

A COMPARISON OF THE RESPONSES OF BENTHIC
AND PLANKTONIC COMMUNITIES TO ENRICHMENT
WITH INORGANIC FERTILIZERS

by

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ABSTRACT

A comparison was made of the responses of benthic and planktonic components of natural water-sediment systems enriched with inorganic fertilizers. Three levels of fertilization were applied to a series of enclosures placed in a shallow (1 m depth) area of Marion Lake, British Columbia and community respiration, primary production, standing algal crop and the distribution of added phosphorus were measured in both the sediment and the water column over a period of two years.

A loss of added phosphorus from the water column corresponded to an increase measured in the sediment. Uptake and release of phosphorus by the sediment was proportional to its concentration in the water column, indicating a water-sediment equilibrium. In an undisturbed system, however, there was a net movement of phosphorus into the sediment.

A sustained high level of planktonic primary production persisted throughout the period of fertilization. However, an initial increase in total benthic primary production returned to pre-fertilization levels following the establishment of an increased standing crop of epipelagic algae. At this point, benthic primary production appeared to be a function of grazing pressure, responding to increased grazing but not to additional fertilization. When grazing was experimentally increased by concentrating grazers in an experimental area,

benthic primary production increased. Fertilized sediment appeared to have a much greater ability to withstand increased grazing pressure than normal sediment, maintaining high levels of primary production even at five times normal grazer density. But despite this potential, measurements of grazer and bacteria populations within the experimental enclosures showed no response to the greater standing crop of epipelagic algae. As a result, sustained increase in benthic primary production could be induced only experimentally.

It was concluded that the benthic community was more stable in its response to enrichment than that of the plankton and by absorbing nutrients from the water column served to dampen the effects of the more pronounced fluctuations of the planktonic community.

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INTRODUCTION

Hutchinson (1957) has described as "one of the most important discoveries that have ever been made in limnology" the conclusion by Einsele (1941) that a lake "operates as a self regulatory system" capable of re-stabilizing itself following the temporary disturbance of one of its operating variables. Margalef (1968) has defined such stability as "the ability of the system to remain reasonably similar to itself inspite of... exogenous variations through agents outside the system.....the system has a greater resistance to changes that are external in their origin". The contemporary importance of Einsele's discovery in terms of Margalef's definition of stability may easily be seen in man's disruption of natural processes in many bodies of fresh water.

The most typically disturbed variable is that of inorganic nutrients. Sewage, agricultural and industrial contributions have increased the phosphorus and nitrogen content of many lakes far beyond normally encountered levels. Where existing community structure was unable to absorb such an increase new communities have developed in the place of the previous ones. These community shifts often produce extremely undesirable consequences (Fruh, 1966).

Natural eutrophication is a long process of gradual nutrient enhancement and corresponding community adjustment. Rapid unnatural additions, however, are more similar to the "temporary

disruptions" described by Einsele. The response to such additions should thus provide a measure of stability in the various components of a fresh water system. Dramatic eutrophication effects are characteristically planktonic: the formation of algal mats, increases in bacterial action and oxygen depletion have all been well documented in the water column (Vollenweider, 1968). But it is commonly believed (Margalef, 1963) that the benthic community is a more mature and therefore more stable one than that of the plankton. In this study a comparison was made of the responses of the benthic and planktonic components of enclosed water sediment systems enriched to various degrees with inorganic fertilizers. The results indicate that such a stability difference does exist and that this confers upon the community as a whole an ability to resist structural changes at least on a short term basis.

METHODS

This study was made in Marion Lake, situated in the coastal mountains of south western British Columbia. This lake is small (13.3 ha.) and shallow (mean depth 2.2 m maximum depth 6 m), and the bottom consists almost entirely of deep soft sediment covered with a mat of algae and chironomid tubes. The small volume of the lake combined with frequently rapid run off results in what is often extremely high flushing: indeed, it has been suggested (Dickman, 1969) that during periods of high flow, the water body more closely approximates the widening of a stream than a lake. A more complete description is given by Efford (1967).

Fertilization experiments were conducted in an area of uniform (1 m) depth and substratum on the east side of the lake (Fig. 1). Eight enclosures constructed of a $\frac{1}{2}$ " pipe framework with polyethylene walls were placed in this area adjacent to two control plots as indicated. These square enclosures were 16 m^2 in area and 2 m in depth; they were pressed into the sediment to a depth of approximately .5 m. Each enclosure thus enclosed a mean volume of 16 m^3 of water subject to lake level fluctuation. Screened openings in two walls of each enclosure (Fig. 2b) were fitted with shutters which allowed the regulation of current flow through the enclosures. Quantitative experiments of PO_4 uptake and release

Fig. 1 Contour map of Marion Lake, B. C. indicating position of experimental enclosures. Figures on contour lines refer to depth in metres. Enclosed numbers in this and subsequent figures refer to enclosure identification.

1 and 2 - external control areas

7 and 3 - enclosed controls

4 and 8 - low-level fertilization

5 and 9 - mid-level fertilization

6 and 10 - high-level fertilization

a and b - small enclosures (see text)

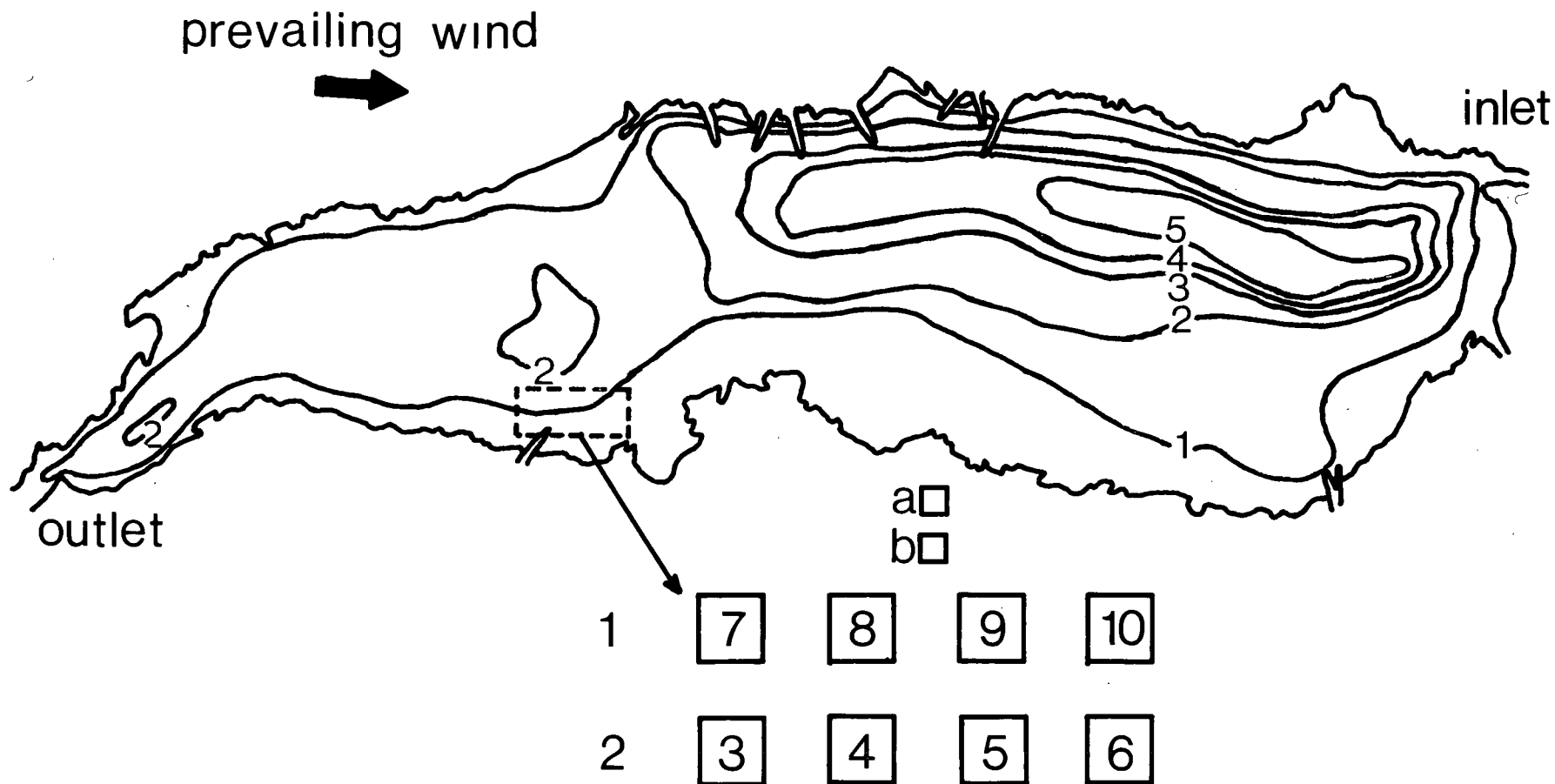
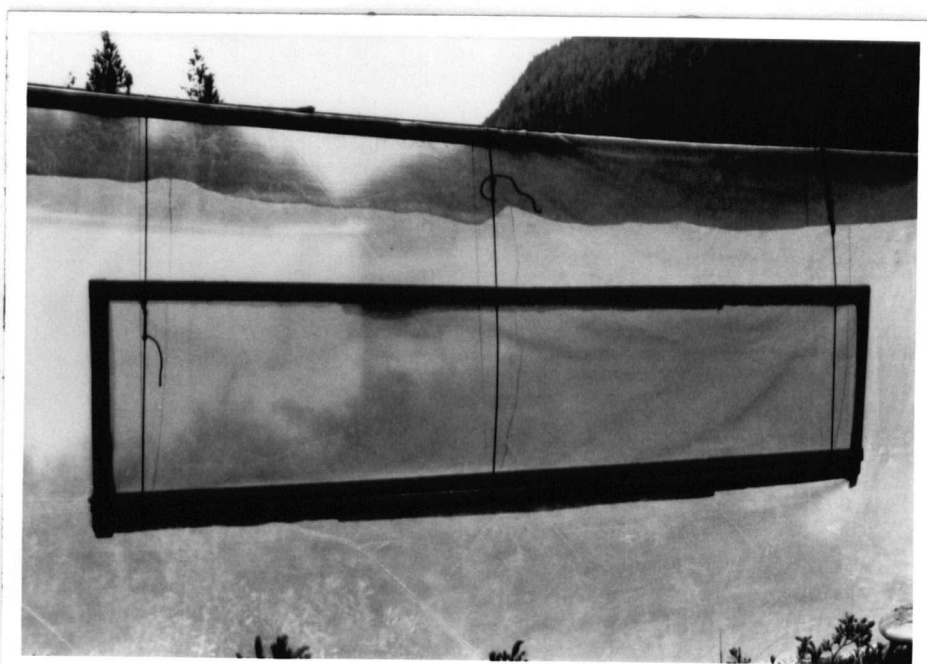


Fig. 2 Screened openings with shutters in the
enclosure walls.



were accomplished in a further set of enclosures. These plywood structures were 1 m square and had sliding sides which could be removed to expose the enclosed sediment to the full flushing of the water current. They were located adjacent to the larger enclosures in positions marked "a" and "b" in (Fig. 1).

Three levels of fertilization were applied, in duplicate, to six of the large enclosures over a period of four months through the summer of 1970. The remaining two enclosures were left untreated as were two adjacent unenclosed plots of sediment: these served respectively as the enclosed and unenclosed controls. As the prevailing winds produced drift in a northerly direction the treatment pairs were aligned in a linear manner such that any fertilizer leakage would only serve to enhance successively higher level enclosures. The fertilizer used was a blend of urea, superphosphate and potash: it contained 1.5% phosphate and 14% nitrate by weight. Phosphates and nitrates were selected for enhancement not only because they are commonly considered to be the most important chemical factors in algal growth (Hutchinson 1967, p. 310) but also because of their low natural levels and previous stimulatory history (Dickman, 1968) in Marion Lake. Commercial agricultural fertilizers such as that used also include a wide spectrum of trace elements not found in reagent quality chemicals. Application

was made bi-weekly during the course of the experiment and was accomplished with the use of a chemical spraying unit. after the appropriate amount of fertilizer had been dissolved in 2 - 3 gallons of lake water. The quantities of fertilizer applied were 32, 160 and 800 g per enclosure for the low, mid and high levels respectively.

