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A. Jassby, M. Dudzik, J. Rees,
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Lawrence Berkeley Laboratory University of California/Berkeley

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Production Cycles in Aquatic Microcosms[1]

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ABSTRACT

Four 700-liter cylindrical containers were filled with demineralized water, enriched with nutrients, and inoculated with 3.5-liter lakewater samples. The microcosms were maintained at a temperature of 18°C under a 12:12 L:D cycle for 6 months and several manipulations of their trophic structure were carried out, including addition of snails (*Physa* sp.), mosquitofish (*Gambusia affinis*), and catfish (*Placostomas placostomas*). Temporal variation of the phytoplankton resembled the bimodal patterns of certain natural systems. Further analysis demonstrated a close analogy with the predator-prey oscillations of temperate marine waters: an initial bloom is terminated by zooplankton grazing; the resulting low phytoplankton levels lead to gradual starvation of the zooplankton; and a second bloom follows the final dieoff of zooplankton. Both decreasing the concentration of initial nutrients or stocking the microcosms with *Gambusia* decreases the time between the "spring" and "fall" blooms. The problem of heavy periphyton growth in microcosms was not solved with the introduction of either *Physa* or *Placostomas*. Possible solutions to this and to other problems peculiar to microcosm research are discussed, and modifications are suggested for increasing the ability of microcosms to simulate natural systems.

INTRODUCTION

The word "microcosm", when used in an ecological context, refers to a collection of chemicals and organisms within well-defined spatial boundaries, generally under controlled physical conditions, and in a volume convenient for laboratory study, i.e., much smaller than ecosystems of interest in nature. Unispecific cultures usually are excluded from this definition. A number of researchers view aquatic microcosms as appropriate experimental objects for the investigation of systemic properties of naturally occuring ecosystems and the delineation of various details concerning trophic interactions, nutrient cycles, and certain other topics (see reviews by Cooke, 1971, and Taub, 1974). In particular, laboratory microcosms have the following desirable properties: (i) the small size permits replication; (ii) the chemical composition of the medium and the trophic structure can be manipulated, so that analogs of qualitatively different ecosystems can be created; (iii) the lack of complicated spatial heterogeneity allows more complete definition of physical, chemical, and biological characteristics; (iv) perturbations of different physical, chemical, or biological variables can be carried out with little effort and expense; and (v) causal relationships often are more easy to deduce than in natural systems, where uncontrolled environmental variability complicates interpretation.

These advantages are not necessarily compelling. A number of drawbacks inherent in the use of aquatic microcosms, such as the high surface-to-volume ratio of the containing structure, can be pinpointed on an

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a priori basis. Although several analogies in biological patterns between microcosms and natural systems do exist (e.g., diurnal gas exchange, Beyers, 1963; long-term community production, Cooke, 1967; nutrient cycling, Whittaker, 1961), it has not yet been demonstrated that microcosms can be made to exhibit most of the features essential to seasonally-varying natural systems of interest, such as the typical succession of phytoplankton and zooplankton, the occurrence of both spring and fall blooms of primary producers, etc. Part of the reason for this lack of information has been an undue concern with creating systems that exhibit steady-state characteristics, a situation almost never observable in nature.

Considering the potential importance of microcosms in determining the macroscopic properties of natural ecosystems, as well as the effect of toxic contaminants on these properties, a need obviously exists for more detailed investigation of the nature of small, synthetic aquatic ecosystems. In this paper, results are presented from a study of 700-liter freshwater microcosms in an attempt to clarify some of the analogies between laboratory microcosms and natural water bodies, as well as to further define the major problems associated with the use of microcosms in environmental research. The microcosms differed in initial chemical composition and in certain features of trophic structure, and a variety of chemical and biological data were collected for periods of up to 6 months. Particular attention is paid to those factors motivating the detailed design of the microcosms.

MICROCOSM DESIGN

Size. The size of freshwater microcosms employed in previous investigations varies from less than 0.01 (Salt, 1971) to 200 liters (Whittaker, 1961), with the large majority less than 20 liters. Size is critical in at least 3 respects: (i) smaller systems have larger container surface-to-volume ratios, rendering surface effects more important than in natural systems; (ii) smaller systems are disturbed to a greater extent when samples are removed for analysis; and (iii) smaller systems support a smaller number of trophic levels. Accordingly, we found it desirable to maximize the size of our microcosms within the boundaries of the available temperature-controlled space. The 4 microcosms are cylinders, 60.9 cm in radius and 75.8 cm in height, constructed of fiberglass with a non-toxic seal. When filled to a depth of 60.1 cm, the water volume is 700 liters in each tank.

Physical conditions. The experiments reported here were oriented toward an examination of microcosm behavior in the absence of seasonal changes in temperature, light, and turbulence, i.e., toward patterns generated solely as a result of the internal interactions between the various components of the microcosms. The systems were maintained in a temperature-controlled room at $19 \pm 1\ ^{o}C$. Each tank was illuminated by a bank of 8 4-ft high-output fluorescent lights on a 12:12 light:dark cycle. The water was agitated by air from a filtered (Dayton Electric Speedaire 2Z435) laboratory supply, passing through a capillary tube 30 cm below the water surface, at a rate of 1.2 liter min^{-1}.

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No attempt was made to create a nonuniform vertical temperature structure within the microcosms. Creation of a thermocline would not be a difficult matter. However, we felt that the presence of the well-illuminated hypolimnion of small volume that would result is so unrepresentative of most naturally-occuring systems that the additional effort was not merited. This scaling problem undoubtedly is one of the outstanding drawbacks of aquatic microcosms. To a certain extent, the function of the hypolimnion as a source of inorganic nutrients resulting from decomposition processes, partially cut off from interactions with the epilimnion, can be replaced by a porous benthic substrate that collects sedimenting organic matter.

There were no inflows or outflows of water during this experiment. Although the trophic state of an aquatic system is dependent markedly on nutrient loading rates and hydraulic retention times (Volenweider, 1975), the biological activity during summer stratification of most temperate lakes appears to be determined by the concentration of dissolved nutrients already present at the onset of spring overturn (Dillon and Rigler, 1974). Because our first concern is with the period of high productivity including and subsequent to the spring bloom, justification therefore exists for setting flowthrough rates to zero. Water loss by evaporation (about 1 cm each week) was compensated for by weekly addition of demineralized water.

Benthic substrate. The choice of substrate presented a difficult optimization problem. If the sediments were too fine in texture, the total particle surface area would support adsorption rates and bacterial

activities that might be unnaturally high relative to the volume of overlying water. On the other hand, sediments of too coarse a texture may preclude establishment of typical benthic macrofauna. Because of the necessarily qualitative nature of these considerations, a compromise between the 2 extremes had to be chosen without an explicit factual basis. The benthic substrate consisted of a layer of river sand (silicates) 4 cm in thickness, and with a particle size ranging from 0.3 to 3 mm. The mean particle size was approximately 1.5 mm.

Sediments from natural ecosystems contain levels of organic matter and inorganic nutrients partially determined by the productivity and depth of the overlying water. When this material is removed into a laboratory microcosm with a smaller depth of overlying water, the danger exists that the sediment will exert a long-term effect on this water surpassing its original effect on the parent system. That is, the material flowing from sediments to water will be diluted far less than in the parent system, and the resulting changes in water quality may proceed over long time periods and to an extent not found in natural systems of interest. Accordingly, we decided to acid-wash the sand thoroughly in concentrated HCl before use, permitting the biological and chemical characteristics of the microcosm water to determine the ultimate organic and inorganic chemical content of the sediments.

Microcosm initiation. Two extremes can be recognized in the initiation of aquatic microcosms in the laboratory. The first appraoch is exemplified by the systems of Taub (1971), in which defined inorganic chemical

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media under controlled physical conditions were inoculated with organisms from pure culture (an alga, a protozoan, and 2 bacteria). Because the community composition is known completely, these microcosms are referred to as gnotobiotic systems. The second extreme is represented by the work of Beyers (e.g., 1965), who removed portions of various naturally-occurring aquatic ecosystems into the laboratory, where they were maintained under controlled conditions of light and temperature. Each approach was chosen to facilitate the attainment of different experimental goals. The gnotobiotic approach was selected for analysis of physical and chemical affects on steady-state community structure, and the confined natural ecosystems for examination of diurnal community gas exchange.

Although both designs were suited eminently for the problems undertaken by the respective investigators, both have certain deficiencies as general tools for the study of macroscopic properties of aquatic ecosystesm. The gnotobiotic systems differ from natural ones in that the initial community lacks diversity and is synthesized by the investigator, not by the natural selection of a diverse community from an even more diverse initial assemblage. Those properties that depend on the existence of a large number of species with subtle properties suitable for their coexistence thus will be lacking.

The laboratory confinement of portions of naturally-occurring systems entails a different set of difficulties. The chemical and biological structure of natural systems depends, in many ways, upon their geometry. When samples of these ecosystems suddenly are confined, the chemical and biological parameters inevitably change in a way not totally representative of the parent system. Although the qualitative behavior of the

sample may resemble that of the parent for some time after confinement, providing valuable information on specific phenomena such as chemical transformations (e.g., Mortimer, 1941-42), the long-term behavior cannot be viewed with any confidence as analogous to the parent unit or, perhaps, to any natural system. As an example, we refer to the discussion in the previous section on the possible consequences of decreasing the depth of water overlying sediments collected from some natural aquatic system.

