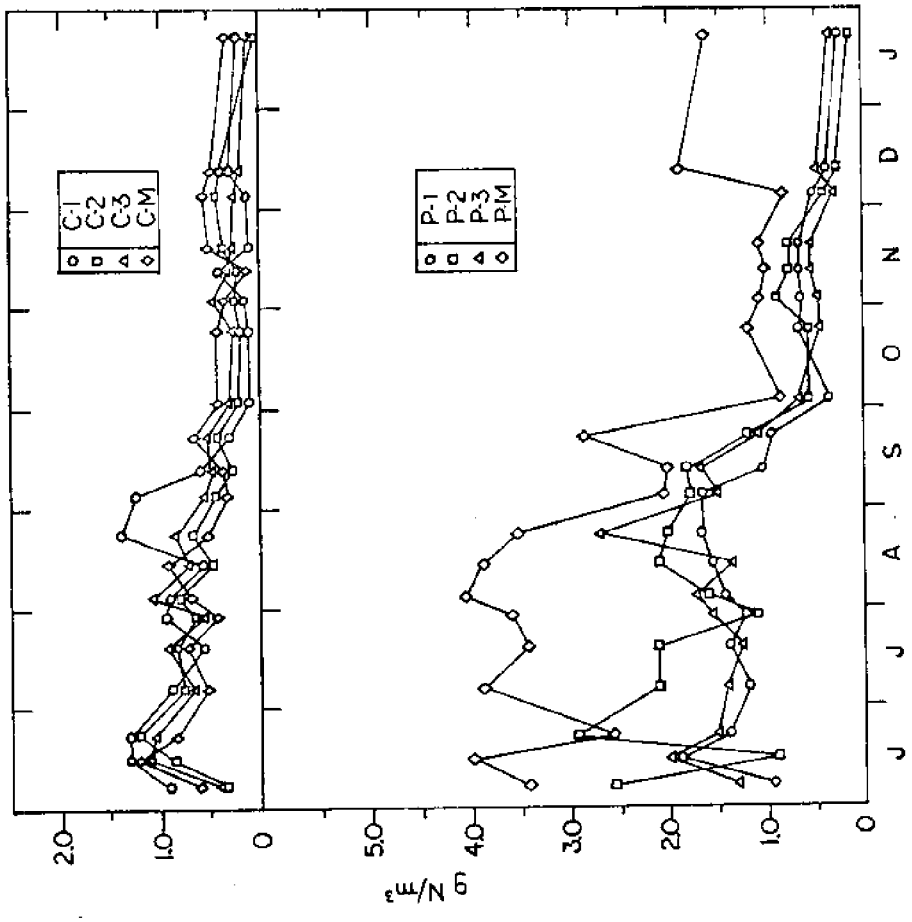


FIGURE 5. TOTAL N (FILTERED)