Four days following application the shutters on the enclosures were opened to maintain an integrity of chemical and temperature conditions between the enclosed and outside water. Evidence of rapid uptake and storage of phosphorus (Hayes et al, 1952, Einsele, 1941) led to the assumption that this was more than sufficient exposure time for sediment uptake. After allowing a period of time for oxygen and temperature equilibration estimates of benthic algal production and community respiration were made according to the method of Hargrave (1969). At each sampling interval six sediment cores were withdrawn from each enclosure. Pyrex cylinders 12.5 cm long were pressed into the sediment to approximately 2/3 of their length: they were then stoppered and withdrawn producing an intact sediment interface with approximately 100 ml of overlying water. Cores were stabilized in the dark for two hours prior to incubation to avoid continuation of oxygen production by algae removed directly from the light. Such a process, as noted by Hargrave (1969) would reduce the apparent community respiration. The overlying water was analyzed for oxygen, and the cylinders then sealed, and incubated successively in the dark and light

portions of a constant temperature chamber. Cooling water for this chamber was drawn from an inlet stream and was at a consistently lower temperature than the lake. This was done in order to prevent the dissolution of oxygen accumulated during incubation in near saturated lake water as discussed by Nielson (1957). For this reason also dark incubation always preceeded that in the light. Inhibition of respiration by reduced oxygen levels in sealed cores as noted by Gessner and Pannier (1958) was avoided by limiting incubation times to 3 hrs in the dark and 2 hrs in the light. At the end of each incubation period oxygen concentrations were again measured. Gross production and community respiration was calculated from these data. Total dissolved oxygen was calculated at each stage with volumes corrected for losses in analysis and re-sealing of cores based upon a single volume measurement at the end of all incubations. The supernatant water, collected in a graduated cylinder, was retained as was the sediment for further analysis.

Similar production and respiration measures were made on the planktonic community on two occasions during the period of enrichment. These measures were made in 300 ml B.O.D. bottles incubated in a manner identical to that previously described. Dissolved oxygen was measured at all times with a modified Winkler technique (Fox and Wingfield, 1938). Samples were withdrawn and analyzed in plastic syringes: for this reason phosphoric acid was substituted

for sulphuric acid in the analysis. Although the possibility of oxygen diffusion through polyethylene was considered, the rapid analysis (2 - 3 min to acidification, 8 - 10 min to titration) makes it unlikely that such an effect could have affected the results. .025 N phenyl arsene oxide prepared by the Hach Chemical Company was substituted for sodium thiosulfate in the titration because of its greater stability.

Once the supernatant water had been poured off it was possible to extrude the sediment core by pressing the bottom stopper up the cylinder. In this manner the top 2 cm of sediment was removed and vacuum filtered with a suction filter apparatus. 2 g samples were weighed out from the resulting semi-dried sediment and recombined with 10 ml of the filtrate: by this process density differences between various samples were minimized. Measurement of the algal standing crop was based upon chlorophyll analysis (Vallentyne, 1955). This type of estimate has been criticized extensively because of the variability of chlorophyll content in algal cells and the inclusion of detrital chlorophyll in the analysis (Edmondson and Edmondson, 1947, Margalef, 1954, summary Strickland, 1960). Hargrave (1969) has, however, established a relation of chlorophyll measured in this manner to algal biomass in the Marion Lake sediments: similarly differences in indicated chlorophyll corresponded to cell counts conducted throughout the course of the experiment.

Chemical phosphate analyses were conducted to monitor

the form and quantity of the added phosphate. Ortho phosphate was measured in the water column just prior to flushing and in sediment and interstitial water at the time of production estimation by the method of Strickland and Parsons (1960). Total phosphate was measured in the sediment from samples taken at the same time. These analyses were performed in conjunction with Can Test Laboratories, Vancouver, B. C.¹ All the sediment measures were expressed in ppm phosphate per gram dry wt of sediment. The difference between total and ortho phosphate estimates is regarded (Ohle, 1939) as representing organic phosphorus in either dissolved or sestonic form.

Within the fertilized enclosures the herbivorous amphipod Hyaletella azteca was monitored as an indicator of grazer response to stimulated algal production. Six sediment samples were taken from each enclosure at bi-weekly intervals and numbers and fresh weights tabulated.

While the above procedure was intended to detect a spontaneous response of grazers to an increase in algae it was also necessary to examine the potential support capacity of the fertilized areas whether or not this potential was utilized. This was accomplished by introducing various densities of grazers into enclosed areas of both natural and enriched sediments and monitoring changes in primary production. In addition, Rana aurora tadpoles were introduced into polyethylene enclosures similar to those used for fertilization experiments.

Note 1: A modification of the method described in Standard Methods (1965) was employed with oxidation of organics achieved by fuming with Nitric and Perchloric acids.

Cores were removed from this area and primary production again estimated under these circumstances.

Densities of Hyaella were manipulated in shorter experiments in the glass cylinder cores themselves: in this instance the primary production of both enriched and unenriched sediments were compared. Cylinders were incubated for 48 hours before production estimation under a light/dark cycle similar to that at the lake. Preliminary experiments indicated that photosynthetic oxygen production maintained oxygen levels close to saturation under these conditions.

Near the end of the experimental period bacteria counts were made from the sediments of the enclosures. Samples were removed from each enclosure with a Kajak core sampler and plated at a 10^4 dilution. Colony counts and identifications were subsequently performed on both these and external control samples.

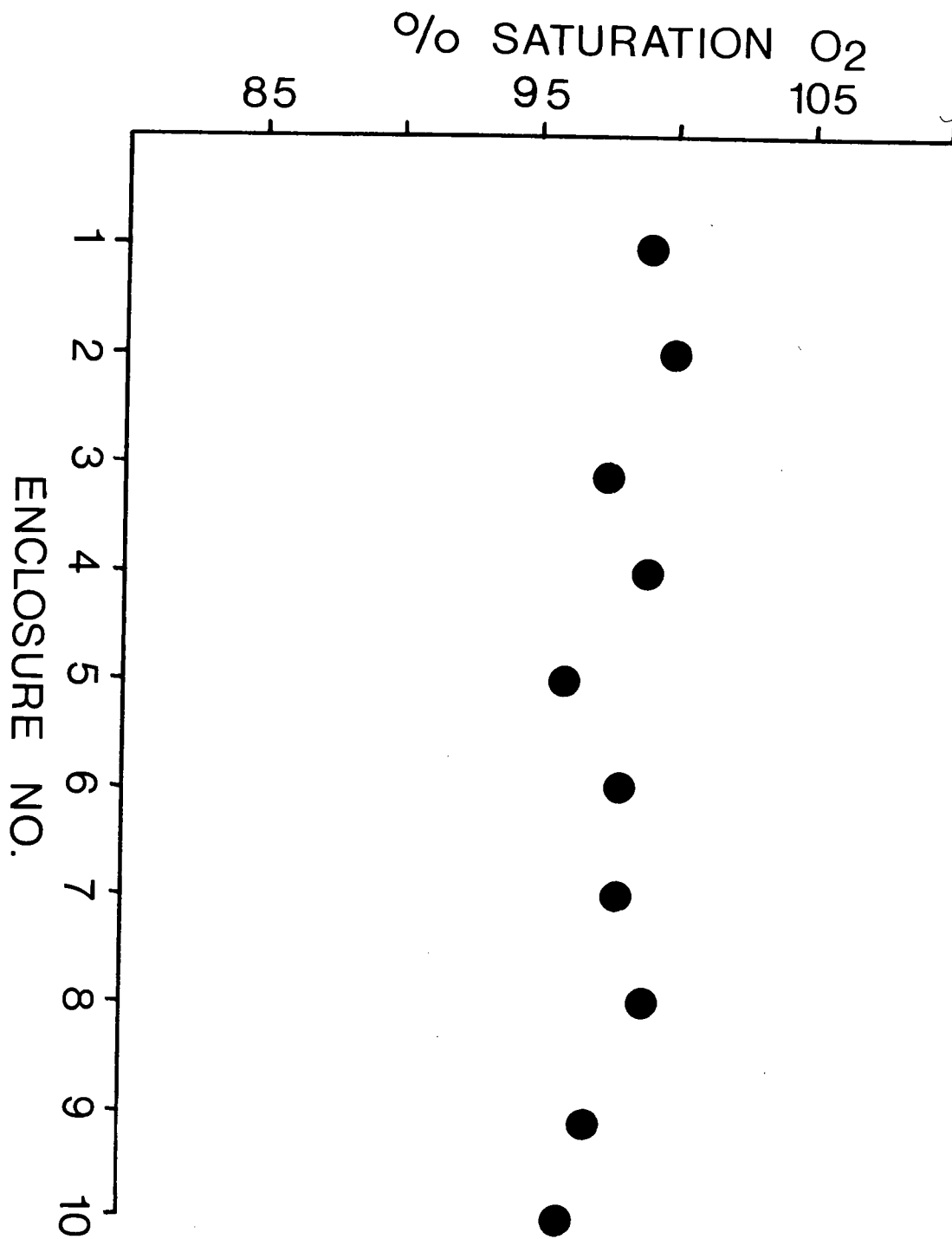
RESULTS

The immediate effect of placing the enclosures was stabilization of the water column in the enclosed area. Drift studies with both dyes and floating markers indicated only a slow circular movement within the enclosures except at times of high winds. Despite this apparent stagnation both temperature and dissolved oxygen concentrations were similar in both the enclosures and the outside water at the time of production estimation (Fig. 3) Bakhtina (1967) in a similar fertilization experiment noted a direct relation between the degree of fertilization and the dissolved oxygen levels in enriched ponds. This was attributed to oxygen production by stimulated algal growth. In this study such accumulations would have been dissipated by the exchange of water through the screens or released from super-saturated solution. As dissolved oxygen in Marion Lake follows the temperature-saturation curve closely (Hargrave, 1969) it would appear that accumulation of oxygen to super-saturation is not favored by conditions in the lake (i.e. turbulence). In any case, as will be demonstrated below, the only increase in oxygen contribution was that of the phytoplankton who were themselves being flushed to an undetermined extent.

DISTRIBUTION OF ADDED PHOSPHORUS

Phosphorus added in the fertilizer was removed rapidly from the water column of both the large and small enclosures.

Fig. 3 Oxygen levels in the experimental enclosures
prior to production estimation (June, 1970)



In the case of the small enclosures which had no openings none of this loss could be attributed to leakage of the contained water. Ortho phosphate measured a week after addition to the large enclosures was at a consistently low level except in the most highly enriched areas (Fig. 4). A phosphate series over time was made in the smaller enclosures (Fig. 5). The resulting curve resembled uptake observed by other workers such as Hayes et al (1952), Einsele (1941), Hutchinson and Bowen (1947). A phosphorus equilibrium appeared to exist within the sediment and water column of the enclosures. The extent of phosphorus removal varied with its initial concentration in the water column (Fig. 6): at higher concentrations a larger amount of phosphorus was lost from the water. Even at the extremely high levels of addition in the small enclosures a quantitative difference in uptake was noted between the two levels (Fig. 5). Conversely a release of phosphorus to the water could be effected upon reducing its concentration in the water by flushing the enclosures (Fig. 5). Azad (1964) and Barchard et al (1964) have demonstrated that algae will not release stored phosphorus once it has been taken up. This would suggest that the sediment retention process has a strong inorganic or physical component which can respond to changes in chemical concentrations of the overlying water. The existence of such an equilibrium is not a new concept: Olsen (1964) has described an equation for the phosphorus equilibrium of oxidized sediments which accounts for both uptake and release from the benthos.

