Effects of Perturbations on Estuarine Microcosms

Donald R. Heinle, David A. Flemer, Rogers T. Huff,
Shelly T. Sulkin, Robert E. Ulanowicz

University of Maryland
Center for Environmental and Estuarine Studies
Chesapeake Biological Laboratory
Solomons, Maryland 20688

Microcosms containing planktonic communities from Chesapeake
Bay responded to enrichment with sewage by developing larger standing
crops of phytoplankton and zooplankton. Data suggest that increased
productivity would be reflected up the food chain but might increase
existing problems with dissolved oxygen and might lead to qualitative
changes in the composition of the zooplankton.

Either phosphorus or nitrogen was removed more rapidly from
solution depending on where and when the experimental water was
obtained. Increases in standing crop of algae were associated with
loss of nitrogen from solution in two experiments and losses of both
nitrogen and phosphorus from solution in one experiment.

Introduction

It is now generally conceded that in most unpolluted fresh
waters phosphorus is the major nutrient that most often limits algal
growth (e.g., Deevey, 1972; Fuhs et al., 1972). Similarly, nitro-
gen is usually considered to be the limiting nutrient in coastal
marine waters (e.g., Ryther and Dunstan, 1971; Thayer, 1971; Flemer,
1972). An estuary with its gradient of fresh to salt water thus
probably contains a spatial gradient in limiting nutrients. In
addition, there are temporal changes in the concentration of nutri-
ents within the saline portion of an estuary (Taft et al., 1976;
Taft and Taylor, 1976). Recent evidence indicates that some tribu-
taries and portions of Chesapeake Bay have already been consider-
ably altered by cultural eutrophication (Jaworski et al., 1972;
Flemer and Heinle, 1974). The first readily observable effects of
eutrophication have been increased standing stocks of algae as

measured by chlorophyll a (Flemer, 1972; Flemer and Heinle, 1974). In Back River, a small tributary of Chesapeake Bay heavily loaded by treated sewage, excess production of algae has led to periods when nighttime respiration by algae has led to anoxic conditions. The tidal freshwater portions of some tributaries have developed noxious blooms of blue-green algae (Brehmer, 1967; Jaworski *et al.*, 1972).

The undesirability of blooms of blue-green algae and anoxic waters is fairly obvious, as they represent or cause major changes in the trophic structure of estuaries that directly affect human use. Less dramatic changes, such as increases in standing stocks and production of algae without apparent qualitative changes, are not as clearly perceived. In this paper we describe some experiments designed to determine the short-term effects of nutrient additions to planktonic ecosystems in Chesapeake Bay. At the time of the experiments, approximately 2.8% of the fresh water entering Chesapeake Bay was sewage (Brush, 1974). Our experiments were designed to determine how the standing crops of phytoplankton and zooplankton might respond to the further addition of sewage and which major nutrients might be limiting standing stocks and productivity, and also to obtain time-series data for use in developing and testing a modeling technique. Methods and results of the modeling have been described by Mobley (1973), Ulanowicz *et al.* (1975), and Ulanowicz *et al.* (1978). This paper describes the data in greater detail, including some of the interrelationships between nutrients, phytoplankton, and zooplankton that were not fully described in the papers on modeling.

Methods and Materials

The three experiments reported in this paper were done from September 16 to November 3, 1972 (experiment 1), July 19 to August 3, 1973 (experiment 2), and September 25 to October 9, 1973 (experiment 3). Experimental procedures were modified slightly each time as a result of experience gained during preceding experiments in an effort to improve replication and reduce day-to-day variations in data. Microcosms were chosen over field studies as they provided a means of repeatedly sampling the same mass of water, albeit small.

We used six 750-liter polyethylene tanks (cylinder 0.94m in diameter by 1.22 m high) for microcosms (Plate 1). Early experiments indicated that mixing was important for sampling purposes and necessary for the retention of realistic planktonic populations in the microcosms. The tanks were stirred four times daily by large polyethylene propellers mounted on flexible fiberglass shafts driven at 24 rpm by gear motors. During the first two experiments each stirring was for one hour, and each was for 20 minutes during the third experiment. Each tank was illuminated by a 500 w quartz-iodide floodlight mounted above the surface of the water on a cycle of 12 hrs. light and 12 hrs. dark. One-eighth inch plexiglass covers attenuated the illumination to about 0.15 g cal $cm^{-2} \cdot min^{-1}$ at the surface of the water. Dawn and dusk were simulated by

Plate 1. CBL estuarine microcosms.
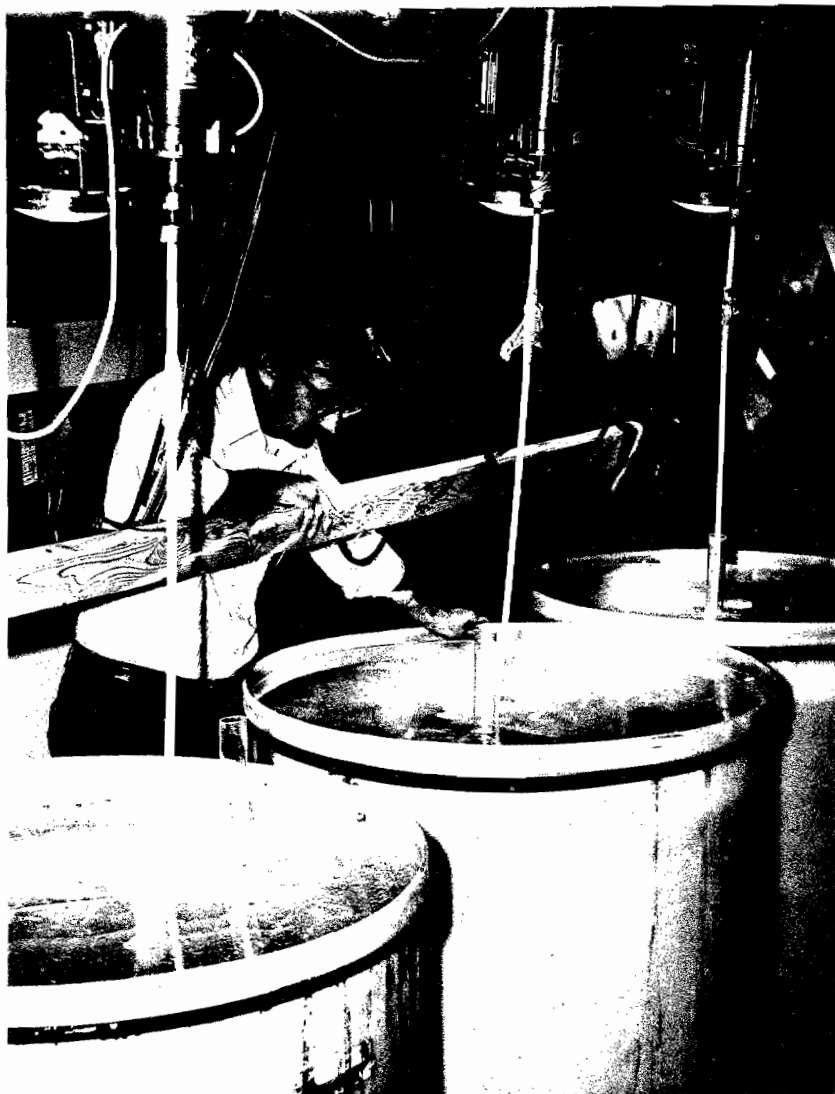
a single 20 w plant-gro flourescent light that burned for 30 minutes
before and after the quartz-iodide lights. Control of temperatures
was achieved by cold water circulated through 0.5cm inside diameter
polyethylene tubing coiled near the perimeter of the tanks at the
surface. The walls and bottoms of the tanks were scrubbed daily with
a string mop of synthetic sponge during the third experiment and
less frequently during the first two experiments. Samples were taken
daily between 0900 and 1000 hours during a mixing period by
vigorously agitating the contents further with a bucket and then
dipping the sample from the surface with the bucket.

Chlorophyll a was measured by a modification of the flourometric
techniques of Yentsch and Menzel (1963) and Holm-Hansen *et al.* (1965)
as described by Heinle and Flemer (1975). Samples collected on
Whatman GF/C filters with magnesium carbonate were frozen for analy-
sis.

Particulate organic carbon was determined by the method of Menzel and Vaccaro (1964) using a Beckman Model IR215 infra-red analyzer.