In parallel with certain other investigations (e.g., Maguire, 1971; Neill, 1975), the method of initiation that we chose represents a compromise between the above two extremes. The 4 tanks (designated I, II, III, and IV) each were filled with demineralized water to a final water volume of 700 liters. The water then was enriched from stock solutions of a modification of a common freshwater algae medium (Woods Hole MBL; Nichols, 1973). Enrichment was identical for each tank (Table 1), except for concentrations of inorganic phosphorous and nitrogen. Systems II, III, and IV were enriched with concentrations of 3.0×10^2, 77, and 19 μmol liter^{-1} $NaNO_3$, respectively. System I was enriched identically to III, and all systems had molar N:P = 16 in the enrichment.

Vitamins were not added, as we preferred that the organisms in each system establish levels of vitamin activity reflecting their own metabolic rates and interactions. Tris buffer also was omitted. The levels of tris normally used for buffering activity (equivalent to ca.10 mol liter^{-1} organic carbon) far exceed the detrital carbon concentrations of any freshwater system. Most unpolluted inland waters have organic carbon concentrations of 0.2 to 3 mmol liter^{-1} (Wetzel, 1975). The EDTA

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in our medium contributes 0.06 mmol liter^{-1} to the detrital carbon pool and plays the essential role of a refractory humic-like chelating agent for trace elements. The demineralized water contains an additional 0.10 mmol liter^{-1} of organic carbon in unknown form. Biological activity in the systems provides up to 0.32 mmol liter^{-1} (see below). The organic carbon in the microcosms thus ranges from 0.26 to 0.58 mmol liter^{-1}, representative of many natural aquatic ecosystems.

On the day following chemical enrichment of each microcosm, the systems each were inoculated with a 3.5-liter water sample collected from the littoral zone of eutrophic Lake Anza in the Tilden Park area of Berkeley, California. Manipulation of the trophic structure was carried out at various times after inoculation. These manipulations, summarized in Table 2, involved additions of juvenile mosquito fish (*Gambusia affinis*), South American catfish (*Placostomas placostomas*), oligochaetes (*Pristina* sp.), midge larvae (*Tanytarsus* sp.), and snails (*Physa* sp.), and were designed to fill niches not necessarily represented in the initial inoculum.

ANALYTICAL METHODS

The following parameters were measured on a weekly basis: temperature, O_2, pH, inorganic carbon (IC), organic carbon (OC), NH_4, NO_3 + NO_2, inorganic phosphorus (IP), total phosphorus (TP), phytoplankton species and number, and zooplankton species and numbers. All chemical measurements were duplicated. Chlorophyll a (Chl a) occasionally was measured in the microcosm water and in periphyton scraped from the tank sides.

Monitoring of phytoplankton and zooplankton continued until Day 198, although all other analyses were terminated on Day 147.

Methods and special instrumentation for each parameter monitored in the microcosms are summarized in Table 4. References to detailed descriptions of the techniques are provided where necessary. Vertically integrated samples were collected for analysis at 1100 h, 3h after the beginning of the light period, by immersing a hollow glass tube to a depth of 5 cm above the bottom of each system. Zooplankton were collected by tows with a plankton bucket (Wildco) fitted with a 64 μm straining net (Nitex).

Total phytoplankton, protozoa, and rotifer volumes were estimated from microscopic measurements on representatives of each species. Crustacean volumes were estimated from the numbers in various length classes for each species, using the length-weight relationships of Pechen (1965) for *Daphnia* and *Simocephalus* and of Kelkowski and Shushkina (1966) for copepods. The *Daphnia* relationship also was applied to *Alona* and *Cypridopsis*.

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RESULTS

No additions of either *Gambusia* or *Placostomas* were carried out for system I, which thus contains the simplest structure of the 4 systems. It is important to note that the biomass of *Gambusia* introduced into systems II, III, and IV is much higher than the level that could develop within any natural system. The 5 fish in each microcosm represent approximately 3 g or 4 mm^3 $liter^{-1}$ wet weight, compared to a maximum crustacean level of less than 2 mm^3 $liter^{-1}$. Although crustacean production apparently was high enough to maintain the *Gambusia* population (see below), the biomass of a planktivorous trophic level in natural systems normally is far less than the maximum biomass of its food supply. Any phenomena attributable to the presence of *Gambusia* thus can be viewed only in a qualitative manner; the effects in natural systems would be far less dramatic. For this reason, and in order to avoid unnecessary duplication in the presentation of results for all 4 systems, we will concentrate on the details of system I. Only those phenomena in II, III, and IV that differ significantly from those in I are presented.

On the basis of phytoplankton and crustacean biomass, the experiment with system I can be divided into 3 periods of differing biological activity (Fig. 2): (i) an initial bloom that terminates by Day 56, (ii) a period of low phytoplankton biomass whose end is marked by the disappearance of the cladocerans on Day 161, and (iii) a secondary bloom beginning on Day 161. A similar division may be applied to

systems II and III (Figs. 4,5), although the second interval terminates on Day 140 and 198, respectively. In IV, the distinction between an initial bloom and a second interval of lower phytoplankton biomass cannot be made (Fig. 6), although the disappearance of crustaceans on Day 77 may be taken as a natural division between the second and third period.

(i) *Initial bloom.* As exemplified by system I, a number of distinctive features characterize the chemical data during the first interval, most of the parameters exhibiting a local extremum by Day 56 (Fig. 1). On Days 28 to 35, pH and OC attained a maximum, and IC and $NO_3 + NO_2$ a minimum for the period. The increase in OC of 0.18 ± 0.03 mmol $liter^{-1}$ over the initial value corresponded in magnitude to the IC decrease of 0.21 ± 0.03 mmol $liter^{-1}$. The minimum $NO_3 + NO_2$ (64 ± 1 µmol $liter^{-1}$ below the initial level) was accompanied by the end of a rapid decline in TP and IP from 3.8 to 0.5 ± 0.1 and 0.4 ± 0.1 µmol $liter^{-1}$, respectively. By the end of the first period, NH_4 had risen to a peak of 23 ± 1 µmol $liter^{-1}$ from previous values of less than 2 µmol $liter^{-1}$. Systems II, III, and IV qualitatively were similar in behavior, although IV exhibited no significant rise in either NH_4 or $NO_3 + NO_2$ after the initial decline in $NO_3 + NO_2$.

A well-defined sequence of plankton pulses was observed during the first interval. In all tanks, the phytoplankton bloom attaining a maximum on Days 28 to 35 was preceded 1 or 2 weeks by a protozoa peak, was more or less coincident with a rotifer peak, and was followed

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by a crustacean maximum within several weeks (Figs. 2, 4, 5, 6). For systems II, III, and IV, the size of the phytoplankton, protozoa, and rotifer maxima were in the same rank order as the initial N and P concentrations. System I, which was treated in the same manner as III until the addition of *Gambusia* on Day 34, exhibited unexpectedly large deviations from III in levels of phytoplankton, protozoa, and rotifers before Day 34.

The initial bloom was dominated by diatoms and green algae. In I, *Cyclotella meneghiniana* and *Oocystis* sp. accounted for 55% of the total volume on Day 28, an unidentified LRGT and *Oocystis* for 84% on Day 35, and *Synedra ulna* and the LRGT for 76% on Day 42. The first protozoa peak was dominated by *Micropseudothorax* sp. and the initial rotifer peak consisted almost solely of *Dicranophorus* sp. The crustacean community up to Day 56 was formed primarily of the cladocerans *Alona guttata* and *Daphnia pulex*, although levels of the only copepod present, *Cyclops vernalis*, also had begun to rise (Fig. 3). As in I, the local maximum of crustacean volume in II, III, and IV that occurred during the first interval was dominated by *Alona*.

Only qualitative observations on periphytic growth and the associated fauna were recorded. However, certain phenomena observed on the container sides were of a dramatic nature, occurring within a well-defined time interval, and offer some insights into the patterns developing in the "pelagic" zone. A light periphytic covering in system I that developed during the first 42 days evolved into a heavy growth by Day 49, the same day the pH levels began to rise a second time (Fig. 1a)

The growth consisted primarily of *Oscillatoria* and *Cladophora* sp., the latter being covered by an epiphytic diatom community. Similar growths appeared in II, III, and IV, although the taxonomic composition was highly variable; the qualitative denseness of the periphytic covering was in the same rank order as initial N and P concentrations. *Tanytarsus* larvae were observed on the container sides of all systems by Day 56. The midge larvae fed on periphytic algae, as could be deduced from the presence of circular patches free of attached algal growth surrounding individual larvae.

(ii) *Period of low phytoplankton biomass.* The second interval in system I was characterized by total phytoplankton volumes of less than 0.12 mm^3 $liter^{-1}$. The NH_4 peak on Day 56 was followed 1 week later by a NO_3 + NO_2 maximum of 73 ± 4 µmol $liter^{-1}$, close to the intial value of 77 µmol $liter^{-1}$ (Fig 1). The subsequent decline in NH_4 and NO_3 + NO_2 was accompanied by an increase in pH to a smaller secondary maximum and a decrease of IC to a smaller minimum. No corresponding change in OC was observed. Secondary peaks in NH_4 and NO_3 + NO_2 occurred on Day 119 and 133, respectively. Systematic problems with the carbon analyzer after Day 105 prevented collection of accurate IC and OC data for the remainder of the experiment. Both IP and TP exhibited only erratic fluctuations at low levels after their initial decline during the first interval. The chemical behavior of systems II and III resembled that of I, except that no secondary maximum for NH_4 or NO_3 + NO_2 occurred in III. System IV exhibited no significant changes in pH, C, N, or P after the first interval.