Fig. 4 Ortho phosphate measurements in the water of
the large enclosures. (mg/l)

a. Concentration of initial addition

b. Concentration 4 days following addition

1 - External control area

3 - Enclosed control area

4 - Low level fertilization

5 - Mid level fertilization

6 - High level fertilization

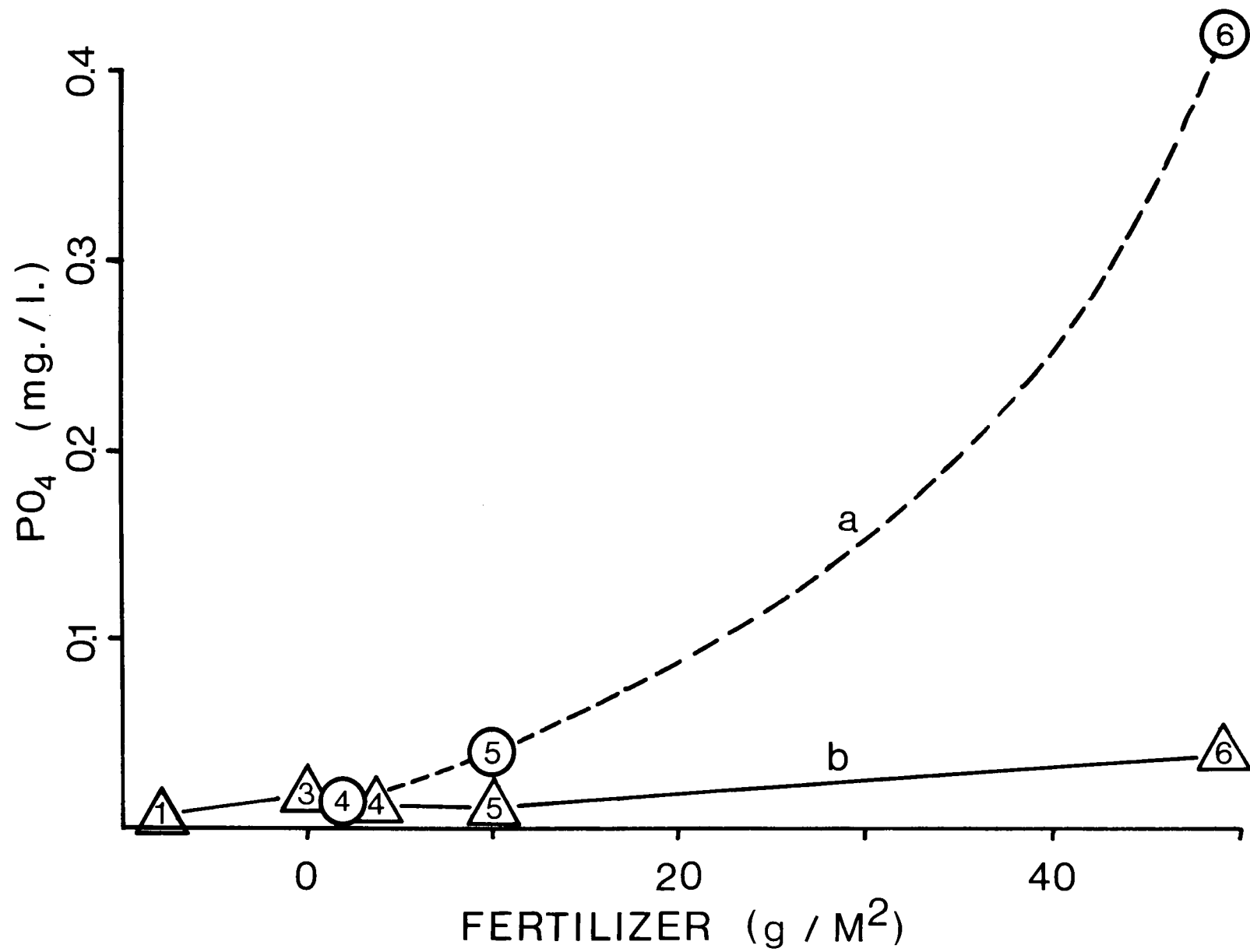


Fig. 5 Ortho phosphate concentrations in the water of the two small enclosures over time following the addition of two different amounts of fertilizer. (enclosures flushed on day 12)

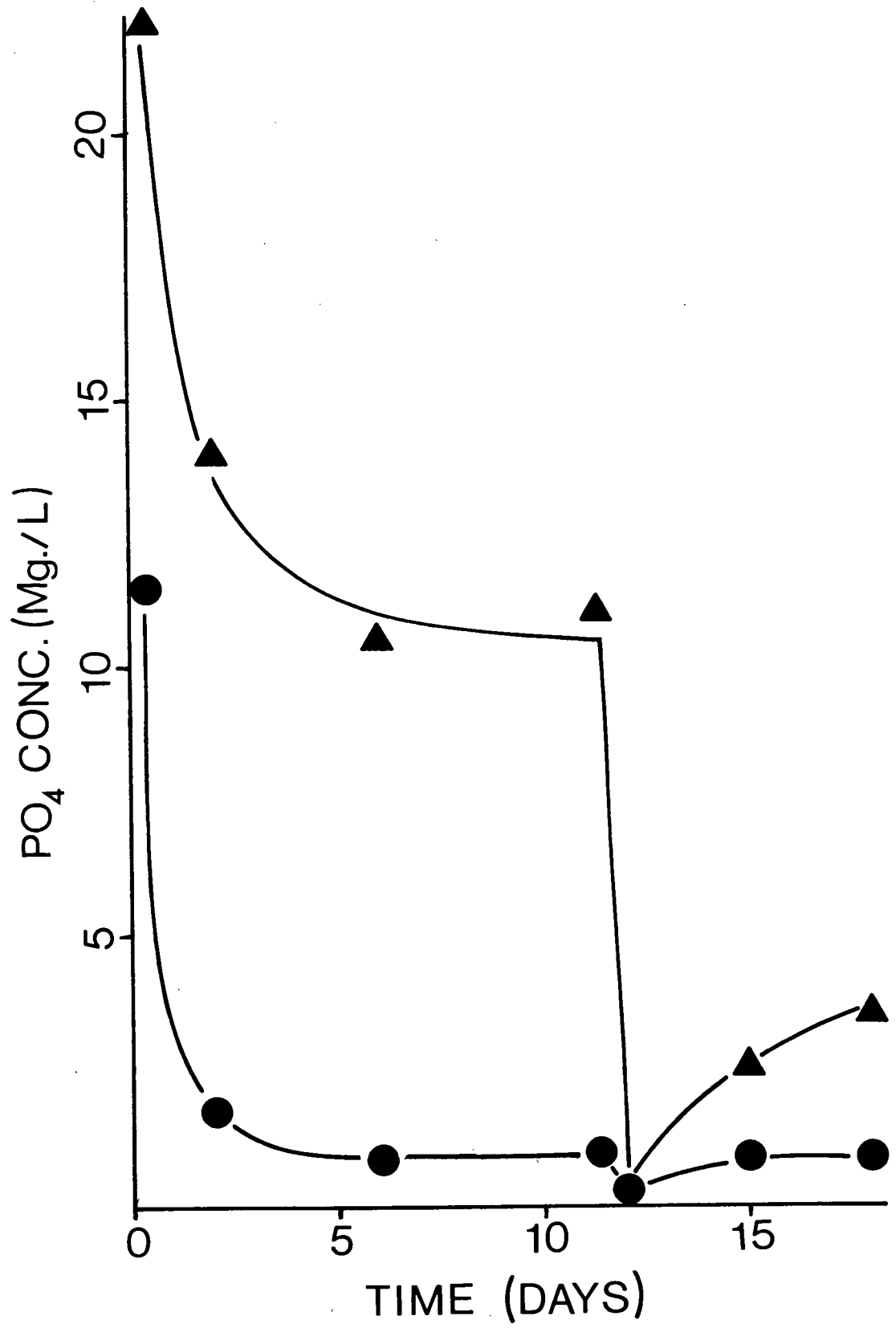
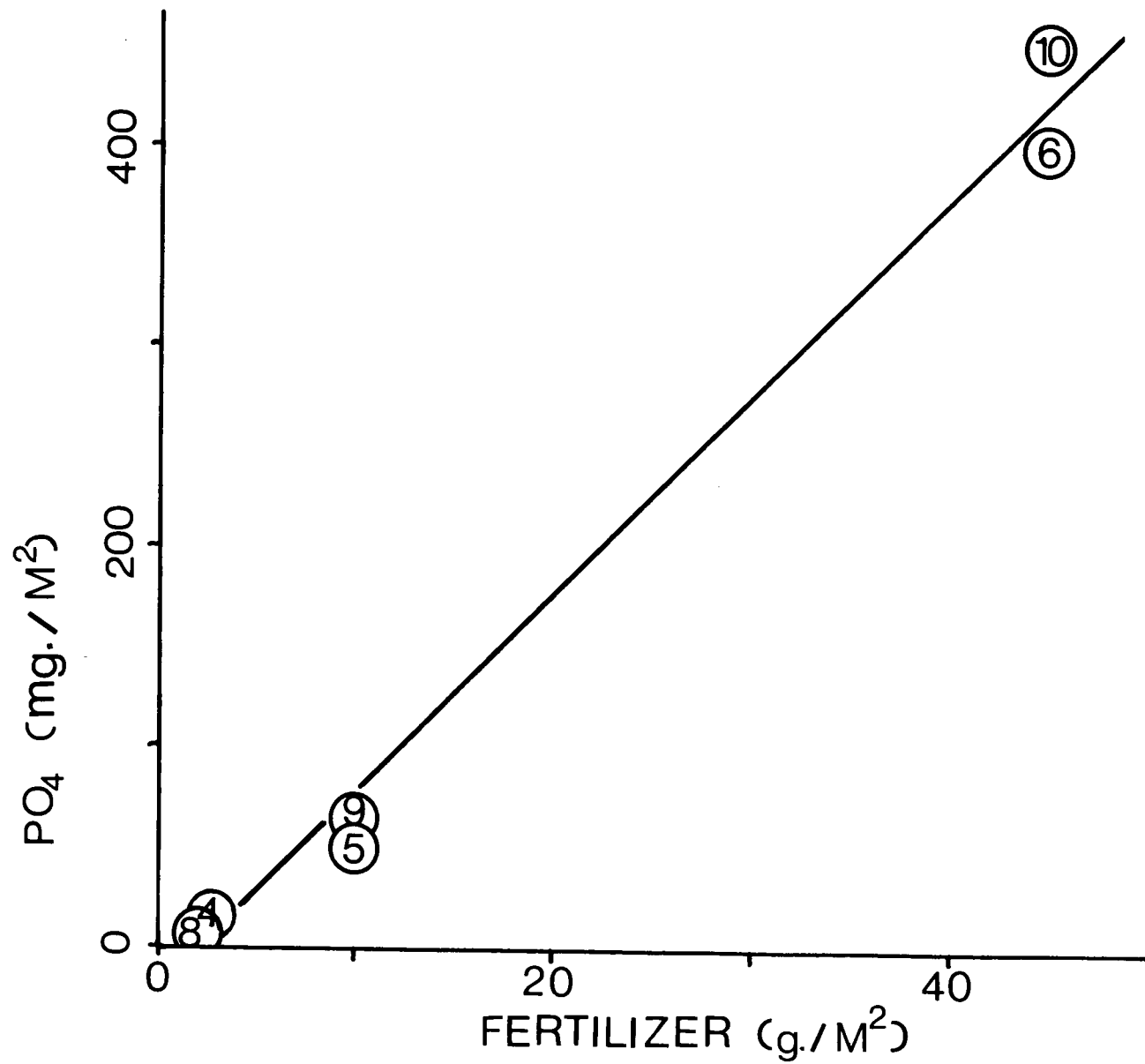


Fig. 6 Loss of phosphorus from water column in a four day
period as a function of initial concentration.

4 and 8 - Low level fertilization

5 and 9 - Mid level fertilization

6 and 10 - High level fertilization



The decrease in phosphorus measured in the water corresponded to an increase in that measured in the sediment: this relation was similar to that noted by Einsele (1941) in the Schleinsee. Here it appeared that the phosphate fraction was being removed from the water column in a manner that was not directly proportional to the concentration of available phosphate (Fig. 7). This observation was, however, partly an artifact of the sampling method which included in the analysis an undetermined amount of the sediments pre-fertilization history. If quantities in the enclosed controls are used as a correction factor for other enclosures the measured sediment phosphorus is more closely proportional to the amount added. In any case phytoplankton in the enclosures would retain phosphorus in particulate form in the water column. This retention would be proportional to the standing crop and the larger standing crops of phytoplankton in more highly fertilized enclosures could in this way account for the balance of added phosphorus.

The ratio of ortho: organic phosphorus was not constant through the various levels of enrichment: with greater fertilization an increasing proportion of the added phosphorus was measured as inorganic phosphate (Fig. 7). If this is indeed the case it would appear that an increasing proportion of the added phosphorus remains unused by the algae at higher levels of addition: that is, that the phosphorus was in excess of the algal uptake capacity. Rigler (1968) has suggested that

Fig. 7 Phosphorus measured in the sediments of the experimental enclosures (/g dry wt sediment). The upper histogram represents total PO_4 while the lower represents ortho PO_4 .