The Dumas method of high temperature oxidation was used to determine particulate nitrogen. Analyses were carried out on a Coleman Model 29A Nitrogen Analyzer equipped with a Model 29 combustion tube and syringe. Particulate material was concentrated on Whatman GF/C filters. These filters were then desiccated and frozen until analyzed. The filters were rolled and put in the combustion tube for oxidation at 850°C.

Total phosphorus was determined with the oxidation method of Menzel and Corwin (1965). The same method was used to oxidize dissolved phosphorus after the sample had been passed through a GF/C filter. Dissolved inorganic reactive phosphorus was determined with the composite reagent method (Strickland and Parsons, 1968).

All samples of dissolved nutrients were quick-frozen and stored for later analysis. Ammonia plus amino acids were measured by the methods of Solorzano (1969). Nitrite and nitrate were analyzed by the methods given in Strickland and Parsons (1968). We employed a modification of the ultra-violet light oxidation method for the determination of soluble organic nitrogen (Strickland and Parsons, 1968). A half-strength seawater solution was used for the solvent for the blanks, ammonium sulfate, and pyridine standards. The seawater solution was made up, according to Strickland and Parsons' (1968) nitrate method, and then diluted by one-half with double distilled, deionized water. This solution was used to dilute river water samples and to add salts to facilitate the uv oxidation. We diluted 20 ml of Bay water sample to 100 ml with half-strength seawater. Two drops of 30% hydrogen peroxide were added to the sample in the quartz tube; the sample was capped and irradiated 7 cm from a 1,200 watt Hanevia-Englehardt 189A lamp for three hours. Strickland and Parsons' (1968) procedure was followed for the remainder of the analysis.

Relative primary productivity was measured by suspending two light and one dark bottles to which ^{14}C had been added just below the surface of the water in each tank. Phytoplankton were exposed to the label for 4 hr. Between 20 and 40 ml, depending on the density of phytoplankton, were filtered through 0.45-μm Millipore filters. Samples were counted on a Nuclear Chicago planchet counter with a model 8707 decode scaler, a model 1042 planchet changer, and a model 470 gas flow detector.

One-liter samples of zooplankton were concentrated in a net cup fitted with a No. 20 (70-74 μm aperture) Monel screen. The concentrated samples were preserved in 4% formaldehyde. The entire content of each sample was later counted by species and major age group (eggs, nauplii, copepodids and adults of copepods; rotifers and eggs of rotifers). All the individuals in sparse samples and representative numbers in dense samples were measured so that herbivore biomass could be calculated from known length-weight relationships of copepods (Heinle, 1969; Heinle and Flemer, 1975) and from the spherical volume of rotifers and an assumed density of 1.0 and dry weight of 20% of mass.

Temperature and concentrations of dissolved oxygen were measured daily with a Yellow Springs Instrument Model 54 oxygen analyzer. These data were taken primarily to assure that the environmental control was adequate, and as little variation occurred, they will not be reported in detail.

The first two experiments were done with water collected from a depth of 1m at the end of the pier at the Chesapeake Biological Laboratory at Solomons, Maryland, near the confluence of the Patuxent River estuary with Chesapeake Bay. The third experiment was done with water collected from a depth of 4m above the outfall of the Broad Neck Sewage Treatment plant at the confluence of the Magothy River estuary with Chesapeake Bay, hopefully to predict effects of that plant on the immediate area (Flemer and Heinle, 1974). For all three experiments water was pumped into a mixing tank and thence into the experimental tank. During the first two experiments, we attempted to regulate the initial population of herbivores by filtering the water through a series of successively finer mesh nets down to 28 um. Copepods were then added from mass cultures. As we were unable to completely regulate the resulting populations, water was filtered through a 0.5mm net only to remove predatory zooplankton (ctenophores and jellyfish) during the third experiment. In all three experiments one to three small carnivorous fish (*Menidia menidia*) were placed in each tank. We attempted to achieve a 10 to 1 ratio of herbivore biomass to carnivore biomass and so used fish 20 to 40 mm long. Fish that died during the second experiment were replaced. No fish died during the first and third experiments.

During the first experiment, two tanks were perturbed by the addition of 10 µg-atoms of nitrogen as NH_4Cl and 1 µg-atom of phosphorus as $KH_2PO_4 \times 1^{-1}$, respectively. There was no replication of the first experiment. A single tank received no additions. We thus attempted to determine if nutrients added singly would affect the ecosystems. During the second experiment, two tanks each received 1% and 10% by volume of treated sewage from the Broad Neck treatment plant. Two tanks received no additions and served as controls. The 10% dose caused changes that appeared to be related to chlorine rather than nutrients, so during the third experiment, two tanks each received 1% and 0.1% by volume of treated sewage from the Broad Neck treatment plant. Two tanks again were unperturbed and served as controls.

Prior to the third experiment, the inner walls of the tanks were scrubbed thoroughly with 70% ethanol to reduce populations of microorganisms.

Table 1 is a summary of the analytical precision we achieved for fractions of nitrogen and phosphorus, using several replicates of individual representative samples. Analytical precision as percent of means was somewhat better for nitrogen compounds than for phosphorus.

Results

During the first experiment, the concentrations of chlorophyll a increased in all three tanks during the first three days (Fig. 1),

Table 1. Analytical precision estimated from replicates of representative samples.

Compound	Mean (μg at /l)	Standard deviation	No. of replicates	95% confidence limits($\pm$)
NO_2	1.12	0.006	10	0.395
NO_3	13.89	0.105	9	0.207
NH_3	9.49	0.202	12	0.395
Total dissolved nitrogen	8.03* 80.3(x10)	0.633 6.33	10 10	1.24 12.4
Dissolved inorganic phosphorus	2.01	0.074	10	0.146
Total dissolved phosphorus	2.79	0.503	13	0.986
Total phosphorus	2.79	0.365	15	0.715
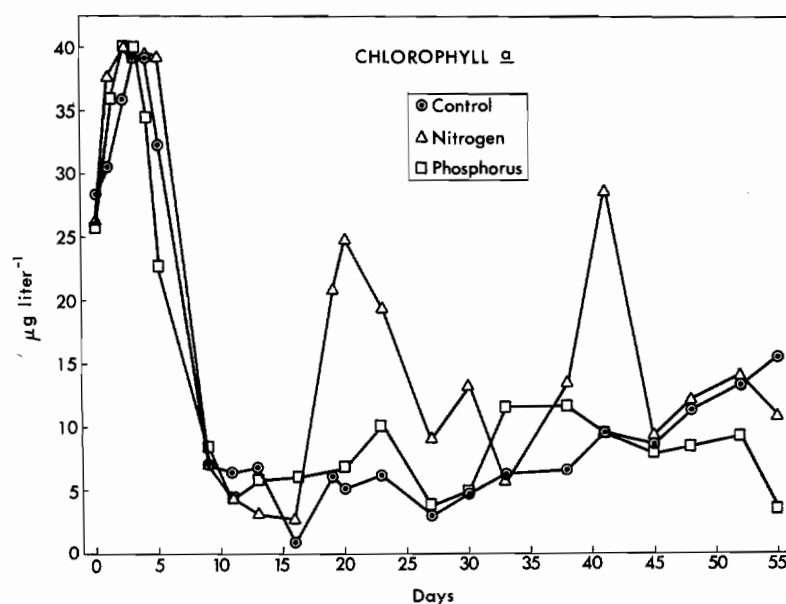
Fig. 1. Concentrations of chlorophyll a (μg l^{-1}) versus time in tanks enriched with nutrients and a control tank, September 16 to November 3, 1972. Experiment 1.

presumably in response to a release from grazing. The rise was most rapid in the tank to which NH_4Cl was added, less rapid in the tank to which KH_2PO_4 was added, and least rapid in the control tank. The concentrations of chlorophyll a were between 37 and 40 μg l^{-1} in the control and phosphorus tanks for only 2 days but remained at those levels for 5 days in the nitrogen tank. Concentrations fell to below 10 μg l^{-1} in all tanks by the ninth day of the experiment. The concentrations in the control and phosphorus tanks then rose gradually with fluctuations until the end of the experiment while the nitrogen tank experienced two more "blooms" on approximately the 20th and 40th days.