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Protozoa volume remained low during the second interval (Fig. 2b); 3 small peaks dominated by *Strombidium* sp. were observed. Rotifer levels also remained depressed, the small community consisting of *Keratella cochlearis* and species of *Lecane*, *Philodina*, *Trichotria*, and *Voronkowia*, until a final rise in rotifers was detected following Day 140. This secondary rise consisted primarily of *Polyarthra* sp., accompanied by *Keratella quadrata* and *Anuraeopsis* sp. Among the crustaceans, (Fig. 3), the major peaks for each group were segregated in time in a clear manner. The first peak of *Alona guttata* was followed successively by *Daphnia pulex*, *Cyclops vernalis*, *Simocephalus vetulus*, and the sole ostracod *Cypridopsis* sp. Smaller peaks for *Daphnia* occurred before and after the maximum for *Simocephalus*. The disappearance of cladocerans on Day 161 coincided with the end of the second interval. Protozoa and rotifer volumes in II, III, and IV also remained at relatively small values during this second interval, but only in II was there a suggestion of a secondary rotifer increase (Figs. 4,5,6). As in I, the crustacean community was dominated by the cladocerans *Alona*, *Daphnia* and *Simocephalus* (except for IV, in which *Simocephalus* was not detected). *Cyclops* and *Cypridopsis* also were present, although never in significant amounts. The maximum levels attained for the various crustacean species in II, III, and IV were in the same rank order as the initial N and P concentrations; the crustacean peaks in I exceeded those in III.

On Day 84, when the pH in system I had ceased to increase and NO_3 + NO_2 levels had stabilized (Fig. 1), the 5 *Physa* individuals, which had grown to adult size, were observed for the first time to be exerting an effect on the periphytic community: snail movement on the container sides was preserved clearly by a complex network of swaths free of algae. On Day 105, a large number of tiny snails (less than 4 mm longest dimension, approximately 1 ind cm^{-2} of side surface), which had hatched from egg capsules adhering to the container sides, were present on the sides; on this same day, NH_4 levels in the water column began a secondary increase (Fig. 1b). By Day 119, the sides essentially were free of algae, aside from scattered patches of *Cladophora*, and, by Day 133, the snail numbers had dwindled and the *Cladophora* again were present in dense amounts. In system II, population increases and the subsequent effects on the periphyton community resembled the corresponding phenomena in I. Although *Physa* egg capsules occurred on the sides of III as early as Day 105, no young snails were observed until Day 140 and the snail population always remained 1 to 2 orders of magnitude less than in I and II. The *Placostomas* fed on the side growth and maintained it at levels lower than in I or II, although the periphyton levels remained high enough to confer a distinct green color to the tank sides. The introduction

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of *Placostomas* resulted in a shift from unicellular to filamentous periphytic forms. In IV, the *Physa* individuals added initially never produced egg capsules, but periphytic growth was removed completely 9 days after the introduction of *Placostomas* on Day 59 . Side growth never reappeared, even when 3 of the *Placostomas* were transferred to system III.

Two *Tanytarsus* maxima were evident in I, the first on Day 56 when water column concentrations of emerging larvae reached 2.0 ind liter^{-1}, the second on Day 133 when levels of 0.7 ind liter^{-1} were recorded. Only the first maximum occurred in the remaining 3 systems. In all systems, ostracod abundance always was far greater on and near the sides and bottom than in the water column; a similar comment applies to adult *Cyclops vernalis*, but not to the nauplius or early copepodid stages. *Daphnia pulex* also occurred near the sides, but appeared to prefer the open water. *Daphnia* ephippia consistently were present on the sides near the water surface from Day 56 on.

(iii) *Second bloom.* The disappearance of *Daphnia pulex* on Day 161 marked the beginning of a smaller secondary bloom in system I, a peak of 1.3 mm^3 liter^{-1} appearing on Day 175 (Figs. 2a, 3b). The bloom was due almost entirely to the cryptophyte *Cryptochrysis* sp., which accounted for more than 85% of the volume on Days 168 to 189. Only a small pulse of protozoa was noted, mostly individuals of a *Paramecium* sp., but the rotifer community (mostly *Polyarthra* sp.) continued the rise that began during the previous period. The crustacean

community was absent completely after *Cypridopsis* finished its decline on Day 168. As in I, large increases in phytoplankton and rotifer levels, with only minor changes in protozoa concentrations, took place after the beginning of the third period in II and III although the maximum volumes attained even exceeded those of the first period (Figs. 4,5). System IV offers a slightly more complicated picture (Fig. 6). Two distinct blooms occurred during the third period, the beginning of the first on Day 77 coinciding with the disappearance of the crustacean zooplankton, the second on Day 147 slightly preceding the disappearance of rotifers. The small quantities of zooplankton sporadically appearing in II, III, and IV during this third period all were either *Cyclops* or *Cypridopsis* individuals.

No dramatic periphyton changes took place after the second interval: I and II retained their heavy growths, III its lighter growth, and IV remained free of visible attachment. All fish remained active and healthy in appearance throughout the experiment. For II, III, and IV, the *Gambusia* lengths increased from 11.8 ± 1.1 to 25.1 ± 4.8 mm. There were no significant growth differences among systems.

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DISCUSSION

The production cycle of system I (Fig. 2a), less so that of II and III, bears a striking resemblance to the bimodal patterns observed in temperate marine waters (Cushing, 1959) and in certain inland water bodies, such as the Laurentian Great Lakes and productive lakes of the English Lake District (Hutchinson, 1967). Even the interval length between the 2 peaks, approximately 5 months in the case of I, is similar to the separation between peaks found in these natural systems. Despite the lack of temperature and irradiance variations, the similarity between microcosm behavior and that of certain naturally-occurring water bodies is marked, and the analogy will be explored further in what follows. As mentioned previously, the presence of fish in II, III, and IV leads to a quantitatively unrealistic trophic structure, so that our attention will be focused primarily on system I.

(i) *Initial period.* Conditions of Day 0 are similar to those at the beginning of the spring bloom – a large reserve of inorganic nutrients and low levels of plankton biomass. The resulting well-defined sequence of plankton pulses is suited particularly well for deducing the trophic relationships that characterize this initial period. In large natural systems, where a given trophic process may start at slightly different times at different locations, horizontal mixing tends to obscure the trophic relationships that can be deduced from sampling at a fixed point. It must be noted, of

course, that this very mixing may influence the subsequent biological development of natural systems in a manner that cannot be captured with the use of isolated microcosms.

The initial increase in phytoplankton productivity is retarded by protozoan grazing, as indicated by the larger standing crop of protozoa during the first 2 or 3 weeks (Figs. 2,4,5,6). The subsequent decline in protozoa appears to be due to grazing by rotifers and not to a decrease in food supply, which continues to rise for 1 or 2 weeks. On the other hand, the decline in rotifers clearly is related to the decreasing phytoplankton levels, although it is not obvious whether the decline in the rotifer community occurs solely as a result of overgrazing by rotifers or is aided by competition with the increasing crustacean community for a common food supply. The fact that the phytoplankton continue their rapid increase between Days 28 and 35, when the rotifers already have attained maximum levels, does suggest that the rotifer downturn is accelerated by competition with *Alona*. Similar conclusions may be deduced for the remaining 3 microcosms.

Whether by rotifers or by crustaceans, grazing must be the dominant factor behind the termination of the phytoplankton bloom; inorganic N and P levels observed on Day 35 (15 ± 1 and 0.4 ± 0.1 μmol liter^{-1}, respectively; Figs. 1b, 1c) clearly are sufficient to support further growth (e.g., Carpenter and Guillard, 1971; Perry, 1976), while all other required nutrients were added in excess (Table 1). The fact that the second major bloom in II, III, and IV, when crustaceans are absent, exceeds the first lends additional weight to this viewpoint.

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Although the "spring" bloom may have been halted by grazing, the size of the bloom must be determined, at least in part, by the quantity of nutrients initially present. The peak phytoplankton levels in II, III, and IV during the first period are 5.7, 0.76, and 0.13 mm^3 $liter^{-1}$, respectively. The protozoa and rotifer peaks in this interval also fall in the appropriate rank order, reflecting the relative levels of their food supply.

The discrepancy in magnitude between the phytoplankton peak of I (2.4 mm^3 $liter^{-1}$) and III is disconcerting, in view of the fact that both systems were treated in an identical manner until the addition of *Gambusia* on Day 34. The protozoa attained a maximum of 2.0 mm^3 $liter^{-1}$ in III, only 0.52 mm^3 $liter^{-1}$ in I, suggesting that the lower phytoplankton levels in III reflect a more intense grazing pressure from protozoa during the first few weeks. In turn, the discrepancy in protozoa may result from a different protozoan composition in the initial inoculum; although the inoculum volume was large (3.5 liter), protozoa were sparse and a significant random variation in the initial protozoa levels may have resulted. The rotifer peaks in I and III (0.12 and 0.082 mm^3 $liter^{-1}$, respectively) show less of a discrepancy than either the phytoplankton or protozoa concentrations, probably a result of the fact that a smaller amount of phytoplankton is compensated for by a larger amount of protozoa in the food supply of the rotifers in III.

The correspondence between the magnitudes of the IC decrease and the OC increase (Fig. 1a) must be viewed as somewhat coincidental. Although the IC decrease should equal the OC increase in a closed homogeneous system, aeration and sinking of particulate organic matter will lower the absolute magnitude of each peak, respectively, and not necessarily by the same amount. Further evidence for significant sinking losses (whether before or after being processed by grazers) is offered by the TP (Fig. 1c) and $NO_3 + NO_2$ concentrations (Fig. 1b).