1 and 2 - External control areas

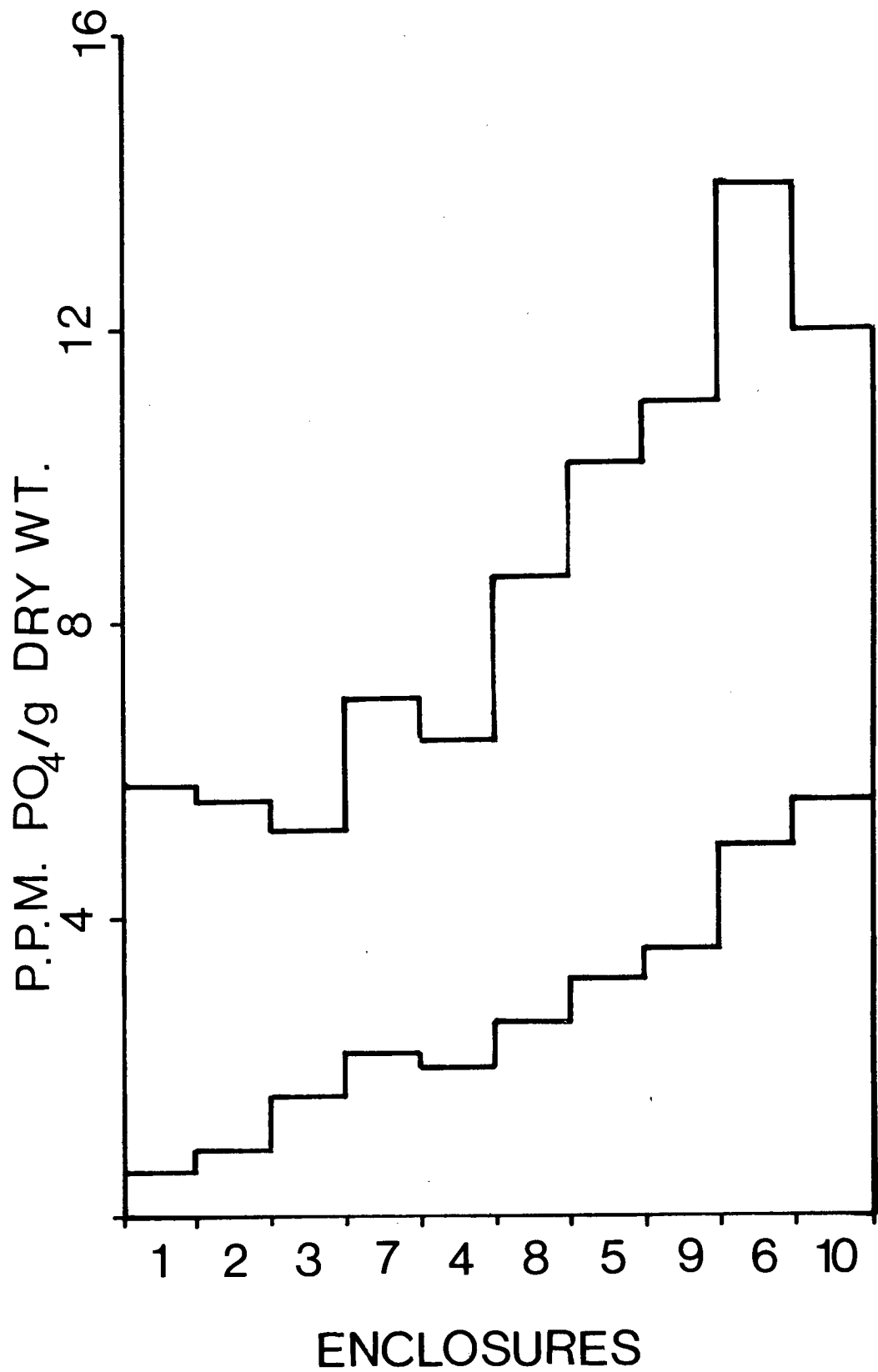
7 and 3 - Enclosed controls

4 and 8 - Low level fertilization

5 and 9 - Mid level fertilization

6 and 10 - High level fertilization

a and b - small enclosures (see text)



the type of analysis (molybdenum blue) used overestimates ortho phosphate measurement by recruitment of organic phosphate through hydrolysis. If soluble reactive phosphorus (Strickland and Parsons, 1960) measures are overestimated to the degree suggested (10-100X) the significance of the observed excesses would be considerably reduced.

RESPONSE OF ALGAE

Algal response was measured in terms of changes in both primary production and standing crop. Differences in the parameters were noted even between external and enclosed control areas. Primary phytoplankton production increased in the control (unfertilized) enclosures (Fig. 8) over that of the outside water. Dickman (1969) made similar observations in his enclosures and attributed the increase to a reduction in phytoplankton cropping by rapid current flow. An initial increase in epibenthic algal production was also noted in the control enclosures (Fig. 8): this increase led, as in the case of increase phytoplankton production, to a higher standing crop in the enclosures than on the outside sediment. A most dramatic response in all enclosures was the proliferation of epiphytic algae on the walls of the enclosures themselves. This growth reached such portions that oxygen production on the enclosure walls approached that of the sediment surface on an area basis: indeed, the addition of increased surface area in the form of the enclosures was one of the greatest factors in algal growth.

The standing crop of epipelagic algae measured as chlorophyll

Fig. 8 Planktonic primary production in the experimental enclosures following fertilization (July, 1970)

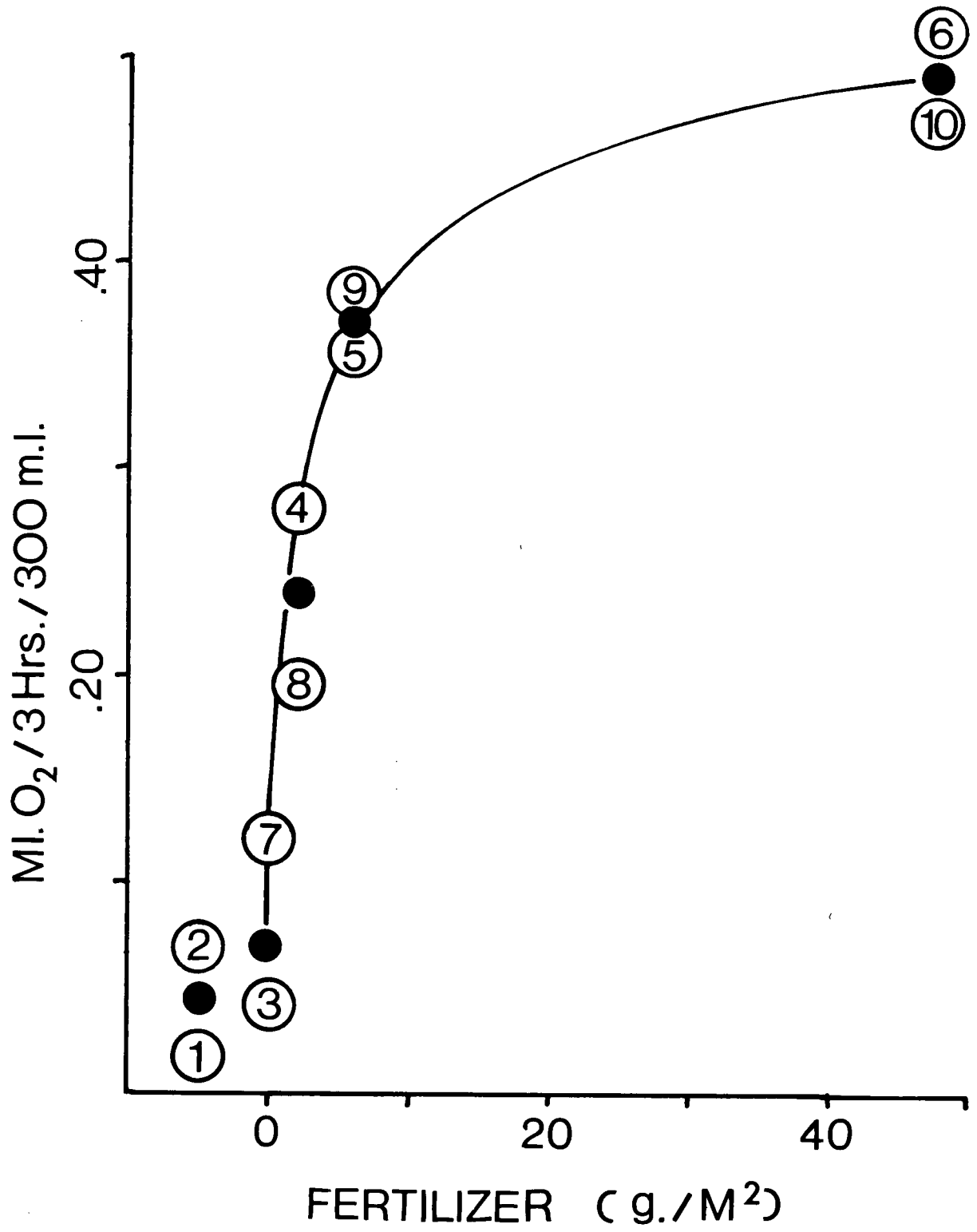
1 and 2 - External controls

7 and 3 - Internal controls

4 and 8 - Low level fertilization

5 and 9 - Mid level fertilization

6 and 10 - High level fertilization



increased in a near linear manner with increased enrichment (Fig. 9). Measured chlorophyll in the most highly enriched enclosures was 10X that of the control areas and measures in each of the treatment pairs were, with the exception of the mid level, closely similar. While no actual measurements were made of phytoplankton standing crop it was visually evident that enrichment produced an increase here as well. At high levels of fertilization plankton blooms formed which lasted almost the entire duration of the experiment in spite of the intermittent flushing of the enclosures.

Primary production in the water column increased with increasing levels of fertilization (Fig. 8). This is a response observed many times in similar studies (Dickman, 1968, Batkina, 1967) as an accompaniment to an increase standing crop. Vinberg (1965) in a discussion of pond fertilization, states (p. 213) "the initial effect of artificial fertilizers in effectively fertilized ponds is to stimulate the vigorous development of phytoplankton". Epipellic primary production, however, did not demonstrate a sustained increase as a response to fertilization (Fig. 10): a stimulated production was noted only in the course of a pilot experiment conducted in the fall of 1969 when initial fertilization was accomplished (Fig. 11). When the program resumed in May 1970 the epibenthic algae had assumed the variation in standing crop noted in the initial measurements (Fig. 9) and O_2 production had returned to pre-fertilization levels in all enclosures.

Fig. 9 Benthic algal standing crop in the experimental
enclosures (chlorophyll units /g dry wt sediment)
July, 1970

1 and 2 - External control areas

7 and 3 - Enclosed controls

4 and 8 - Low level fertilization

5 and 9 - Mid level fertilization

6 and 10 - high level fertilization

a and b - small enclosures (see text)

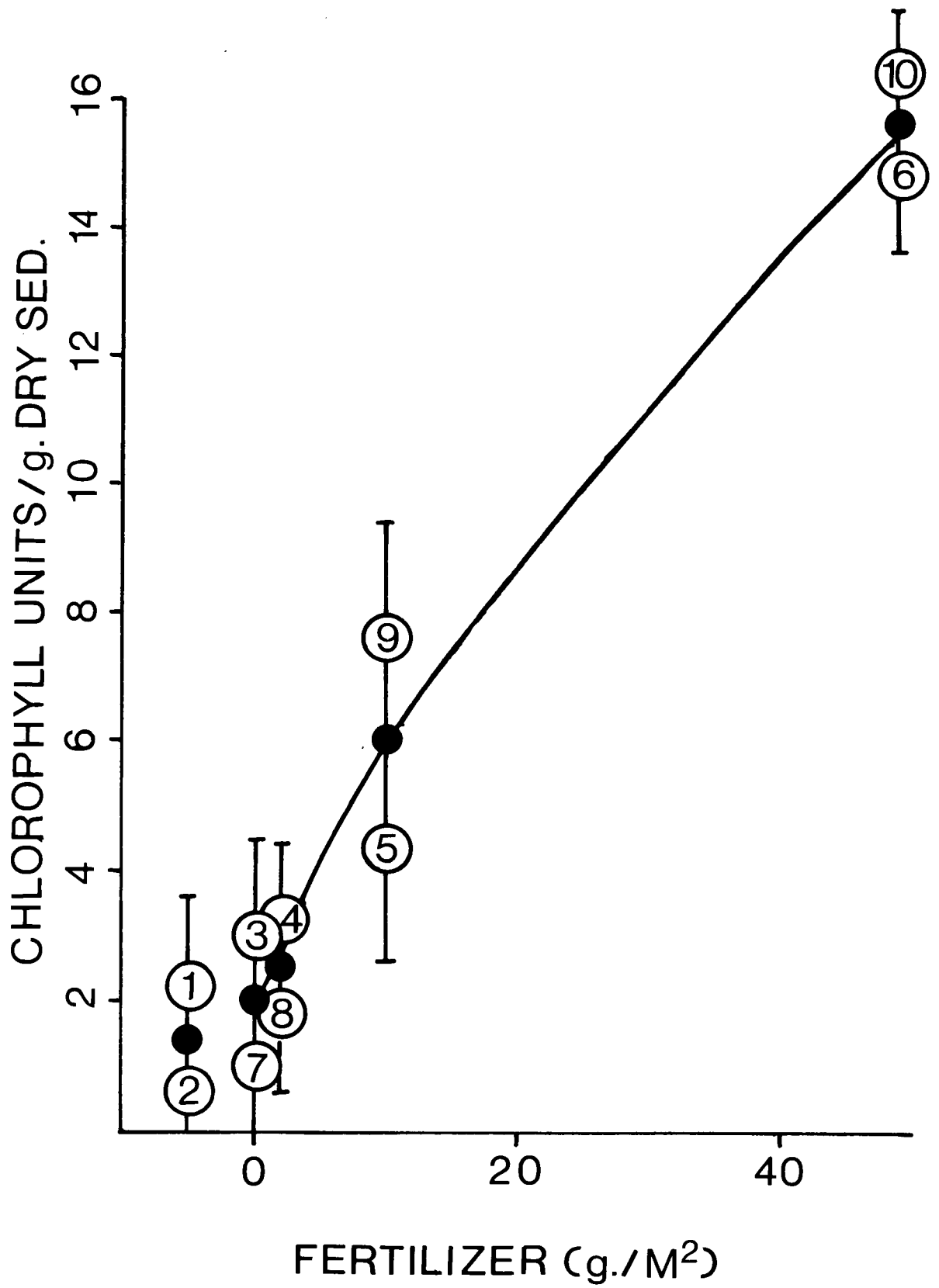


Fig. 10 Benthic primary production in the experimental enclosures (July, 1970)