The nitrogen tank consistently had the lowest biomass of copepods through the first 40 days (Fig. 2). The species copepod that was added to the tanks on day 5 was *Eurytemora affinis*, but as

Acartia tonsa always was much more abundant, the herbivore popula-
tions developed primarily from eggs that were accidentally intro-
duced into the tanks. The increase in biomass of copepods during
the gap in sampling between days 4 and 9 was due mainly to growth
and reproduction of *A. tonsa*. Most of the decline in concentrations
of chlorophyll a between days 4 and 9 was presumably the consequence
of grazing by the copepods.

The concentrations of ammonia are reflected on day 0 in Figure
3. The tank to which NH_4Cl was added had an ammonia concentration
of 5.35 μg at 1^{-1} prior to the addition, nearly identical to the
control tank. Concentrations of ammonia remained reasonably high,
but variable, in the nitrogen tank through day 13 and then fell to
less than 1 μg at 1^{-1} by day 20. After an initial drop, the con-
centrations of ammonia rose between days 3 and 13 in both the con-
trol and phosphorus tanks, presumably as a result of excretion by
the large population of copepods. Concentrations of ammonia also
fell in the control and phosphorus tanks by day 20. With the ex-
ception of one sample from the control tank, less than 2.5 μg at 1^{-1}
of ammonia were observed in all three tanks for the duration of the
experiment.

The concentration of nitrate varied slightly around 3 μg at 1^{-1}
in the nitrogen tank until day 13 while it fell very rapidly in the
phosphorus tank and equally rapidly but from a higher initial value
in the control tank (Fig. 4). Preferential use of ammonia added to
the nitrogen tank probably accounts for the relatively constant con-
centration of nitrate in that tank. The timing of the removal of
nitrate in the phosphorus and control tanks may reflect, in part,
the one day difference between them in algal growth (Fig. 1).

The phosphorus added was used rapidly, and concentrations were
similar in all tanks by the 4th day (Fig. 5). The concentrations of
phosphorus rose again in the phosphorus and nitrogen tanks on days
11 and 13, but were low again by day 20. The source of the large
pulse of phosphorus in the control tank on day 38 is unknown, but
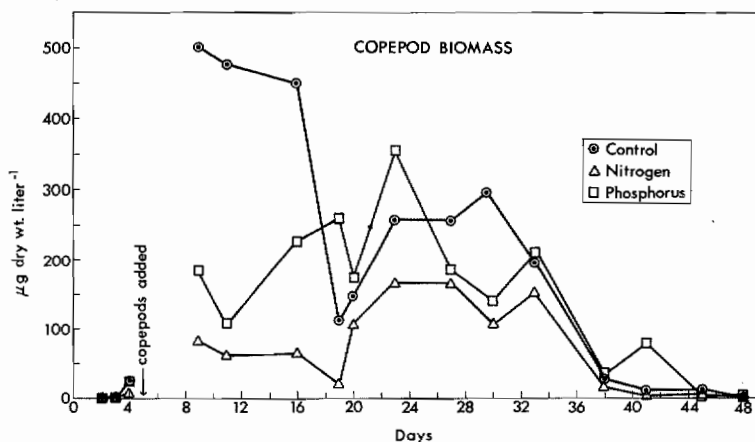apparently real. By the 41st day most had been converted to dis-

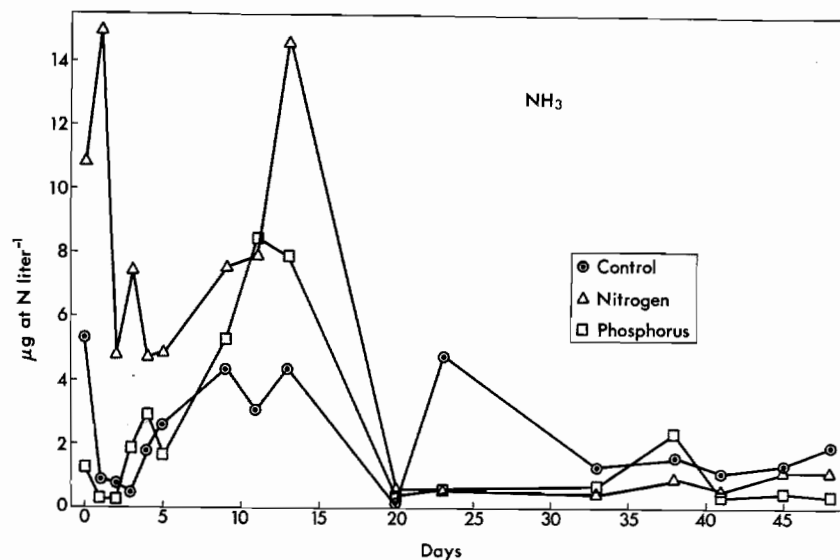Fig. 2. Biomass of herbivores (μg dry weight 1^{-1}) versus time.
Experiment 1.

Fig. 3. Concentrations of ammonia (μg at 1^{-1}) versus time. Experiment 1.
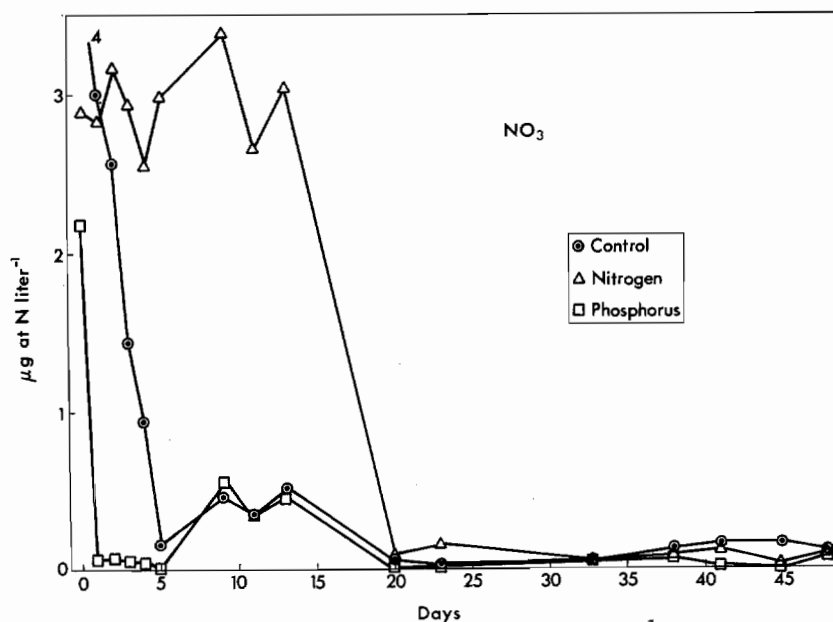

Fig. 4. Concentrations of nitrate (μg at 1^{-1}) versus time. Experiment 1.

solved organic phosphorus (the difference between total dissolved phosphorus and dissolved inorganic phosphorus), and concentrations were similar in all three tanks after day 45. Phosphate concentrations that we measured were higher than those for total dissolved phosphorus, probably a result of arsenate interference (Goulden and Brooksbank, 1974) and therefore not reported here.

Three fish were added to each of the tanks on the 28th day of the experiment. The fish were between 20 and 42 mm long. Comparison of length-weight relationships at the end of the experiment with those of the wild fish placed in the tank indicated that the fish
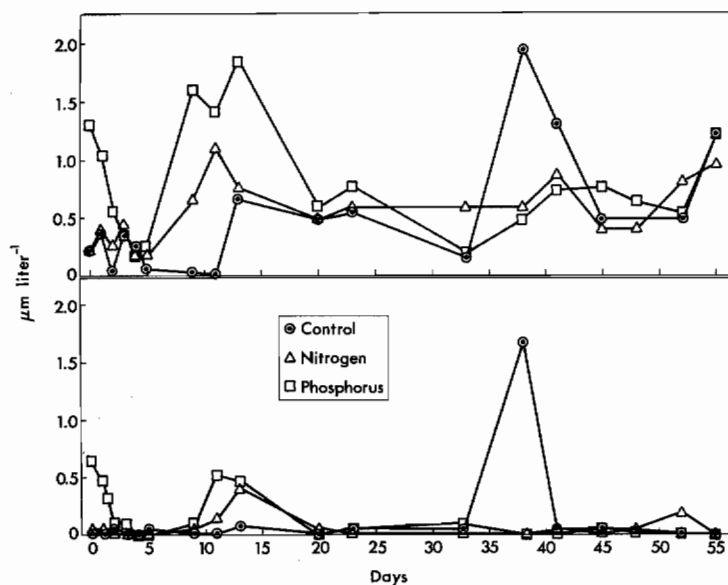
Fig. 5. Concentrations of dissolved inorganic phosphorus (lower panel) and total dissolved phosphorus (upper panel) (μg at 1^{-1}) versus time. Experiment 1.

were slightly fatter than wild fish in all three tanks, but fattest in the control tanks (Table 2). The final condition of the fish thus indicated feeding during the experiments.