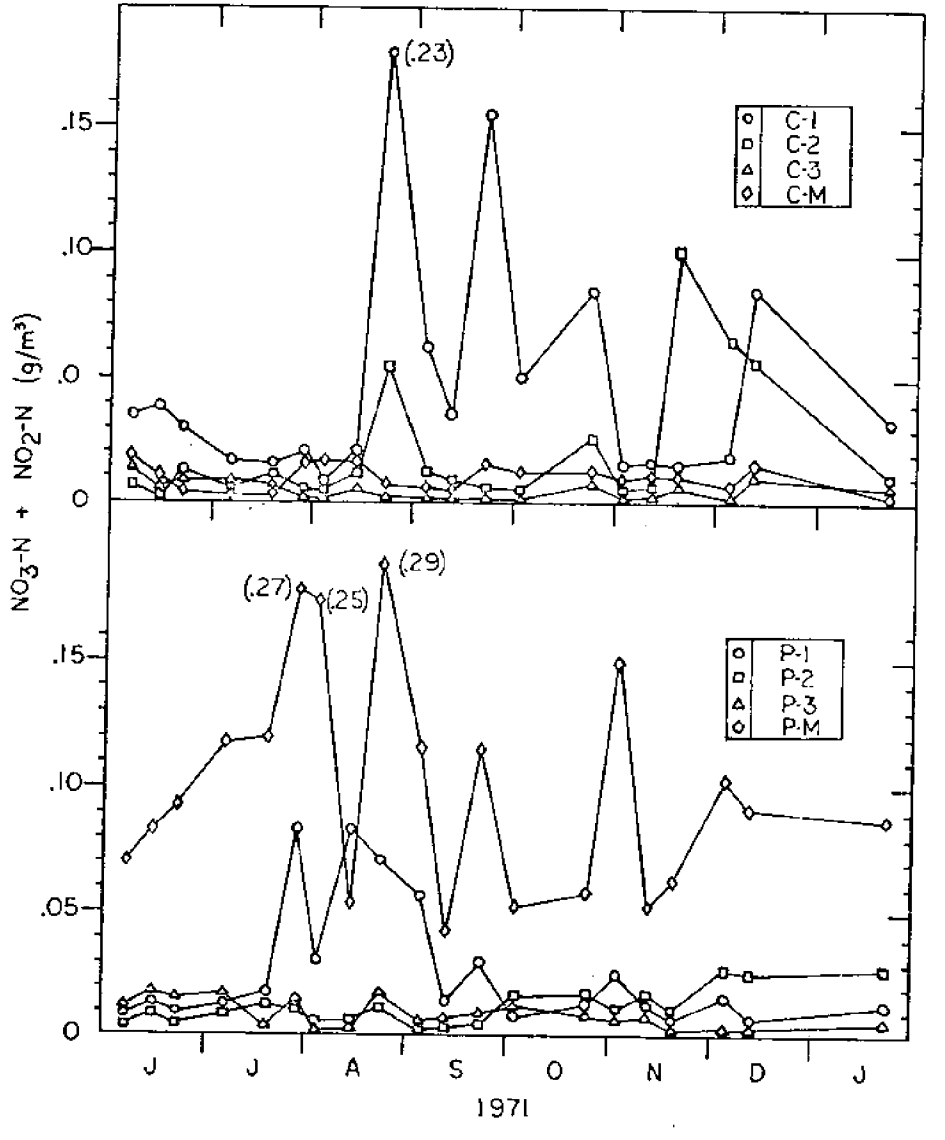


FIGURE 7. NO_3-N + NO_2-N

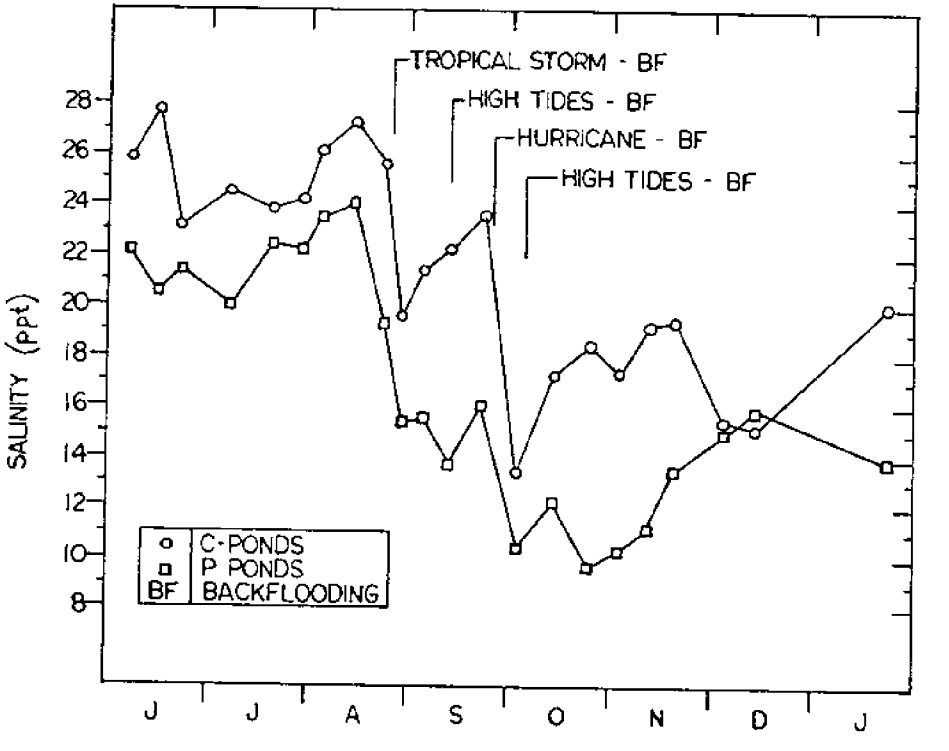


FIGURE 8. AVERAGE POND WATER SALINITY

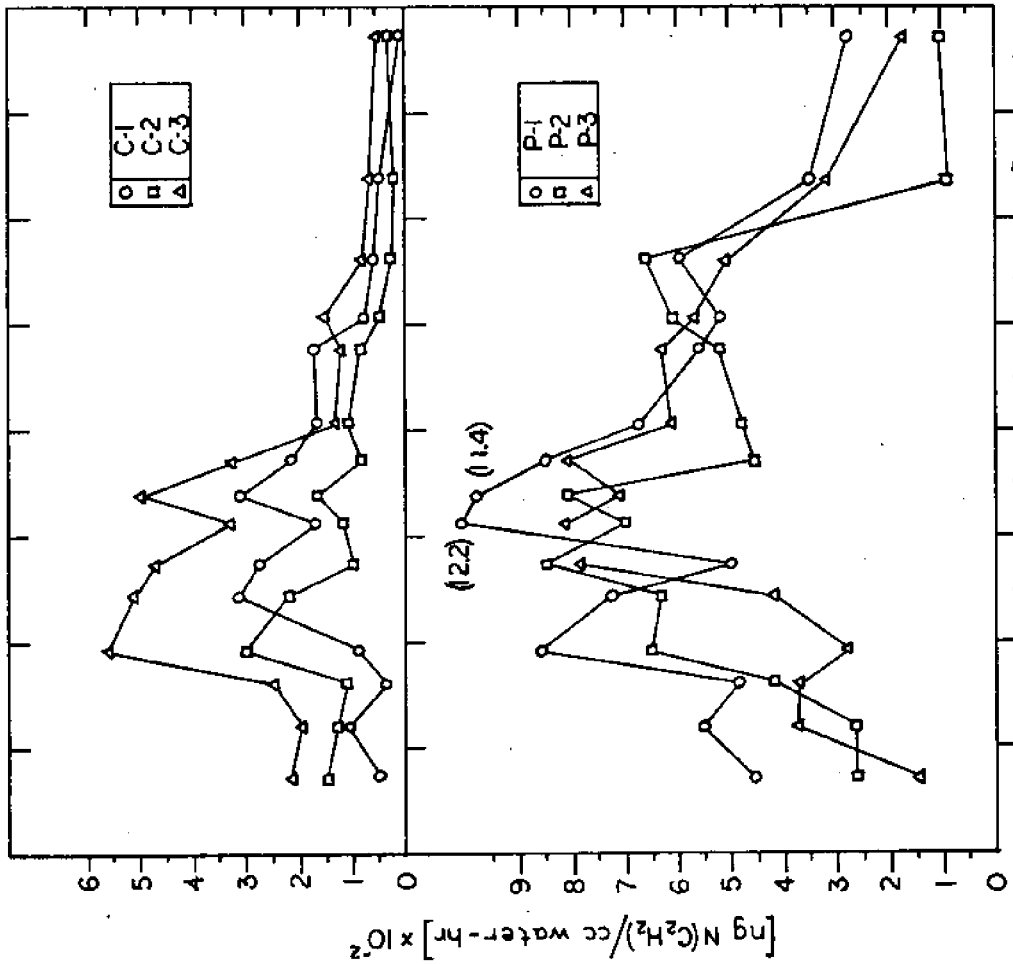


FIGURE 9. N-FIXATION IN POND WATER

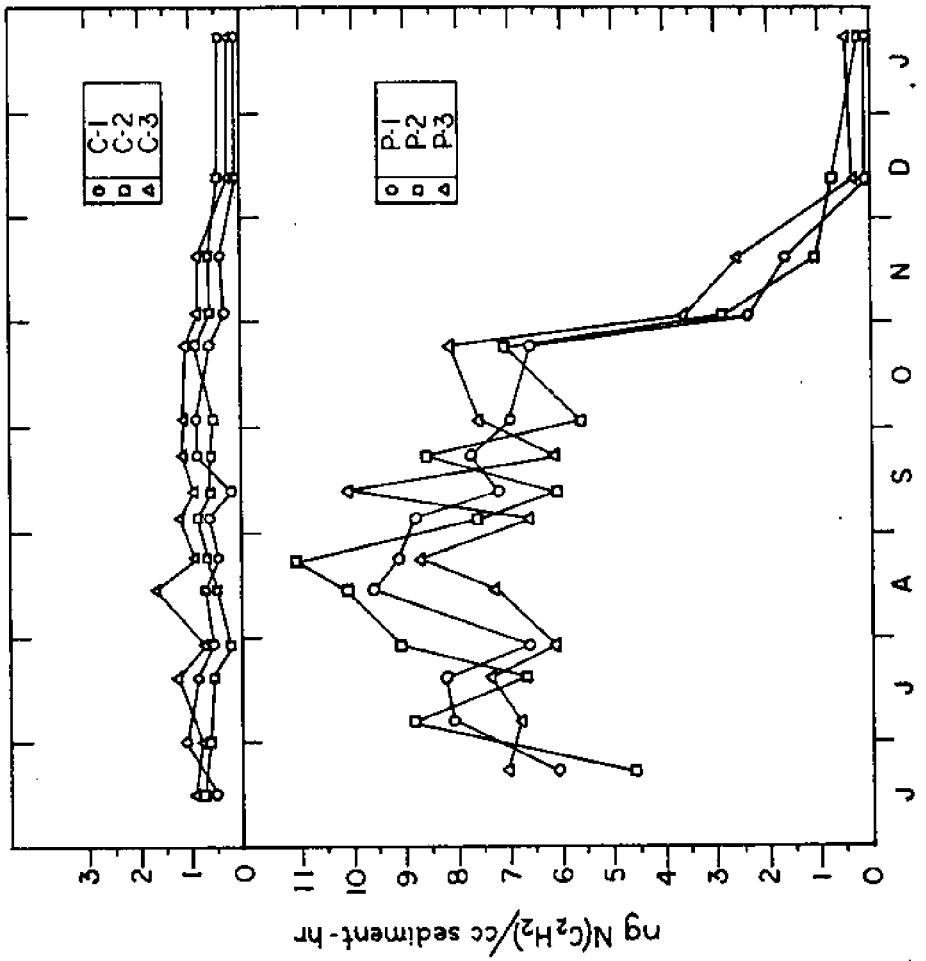