In the case of TP, a drop of 4.3 μmol liter^{-1} in the water column takes place by Day 35, although some of these phosphorus losses may be due to adsorption by the sand and tank sides and periphyton uptake, not necessarily to the sinking out of phytoplankton. The rate constant for TP loss estimated for the first 35 days in system I is 22 a^{-1} ($r^2 = 0.81$); using Vollenweider's (1975) empirical approximation of $10/\bar{z}$ a^{-1} for the rate constant, where $\bar{z}$ = mean lake depth (m), a value of 18 a^{-1} is obtained for the microcosm. The agreement between the 2 numbers provides some evidence that the loss processes for TP in the microcosms resembles those of natural systems.

As no total nitrogen determinations were carried out, the exact fate of the $NO_3 + NO_2$ losses is more difficult to pinpoint. If all of the $NO_3 + NO_2$ decrease up to Day 35 (64 μmol liter^{-1}) represented a transformation to organic form remaining in the water column, the OC increase of 0.18 mmol liter^{-1} would imply a C:N molar ratio of 3 in this organic matter. Although C:N ratios of this magnitude have

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been observed for marine deep water ditritus (e.g., Duursma, 1960), the detritus of lakes more generally is characterized by C:N ratios exceeding 10 (Birge and Juday, 1934). In fact, assuming a value of 0.1 for the ratio of phytoplankton carbon to wet weight and a value of 3 to 6 for the molar C:N ratio in phytoplankton (e.g., Antia *et al.*, 1963), only 3 to 7 μmol liter^{-1} N is required to account for the nitrogen contained in the phytoplankton peak, a small proportion of the actual decrease in $NO_3 + NO_2$. Accordingly, we can conclude that a significant proportion of the $NO_3 + NO_2$ decrease results from sinking of biological material (including that which may have been grazed upon) onto the sediments or from uptake by periphyton. It is interesting to note that the molar N:P ratio for the lost nutrients by Day 35 is 15, close to the mean value traditionally applied to phytoplankton (e.g., Antia *et al.*, 1963), and it is tempting to conclude that the decrease in $NO_3 + NO_2$ and TP is due primarily to uptake by phytoplankton and periphyton, and subsequent sinking of living and grazed phytoplankton. However, the lack of a correspondence between N and P changes in the intervals preceding Day 35 precludes such a simple explanation. Whatever the exact mechanism behind the decrease in $NO_3 + NO_2$, bacterial and zooplankton processing of nitrogenous organic matter leads to a rapid building up of NH_4 as the OC decreases after Day 35; nitrification of the NH_4 results in the reappearance of almost all the $NO_3 + NO_2$ shortly after the start of the second period.

The large loss in N and P and the OC increase of 0.18 mmol liter^{-1}, when compared to the phytoplankton standing crop of approximately 20 µmol liter^{-1} on Day 35 (using the C:wet weight ratio of 0.1), all suggest that the maximum standing crop is only a small fraction of the actual phytoplankton production during the first half of the bloom. Assuming extracellular production of photosynthate is negligible compared to particulate phytoplankton production, the increase in OC represents a minimum estimate of the quantity of phytoplankton produced up to Day 35 (uncorrected for respiration and sinking losses). A minimum estimate for zooplankton grazing thus is obtained by subtracting the standing stock of phytoplankton, and it then appears that at least 90% of the particulate primary production was processed by zooplankton.

A crude estimate of the loss rate in each interval can be obtained by assuming that phytoplankton generation rates and loss rates are constant within any given interval, and by choosing a reasonable value for generation rates. Because

$$\dot{x}_p = (c_r - c_l)x_p \quad , \tag{1}$$

where x_p = phytoplankton biomass (mm^3 liter^{-1}), c_r = generation rate (d^{-1}), and c_l = loss rate (d^{-1}), c_l can be determined from

$$c_l = c_r - \frac{1}{(t_1 - t_o)} \ln \left[\frac{x_p(t_1)}{x_p(t_o)} \right] \quad , \tag{2}$$

where t_o and t_1 (d) mark the endpoints of the interval. A reasonable estimate of the generation rate c_r can be determined from the maximum rate of increase of phytoplankton standing crop, usually at times when the zooplankton are at lowest levels. This maximum rate corresponds to a

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doubling time of 1.0 to 2.9 d in the various systems; accordingly, we chose $c_r = 0.7\ d^{-1}$, equivalent to a doubling time of 1 d. The resulting values of c_1 for the first period in system I range from 0.1 to 1 d^{-1}. The correlation between c_1 and x_z, where x_z = zooplankton volume ($mm^3\ liter^{-1}$), is 0.75 (d.f. = 8, $p < 0.05$), suggesting that the losses are due primarily to zooplankton grazing, although the grazed material subsequently may sink out. Note that the correlation is independent of the value chosen for c_r, providing that c_r remains constant for all intervals; the fact that irradiance and temperature is constant may be construed as partial evidence for this assumption.

(ii) *Period of low phytoplankton levels.* After the termination of the spring bloom by zooplankton grazing, the phytoplankton remain at low levels due to continued grazing pressure. The continuing importance of the crustaceans is illustrated in Fig. 7, which depicts the results of removing part of the zooplankton community with a 64-μm mesh size plankton bucket from a 4-liter sample of system I. After a delay of several days, a rapid increase in fluorescence takes place with respect to a control beaker in which the zooplankton are left undisturbed. When the zooplankton are removed similarly from the control beaker on the ninth day and transferred to the beaker containing no zooplankton, fluorescence rises in the control beaker and decreases in the other. Apparently, the generation rate of phytoplankton and the grazing pressure of zooplankton remain more or less in balance during the second period.

Further evidence that the production losses after the bloom reasonably may be attributed to zooplankton grazing is obtained by consideration of the quantity c_1/x_z' (liter ind^{-1} d^{-1}), where x_z' = zooplankton number (ind liter^{-1}). This quantity is the filtering rate that would be required to explain production losses in terms of crustacean grazing pressure alone. The mean value for Days 56 to 154, that portion of the second period when crustaceans dominate the zooplankton (Fig. 2b), is 0.01 ± 0.01 liter ind^{-1} d^{-1}. Lowest values of 0.002 liter ind d^{-1} are obtained when *Cyclops vernalis* is at its maximum; intermediate values of 0.007 ± 0.004 liter ind^{-1} d^{-1} when *Alona guttata* and *Daphnia pulex* are dominant; and highest values of 0.02 ± 0.02 liter ind^{-1} d^{-1} when *Simocephalus vetulus* is the dominant species. For the low food concentrations during this period (<10^3 cell liter^{-1}), these values correspond to the results of various zooplankton feeding studies (e.g., Wetzel, 1975, Table 16-8, for copepods; Infante, 1973, for *D. pulex*; Sushtchenia, 1958, for *S. vetulus*).

Because the magnitude of the peaks for all 5 zooplankton species in systems II, III, and IV fall in the same rank order as initial N and P levels, the peak sizes clearly are dependent upon food supply in the microcosms. The limiting nature of the food supply is suggested also by the presence of cladoceran ephippia in all 4 microcosms after the initial bloom. The larger zooplankton peak sizes in I compared with III imply that fish planktivory also is a factor in determining

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the maximum development of zooplankton species in these systems. *Simocephalus*, a large zooplankter and presumably one of the most susceptible to *Gambusia* feeding, was absent completely from IV, the least productive microcosm. In addition, a second *Tanytarsus* maximum occurred only in I, where fish were absent.

The clear temporal separation between the zooplankton peaks in system I (Fig. 3) is probably not just a reflection of life cycle timing. In particular, the manner in which the *Daphnia* peaks are interspersed between those for the remaining species suggests significant competition between members of the zooplankton community. However, it is difficult to deduce the exact causal mechanisms that lead to the temporal segregation of zooplankton peaks in Fig. 3. Damped oscillatory populations often are observed in unispecific *Daphnia* cultures (Pratt, 1943; Slobodkin, 1954), and frequently the period of oscillation is approximately 40 d. One way to view the *D. pulex* data is to hypothesize a series of 3 damped oscillations generated as a result of interactions between *Daphnia* and its food supply, with peaks that would have occurred around Days 50, 90, and 130 in the absence of other crustaceans. The presence of *Alona* suppresses the first peak, and that of *Simocephalus* the second; *Cyclops* and *Cypridopsis* then take advantage of those times when *Alona* and *Daphnia* are suppressed. The exact cause-effect relationships undoubtedly are more complicated. For example, the raptorial food habits of *Cyclops* may initiate or accelerate the depression of *Daphnia* after Day 63. In

any case, the value of this microcosm design for competition studies is clear; many of the details of competition phenomena probably would reveal themselves by using an inoculum for the microcosms in which the crustacean composition was manipulated artifically.

The rise in pH and the decrease of IC and $NO_3 + NO_2$ during the second period (Fig. 1) appear to result from periphyton photosynthesis, as evidenced by an increase in periphyton density from Days 49 to 84. Periphyton scraped with a razor blade off small areas of the tank sides revealed densities as high as 60 mg m^{-2} Chl *a* by Day 84, equivalent to 38 µg $liter^{-1}$ Chl *a* if dispersed throughout the water. Chl *a* measurements occasionally collected in the water column during the second period never exceeded 4 µg $liter^{-1}$, so that the phytoplankton biomass was far less than the periphyton biomass by Day 84. It is of some interest to estimate the maximum density that can be attained by periphyton packed on the tank sides. Assuming that the thickness of the periphyton layer cannot exceed the distance for which incident irradiance would be reduced to compensation levels of irradiance, the following relationship must be satisfied:

$$I_c = I_o e^{-k_c C d} \quad , \qquad (3)$$

where I_c = compensation irradiance (W m^{-2} PAR); I_o = incident irradiance (W m^{-2} PAR); k_c = specific extinction coefficient for Chl *a* (m^2 mg^{-1}); C = Chl *a* concentration on the sides (mg m^{-3}); and d = thickness of periphyton layer (m). The mean of I_o incident on the sides is approximately 7 W m^{-2} PAR. Using a value of $k_c = 0.02$ m^2 mg^{-1} and $I_c = 1$ W m^{-2} PAR (Platt and Jassby, 1976), an areal Chl *a* concentration

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(= Cd) of 100 mg m^{-2} is obtained, close to the value observed. The periphyton thus appear to have built up to their maximum light-limited density. The same argument does not apply to forms capable of developing filaments, which avoid light limitation by extending into the water column where individual filaments can avoid significant shading effects.