From the first experiment we learned that rapid changes in populations of primary producers, grazers, and nutrients occurred during the first two weeks. A limiting nutrient could not be clearly detected in this experiment although phosphorus was reduced to low levels more rapidly than nitrogen. Large effects related to the addition of nutrients were not apparent in either the concentration of chlorophyll a (Fig. 1) or the biomass of herbivores (Fig. 2).

In the second experiment, the concentrations of chlorophyll a fell from about 30 μg 1^{-1} to about 10 μg 1^{-1} in the tank that received 10% sewage (Fig. 6). The treated sewage had a chlorine concentration of "higher than 3 parts per million," according to the operator of the sewage treatment plant. We could smell chlorine over the tanks that received the 10% by volume of sewage for a few hours after the addition. The concentrations of chlorophyll a remained lower in those tanks until day 7. After day 7 the concentrations in one tank (Tank B) were similar to those in the control

Table 2. Length-weight relationship of *Menidia menidia* placed in the tanks during experiment 1 (wild fish) and after 16 days in the tanks. Dry weight (mg) = b length (mm)-a, r = linear correlation coefficient, n = number of fish.

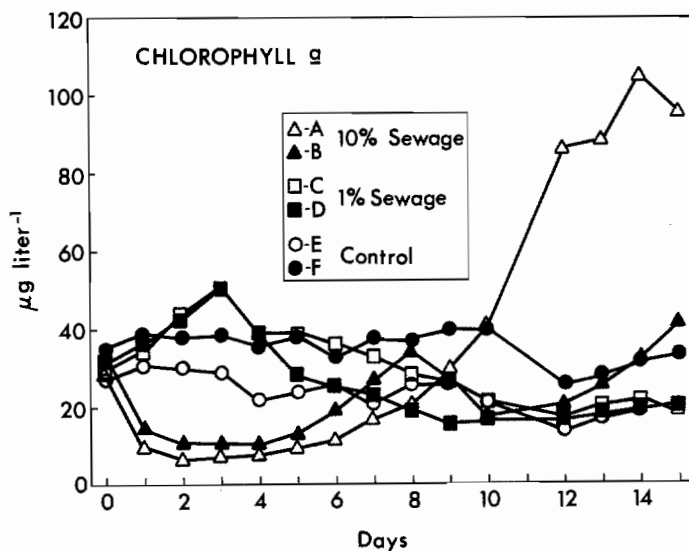|	b	a	r	n
Wild fish	3.48	− 59.4	0.995	5
Control tank	5.57	−109.4	0.998	3
Nitrogen tank	4.25	− 89.2	0.996	3
Phosphorus tank	4.51	− 87.0	0.996	3

Fig. 6. Concentrations of chlorophyll a (µg l^{-1}) versus time in tanks perturbed by 10% and 1% sewage and in controls July 19 through August 3, 1973. Experiment 2.

tanks and the tanks that received 1% sewage while those in the other tank (Tank A) rose to over 90 µg l^{-1}. The concentrations of chlorophyll a rose to 50 µg l^{-1} by day 3 in the tanks that received 1% sewage and then declined gradually for the remainder of the experiment. The concentrations of chlorophyll a in the control tanks remained between 20 and 40 µg l^{-1} for most of the experiment. Primary productivity followed the trends of chlorophyll a.

Eurytemora affinis were added from mass cultures on the first day of the experiment. After day 7, Tanks A and B (10% sewage) still contained primarily *E. affinis*, Tanks C and D (1% sewage) were mixed *E. affinis* and *Acartia tonsa*, and the control tanks (E and F) had sparse populations of nearly pure *A. tonsa* with some *Oithona col-carva*.

The biomass of copepods remained low in all tanks until day 7 (Fig. 7). The biomass of the copepods increased to over 600 µg dry weight per l in Tank B (10% sewage) and Tanks C and D (1% sewage) during the last seven days of the experiment. Biomass of copepods remained low in the other tank that received 10% sewage (Tank A) and in the two controls for the duration of the experiment.

Two fish were added to each tank on day 0. By day 3 all of the fish had died in the tanks that received 10% sewage, one died in one of the tanks that received 1% sewage, and one died in each of the controls. All were replaced on day 3. The fish were not observed on day 5 and presumed dead, but not replaced. A live fish was later observed in Tank A (10% sewage) and removed on day 11.

In this experiment the tanks that received sewage developed either greater concentrations of chlorophyll a (Tank A), greater biomass of copepods (Tank B), or both (Tanks C and D). The failure of the copepods (*E. affinis*) to outgrow the predation by fish in Tank A apparently caused that tank to develop quite differently from its repli-
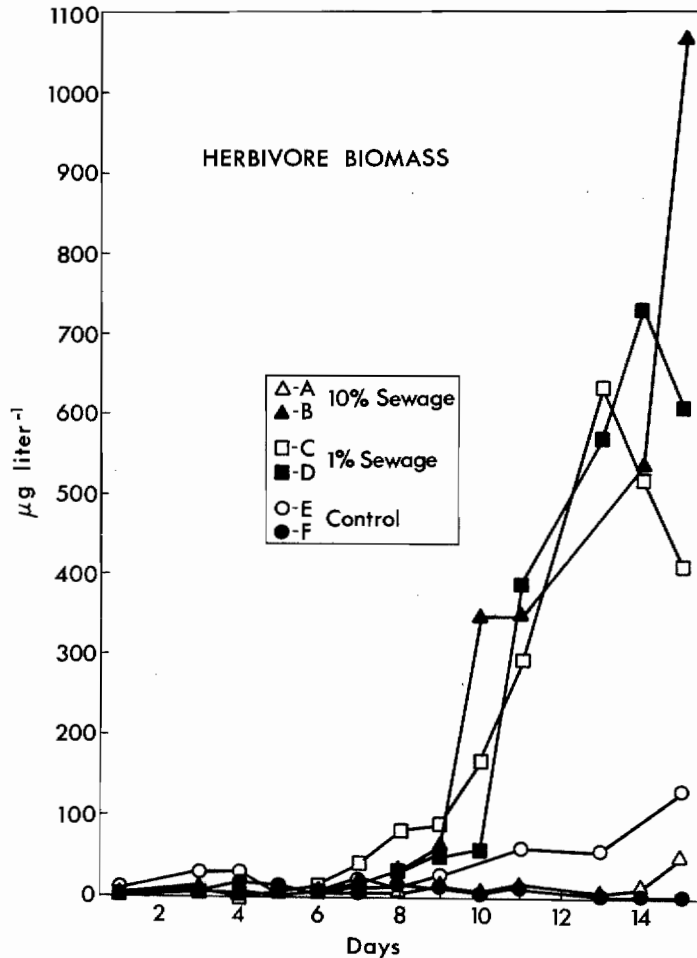
Fig. 7. Biomass of herbivores (μg dry weight l^{-1}) versus time.
Experiment 2.

cate (Tank B). The concentrations of total dissolved phosphorus
in all six tanks reflected the addition of sewage between days 0 and
1 and then remained nearly constant for the duration of the experi-
ment (Fig. 8). Clearly, there was no net removal of phosphorus re-
lated to the increases that occurred in algal or copepod biomass in
the tanks that received sewage. The concentrations of dissolved in-
organic phosphorus and total phosphorus were similar to those for
total dissolved phosphorus shown in Figure 8 and therefore are not
presented.