FIGURE 10. N-FIXATION IN TOP SEDIMENTS

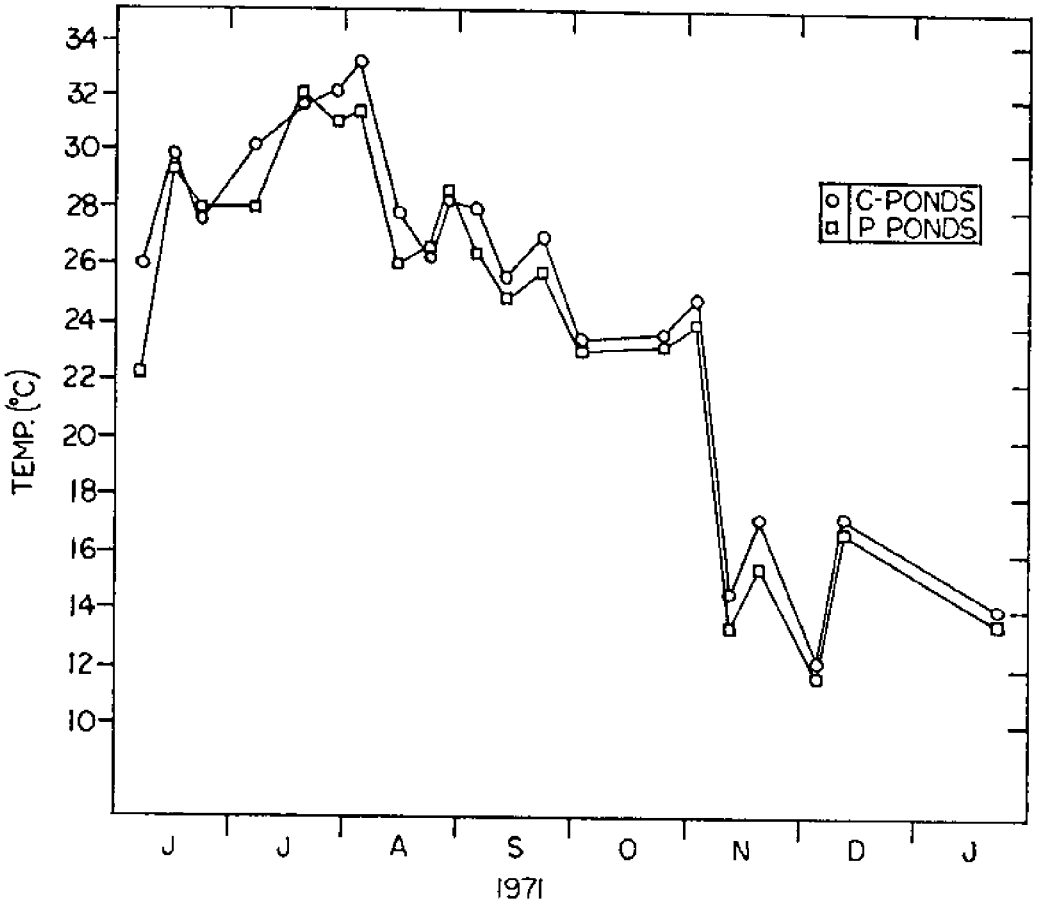


FIGURE 11. AVERAGE MID-DAY WATER TEMPERATURE

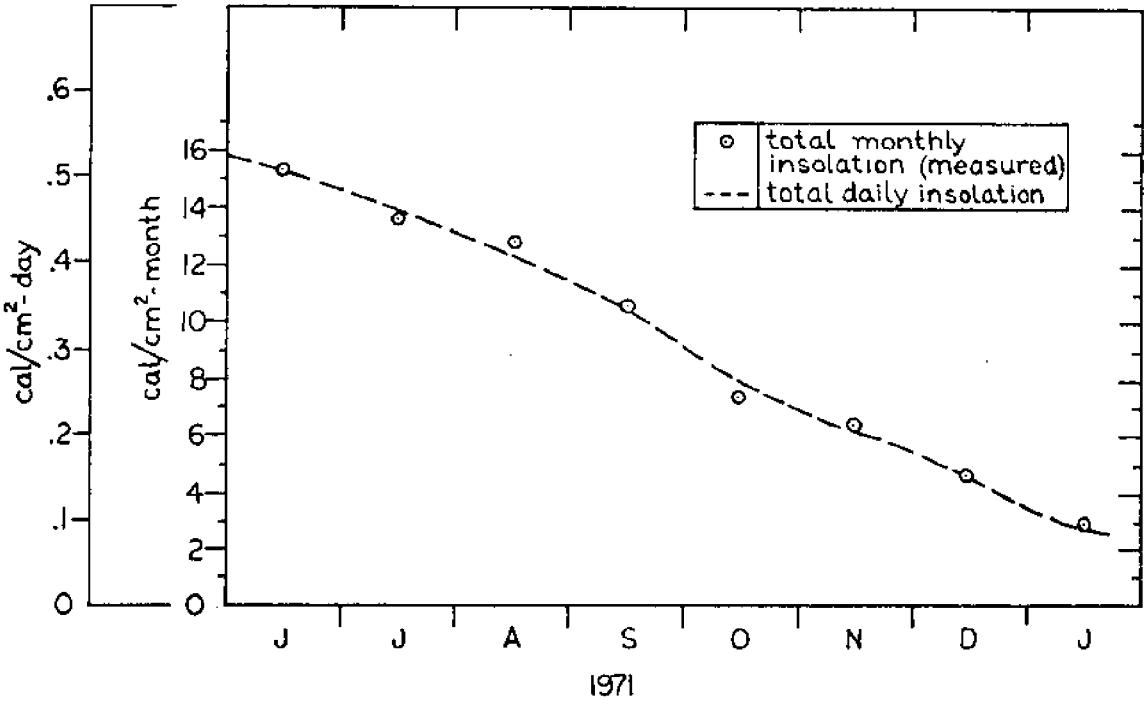


FIGURE 12. INSOLATION

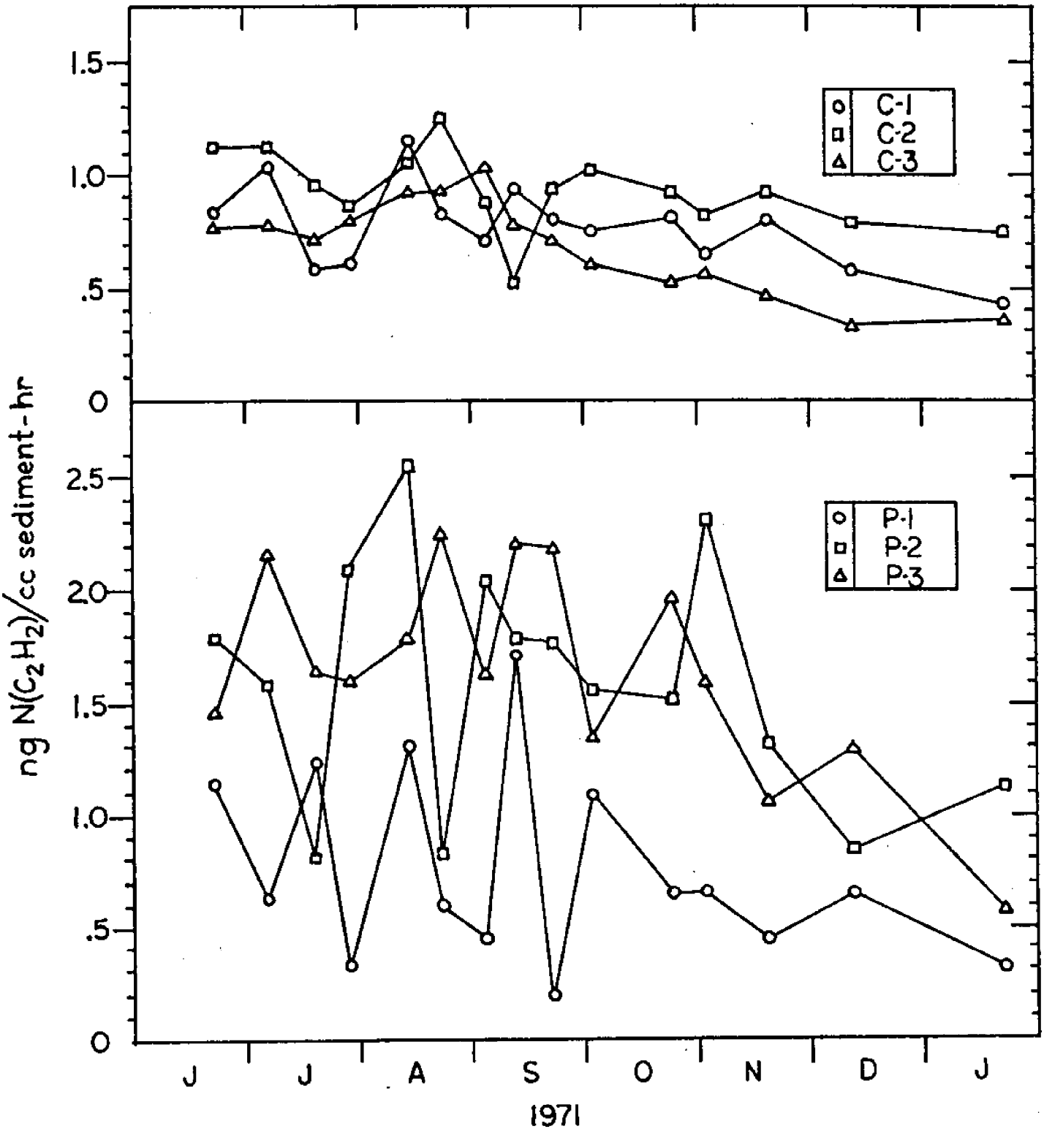


FIGURE 13. N FIXATION IN LOWER SEDIMENTS