The consequences for studies of nutrient cycles and trophic dynamics in microcosms are overwhelming. Once the periphyton are established, temporal changes in nutrient concentrations (such as the second NO_3 decrease illustrated in Fig. 1b), no longer can be attributed to the behavior of planktonic organisms alone. In addition, the explanation of population fluctuations in organisms that partition their time between the "littoral" and "pelagic" zones (such as observed for *Cypridosis*, adult *Cyclops*, and *Daphnia*) requires more complicated considerations of food supply. Although the littoral zone fulfils a similar complicating role in natural systems, the productivity of non-phytoplanktonic vegetation exceeds phytoplankton productivity by an order of magnitude or more only in small, shallow lakes (e.g., Wetzel, 1975, Table 15-15). The periphyton problem thus limits severely the use of microcosms as more general analogs of inland water bodies or for investigation of purely planktonic relationships.

The introduction of *Physa* and *Placostomas* into the microcosms represents an attempt to decrease the periphyton abundance by herbivory. In the case of *Physa*, the initial individuals grew continuously but did not make their presence felt until Day 84, when visible effects of their grazing coincided with the second decrease in pH (Fig. 1a). The resulting decrease in periphyton production apparently permitted aeration

to return the pH to its initial level. The appearance of large populations of young snails on Day 105 coincided with the second NH_4 increase, presumably because of snail excretion of grazed periphyton nitrogen. The snails subsequently died from overgrazing after Day 119 and the periphyton quickly reappeared. The increase in pH accompanying the reappearance of periphyton is suppressed possibly because of CO_2 production from the decomposition of starved *Physa*. The lower concentrations of young *Physa* in III, compared with I and II, can be attributed to *Placostomas* feeding on snail egg capsules attached to the sides, and no secondary NH_4 peak from snail excretion was observed. In I, productivity was not sufficient to support *Physa* reproduction. Because of the time lag between periphyton growth and snail reproduction, and because of overgrazing, the snails generally proved incapable of maintaining sides consistently free of periphyton. Accordingly, we do not feel that introduction of snails offers even a partial solution of the periphyton problem. In fact, their short-term effects on the pelagic N concentrations make interpretation of nutrient cycles even more complicated.

The presence of *Placostomas* in IV proved to be completely effective in removing periphyton from the sides and preventing recolonization. However, system IV had the lowest level of nutrients and the least dense periphyton growth. In III, where initial N and P levels were 4 times higher, the 3 *Placostomas* never succeeded in eliminating periphyton growth, even with the aid of the snails present. Although

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growth was always less dense than in I or II except when the snails temporarily had eliminated the periphyton in these latter 2 systems, the color suggested that Chl a densities were not an order-of-magnitude lower than in I or II and probably still exceeded phytoplankton biomass. The *Placostomas* appeared to be particularly ineffective against the long *Cladophora* strands that developed in III during the third period.

Accordingly, neither the snails nor catfish turned out to be an adequate method for dealing with dense periphyton communities. This difficulty still remains unsolved and is the most serious hindrance to long-term studies in microcosms.

(iii) *Second bloom.* In all 4 microcosms, the beginning of the second bloom coincides precisely with the disappearance of crustaceans from the water column. The release from grazing pressure permits the phytoplankton community to take full advantage of the nutrients available and increase to levels approaching or exceeding those of the initial bloom. The timing of the crustacean dieoff reflects the effects of both food supply and predation. In systems II, III, and IV, the dieoff is postponed more when the initial N and P levels are increased; in I, the lack of a planktivorous fish allows the intermediate period between blooms to continue for the longest time of all 4 systems. The bloom starting on Day 77 in IV appears to be prematurely destroyed by the rotifer community, and it is only when the rotifers disappear between Days 147 and 154 that a major phytoplankton rise takes place (Fig. 6).

The termination of the "spring" bloom by grazing, and the onset of the "fall" bloom after starvation of the zooplankton, both suggest that the production cycles in these microcosms reflect a predator-prey oscillation (although not necessarily of the Lotka-Volterra type). The oscillations are forced by the initial conditions favoring high phytoplankton growth rates with little interference from crustacean grazers. At a later time, the crustaceans in the inoculum spawn and the grazing capacity of the zooplankton community increases. Subsequent grazing mortality among the phytoplankton results in primary production levels too low to support the zooplankton, whose levels gradually decrease through starvation. Once the cladocerans, in particular, have disappeared, conditions resemble those at the start and a new sequence of production begins.

The course of events is similar, in many ways, to that observed in certain temperate marine waters where production cycles essentially are a reflection of predator-prey relationships, and nutrient levels respond to, rather than cause, the cycle (see discussion by Cushing, 1975). As in the microcosms, the quantity of phytoplankton produced is many times the maximum standing crop, and about 1 month is required before crustacean grazing becomes effective enough to terminate algal increases in the spring. Although tropical and polar production cycles also can be viewed as resulting from predator-prey interactions (Cushing, 1959), latitudinal differences in the annual temperature pattern result in differences in the annual production pattern. In arctic waters, the

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colder temperatures result in a longer delay time between the onset of spring phytoplankton increases and the onset of effective grazing, and only a single phytoplankton peak of high amplitude occurs before production is halted by winter conditions. In tropical waters, the short delay time results in low amplitude phytoplankton changes without the discontinuity characteristic of higher latitudes. In general, the longer the delay time, the greater the phytoplankton peak (up to the onset of nutrient limitation) and the greater the proportion of phytoplankton biomass lost to sinking and respiration without being grazed. Thus, the delay time between phytoplankton increases and the beginning of effective grazing is an extremely important factor governing the amplitude of production cycles and the ratio of secondary to primary production. The use of microcosms offers a unique opportunity for exploring the precise conditions under which different production patterns result; irradiance, temperature, initial nutrient levels, and initial (i.e., "overwintering") levels of crustacean females or resting eggs all can be manipulated to analyze effects on delay times and subsequent levels of primary and secondary production.

The analogy between the microcosms and inland water bodies is less general. Evidence for predator-prey oscillations dominating production cycles is clear only in the simplified associations of extreme habitats, such as highly saline or alkaline lakes (Anderson *et al.*, 1955; Anderson, 1958). In most well-studied cases, nutrient depletion is a more significant factor than grazing in causing the spring decline of phytoplankton;

for example, silicate depletion plays this role in parts of the English Lake District (Lund *et al.*, 1963), and phosphate depletion in certain Laurentian Great Lakes (Schelske *et al.*, 1972). In addition, upwelling of subthermocline nutrients or algae (Fee, 1976) during the fall overturn, rather than zooplankton disappearance, appears to be the main cause of the fall bloom.

The possibility of a more realistic simulation of inland waters with microcosms cannot be ruled out. As pointed out previously, protozoa grazing is a major factor in suppressing initial phytoplankton increases in the microcosms. In natural systems, protozoa usually constitute only a minor portion of the zooplankton and achieve maximum levels after the spring bloom. (e.g., Schönborn, 1962; Sorokin and Paveljeva, 1972). Initial microcosm conditions apparently allowed the inoculated protozoa to rapidly outgrow predators and exert an undue effect on the spring bloom. It is possible that, if protozoa could be excluded from the inoculum, phytoplankton increases would proceed at a rate sufficient to exhaust nutrients before crustacean grazing became effective enough to terminate the bloom. In addition, certain aspects of the fall overturn perhaps could be simulated by vigorous mixing of the benthic substrate into the water column for a short time, releasing the accumulation of nutrients from decomposed organic matter and returning viable algae that have settled out. Neither modification was attempted in the present set of experiments, but both bear serious consideration for any future work of this nature.

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CONCLUSIONS

A number of important drawbacks deserving further discussion have been made clear to us in the course of this work. Some of these drawbacks are inherent difficulties in size scaling. For example, the shallow depth results in higher sinking losses, smaller vertical irradiance changes, smaller migration distances for zooplankton, etc., as compared to natural systems. Little can be done about such scaling problems and their possible interference must be interpreted within the context of each individual experimental aim.

Other major problems, however, do have potential solutions:

(i) It is not possible to stock planktivorous fish in realistic concentrations, and their presence results in premature disappearance of the cladocerans. The obvious answer to this difficulty is the complete exclusion of fish from the microcosms. Their exclusion also permits the use of smaller systems, although proper zooplankton sampling sets a lower limit on microcosm volumes somewhere between 10 and 10^2 liters.

(ii) Poor replication will result unless all inoculated organisms that can proliferate in the microcosms are present in sufficiently high numbers in the inoculum; also, protozoa appear to play an unnaturally important role. Both of these problems suggest the use of gnotobiotic systems, although experiments then are subject to the lack of realism inherent in the gnotobiotic approach (see section on microcosm design). Perhaps a compromise is suitable, in which the inoculum is prepared from several unialgal (although not axenic) cultures, along with, say, 1 rotifer species and 1 crustacean species (with large numbers of individuals in each case). Greater ability to exclude contamination by other

zooplankton will be obtained in the smaller systems without fish.
(iii) Periphyton growth is too high and can dominate events in the microcosms. The periphyton problem can be dealt with in part by limiting experiments to less than about 1.5 months, the time when the consequences of side growth first become apparent. This limited time still permits a detailed examination of the circumstances surrounding the spring bloom. An alternative method is mechanical removal of periphytic attachment, although daily manual scraping of the tank sides is too tedious for routine work and an automated method is desirable. In any case, the solution of the periphyton problem deserves the most serious consideration in any further work of this nature. The dominance of biomass by side growth precludes the simplified study of nutrient cycles and energy flow that microcosms potentially can offer.