The concentrations of ammonia did not reflect the addition of
sewage and varied widely--between 1 and 20 μg at l^{-1} in all six
tanks with no apparent patterns. The concentrations of nitrate did
reflect the addition of sewage, however, and rose to 180 - 193
μg at l^{-1} in the tanks that received 10% sewage and to 15.5 - 16.5
μg at l^{-1} in the tanks that received 1% sewage (Fig. 9). Concentra-
tions of nitrate in the controls varied between 0.02 and 0.24 μg at
l^{-1} throughout the experiment with no apparent trends with time.
Concentrations in the tanks that received 10% sewage remained over

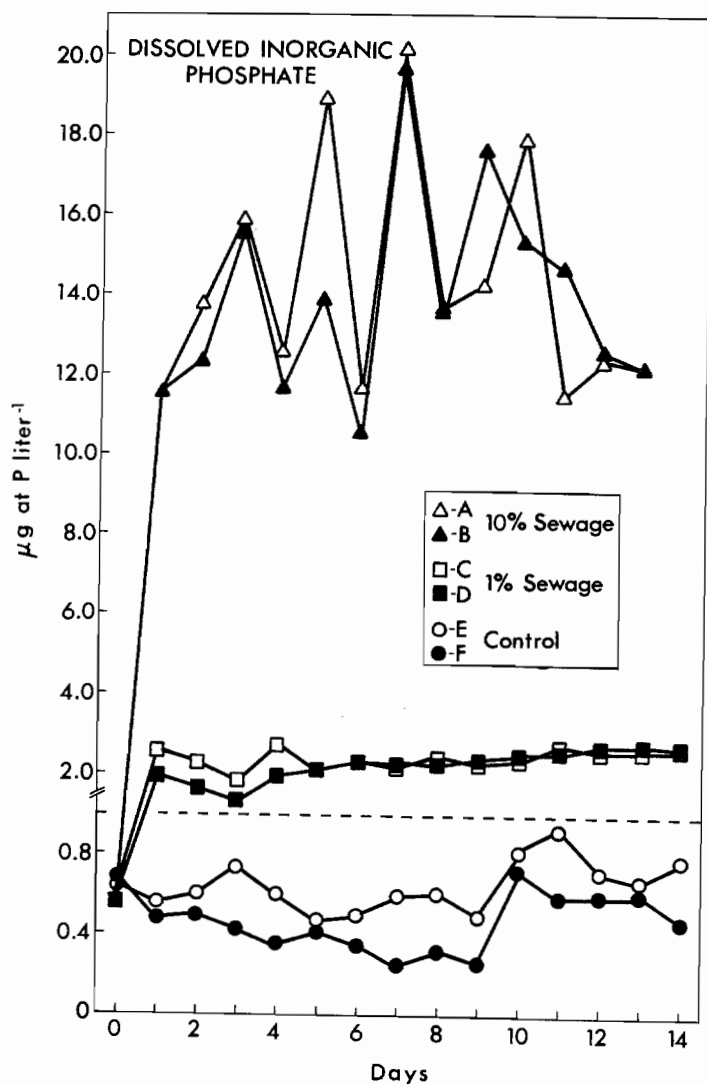Fig. 8. Concentrations of total dissolved phosphorus (µg at P
1^{-1}) versus time. Experiment 2.

130 µg at 1^{-1} for the first six days, fell briefly to about 60 µg
at 1^{-1} during days 7 and 8, and then varied between 116 and 152 µg
at 1^{-1} for the remainder of the experiment. The events in the tanks
that received 1% sewage were more interesting. Concentrations of
nitrate fell to between 1 and 4 µg at 1^{-1} by day 4 (coinciding with
the increase in concentration of chlorophyll a in those tanks) and
then remained relatively constant (Fig. 9) as the algal biomass
(Fig. 6) was converted to copepods (Fig. 7) during the remainder
of the experiment. Concentrations of nitrite were below (usually
well below) 1 µg at 1^{-1} during the entire experiment with no appar-
ent trends. Concentrations of total dissolved nitrogen reflected
the differences caused by the initial addition of sewage, but the
controls and the tank that received 1% sewage tended to converge
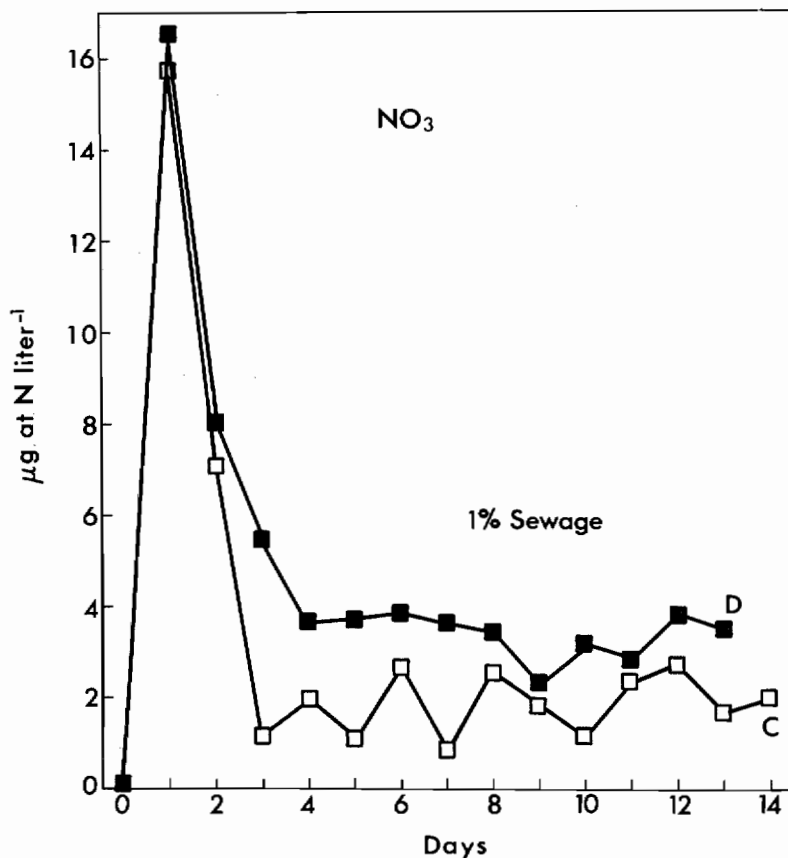somewhat by about day 7. Concentrations of total dissolved nitro-

Fig. 9.   Concentrations of nitrate (μg at N 1^{-1}) versus time in tanks perturbed by 0.1% sewage.   Experiment 2.

gen were rarely below 20 μg at 1^{-1} in the tanks that received 1% sewage, reflecting the high concentrations of nitrate in those tanks. Patterns of concentrations of particulate nitrogen generally followed those of chlorophyll a (Fig. 6) and are not reported here in detail.

The constancy of the concentrations of phosphorus (Fig. 8) and the decline in concentration of nitrate (Fig. 9) which accompanied the increases in concentrations of chlorophyll a in the tanks perturbed by 1% sewage (Fig. 6) suggest that nitrate-nitrogen was a major limiting nutrient in the water used.   Phosphorus was apparently present in surplus as there was no net removal of phosphorus from solution in any of the tanks.

The third experiment was done between September 25 and October 9, 1973, at temperatures of 25 to 30°C.   These data have been reported in part by Flemer and Heinle (1974) and Ulanowicz et al. (1977).   The remaining results are all from the third experiment.