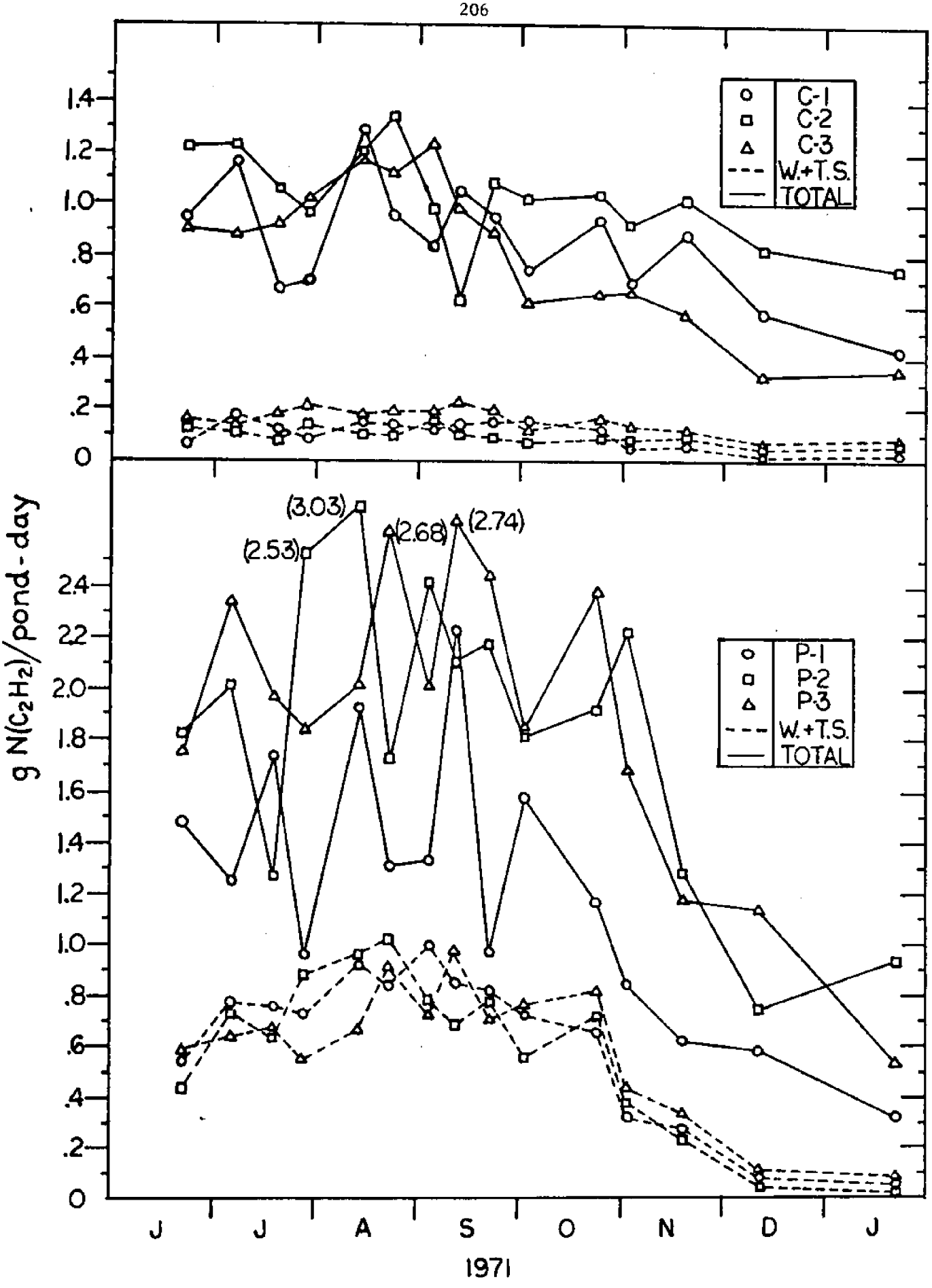


FIGURE 14. TOTAL POND N-FIXATION

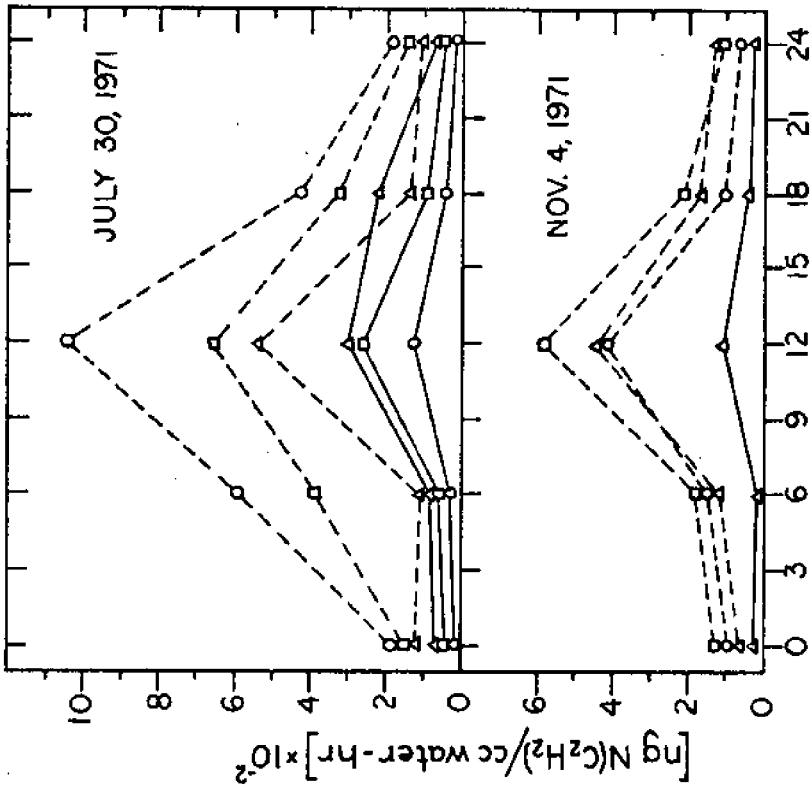


FIGURE 15 N-FIXATION IN POND WATER VS. TIME-OF-DAY

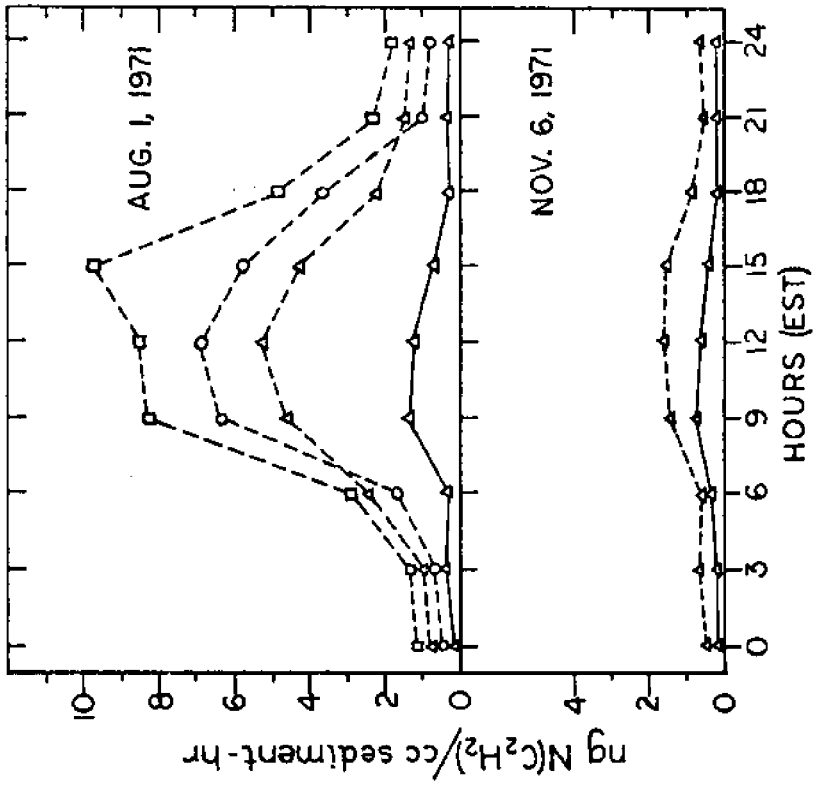
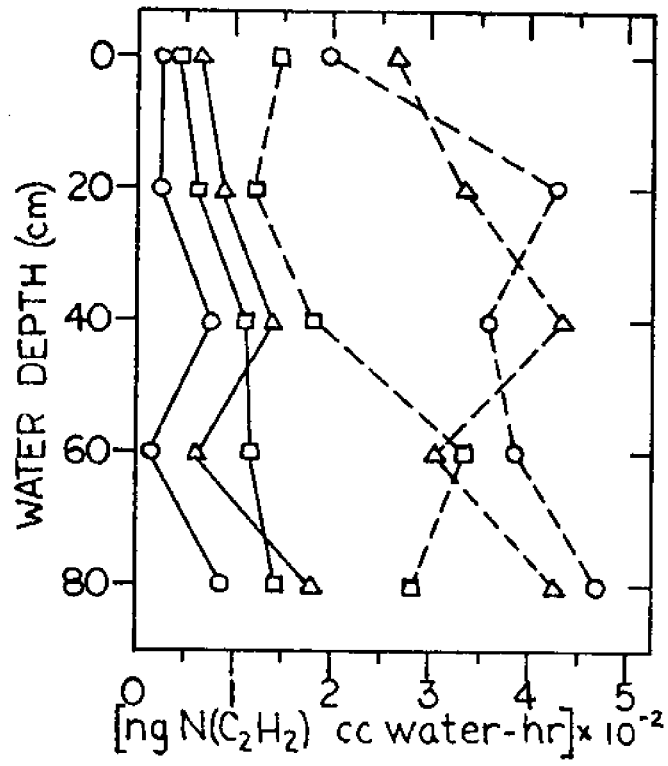
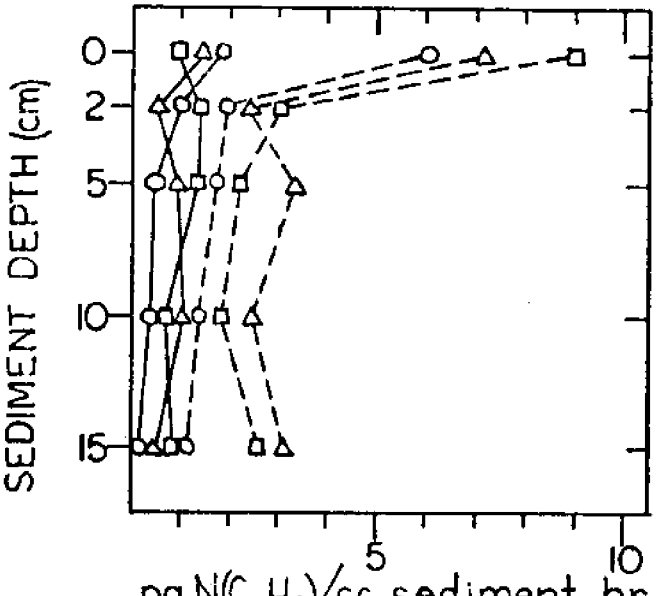