Despite these drawbacks, this study has demonstrated certain resemblances between microcosms and natural water bodies where production cycles are determined by predator-prey relationships. Also, modifications have been suggested to increase the generality with which these microcosms can be made to simulate natural systems. Because of the resemblance to certain natural production cycles, and because of the clear sequence of plankton pulses in the absense of spatial heterogeneity, we feel that the microcosm method described here offers a powerful tool for assessing environmental effects (whether of human origin or not) on production patterns, trophic relationships, and competition phenomena.

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TABLE HEADINGS

Table 1. Composition of the inorganic enrichment medium for the 4 700-liter microcosms. The stock solution is diluted 1:700 in the microcosms.

Table 2. Manipulation of the biological structure in the 4 700-liter microcosms.

Table 3. Methods of analysis for the parameters monitored.

Table I.

Nutrient	Stock solution (g liter^{-1})	Microcosm concentrations (μmol liter^{-1})
$CaCl_2 \cdot 2H_2O$	37	3.6×10^2
$MgSO_4 \cdot 7H_2O$	37	2.1×10^2
$NaHCO_3$	37	6.3×10^2
$Na_2HPO_4 \cdot 7H_2O$[1]	--	--
$NaNO_3$[1]	--	--
$Na_2SiO_3 \cdot 9H_2O$	30	1.4×10^2
KCl	10	2.0×10^2
Na_2EDTA	1.5	5.7
$FeSO_4 \cdot 7H_2O$	1.0	5.3
$CuSO_4 \cdot 5H_2O$	0.010	0.057
$ZnSO_4 \cdot 7H_2O$	0.020	0.10
$CoCl_2 \cdot 6H_2O$	0.010	0.060
$MnCl_2 \cdot 4H_2O$	0.20	1.4
$Na_2MoO_4 \cdot 2H_2O$	0.010	0.060

[1] see text

Table II.

Date	Day	Manipulation
05-06-76	0	Inoculation of each of I, II, III, IV with 3.5-liter sample of lakewater
05-21-76	15	Addition to each of I, II, III, IV of 5 *Pristina* and 5 *Tanytarsus* larvae
06-09-76	34	Addition to each of I, II, III, IV of 5 *Gambusia* of length 1.2 cm
06-24-76	49	Addition to each of I, II, III, IV of 5 *Physa* of length 2.5-5.0 cm
07-04-76	59	Addition to IV of 4 *Placostomas* of length 1.0 cm
08-15-76	101	Transfer of 3 Placostomas from IV to III.

Table III.

Parameter	Method	Special equipment	Reference
O_2	polarography	O_2 meter (YSI 57)	--
pH	electrometry	pH meter (Orion 601)	--
IC	infrared absorbance	IR analyzer (Beckman 865)	--
OC	combustion to IC	TOC analyzer (Beckman 915A)	--
NH_4	blue indophenol reaction	spectrophotometer (Zeiss PM2 DL)	Solorzano, 1969
$NO_3 + NO_2$	reduction, diazotization	"	Golterman, 1969
IP	ascorbic acid reduction	"	APHA, 1971
TP	persulfate digestion to IP	"	APHA, 1971
Chl *a*	fluorometry	fluorometer (Turner 111)	Strickland and Parsons, 1968
phytoplankton	Sedgewick-Rafter cell	phase microscope (Reichert Zetopan)	Guillard, 1973
zooplankton	64-µm tow	dissecting microscope (AO 570)	--

FIGURE LEGENDS

Figure 1. Chemical measurements from System I. Vertical lines indicate the standard error for duplicate measurements. (a) pH, inorganic carbon (IC), and organic carbon (OC); (b) NH_4 and NO_3 + NO_2; (c) inorganic phosphorus (IP) and total phosphorus (TP).

Figure 2. (a) Total volume of phytoplankton in system I; (b) total volume of protozoa, rotifera, and crustacea in system I.

Figure 3. (a) Resolution of the crustacea in Fig. 2b into cladocera, copepoda, and ostracoda; (b) resolution of the cladocera in Fig. 3a into component species.

Figure 4. (a) total volume of phytoplankton in system II; (b) total volume of protozoa, rotifera, and crustacea in system II.

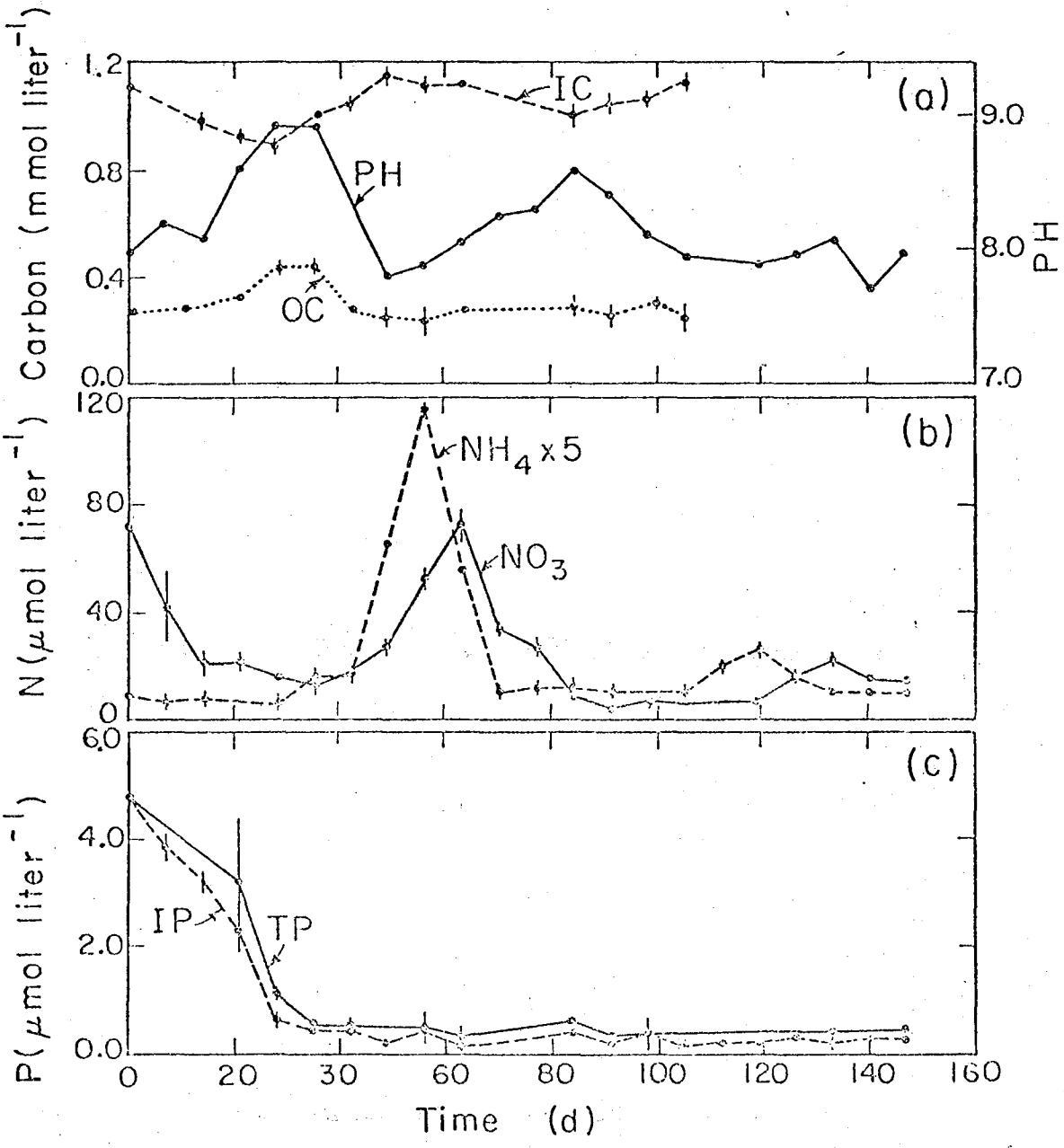
Figure 5. (a) Total volume of phytoplankton in system III; (b) total volume of protozoa, rotifera, and crustacea in system III.

Figure 6. (a) Total volume of phytoplankton in system IV; (b) total volume of protozoa, rotifera, and crustacea in system IV.

Figure 7. Importance of zooplankton grazing in 4-liter samples collected from system I on Day 63. Removal of zooplankton with 64 μm net (solid line) results in increased fluorescence with respect to a control beaker (dashed line). When zooplankton are transferred from the control beaker to the beaker that is zooplankton-free, fluorescence trends reverse.