Concentrations of chlorophyll a rose from 20 to 100 μg 1^{-1} within 3 days in the tanks that received 1% sewage and from 35 to 40 μg 1^{-1} within 3 days in the tanks that received 0.1% sewage. Concentrations then fell gradually in both pairs of perturbed tanks (Fig. 10).   The concentrations of chlorophyll a rose from 20 to 30 ug 1^{-1} in the control tanks on the second day and then declined to
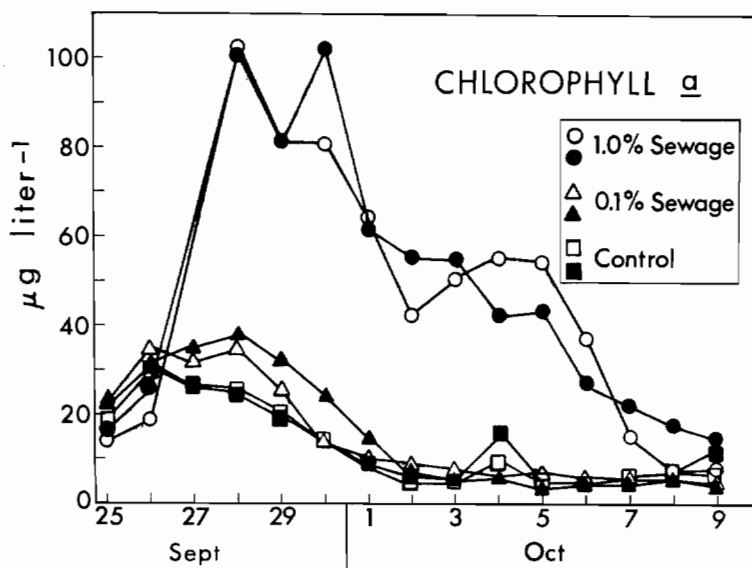
Fig. 10. Concentrations of chlorophyll a (µg l^{-1}) versus time
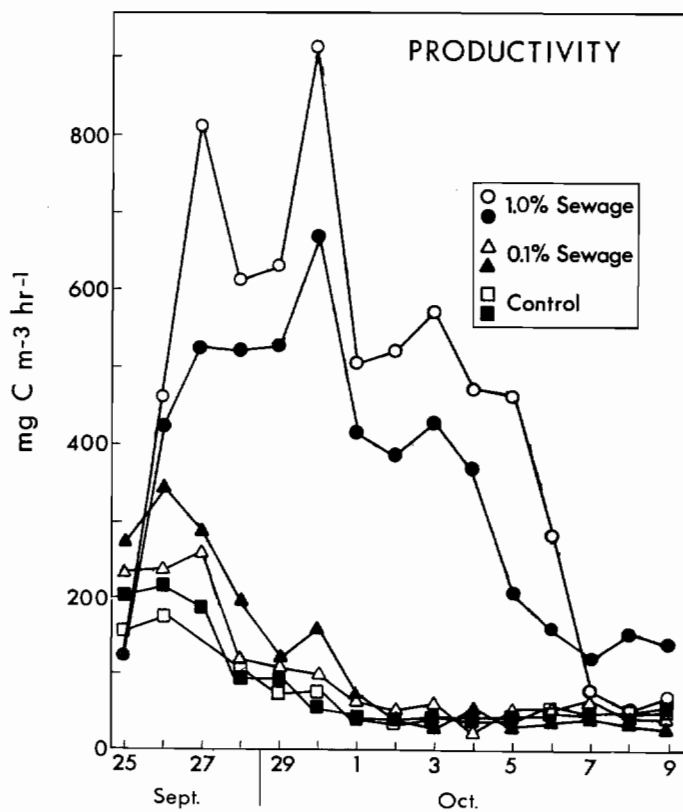September 25 through October 9, 1973. Experiment 3.

Fig. 11. Primary productivity (mg C m^{-3}l^{-1}) versus time.
Experiment 3.

about 5 μg l^{-1} by day 7 (October 2) and remained low thereafter. Concentrations of chlorophyll a were similar in the control and 0.1% sewage tanks after day 7, but the tanks that received 1% sewage continued to have higher concentrations until the end of the experiment.

Primary productivity followed chlorophyll a in pattern (Fig. 11) although values differed more in replicate tanks. Maximum productivities of 650 to 900 mg C $m^{-3}hr^{-1}$, 250 to 350 mg C $m^{-3}hr^{-1}$, and 150 to 210 mg C $m^{-3}hr^{-1}$ were achieved in the 1% sewage, 0.1% sewage, and control tanks, respectively.

The biomass of zooplankton (*A. tonsa* and rotifers) rose in all tanks until day 8 with considerable overlap and variation. By day 8 (October 3), the biomass in the perturbed tanks was up to twice as great as in the controls (Fig. 12). After day 8 the biomass of zooplankton declined in the control tanks. In the tanks that received 0.1% sewage, the biomass of zooplankton was highest on days 9 and 10. The one very high value of 625 μg carbon l^{-1} in Tank A (1% sewage) is probably the result of sampling error. Ignoring that point, the biomass of zooplankton in the tanks that received 1% sew-
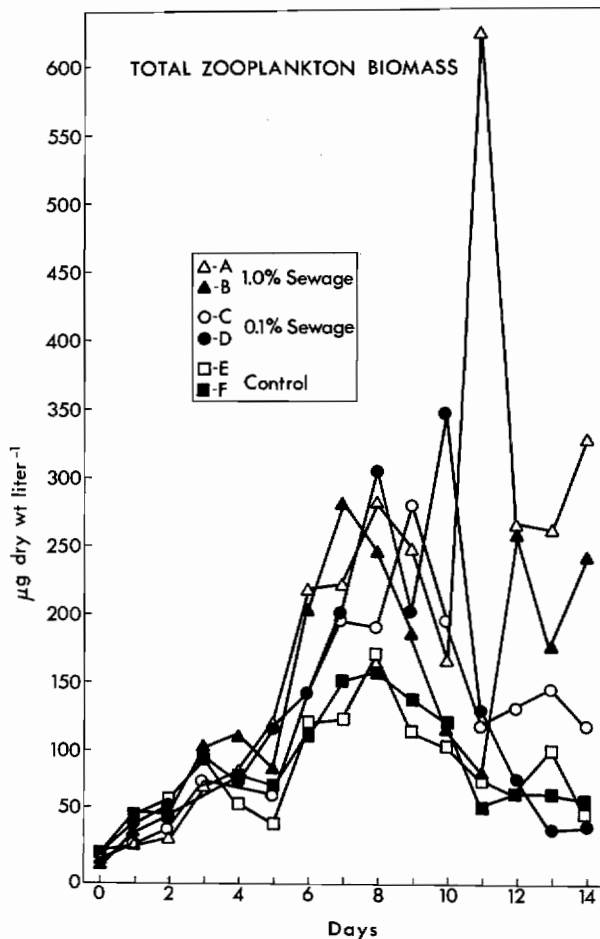

Fig. 12. Biomass of herbivores (μg dry weight l^{-1} of copepods plus rotifers) versus time. Experiment 3.

age declined from days 7 and 8 to days 9 and 10 and then rose again until the end of the experiment. We believe that the final rise in biomass of zooplankton (essentially pure *A. tonsa*) during days 10 through 14 was an artifact of calculating biomass from the length-weight relationship of wild copepods. Production of eggs by the copepods ceased in all tanks by day 10, suggesting inadequate food (Dagg, 1977), and adult copepods collected at the end of the experiment weighed less than half as much as calculated from the length-weight relationship. The biomass of rotifers peaked at 30 µg C 1^{-1} in the control tanks on day 2 (September 27), at 44 to 61 µg C 1^{-1} in the 0.1% sewage tanks on day 3, and at 140 to 184 µg C 1^{-1} on days 7 and 8 in the 1% sewage tanks (Fig. 13). Rotifers declined to 1 to 5 µg C^{-1} in the control tanks and one of the 0.1% sewage tanks by day 6 and rose gradually after day 8. The biomass of rotifers never fell below 9 µg C 1^{-1} in the other tank that received 0.1% sewage. The biomass of rotifers diverged after day 7 in the tanks that received 1% sewage, falling rapidly in Tank B and remaining much higher in Tank A. The total biomass of zooplankton on day 7 was thus over 50% rotifers in the tanks that received 1% sewage and predominantly copepods in the other tanks.

Concentrations of particulate carbon rose from 1.0–1.5 mg 1^{-1} to 2.0 mg 1^{-1} in all tanks between days 0 and 2 and then fell gradually to 1.5 mg 1^{-1} in the control and 0.1% sewage tanks (Fig. 14). Particulate carbon continued to rise over 4 mg 1^{-1} on day 6 in the
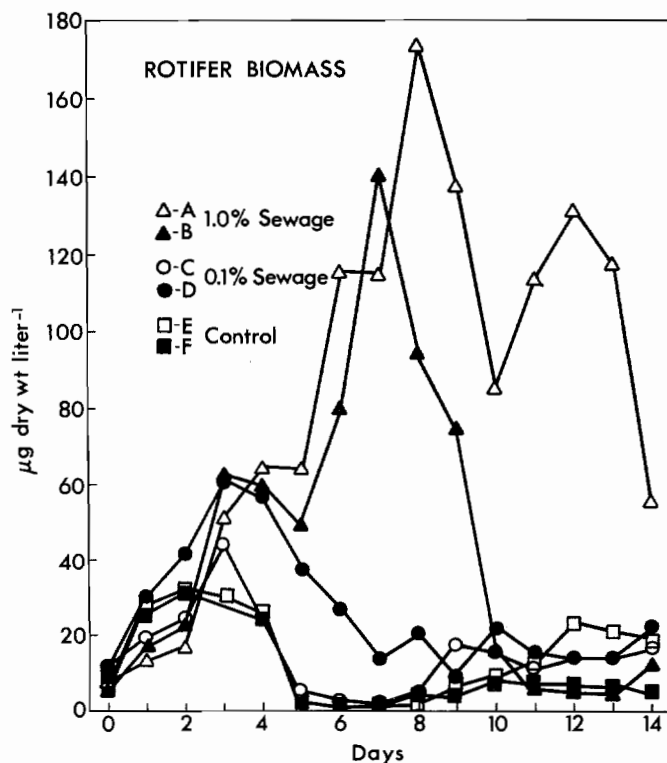