FIGURE 16. N-FIXATION IN TOP SEDIMENTS VS. TIME-OF-DAY



a. POND WATER (JULY 8, 1971)



b. SEDIMENTS (JULY 10, 1971)

FIGURE 17. N-FIXATION VS. DEPTH

C-1		P-1	
C-2		P-2	
C-3		P-3	

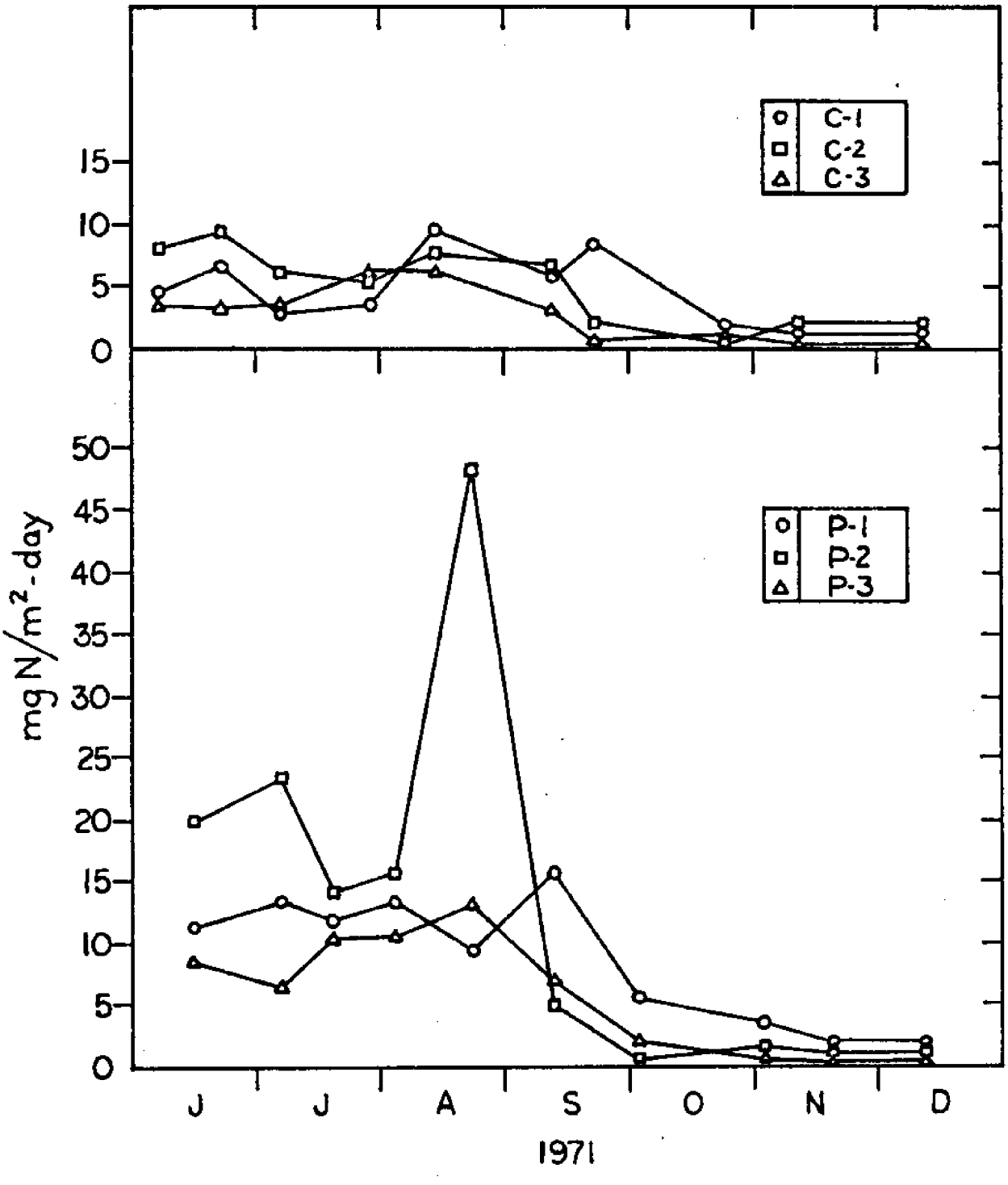


FIGURE 18. AMMONIA DIFFUSION

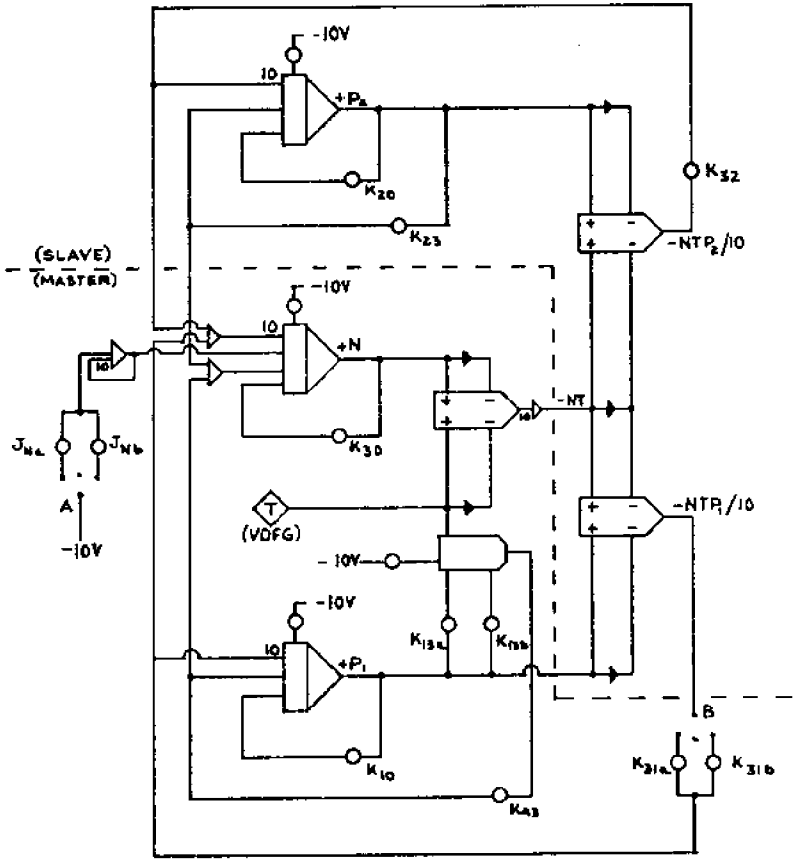


FIGURE 20. ANALOG COMPUTER PROGRAM OF P-POND SEASONAL NITROGEN

$$\frac{dN}{dt} = J_N + K_{13}P_1 + K_{23}P_2 - K_{31}NTP_1 - K_{32}NTP_2 - K_{30}N$$