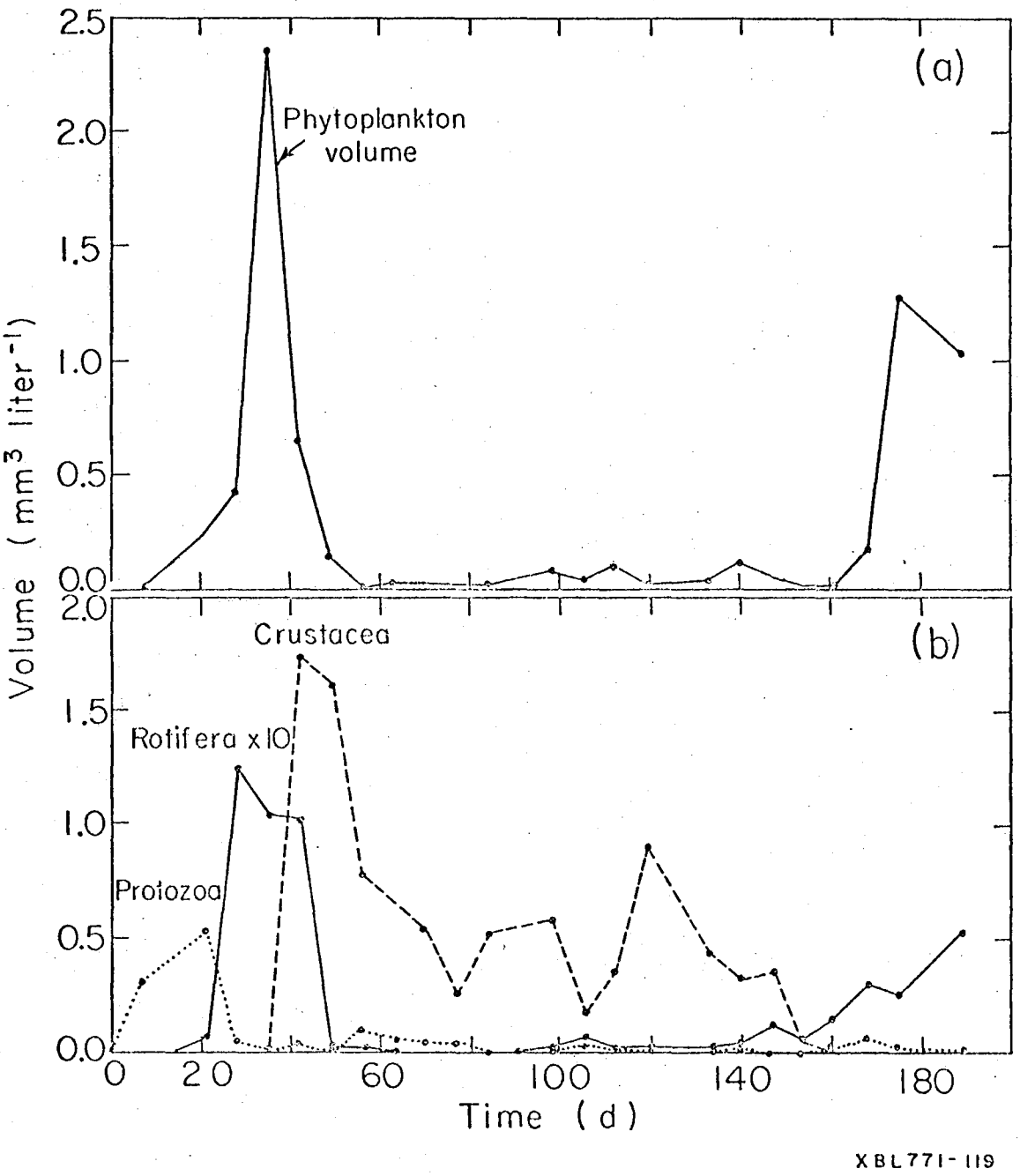
0 0 0 0 4 7 0 6 6 3

Figure I



XBL771-120

Figure II


(a)
Phytoplankton volume
(b)
Crustacea
Rotifera x10
Protozoa
Volume (mm3 liter-1)
Time (d)
0
20
60
100
140
180
0.0
0.5
1.0
1.5
2.0
2.5
XBL771-119

0 0 0 0 4 7 0 0 6 6 4

Figure III

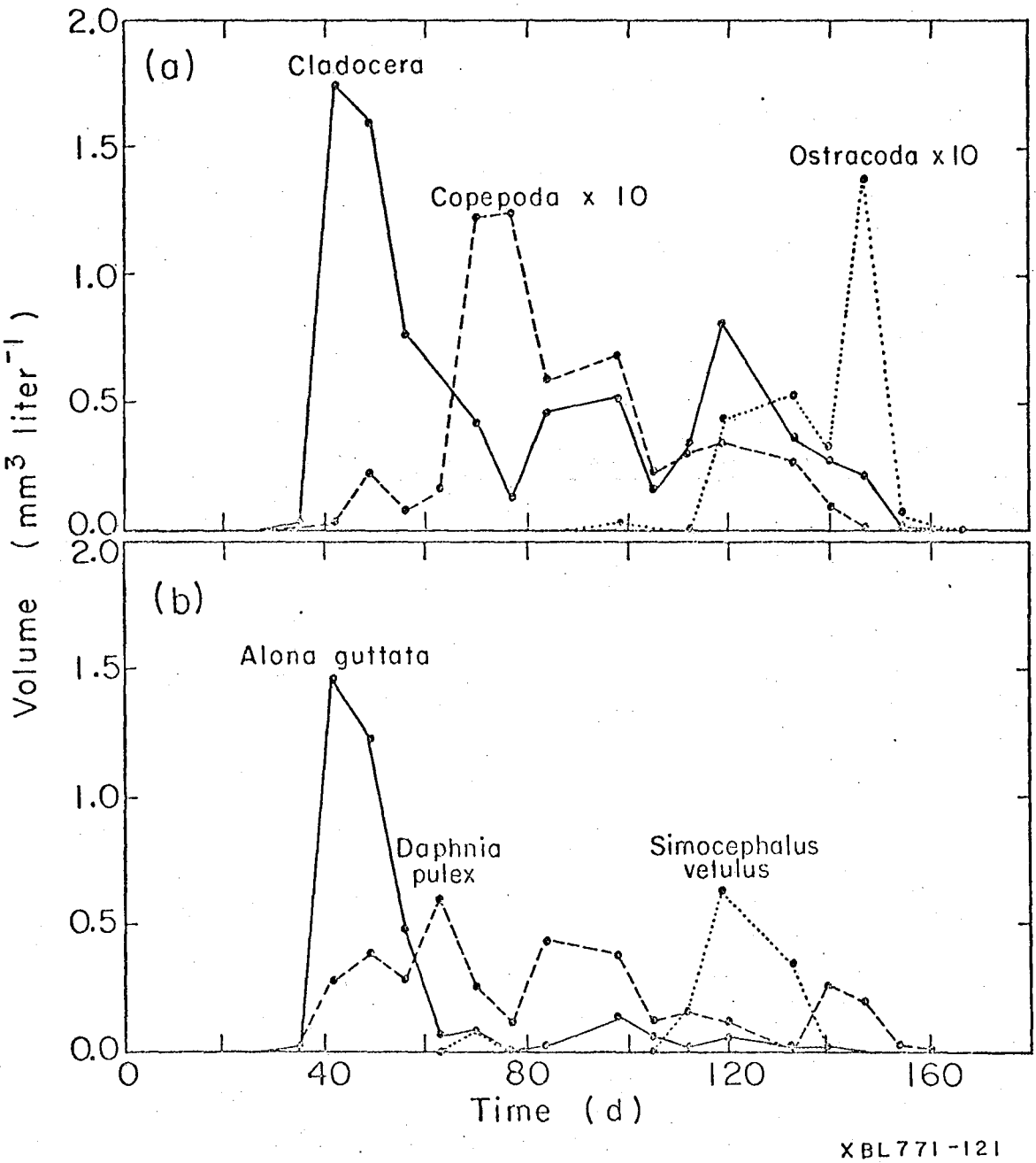
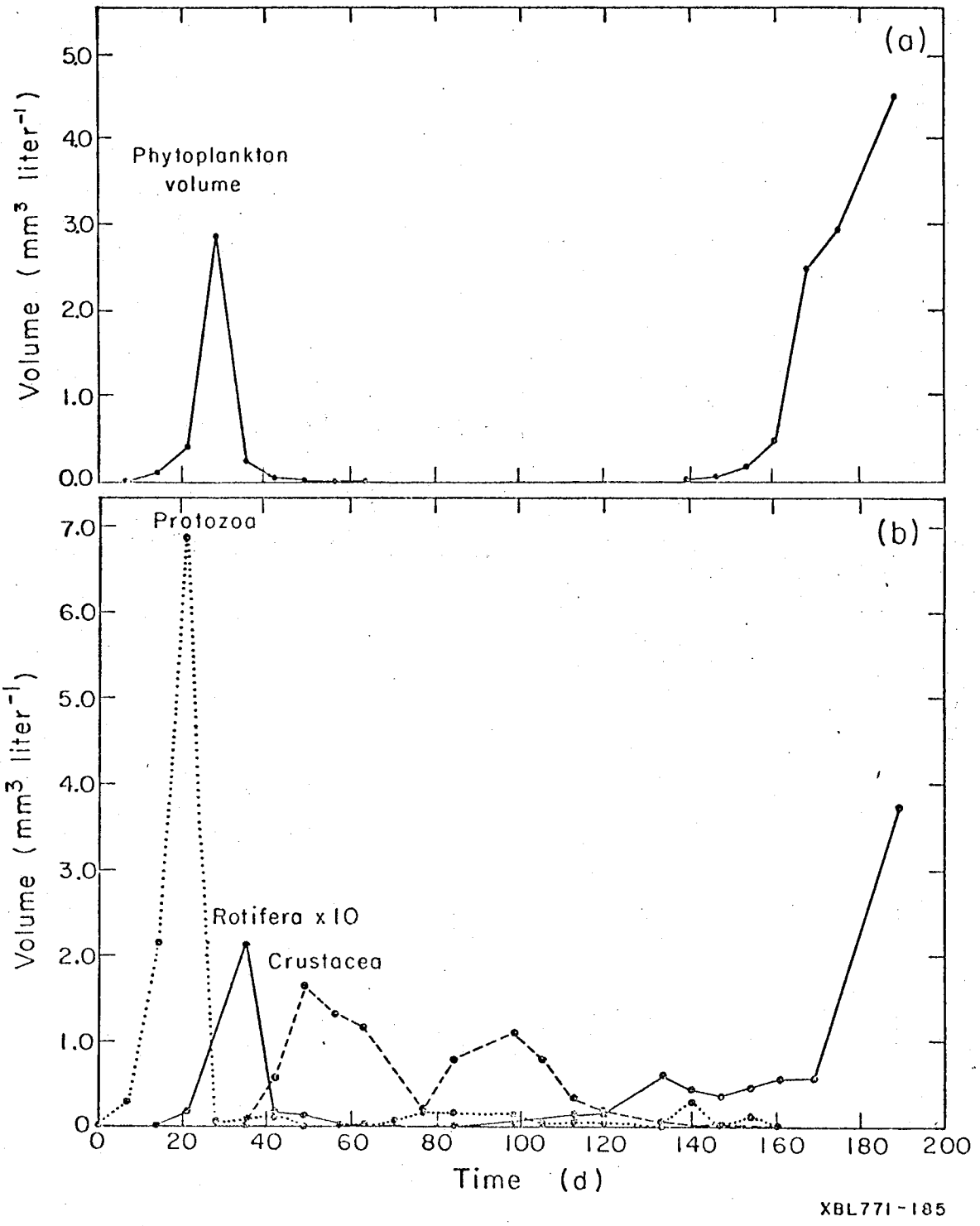
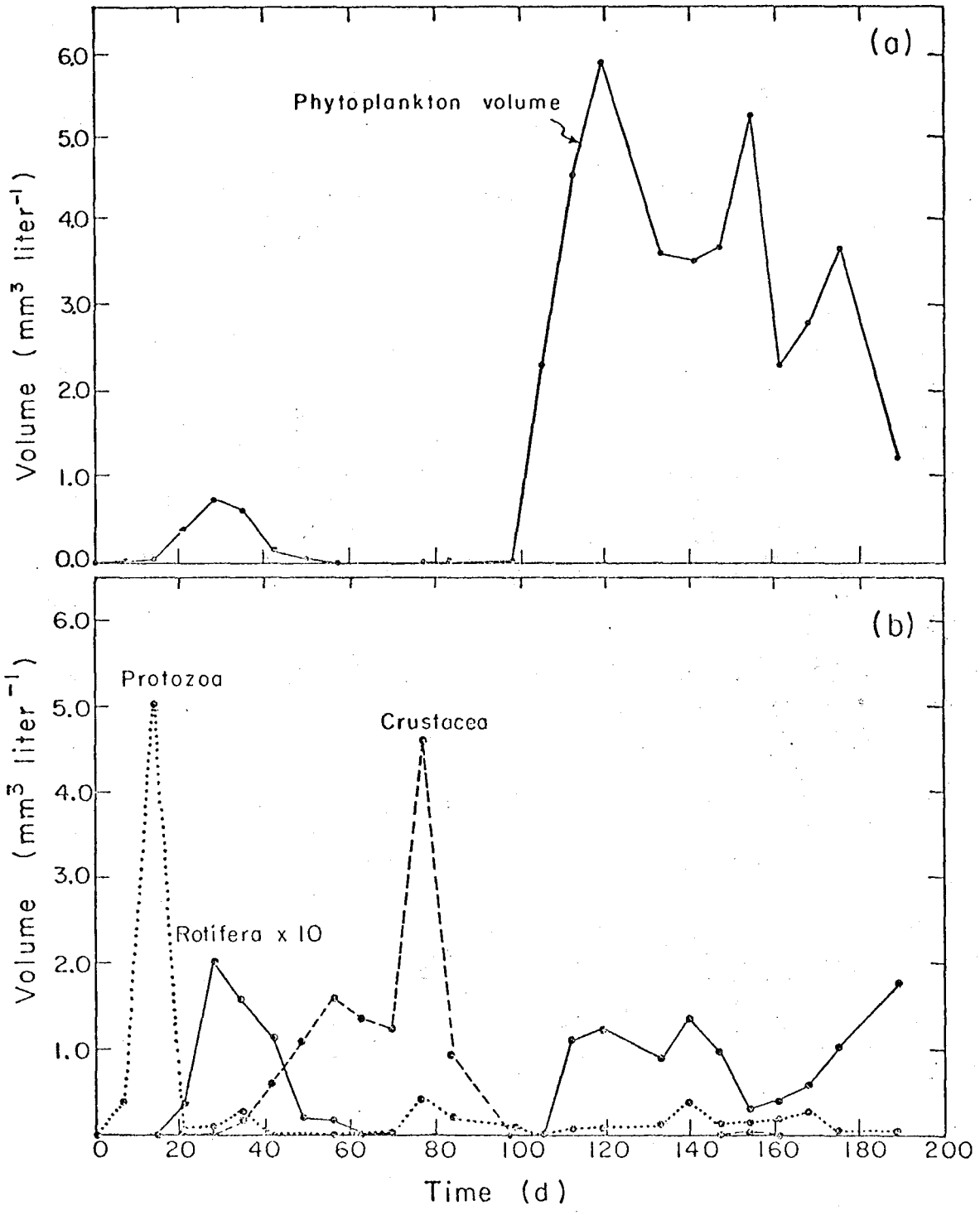