Fig. 13. Biomass of rotifers (µg dry weight 1^{-1}) versus time. Experiment 3).
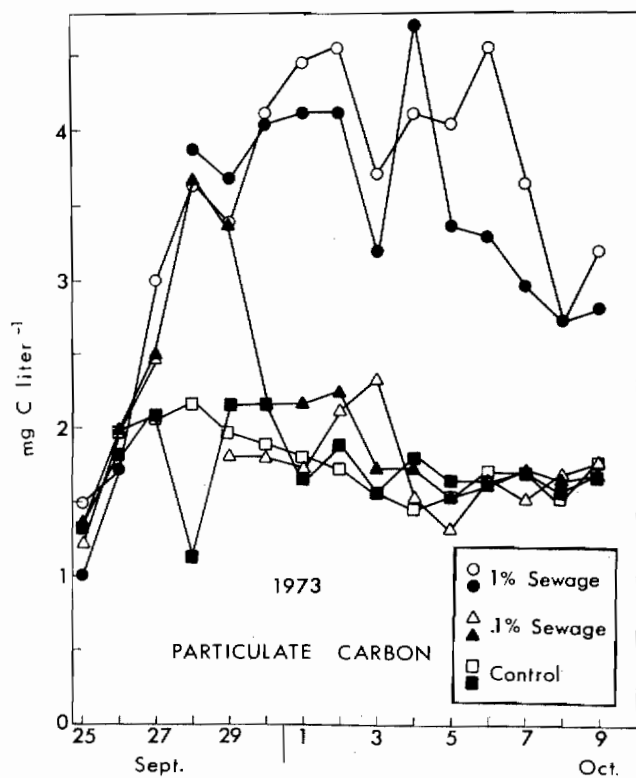
Fig. 14. Concentrations of particulate carbon (mg C 1^{-1}) versus time. Experiment 3.

tanks that received 1% sewage and remained above 3 mg 1^{-1} throughout the remainder of the experiment.

Concentrations of particulate nitrogen (Fig. 15) were relatively constant (about 0.28 mg N 1^{-1}) in the control tanks, less constant and higher in the tanks that were perturbed by 0.1% sewage, and highest in the tanks that received 1% sewage. The concentrations of particulate carbon and nitrogen reflect primarily the combined algal and zooplankton biomass. Estimating algal carbon by 50x chlorophyll a (Strickland, 1965) and summing with zooplankton carbon suggest that about 75% of the particulate carbon was in those fractions on day 7.

One large fish (about 60mm) was added to each tank on day 0. The fish lived for the duration of the experiment and appeared to be healthy throughout.

The concentrations of dissolved inorganic phosphorus reflected the addition of sewage on day 0 (Fig. 16). Concentrations in the control tanks were 0 to 0.05 µg at 1^{-1} on days 0 through 3 and remained so low they were not graphed. Concentrations in all tanks fell to 0 by day 6 and remained 0 through day 9. Concentrations rose in all tanks after day 10 but only reached 0.1 µg at 1^{-1} in the control tanks while rising to 0.20 to 0.36 µg at 1^{-1} in the 0.1% sewage tanks and 0.78 to 0.88 µg at 1^{-1} in the 1% sewage tanks.

Nitrates similarly reflected the addition of sewage (Fig. 17) but declined to lower values in the 0.1% sewage than in the con-
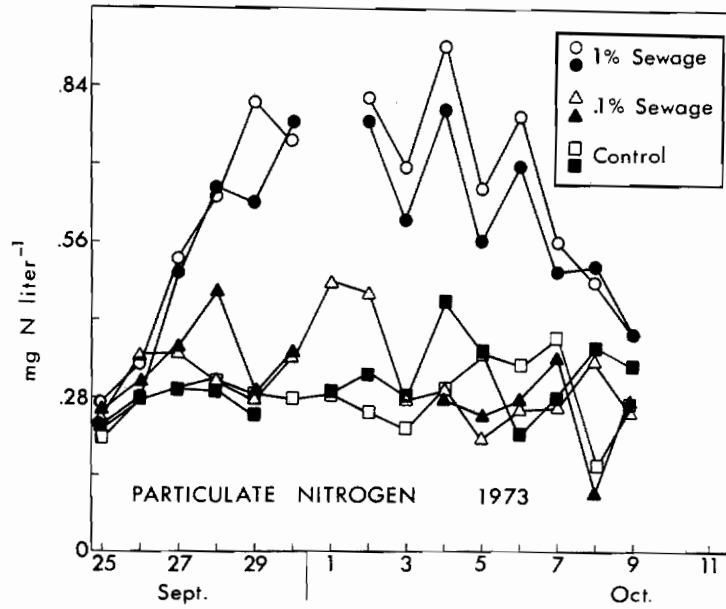
Fig. 15. Concentrations of particulate nitrogen (mg N 1^{-1}) versus time. Experiment 3.
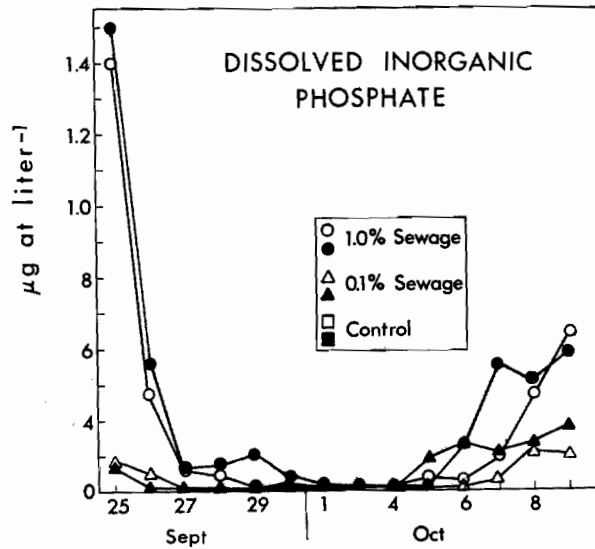

Fig. 16. Concentrations of dissolved inorganic phosphorus (ug at P 1^{-1}) versus time. Experiment 3.

trols and to lowest values in the 1% sewage tanks. Concentrations of nitrates in the controls remained between 10 and 15 µg at 1^{-1} as N throughout the experiment, with the exception of one point on day 8.

Nitrite concentrations were not increased by the addition of sewage (Fig. 18). Like the concentrations of nitrate, those of nitrite decreased in the tanks that were perturbed by sewage. Lowest concentrations were observed in the tanks that received 1% sewage and highest concentrations in the controls.
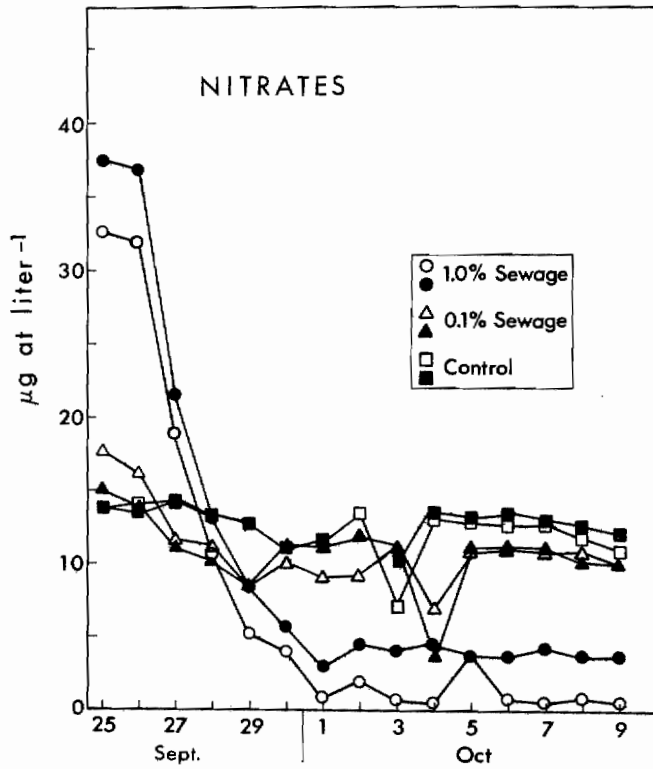
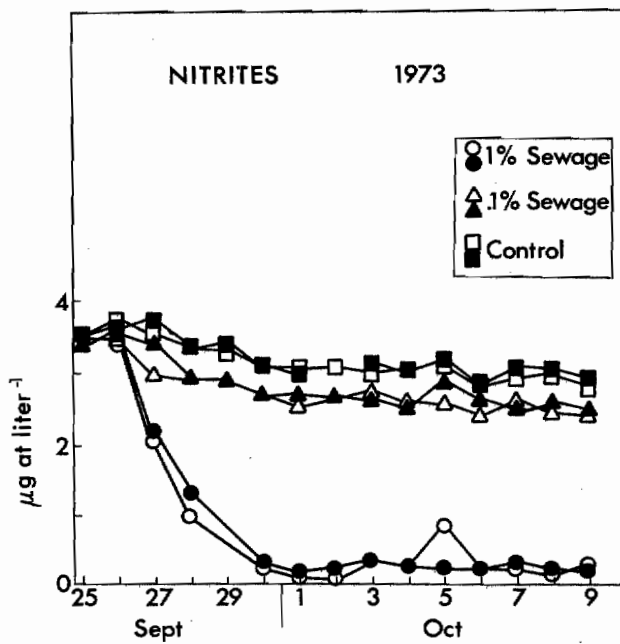Fig. 17.   Concentrations of nitrates (µg at N 1^{-1}) versus time.
Experiment 3.