$$\frac{dP_1}{dt} = K_{31}NTP_1 - K_{13}P_1 - K_{10}P_1$$

$$\frac{dP_2}{dt} = K_{32}NTP_1 - K_{23}P_2 - K_{20}P_2$$

Where N = nitrogen pool
P_1 = summer phytoplankton
P_2 = winter phytoplankton (Monodus)
T = water temperature
J_N = nitrogen inflow
K_{10} = P_1 outflow rate
K_{20} = P_2 outflow rate
K_{30} = N outflow rate
K_{13} = transport coef (P_1 to N, autolysis & grazing deaths)
K_{31} = transport coef (P_1 growth)
K_{23} = transport coef (P_2 to N, autolysis & grazing deaths)
K_{32} = transport coef (P_2 growth)

Figure 19. Differential equations for P-pond nitrogen and phytoplankton model.

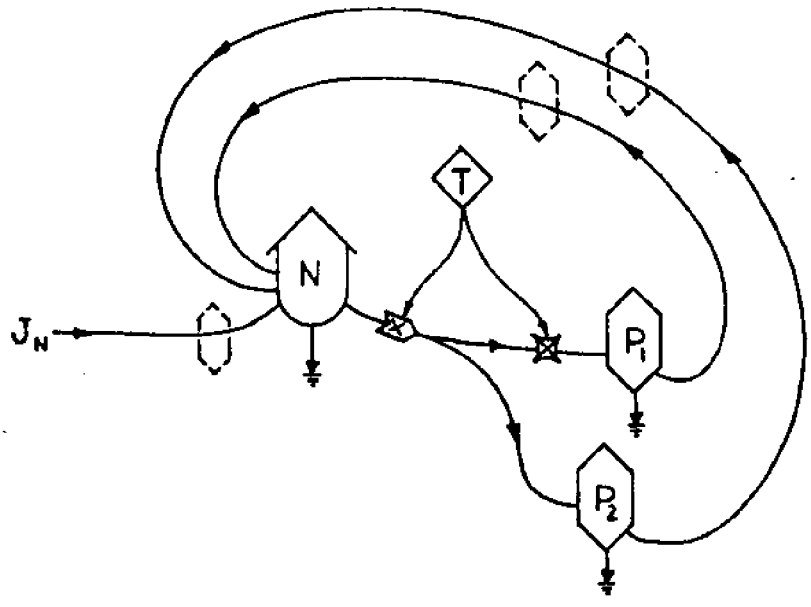


FIGURE 21. SIMPLIFIED FLOW DIAGRAM OF P-POND SEASONAL NITROGEN

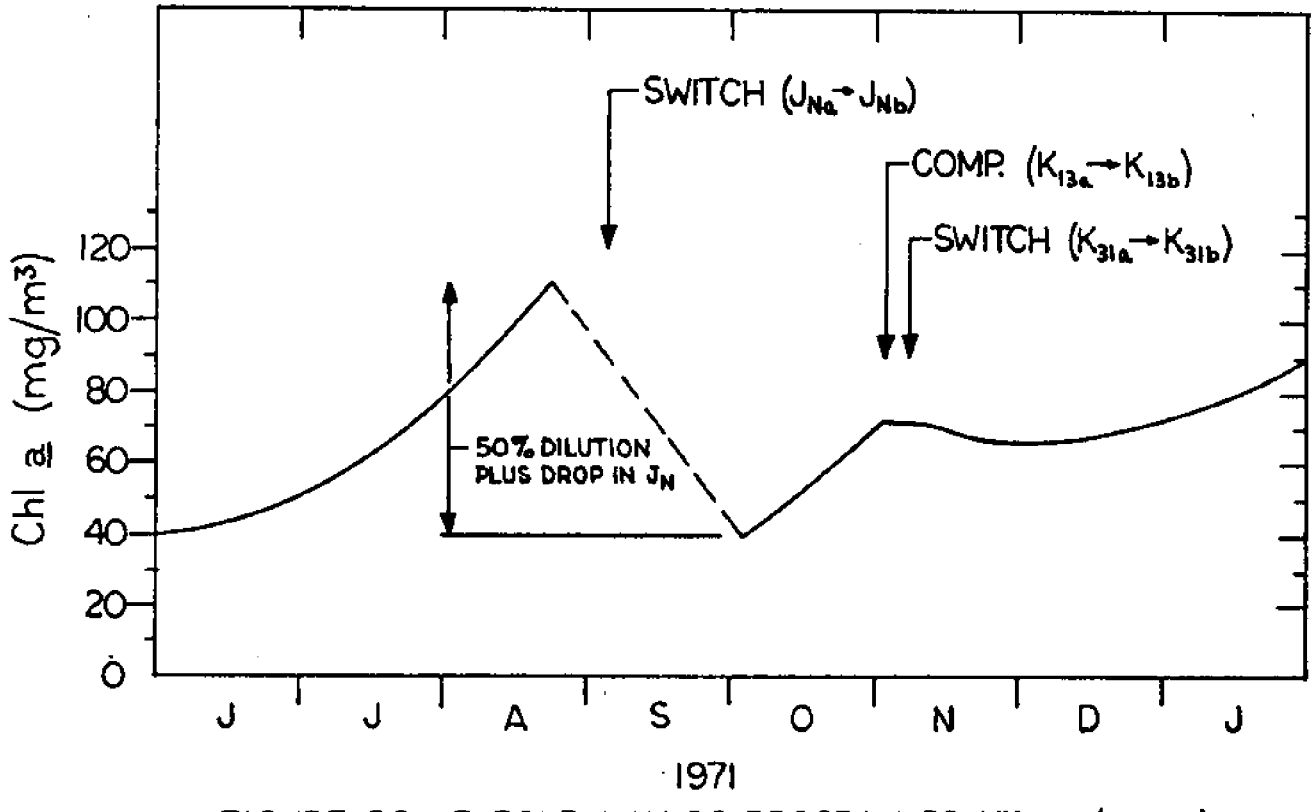


FIGURE 22. P-POND ANALOG PROGRAM SOLUTION ($P_1 + P_2$)

EFFECTS OF TREATED SEWAGE ON BRACKISH POND ECOSYSTEMS 1968-1972; SUMMARY AND CONCLUSIONS

Edward J. Kuenzler

INTRODUCTION

Estuaries and Man. Many of the estuaries in the United States are compromise ecosystems in the sense of Odum (1969).[1] We expect them to serve productive, urban-industrial, and protective functions, usually without adequate understanding of their capacity to do any one of these at a sustained level, nor of the influence of one usage on the others. In particular, we need to know in much greater detail the effects of urban and industrial wastes upon the functions of estuarine and salt marsh systems.

Estuaries have developed and evolved over geological time. Their form, their hydrographic characteristics, their sediment distributions, their communities of organisms have all been "self-designed". The communities of organisms have developed to maximize productivity and diversity, with certain compromises, in the face of relatively large excursions of environmental factors--changes in salinity, changes in inundation by tides, wide temperature excursions, and strong current flows and associated scouring.

Man is a newcomer, geologically speaking, to estuaries. He has, however, affected current flows, turbidity, and sediment distribution; he has decimated some populations; and he has contributed large amounts of numerous pollutants. His effects on estuaries are many, but given a reasonable chance, that is to say, if shocks are not too severe and if certain standards are not exceeded, estuaries will adapt to the new conditions imposed by man. Functioning ecosystems will develop, although the populations may be far different from those of undisturbed estuaries and the selection of seafoods available for harvest may be poor. The successional patterns, the final plant and animal populations, and the metabolism of stressed estuaries are of great scientific and practical interest.

Self-design. The "self-design" of smaller systems is also of considerable interest. Dr. H.T. Odum and Dr. A.F. Chesnut accordingly initiated this research effort to learn the nature of the ecological systems which develop when small brackish water ponds are subjected to domestic sewage. Six ponds were built, three of which received sewage wastes from the town of

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[1]Odum, E.P. 1969. The strategy of ecosystem development. Science 164:262-270.