- 1 and 2 - External control areas
- 7 and 3 - Enclosed controls
- 4 and 8 - Low level fertilization
- 5 and 9 - Mid level fertilization
- 6 and 10 - High level fertilization
- a and b - small enclosures (see text)

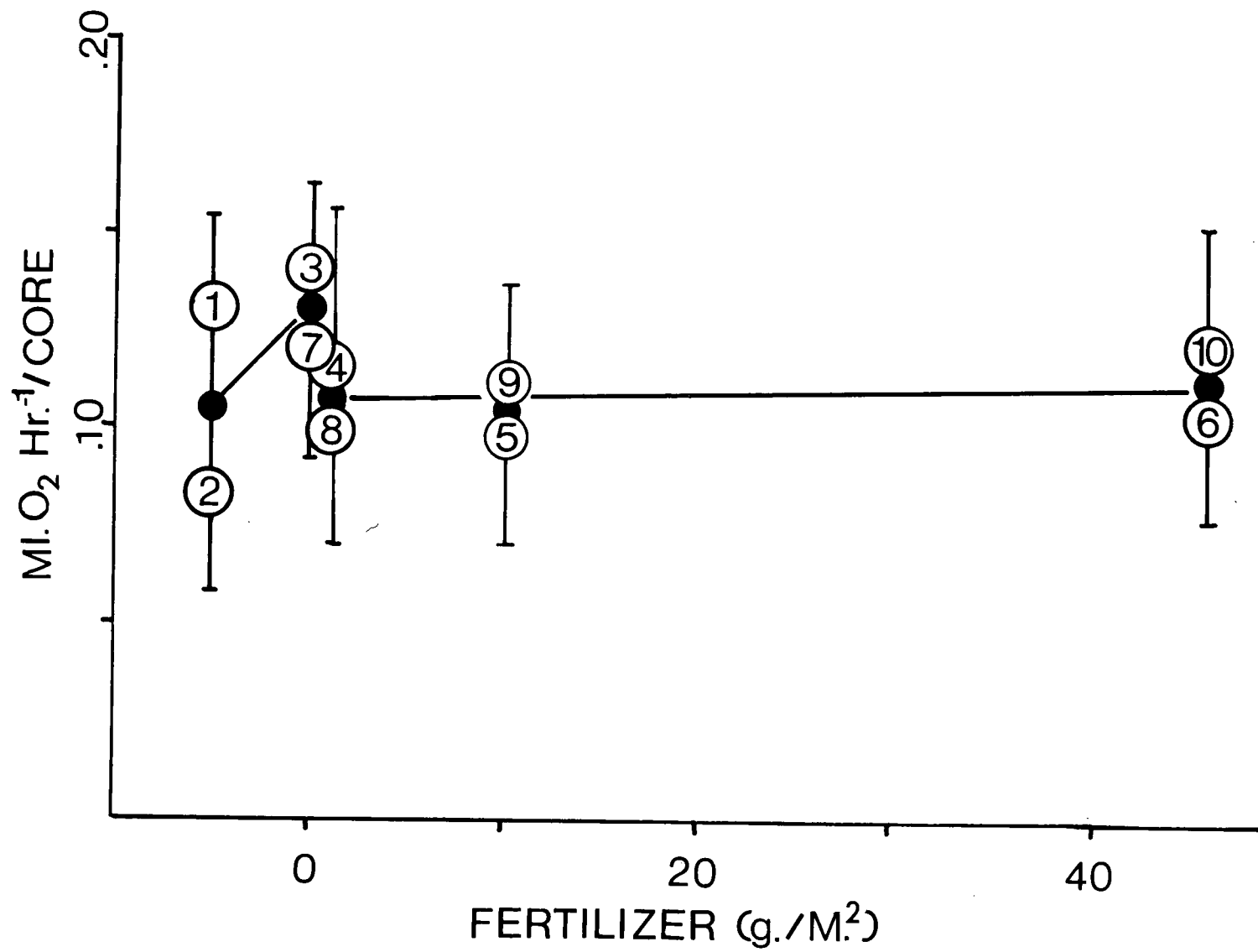
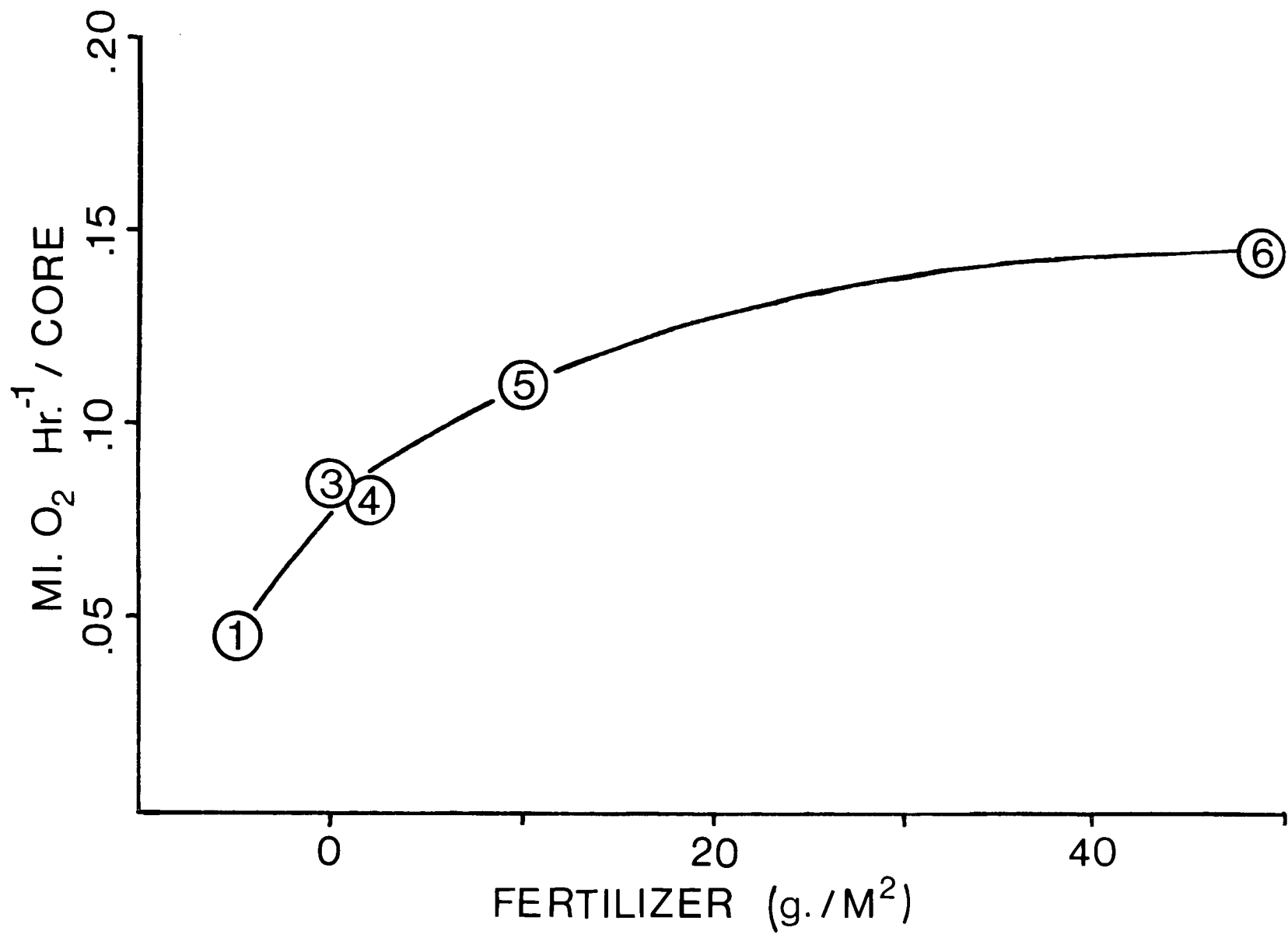


Fig. 11 Benthic primary production in the experimental enclosures (September, 1969)

- 1 - External control area
- 3 - Enclosed control area
- 4 - Low level fertilization
- 5 - Mid level fertilization
- 6 - High level fertilization



This variation in standing crop continued to exist for the duration of observation (September, 1970). It can only be assumed that each particular standing crop developed during the winter and spring (1969-70) through the stimulation of fertilizer added during the course of the pilot experiments in the fall of 1969. An indication of this likelihood may be seen in the proportionally stimulated oxygen production measured at this time.

The significance of primary production and standing crop measures may be more clearly seen by examining the relation of these two factors under various conditions.

While phytoplanktonic primary production increased as a function of increased standing crop that of the epipellic algae appeared to remain constant through a wide range of standing crop (Fig. 12). In terms of a single algal unit this means that the production of a phytoplankter remains constant while that of an epipellic alga decreases with an increase in standing crop (Fig. 12). This inverse response is not limited to nutrient induced standing crop changes. Where grazing was artificially increased by unnaturally high concentrations of grazing tadpoles the production of individual algae in the reduced algal population was increased (Fig. 12): the total production on an area basis was the same under these circumstances as that in the undisturbed sediments. In a similar experiment by Dickman (1968) heavy grazing by hatching tadpoles produced a similar result in epiphytic algae: under these intense

Fig. 12 Relation of benthic algal standing crop to production of individual algal unit.

Note: "G" refers to observations made in enclosures with high tadpole density.

1 and 2 - External control areas

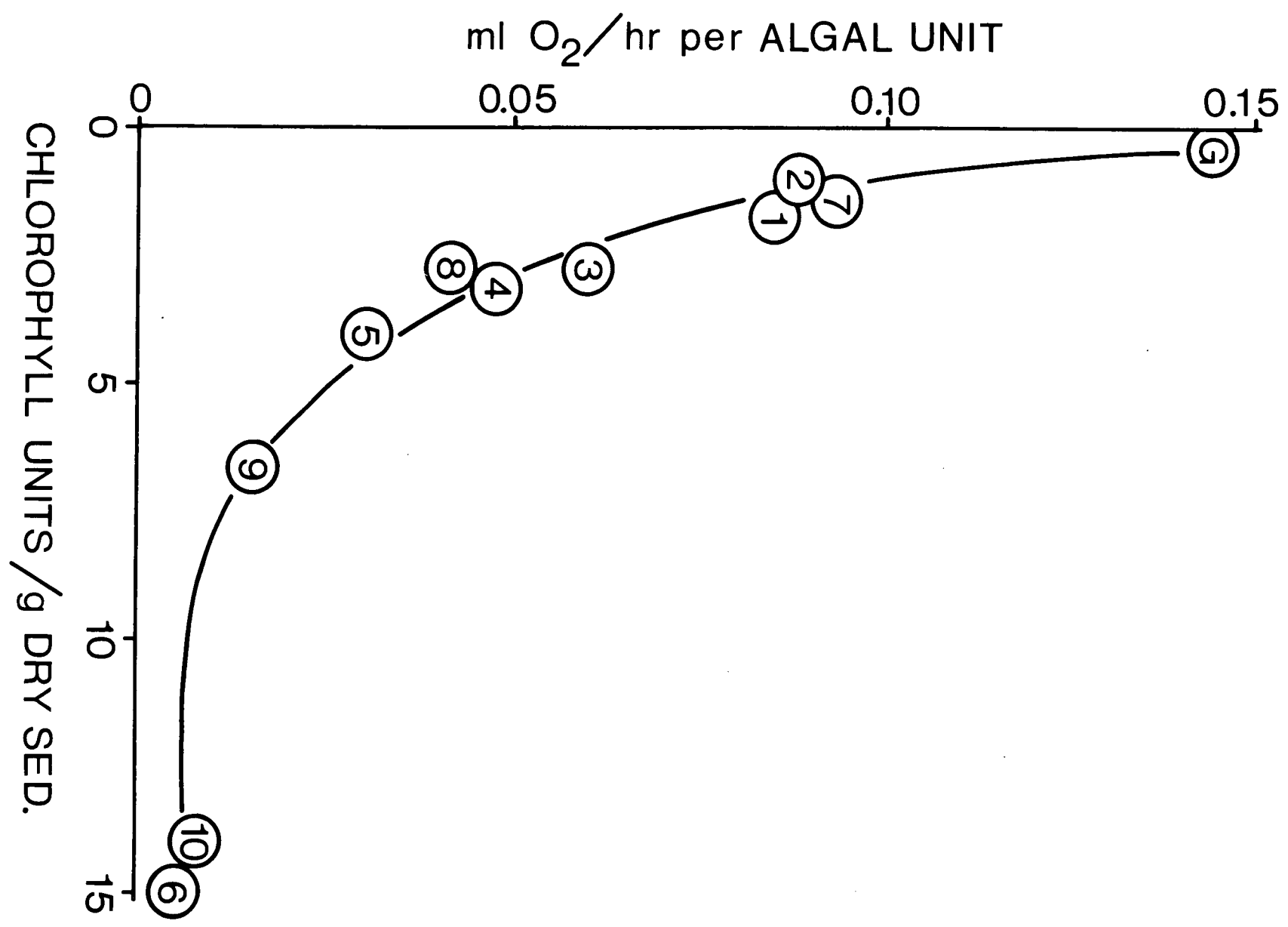
7 and 3 - Enclosed controls

4 and 8 - Low level fertilization

5 and 9 - Mid level fertilization

6 and 10 - High level fertilization

a and b - small enclosures (see text)



grazing pressures, algae species present were those with the most rapid rate of growth. Only in the Hyalella grazing experiments did total primary production as measured in a cylinder core drop below natural levels (Fig. 13). But even in this case the reduction was due not to a drop in individual O_2 production but rather to the drastically reduced algal crop.