Fig. 18.   Concentrations of nitrites (µg at N 1^{-1}) versus time.
Experiment 3.

The concentrations of ammonia also failed to reflect the addition of sewage and, while somewhat variable, dropped from 4.5 to 8.0 µg at 1^{-1} on day 0 to 1.0 to 2.3 µg at 1^{-1} on day 3 in all tanks except one control with no clear distinction between treatments except that concentrations in the 1% sewage tanks were toward the lower part of the range (Fig. 19). Thereafter, the concentrations of ammonia varied considerably with low (4.0 µg at 1^{-1}) and high (15 µg at 1^{-1}) concentrations being observed in all tanks at various times. After day 7 (October 2) the concentrations of ammonia trended upward from 0 to 2 µg at 1^{-1} in all tanks.

It appears that the addition of phosphorus with the sewage stimulated the growth of algae that caused nitrogen concentrations to decrease below those in the controls. While all forms of nitrogen may have been used, nitrate and nitrite nitrogen, both of which were present in moderately high concentrations in the Bay water, were decreased more rapidly than ammonia in the enriched tanks (Figs. 17 and 18).

Increases in algal standing crops were roughly proportional to the amounts of sewage added (Fig. 11). Some of the algal increase was reflected at the herbivore level (Fig. 12), but stimulation of the ecosystem by sewage considerably influenced the composition of the zooplankton (Fig. 13).
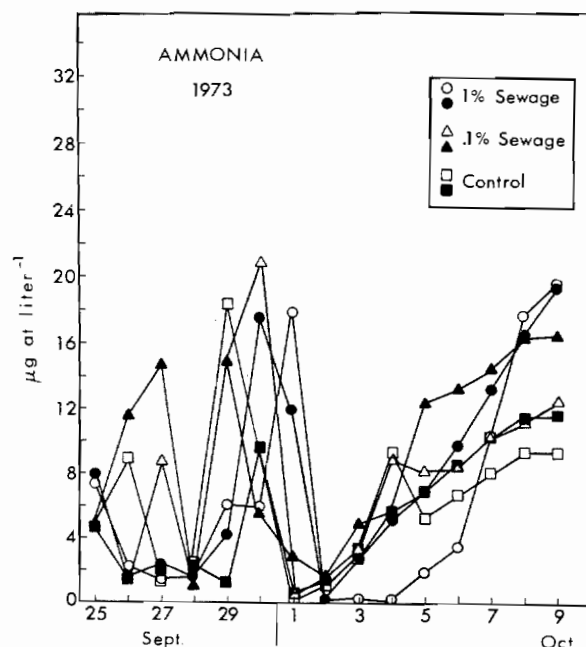

Fig. 19. Concentrations of ammonia (µg at N 1^{-1}) versus time. Experiment 3.

Discussion

Our results suggest that continued cultural eutrophication of Chesapeake Bay will lead to additional increases in primary production and standing stocks of algae. Secondary producers might also be increased. As we were unable to identify the species of algae in our experiments, we do not know if the qualitative changes we observed in the zooplankton (more rotifers in enriched microcosms) were caused by some unknown qualitative change in the phytoplankton or by the increased quantity of phytoplankton.

Our microcosms were not large enough to allow detailed study of the carnivores we added. It seems likely that increased secondary production without changes in species composition must result in increased biomass of zooplankton as most species in Chesapeake Bay are often growing at physiologically maximum rates (Heinle, 1966, 1974; Heinle and Flemer, 1975).

Qualitative changes in zooplankton, as observed in experiment 3 (Fig. 13), could, however, lead to increased secondary production without increases in the biomass of herbivores, as rotifers are generally more active metabolically than copepods (Allan, 1976). As prey density and composition may directly affect the efficiency of planktivorous fish (Ivlev, 1955), increased productivity should be reflected up the food chain.

We have not attempted to evaluate quantitatively the effects of increased productivity of surface waters on the duration or extent of the mass of deep water in Chesapeake Bay that is nearly anaerobic during the summer months. It seems likely, however, that increased productivity would enhance the depletion of oxygen in deep waters as organic material sinks through the halocline. Increases in productivity of plankton might thus have serious negative effects on existing benthic communities in Chesapeake Bay.

Ulanowicz *et al.* (1978) concluded from the modeling of data from the third experiment that increases in chlorophyll a were most closely related to the rates of change in concentration of nitrates and nitrites. Their model, derived from the tanks that received 1% sewage, did not predict the changes in the concentrations of nitrate and nitrite in the other four tanks, however. While nitrite and nitrate nitrogen disappeared from solution more rapidly in the enriched tanks during the third experiment, all forms including ammonia reached minimum concentrations on day 6 or 7 (October 1 or 2) (Figs. 17, 18, 19) coincident with a decrease in primary productivity in the tanks enriched with 1% sewage (Fig. 11). It appears that phosphorus was the "limiting nutrient" in an absolute sense at the beginning of that experiment, but it was removed from solution so rapidly during the first 3 days of the experiment that later changes in chlorophyll a did not correlate well with concentrations of phosphorus. Nitrogen may have limited primary productivity on days 6 and 7. Increasing concentrations of ammonia (Fig. 19) and biomass of herbivores (Fig. 12) toward the end of experiment three suggest that grazing rather than nutrients limited primary production during that period.

By contrast, forms of nitrogen appear to be the limiting nutri-

ents in the second experiment, and possibly the first. In some cases, the "preferred" forms of nitrogen appeared to be nitrate and nitrite rather than ammonia, as these forms were removed from solution more rapidly than ammonia (second and third experiments). The biomass of herbivores was sufficiently low during the early part of all experiments so that regeneration of ammonia was not a major source of nitrogen. Past day 7, however, ammonia excreted by zooplankton may have been the major source of nitrogen in some tanks. Other studies on Chesapeake Bay have shown that ammonia is generally the preferred form of nitrogen, based on rates of uptake (McCarthy et al., 1975, 1977). Our results are not inconsistent with those of McCarthy et al. (1975, 1977). Our experiments were done during August and October when nitrogen preferences were less distinct. We believe that all forms of nitrogen may have been used by algae in our experiments, but ammonia was excreted by zooplankton, resulting in greater variation in the concentrations of that form.

Our experimental design did not allow us to evaluate the role of benthic community processes in nutrient regeneration. That role is known to be important in weakly stratified, aerobic estuaries (Nixon, Oviatt, and Hale, 1976). Future studies of microcosms should include a benthic component (see for example papers by Nixon et al. and Oviatt et al. in this volume).

Our results suggest that Chesapeake Bay, while already impacted by cultural eutrophication (Flemer and Heinle, 1974), is capable of responding to the addition of increased amounts of sewage with increased primary production. Production at higher trophic levels will probably increase also, but qualitative changes may occur whose consequences are difficult to predict.

We are convinced that the keys to the excellent replication obtained in the third experiment were the careful cleaning of the tanks prior to the experiment, rigorous control of environmental variables, and minimum manipulation of the composition of the planktonic community.

Acknowledgments

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