Morehead City, North Carolina. Details of construction of these ponds are given in Odum and Chestnut (1970), but the essential features are as follows. They were small, averaging about 510 m^2 or 0.12 acres. The bottom of each was covered with salt-marsh mud and peat; the bottom sloped down from the edge of each pond to a maximum depth of 0.8-1.0 m, with the average depth of the ponds varying from 0.4 to 0.5 m. Pumps mixing tanks, and distribution lines were installed so that seawater and tapwater or seawater and treated sewage flowed into the control or polluted ponds, respectively. The sewage came from the Morehead City treatment plant; it was the chlorinated effluent of a trickling filter. The flow of each kind of water into each pond was regulated such that salinities were usually in the range 15-20 and the retention times were usually 15-30 days. The ponds were seeded by larvae and microscopic plants and animals that passed through the pumps, and also by dumping in bucket-loads of freshly caught fish and macroinvertebrates. Clams and oysters were placed in the ponds and Spartina alterniflora was planted around the pond margins.

In essence, the waste ponds were tertiary treatment or oxidation ponds. Brackish oxidation ponds have seldom been studied and consequently there was a need to examine, insofar as possible, the entire system, the physical factors, the water chemistry (especially plant nutrients), the plant and animal populations which developed, the metabolic processes of the pond systems, the effects of the pond system on water quality, and the aquaculture potentials.

Measurements on the pond system have been made over about four years -- temperature, salinity, insolation, and plant nutrients; standing crops of phytoplankton, zooplankton, fish, crabs, snails, oysters, bacteria, and plankton primary productivity, assimilation and respiration of organic substrates by microbes, nitrogen balances and phosphorus kinetics, bottom algal productivity, and growth rates of selected species of crabs, fish, and shrimp.

Details of the investigations supported by this grant are available in annual reports to Office of Sea Grant Programs (Odum and Chestnut 1968; Odum and Chestnut 1970; Kuenzler and Chestnut 1971A; Kuenzler and Chestnut 1971B; and this report). Only the broad conclusions will be discussed here and reference will be made to the authors of the detailed reports. A manuscript for publication is presently in the final stage of preparation; it will consolidate all of the prior individual reports and provide analysis of the entire ecological systems which developed in these ponds.

PLANT NUTRIENTS AND PRODUCTIVITY

The most obvious initial difference between the waste ponds, designated P, and the Control ponds, designated C, is that the former receive large amounts of plant nutrients in the sewage plant effluent. Total phosphorous (soluble and particulate) was usually at least 10 times higher in the P-ponds than in the C-ponds (Woods 1970; Kuenzler, McKellar, and Muse 1970; McKellar 1971). Total nitrogen was also more

abundant in the P-ponds, although not by such a large factor as for phosphorous (Woods 1970; Hebert 1970; Masarachia 1971). In short, the waste ponds were highly eutrophic.

The ponds were productive. The productivity varied, of course, from day to day and was higher in the waste ponds than in control ponds. The values of net daytime production and night respiration were high (Table 1). Assuming that night respiration is the same as daytime respiration, the approximate value of gross productivity was obtained by addition (Table 1). Most of the production appeared to be planktonic; one series of measurements in July indicated that benthic productivity may represent only 10% of the total in the C-ponds and only 1% in the more turbid P-ponds.

Table 1. Approximate annual metabolic rates for brackish ponds (after Day, 1971).

	CONTROL PONDS	WASTE PONDS
NET DAYTIME PHOTOSYNTHESIS (g O_2 m^{-2} yr^{-1})	446	944
NIGHTTIME RESPIRATION (g O_2 m^{-2} yr^{-1})	348	863
GROSS PRIMARY PRODUCTIVITY (g O_2 m^{-2} yr^{-1})	794	1,807
NET PRODUCTION EFFICIENCY	0.21%	0.45%

The diversity of phytoplankton in the control ponds was high and the population densities were greater in summer than in winter (Kuenzler 1970; Campbell 1971). On the other hand, in the waste ponds there was a marked seasonal pattern of diversity and density. During the summer the population levels were lower than in winter, but the diversity was relatively high. The winter period was dominated by a dense bloom (10^6-10^7 cells/cc) of a unicellular xanthophyte Monodus guttula with a concomitant deop in diversity (Kuenzler 1970: Campbell 1971). Chlorophyll concentrations in the particulate matter of the ponds gives an index to the population densities. The control ponds generally had chlorophyll-a levels of 1-10 mg/m^3 during winter and 10-40 mg/m^3 during the summer. The waste ponds usually had 300-500 mg/m^3 of chlorophyll-a during the winter and 60-200 mg/m^3 during the summer (Kuenzler 1970; McKellar 1971). There was a striking crash of the Monodus population each spring. An interesting feature of the control ponds was the general decline in the phytoplankton populations in C-2 and C-3 in recent years as the benthic widgeongrass, Ruppia maritima, developed. Beginning in 1970 the turbidity and the chlorophyll concentrations clearly show this decline (McKellar 1971; Laughinghouse and Kuenzler 1971B).

Laboratory studies of Monodus were undertaken in an effort to link its physiological and its ecological aspects. It is a euryhaline species

(about 0-30°/oo), with a requirement for high pH (>6.0) (Hommersand and Talbert 1971). Axenic cultures were grown on a mineral medium. It would grow on nitrate, but better growth took place when ammonium was supplied as the nitrogen source. It was proposed that the spring crash might result from rapid settling and sinking of Monodus as increased respiration at the rising temperatures quickly consumed its oil reserves. Cytological, biochemical, and taxonomic studies of Monodus have been completed (Hommersand and Huang 1973).

The very dense phytoplankton populations in the waste ponds markedly affected the oxygen concentrations and the pH of the water. The oxygen levels in the waste ponds not infrequently exceeded 20 mg/l (Smith 1971). During spring at the time of the crash of the Monodus bloom, and sometimes also during the summer, oxygen levels became undetectable (Smith 1971). The pH of the control ponds was generally between 7.9 and 8.3. The pH of the waste ponds was normally higher, but during November-May, the period of the Monodus bloom, it was usually between 9.5 and 10.0 (Day 1971) because CO_2 diffusion from the atmosphere could not meet algal demands.

A study of the carbon budgets of the ponds (Day 1971) demonstrated major differences between the experimentals and the controls. In the control ponds, the bulk of the carbon was inorganic and the concentration did not change markedly throughout the year. There was generally an outward diffusion of CO_2 from the ponds. In the waste ponds, however, the organic carbon concentrations exceeded the inorganic concentrations for most of the year, but especially during the cold months when Monodus dominated. During this bloom period there was a constant diffusion of CO_2 into the ponds; during the non-bloom period CO_2 diffused in during the day and out at night. In contrast to the control ponds, the major loss of carbon from the waste ponds was as organic carbon that went out via the overflow pipe. (Day 1971)

The phosphorus budgets of the two sets of ponds were quite different. In the control ponds total phosphorus concentrations were usually less than 2 µgat/l . Most of this was particulate phosphorus, largely phytoplankton; most of the remaining, soluble phosphorus was organic. Dissolved inorganic phosphorus was usually very scarce; it was usually less than 0.1 µgat/l, and was often undetectable (<0.03 µgat/l). The relative uptake rate of dissolved inorganic phosphorus by phytoplankton was highest in summer when the populations were densest, with uptake rates greater than 20% / minute not being uncommon (Kuenzler, McKellar, and Muse 1970; McKellar 1971). In the waste ponds, on the other hand, total phosphorus was usually about 50 µgat/l, or about 25 times more than in the control ponds. The phosphorus was mostly dissolved inorganic phosphorus in summer, but mostly particulate phosphorus in winter. Dissolved organic phosphorus concentrations were generally low and fairly constant at about 5 µgat/l, about 10 times higher than in the coastal ponds. The relative uptake rates of dissolved inorganic phosphorus were highest during the periods of phytoplankton blooms, but nevertheless were much lower than the control ponds; they seldom exceeded 0.2% / minute (Kuenzler, McKellar, and Muse, 1970). The

dissolved organic phosphorus reservoir in the ponds proved to be quite labile and evidence was presented that the phytoplankton contributed both to its production and decomposition (Kuenzler 1971B).