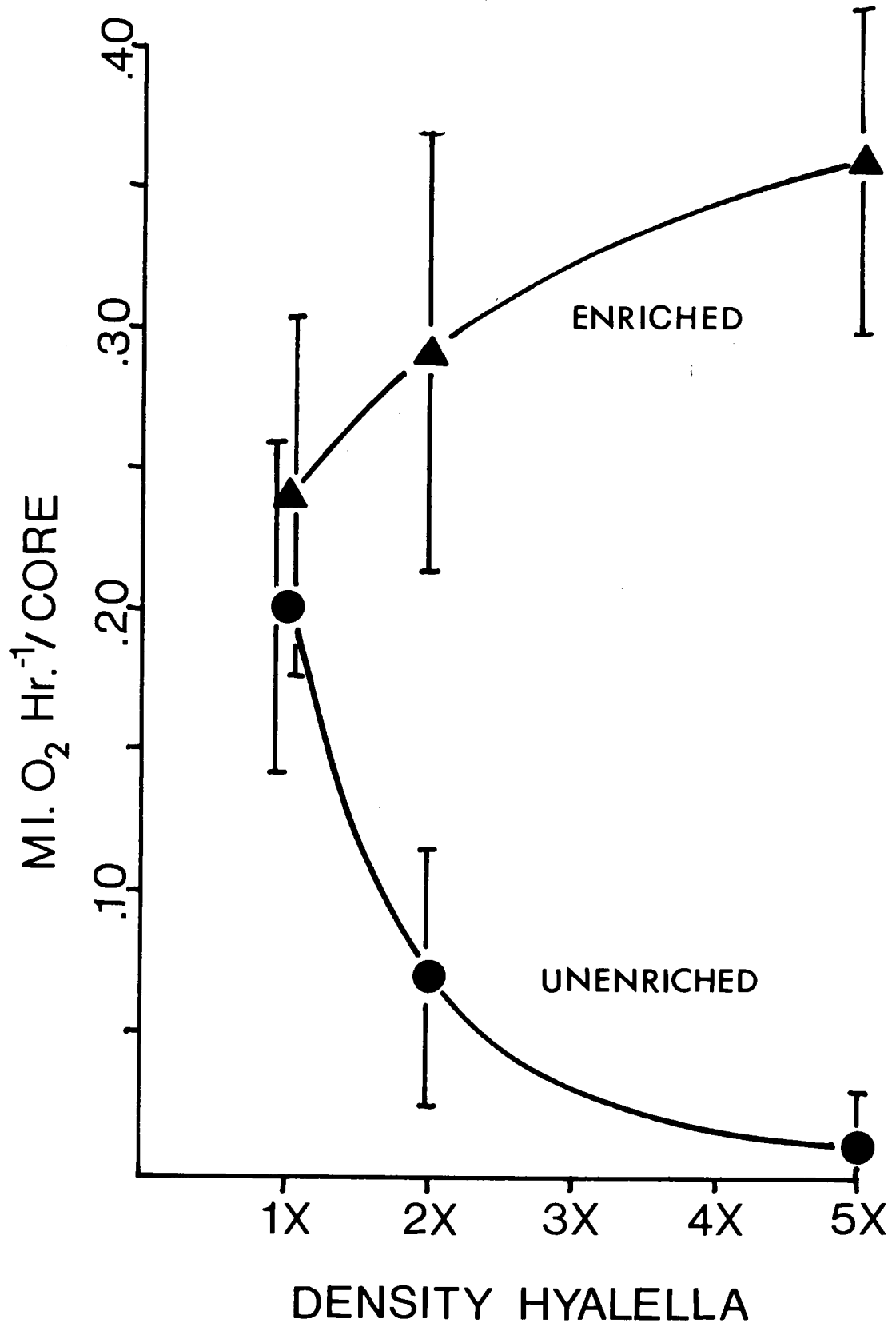
The response of enriched sediment to heavy grazing was quite different from that of the natural community. As already indicated the drastic reduction by grazers of the natural algal population resulted in a drop in primary production. In enriched sediments, however, the heavier algal standing crop responded to grazing with an increase in total production (Fig. 13). Assuming that the production capacity of any individual alga is dependent only upon the availability of growth requirements it seems quite obvious that the total production potential is much greater in a situation of high standing crop. The algae removed by grazers in the enriched sediment represent a much smaller proportion of the total community than in the natural situation. This removal apparently provides the survivors with sufficient stimulation that individual production is able to increase. In this way a larger standing crop appears to possess a higher production potential which may be expressed only when increased grazing gives the opportunity for growth.

Fig. 13 Response of benthic primary production to increased grazing by Hyaella

Closed circles represent unenriched sediments

Closed triangles represent enriched sediments

from enclosure 6 (high fertilization level)



RESPONSE BY GRAZERS

The next question is quite obviously that of whether this production potential is being utilized. Evidence from several directions would indicate that it is not.

The first indication may be seen in the consistency of primary production through the various enclosures. As was demonstrated in the Hyalella grazing experiments the immediate response of enriched sediments to increased grazing was an increase in primary production. The fact that algal production did not increase in the fertilized enclosures suggests that removal of epipellic algae was similar in all areas.

Measures of community respiration were made at the same time as primary production estimation. The respiration of the benthic community makes demands upon the dissolved oxygen in the overlying water: these demands are proportional to the metabolism of the community as a whole. As may be seen in (Fig. 14), there was very little increase in community oxygen uptake with an increase in fertilization. Small increases are likely attributable to the larger algae community in the highly enriched enclosures: Hargrave (1969) has stated that algae account for 25% of the total benthic respiration although this proportion, estimated by difference from the remainder of the community, is likely to be overestimated as a result of an underestimation of the bacterial contribution (see Appendix 1).

Hyalella azteca was examined directly in an attempt to

Fig. 14 Benthic community respiration in experimental enclosures (July, 1970)

1 and 2 - External control areas

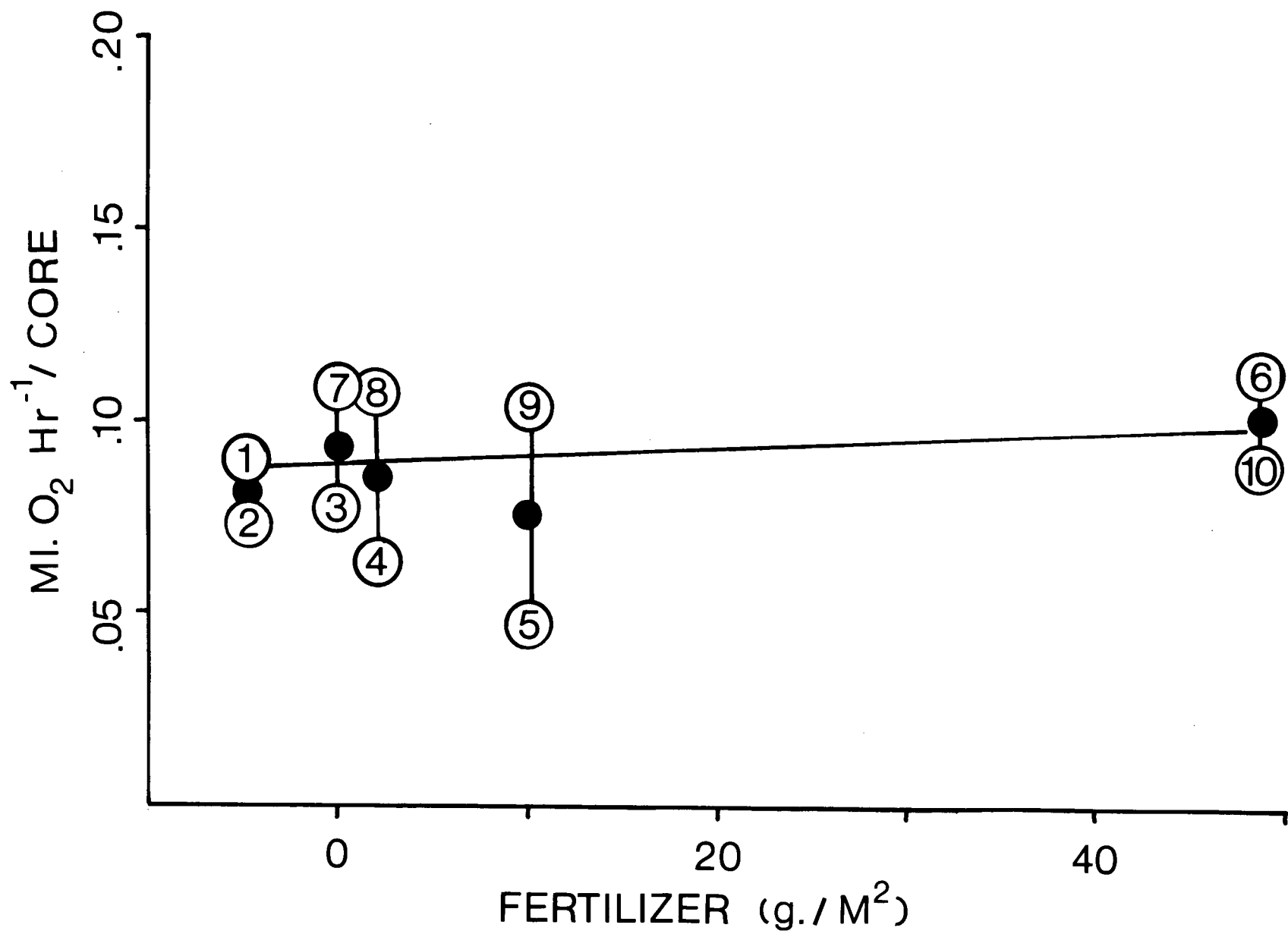
7 and 3 - Enclosed controls

4 and 8 - Low level fertilization

5 and 9 - Mid level fertilization

6 and 10 - High level fertilization

a and b - small enclosures (see text)



detect a grazer response to the increased food supply. Numbers and fresh weights of individuals removed in enclosure samples showed no statistically significant differences between the various enclosures: this evidence reinforces that of the respiration estimation in indicating that the grazing component of the benthic community has not responded to the algal increase. This is not an isolated observation. Vinberg (1965) in a survey of pond fish culture, states "In many experiments in the fertilization of ponds the stocks of benthonic food for the fish failed to show any increase" (p. 338). Batkina, (1967) states that the dynamics of chironomid larve "was the same during the season in all (fertilized and control) ponds and did not depend on fertilizer dosage." (p. 11).

The results of the bacterial plate counts are given in (Fig. 15). Again there is no significant difference apparent among the various enclosures. This indicates two things. First, it would appear that the bacteria present in the sediment are incapable of responding directly to an increase in inorganic nutrients. Finally, as a likely pathway for bacterial nutrition is via algal exudates (Kuznetzov, 1968) and the release of such materials is proportional to algal production (Fogg, 1956) this observation is somewhat of a confirmation of the similar primary production rates observed in the various enclosures.