SECONDARY PRODUCTIVITY

Quantitative collections of zooplankton were made during 1968-69 (McCrary 1970). The copepod Acartia tonsa was abundant in all ponds during the warm months. Oithona sp. was also present in all ponds, but became extremely abundant (10^5-$10^6/m^3$) in the waste ponds during the warm months. Several other species of adult copepods were present and unidentified copepod nauplii were also very abundant. Other invertebrate larvae were often in the plankton: crab zoeae (Uca, xanthids, and others), Palaemonetes, barnacles, and annelids (nereids, capitellids, and spionids). There were also occasional catches of mysid shrimp, gammarid amphipods, and insects.

The biomass of the larger animals varied from year to year. Beeston (1970) reported that blue crabs (Callinectes sapidus) were the dominant crustaceans in control and waste ponds in (1969); peneid shrimps were important in the control ponds and Palaemonetes in the waste ponds. He found killifish (Fundulus heteroclitus) to be the most important fish in all ponds, but spot (Leistomus xanthurus) were relatively important in the control ponds. The total abundance of macrofauna in the ponds was determined in June 1971 by intensive seining of each of them (Table 2). At this time Palaemonetes had become the most important crustacean, followed by Callinectes (especially in the waste ponds). There was greater variety of important fishes, particularly in the control ponds (Table 2), where the flounder Paralichthys lethostigmus was well established. Oysters which had been planted in both sets of ponds were still surviving, but survival and growth was poorer in the waste ponds in spite of abundant particulate matter which might appear to be desirable food.

Table 2. Weight (Kg) of the most abundant macrofauna in each pond in June 1971.

Species	CONTROL POND 1	CONTROL POND 2	CONTROL POND 3	WASTE POND 1	WASTE POND 2	WASTE POND 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 variegatus	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

The infauna of the sediments in the waste and the control ponds were dominated by the polychaete worms *Capitella capitata* and *Laeonereis culveri* (Farris and Williams 1973). An intensive study of various aspects of foraminiferal ecology (LeFurgy and St. Jean 1973) showed higher diversities and standing crops in the control ponds than in the waste ponds, although the same species, *Elphidium clacatum* was the most commonly occurring species in both sets of ponds. Municipal sewage effluent was not in itself deleterious to the foraminiferans, but microenvironmental differences in oxygen, pH, and food concentration made their distribution erratic.

AQUACULTURAL POTENTIAL

The yields of oysters, shrimps, and fishes which might be obtained from the ponds, as indicated by the biomass in 1971 (Table 2) are not encouraging from the standpoint of aquaculture. It is evident that self-design under the conditions of this study, is unlikely to result in a profitable venture unless yields can be markedly increased or a very high priced specialty crop can be grown.

One factor which may severely limit aquaculture in enriched ponds is the oxygen decline to near-zero levels which often occurs. Richards and Williams (1973) showed that this factor could be counteracted by aeration. Air was bubbled through one of the waste ponds, resulting in a much higher minimum oxygen concentration (3.4 mg/l) and lower daytime pH (8.3). Penaeus setiferus stocked in the aerated waste pond survived and had growth rates greater than shrimp in a control pond and comparable to growth rates in natural populations in North Carolina. Unfortunately this work could not be continued because of discontinuation of the grant. Oysters grown in the waste ponds had unacceptably high coliform counts when sewage plant effluent was flowing into the ponds; depuration time for oysters averaged 12.5 days (Herbert 1971).

CALICO CREEK AND NEARBY SALT MARSHES

Calico Creek, the source of salt water for the waste ponds, is the recipient of all of the treated sewage from Morehead City. A study in the summer of 1970 established that a diatom bloom may be present comparable to the phytoplankton levels of the waste ponds. (Kuenzler, Wyman, and McKellar 1971B). This bloom appeared to be associated with a volume of seawater which had been enriched by sewage plant effluent; this intermediate-salinity water oscillated back and forth on each tidal cycle without being lost from the creek.

The grass Spartina alterniflora grew significantly better in the marshes along the margins of Calico Creek than it did in marshes which were flooded by unenriched tidal flows (Marshall 1970). This lends support to the hypothesis that Spartina may be nutrient limited in North Carolina marshes. Furthermore, the snail Littorina irrorata was more abundant in Calico Creek salt marshes than elsewhere (Marshall 1970; Stiven and Hunter 1971) and the individual snails were also larger (Stiven and Hunter 1971). Experimental studies on the effects of crowding on snail growth and survival are continuing (Hunter and Stiven 1973).

The microarthropods of enriched and normal salt marshes were carefully collected by a vacuum-cleaner technique and the populations of enriched and unenriched marshes were compared (McMahan, Knight, and Camp 1971). For the most part populations of microarthropods were similar in both types of marsh, although spiders and amphipods were somewhat more abundant in waste-enriched marshes. Standing crops of microarthopods (about $0.14 g/m^2$) was low and annual productivity appeared to be much lower than has been reported for other marshes. On the other hand the species diversity was higher. A study of fiddler crab populations showed that Uca pugnax, and U. minax were present in waste-receiving marshes whereas only U. pugnax was found in control marshes (Camp, Knight, and McMahan 1971). There were generally more fiddler crabs in the control marshes.

EFFECT OF WASTE PONDS ON WATER QUALITY

Data are not yet available on the performance of the ponds which receive sewage effluent in regard to their removal of organic matter (BOD) or nitrogenous compounds, but they do seem to reduce the amount of phosphorus being released to Calico Creek (Table 3). If one assumes that the effluent from the ponds is very similar to the water in the ponds, then the control ponds appear to act as positive estuaries in the sense that they accumulate total phosphorus, mostly in the form of phytoplankton. DOP levels in the C-ponds are not much different from levels in the supply. The polluted ponds, however, reduce the total P to about half the influent levels, again largely by plankton production, probably followed by its settling to the bottom. DOP levels are even more sharply reduced; the lower DOP levels where phytoplankton is abundant implies that they are more important as destroyers of DOP in the waste ponds than as producers.

The waste ponds were effective in reducing levels of coliform bacteria, with an average reduction of 79% from the levels in the influent to the ponds (Herbert 1973).

Table 3. Concentrations of Total-P and DOP in the influent and in the ponds. Values are averages (ug at. l^{-1}) February - July 1970.

		CONTROL PONDS		WASTE PONDS	
	n	TP	DOP	TP	DOP
Influent concentrations	5-6	1.50	0.28	130	19
Pond concentrations	12-16	1.90	0.24	60	3.2
Influent exceeds pond concentration		5/17	9/15	9/12	9/12

RELATIONSHIP OF THIS STUDY TO PROBLEMS OF ESTUARINE EUTROPHICATION

Conditions in the brackish water control ponds are quite different from a natural estuary in many regards--total area; flushing rate; turbulence, access for migrants, or even occasional wanderers, too large or delicate to pass through the pumps; and community structure. On the other hand a viable ecosystem has developed with high productivity and rapid nutrient cycling. Viable systems also developed in the waste ponds with high primary productivities.

There are several ways in which results from our pond studies may bear on the problem of estuarine eutrophication. It is possible that

relatively small, brackish oxidation ponds may help prevent deterioration of the quality of the receiving water; organics and algal nutrients stripped out in the ponds would not cause troubles downstream. Unfortunately time did not permit the development of a flow regime or other engineering solution to the two major problems in the waste ponds, (1) apparent unsuitability of Monodus as a prime food for oysters and (2) the unstable winter and spring conditions of pH and oxygen associated with the Monodus bloom. Furthermore it would be desirable to establish whether brackish water ponds have any real advantage over freshwater oxidation ponds in terms of effluent quality.

Secondly, larger brackish ponds might be desirable. Very large impoundments are presently being constructed in the high Juncus marshes of North Carolina for mosquito control, sport fishing, and waterfowl hunting. Addition of reasonable amounts of sewage wastes from nearby cities might not prove harmful to these other uses. Studies of yields of oysters, shrimp, crabs, and fish from these ponds are proposed in order to assess the possibility of converting the high primary productivity into a commercial product. Unfortunately, the problem of possible pathogenic virus contamination of shellfish has not been solved.

Finally, we hope that some of our results will be applicable to the problems that result when domestic wastes are dumped directly into estuaries. There is an urgent need for data on levels of nutrients that can be added without harming our traditional and highly desirable uses, as well as the trade-offs that must be made if waste removal is, for some particular estuary, an important consideration. Cost benefit analysis, preferably not limited to economics, is necessary and we trust that planners in the future will consider ecological costs and benefits in their balances.

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