Fig. 15 Bacterial plate count in the experimental enclosures 10^4 dilution. (August, 1970)

1 and 2 - External control areas

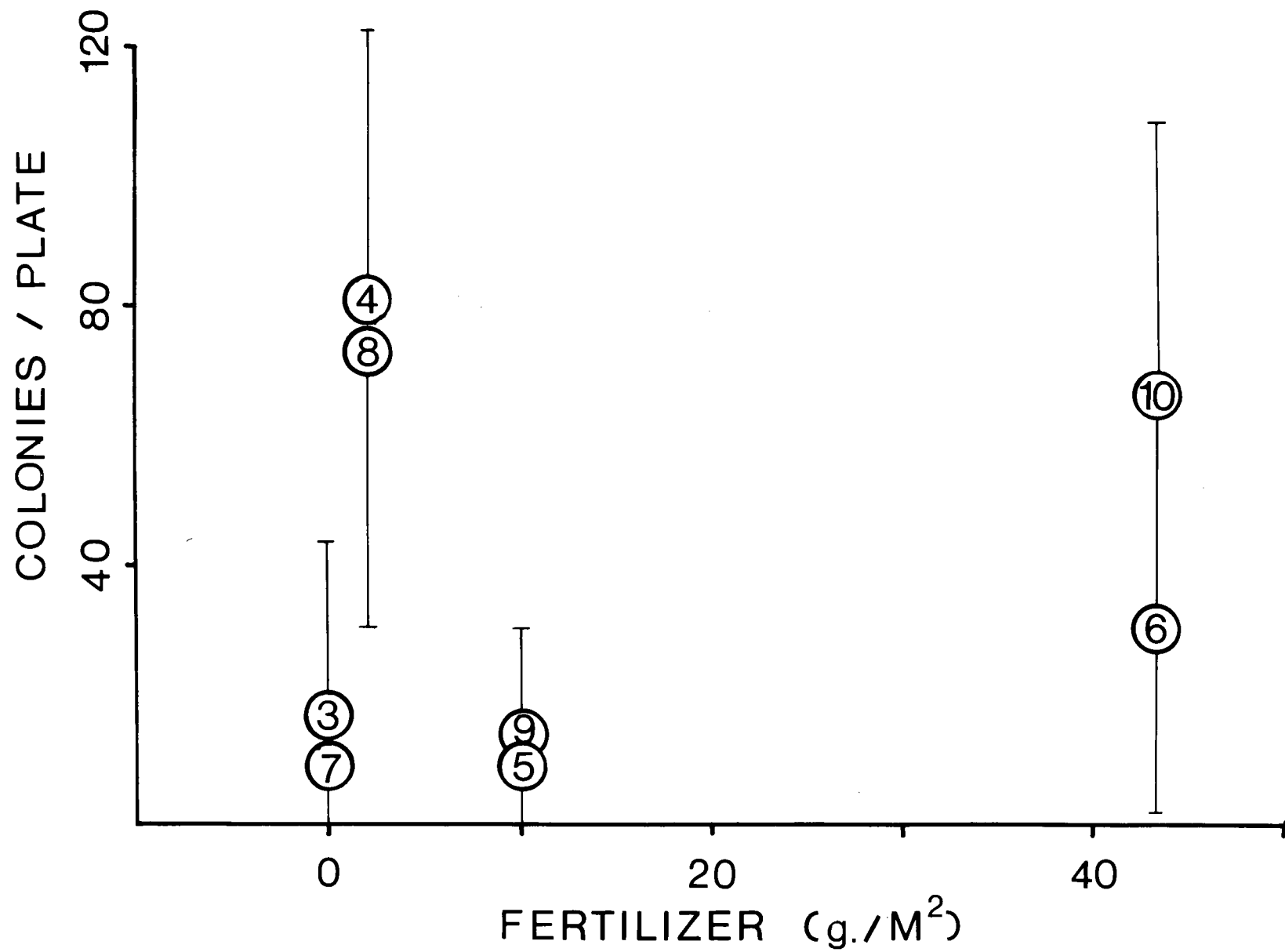
7 and 3 - Enclosed controls

4 and 8 - Low level fertilization

5 and 9 - Mid level fertilization

6 and 10 - High level fertilization

a and b - small enclosures (see text)



DISCUSSION

The response of an aquatic system to inorganic fertilization may involve either proportional stimulation of entire system or "the excessive activity of any group or groups of organisms (which) can lead to an ecological unbalance" (Fruh, 1966). A sustained imbalance may develop conditions under which the original community structure cannot exist. These conditions are typically the result of an increase in algal production which in turn leads to changes in both other community components and in water chemistry.

The maintenance of an imbalance requires that the primary production increase is a lengthy one. But nutrient induced production increases are transitory processes: they are the means to an end which is a standing crop appropriate to the new resource conditions. A higher rate of primary production can only be maintained until the standing crop has reached an equilibrium with the available resources. This is not simply a matter of nutrient supplies but of conditions which determine the disposition of algal material produced. Where the potential increase in standing crop is limited in its accumulation then high production rates may be sustained only with a proportionate increase in removal by the grazing components. The results of this study suggest that the response of benthic grazers imposes a limit on the extent of primary production increases and maintains a proportionality between the two components.

Phosphorus uptake in the various enclosures demonstrated the rapid removal observed by many investigators and reviewed by Hutchinson (1967). Evidence of a sediment-water equilibrium, however, has particular significance in Marion Lake. Olsen (1964) in describing an equilibrium process in similar oxidized sediment states: "the bottom deposits must be regarded as phosphate reservoirs from or to which phosphate will be transferred whenever the equilibrium is disturbed". This certainly appeared to be the case where either fertilizer addition or flushing disturbed the phosphorus balance of the enclosures. But within the lake itself a different problem arises: as the specific conductivity of Marion Lake water is in the range of 6 to 10 micro ohms (Dickman, 1968) the potential for leaching of sediment nutrients particularly at times of rapid flow becomes apparent. Although difficulties of measurement of low phosphorus concentrations prevented further release experiments at more realistic nutrient levels the possibility exists that not only phosphorus but other inorganic and organic nutrients may be lost to the benthos either by equilibration or actual mechanical flushing. Such a phenomenon may account for the increase in epipellic standing algal crop in control enclosures. Dickman (1968) noted an increase in phytoplankton under similar conditions and attributed that observation to a relaxation of cropping by current flow.

An increasingly large proportion of sedimentary phosphorus was present in inorganic form at higher fertilization levels.

As algal cells can remove phosphorus from the water to some 6X that required for maximum growth (Azad and Borchardt, 1970) the presence of large quantities of ortho phosphate in these enclosures suggests that addition has exceeded algal growth requirements (p. 22). The observed stabilization of production in benthic algae cannot, as a result, be explained on the basis of nutrient supply. It would appear that the expanding algal standing crop has equilibrated with the limitations of accumulation as discussed previously. Further evidence for this conclusion is the response of enriched sediment to increased grazing: the dramatic increase in primary production in these experiments was brought about simply by removal of a portion of the algal standing crop.

Limitations on the range of primary production are very different in the benthic and planktonic communities subjected to the same stimulation. There are many instances of vast phytoplankton increases resulting from nutrient stimulation (Vinberg, 1965) and in Marion Lake a ten-fold increase in primary production followed the addition of fertilizers to an enclosed area (Dickman, 1968). The sudden availability of nutrients following spring and fall turnovers is believed to be a primary factor in the characteristic blooms of many dimictic lakes (Hutchinson, 1967). The benthic community, however, is more limited in the range of its response. Margalef (1963) relates this difference to a difference in maturity. He states "Planktonic communities retain always a less mature character

than benthic communities and it is to be expected, in good agreement with observation, that fluctuations in planktonic populations are of shorter period and wider ranges" and further more that "fluctuations in less mature systems are more related to environmental changes".

Limitations on the growth of an algal population are ultimately limitations on the standing crop. A change in an input such as inorganic nutrients changes the potential standing crop as determined by available resources and until this potential is realized production will increase. Only when cropping pressures are such that the potential standing crop cannot be reached does such a stimulation operate on the rate of production in a sustained manner. This is a situation equivalent to Slobodkin's (1960) grazer limited population where a small number of organisms with an abundance of resources have a high individual production. An example of such a situation was seen where artificially increased grazer density resulted in a lowered standing algal crop. Individual production here was the highest recorded in unenriched conditions.

Where grazers do not limit algal production the standing crop of algae will increase until it is in proportion to its resources. At this time further production can only be a function of the replacement of materials removed regardless of the level of standing crop. This situation has been described by Slobodkin as a resource limited one where there is a high standing crop with low individual production. A situation similar to this developed in the sediment of the enriched

enclosures. When standing crop of algae increased, total production remained constant; a function of maintenance rather than expansion. If this is indeed the case it is obvious by the excess of nutrients present that some other factor has replaced these as the limiting resource. Margalef (1963), on the other hand, describes such a situation as being an example of the more efficient energy conversion by a more mature, complex system where "a reduced waste of energy allows maintenance of.....a higher biomass with the same supply of energy". This may thus be a further indication of the relative maturity of the benthic community.

In the plankton, however, production increases as a function of enrichment and Dickman noted that "changes in standing crop and primary productivity were closely correlated ($r = 0.89$) ... an increase in standing crop results in an increase in primary productivity". If similar treatment can produce such different results then factors which limit the extent of algal increase must differ in the two communities.

If sufficient resources are available the question of whether or not a high level of algal production can persist depends upon the disposition of the material produced. An accumulation is a simple solution. Where the potential standing crop as determined by nutrient resources is much greater than that of the original situation the production increase may be a lengthy one and the increased algal crop may simply accumulate to high levels. This occurs in a phytoplankton bloom.

If the degree of accumulation is limited then consumption must increase along with production to sustain a high production rate. Both of these factors operate in the water column. The difference between actual and potential phytoplankton crop is often several hundred times (Hutchinson, 1967). At the same time energy is required to maintain the plankton in the water column and there is a net movement into the benthos of potential energy in the form of settling plankters, (Margalef, 1963). This energy requirement increases proportionately with the standing crop and consumes a portion of the increased production.

The situation on the sediment surface is quite different. Accumulation is limited by the available space and this alone may halt the expanding algae. Indeed, the introduction of a greater surface area in the form of enclosure walls increased the standing crop of the area immediately as this new space was colonized. Furthermore energy for movement is not required by sessile algae: only energy for the retention of high phosphorus concentrations could increase as a function of enrichment but it is generally held (Kamen, 1963) that this retention is through conversion to organic or polyphosphates rather than by an active process. The only possible increase in consumption of algal material produced in the sediment must, therefore, be extrinsic to the algae themselves; that is a response of the other components of the epibenthic community.

It would appear that the conversion of inorganic nutrients in the enclosed systems has been an appropriate one in terms of

the requirements of the existing community. Partly through design and partly through chance factors which may divert such inputs from the existing trophic chain were avoided. One such factor is maintenance of oxygen levels. The low oxygen levels of stagnation are a familiar step in diverting nutrient inputs through anaerobic or near anaerobic invertebrates and bacteria. Batkina (1967), on the other hand, has suggested that an oversaturation of oxygen was responsible for the inhibition of invertebrate grazers in a similar enrichment study. Both such eventualities were avoided by the circulation of water through the enclosures. The excessive development of undesirable blue-green algae may also divert primarily fixed energy from usable channels: at no time did such a development occur in the enclosures probably as a result of the high N:P ratio of the fertilizer.

Yet all the evidence accumulated in this study indicates that the grazing component of the benthic community has not responded to the increase in algal supply with a proportionate increase in removal. This may be more a result of the duration of the experiment than of actual response of the community. A population increase would likely be by small increments resulting from increased fecundity and survival: as this study spanned only a single reproductive season for most of the benthic invertebrate grazers it is most unrealistic to speculate on the long term response to increased nutrients. In any case the assumption of an increase in grazing pre-supposes that the grazers involved

are food limited: discussions with individuals examining the grazers in Marion Lake have indicated that this is not likely the case.

But even if a grazer response is taking place the fact that it is not an immediate one is significant. In short term disturbances a time lag in structural change is as important as no response at all. This is because it gives sufficient time for the disturbance to be corrected before permanent community adjustments take place.

Admittedly some of the conditions which avoided community changes were artificially controlled as previously indicated. But the significant point is that a sudden disproportionate input has been dealt with in a manner which has made it available to the existing community. Furthermore, a mechanism is apparently operating in the benthic community to maintain the proportions of the various existing components: the extent of algal production is limited by the fact that it is not being utilized by the rest of the community.

Resistance to structural alteration in the face of changing nutrient conditions is evidence of stability as previously defined. In this respect the benthic community may be considered a more stable structure than that of the plankton. But the nature of the relation between these two communities is such that benthic stability is conferred upon the system as a whole. This results from the fact that "a net transfer of energy exists from plankton to benthos" and "strong fluctuations in plankton populations

represent a heavy export towards.....the benthos" (Margalef, 1963). It is likely that at least a portion of the benthic chlorophyll measured in the experimental enclosures resulted from sedimentation of enhanced plankton populations. In this way the wider fluctuations of the plankton populations are absorbed by the benthic community. This transfer provides energy to further increase the maturity and stability of the benthic component at the expense of the plankton: "what the one does in excess production", states Margalef, "is put in use by the other".

In Marion Lake the planktonic community is held in a state of immaturity through intensive cropping by current flow, (Dickman, 1969). As such it may be expected to show a more dramatic response to changing nutrient conditions than the more mature benthic community. The results of this study confirm these predictions and further indicate that the benthic component can dampen phytoplankton fluctuations through sedimentation of nutrients and particulate matter. This energy transfer appears to enhance even further the stability of the benthic community and, thus, of the entire system.

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SOME PROBLEMS IN THE USE OF ANTIBIOTICS
TO MEASURE RESPIRATION IN LAKE SEDIMENTS

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Contribution No:

ABSTRACT

The antibiotics, neomycin and streptomycin, failed to block bacterial respiration and growth in vitro and in situ experiments on lake mud. It is suggested that antibiotics cannot be used to partition bacteria from total community respiration.

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INTRODUCTION

The use of antibiotics to selectively eliminate bacteria from algal cultures is a well established practice (Hunter and McVeigh, 1961). More recently, it has become a feature of certain methods of partitioning community respiration into component parts (Hargrave, 1969; Smith et al., 1972). In these methods bacterial respiration is defined by the difference in oxygen consumption of natural sediments and those to which antibiotics have been added. It must be assumed that this difference is due singularly to the elimination of the bacterial component of total community respiration. An attempt to use this method in the course of benthic community studies suggested that the response of the community is not as simple as this assumption indicates. The results of these and further laboratory experiments are presented to support the contention that the antibiotic effect is a complex one, which cannot be directly related to bacterial metabolism.

METHODS

Initial observations were made with the same experimental procedure and mud from the same lake used by Hargrave (1969). Samples of an intact sediment interface with approximately 100 ml of overlying water were removed from the lake bottom at a depth of 1 m in 12.5 cm x 5.0 cm glass cylinders. A combination of neomycin (SO_4) and streptomycin (SO_4) was added to half of the cores to produce a total concentration of 50 mg/l. After measuring oxygen concentrations in the supernatant water the cores were sealed and incubated at constant temperature in darkness. In three hours the water was gently stirred and oxygen again measured in each cylinder. A comparison was then made of the oxygen consumption of treated and untreated sediments: this entire process was carried out at the lake site and was initiated within 20 min of taking the samples.

As a result of inconsistent and contradictory observations the method was modified for further experiments in June, 1970. To give additional time for antibiotic action injected samples were placed in the dark for 2 hr prior to the experiment. The total time of exposure to antibiotics was thus increased to 5 hr. Both respiration and primary production measurements were made successively in the same core. The effect of antibiotics on algal production could then be evaluated by comparing treated and natural cores incubated in the light.

A direct measurement was made of the respiration of bacteria isolated from lake sediment and cultured both singularly and as a combination. Suspensions of bacteria were injected with various concentrations of antibiotics to a maximum of 150 mg/l. The effect of antibiotics on the respiration of ten individual species was measured in this way as was the effect upon a combined culture of the dominant bacteria types found in Marion Lake.

Finally the observed changes in community respiration were compared with the growth of bacteria populations. This was done on the assumption that actively growing bacteria would be utilizing greater amounts of oxygen than static bacterial populations and such a measure would thus provide a means of checking the observations of respiration. Samples removed from both treated and untreated sediments were plated and counted to determine whether the growth of bacteria populations had been inhibited. Changes in species composition were not examined as the object of the experiment was to examine the net effect of antibiotic treatment.

RESULTS

Initial observations with intact sediment demonstrated no consistent reduction in community respiration as a response to antibiotic treatment. Cores which had been treated with antibiotics showed either similar or higher respiration than

equivalent untreated cores (Fig. 1). This latter result was quite opposite to what would have been expected from a selective elimination of the bacterial component.

Further observations in June, 1970 produced similar results despite a 2 hr increase in the time of exposure to antibiotics (Table 1). As both respiration and primary production were measured in the same sample core a further effect of antibiotic treatment became evident: cores to which antibiotics had been added for respiration estimates showed a drastic reduction of net oxygen production in subsequent primary production measurement (Table 1). An increase in the concentration of streptomycin and neomycin to 200 mg/l produced no significant change in either of these observations. This was true also for changes in the mode of antibiotic addition, the degree of sample agitation and other modifications of the method.

Antibiotic treatment of bacteria cultures produced a wide variety of effects on respiration. While the respiration of combined culture of several common bacteria was reduced only 20 % by antibiotics, individual cultures varied in response from an 80 % to a 30 % reduction in their oxygen uptake. A most important feature of these results is that the reduction in respiration was a gradual one whatever its final extent. This means that regardless of the degree to which the bacteria are eventually affected the assumption

Fig. 1 Oxygen consumption of sediment core samples in the fall of 1969

curve a : cores treated with antibiotics at 50 mg/l

curve b : control cores

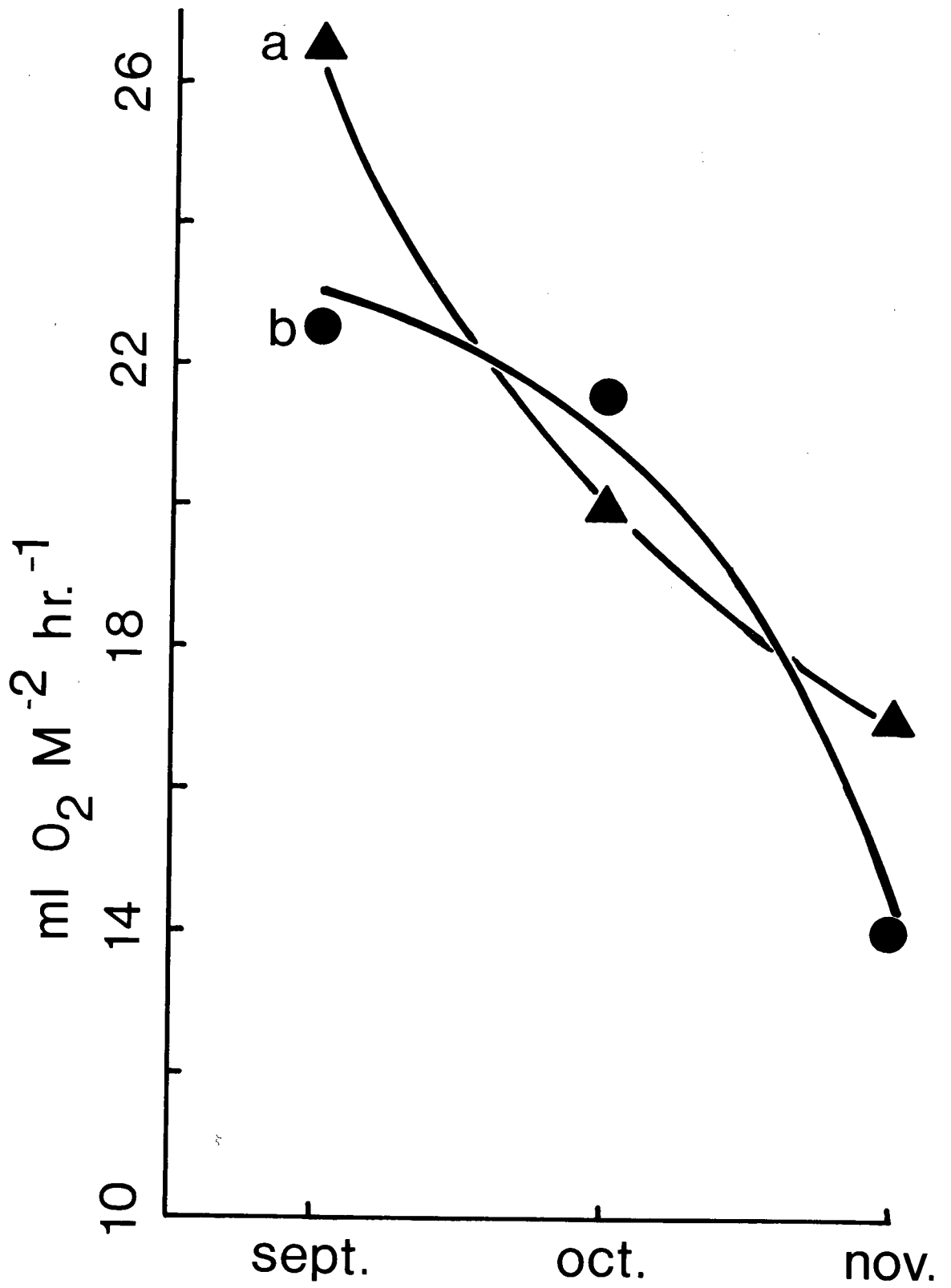


Table 1 : Oxygen consumption and algal oxygen production in sediment core samples (June 1970)

Community respiration (m.l. O_2 hr. $^{-1}$ / core)

Control (untreated sediment) Antibiotic (50 mg. / l.)

area 1	.067	.098
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area 2	.087	.101
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Primary production (ml. O_2 hr. $^{-1}$ / core)

Control (untreated sediment) Antibiotic (50 mg. / l.)

area 1	.126	.002
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area 2	.162	.001
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of an immediate effect is not a valid one.

Results of bacteria plate counts showed a similarly variable effect of antibiotic addition. A comparison of untreated bacteria with bacteria exposed for 5 hr to 50 mg/l of the combined antibiotics demonstrated a variety of response in both (Table 2). In one instance an actual increase resulted from a 5 hr exposure. Of twelve individual species tested (Table 3) nine grew well on a medium including streptomycin at 50 mg/l and three grew well on a medium containing 50 mg/l each of streptomycin and neomycin.

DISCUSSION

Two assumptions must be made in relating the observed effect of antibiotic addition to the respiration of bacteria. The first of these is that bacterial respiration will be totally or at least largely eliminated by the action of the antibiotics. The second assumption is that the response of a community to antibiotics is a singular one due simply to the selective elimination of the bacteria component. Evidence presented here suggest that neither of these assumptions is valid.

The variability of antibiotic inhibition indicates that there is no response common to all bacteria species present in the sediment. This has two potential effects on the interpretation of results at the community level. The first is that if laboratory studies are used to calibrate the impact

Table 2: The effect of antibiotics on the growth of bacteria in sediment samples taken from Marion Lake

Set	Treatment	Plate counts made at times:		
		0 (start)	2 $\frac{1}{2}$ hrs.	5 hrs.
1	W. A.	7	5	28
	W/o. A.	10	11	10
2	W. A.	26	5	4
	W/o. A.	4	4	3
3	W. A.	8	6	4
	W/o. A.	11	3	4
4.	W. A.	24	8	10
	W/o A.	22	7	8

N.B. W. A. signifies treatment with 50 mg/l streptomycin and neomycin before incubation; W/o A. signifies no antibiotic treatment.

Data courtesy of P. Fraker

Table 3: The effect of antibiotics on the growth of individually cultured bacterium species (96 hr. exposure)

Species Code	Control	Streptomycin: 50 mg/l	150 mg/l	Streptomycin & Neomycin 50 mg/l
3	h	h	h	-
7	h	h	h	-
8	m	m	m	-
21	h	h	h	l
42	h	m	h	h
43	h	h	h	-
46	h	m	m	-
21	h	h	h	h

- : no growth

l : light growth

m : medium growth

h : heavy growth

Data courtesy of R. Tyhurst

of antibiotics on bacterial populations as a whole the result will be distorted according to which species are measured individually. If, for example, a more susceptible population is tested the observed results will not be applicable to the bacteria component as a whole. Furthermore, the combined response of even a representative portion of the dominant species is not likely to be simply the sum of the individual responses. If some affected species are releasing their cell contents into a medium where other unaffected bacteria are still growing it is most likely that the remaining bacteria will be stimulated by the sudden availability of nutrients. The resulting total respiration would in such a case be a complex of declining and expanding populations and bear no direct relation to natural bacterial metabolism. The plate counts show a similar situation. While some populations declined with exposure to the antibiotics, others were either unaffected or, in at least one instance, showed an actual increase. It may be argued that experiments conducted under laboratory conditions with growing bacterial cultures may show responses not typical of the situation in the lake itself. However, observations made in this way were quite consistent with those made with intact sediment and do serve to indicate at least the potential response of the individual components.

A further complication exists in the time required for the antibiotics to take effect as the action of even those

antibiotics which did eventually result in a respiration reduction was a gradual one. The assumption of a constant effect throughout the experiment demands, however, an immediate action.

There is also little doubt that the antibiotics used affect more than simply the bacterial component of the benthic community. The disruption of algal activity observed in this study is well substantiated by observations of other workers; Foter et al (1953) in a study of the effects on algae of antibiotics at concentrations (20 mg/l) less than those used in these experiments stated "of the five antibiotics tested against six representative algal cultures, streptomycin and neomycin were the most effective in preventing growth", and Vance (1966) reached the same conclusion in particular reference to blue-green algae. It is thus likely that at least a portion of the observed antibiotic effect is one on the algal rather than bacterial component. This further confuses the already complex response of the bacteria themselves.

It is not my intention to draw conclusions as to the exact effect of antibiotics on the various components of the benthic community. The evidence presented here demonstrates simply that the net effect of antibiotics is the result of a complex of responses not only in the bacterial population but in other parts of the community as well. As such it is most unrealistic to measure bacterial respiration by examining the response of a mud community to antibiotics.

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