Figure IV


(a)
Phytoplankton volume
Volume (mm³ liter⁻¹)
5.0
4.0
3.0
2.0
1.0
0.0
(b)
Protozoa
Rotifera x 10
Crustacea
Volume (mm³ liter⁻¹)
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
0
20
40
60
80
100
120
140
160
180
200
Time (d)
XBL771-185

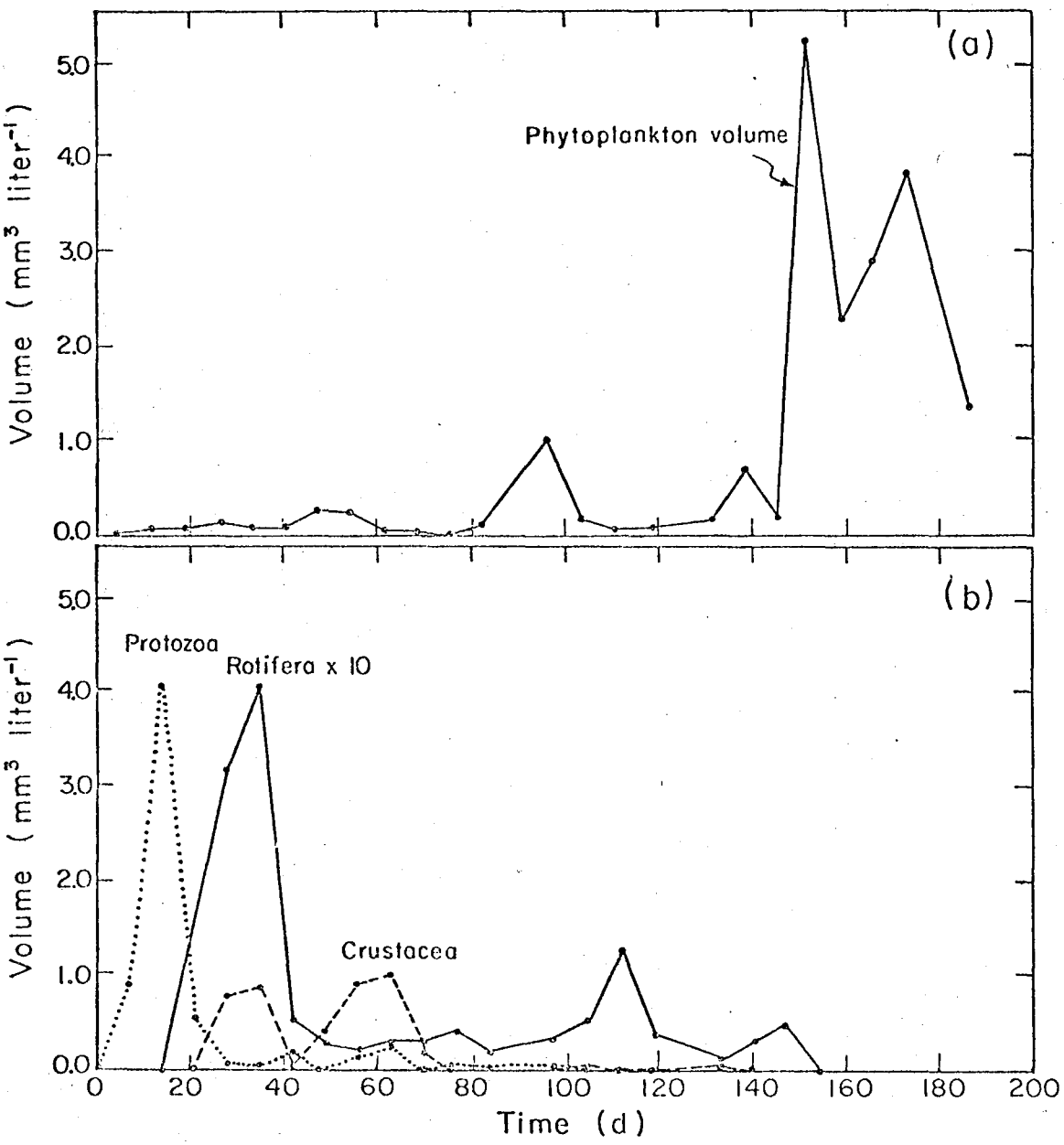
0 0 0 0 4 7 0 0 6 6 5

Figure V



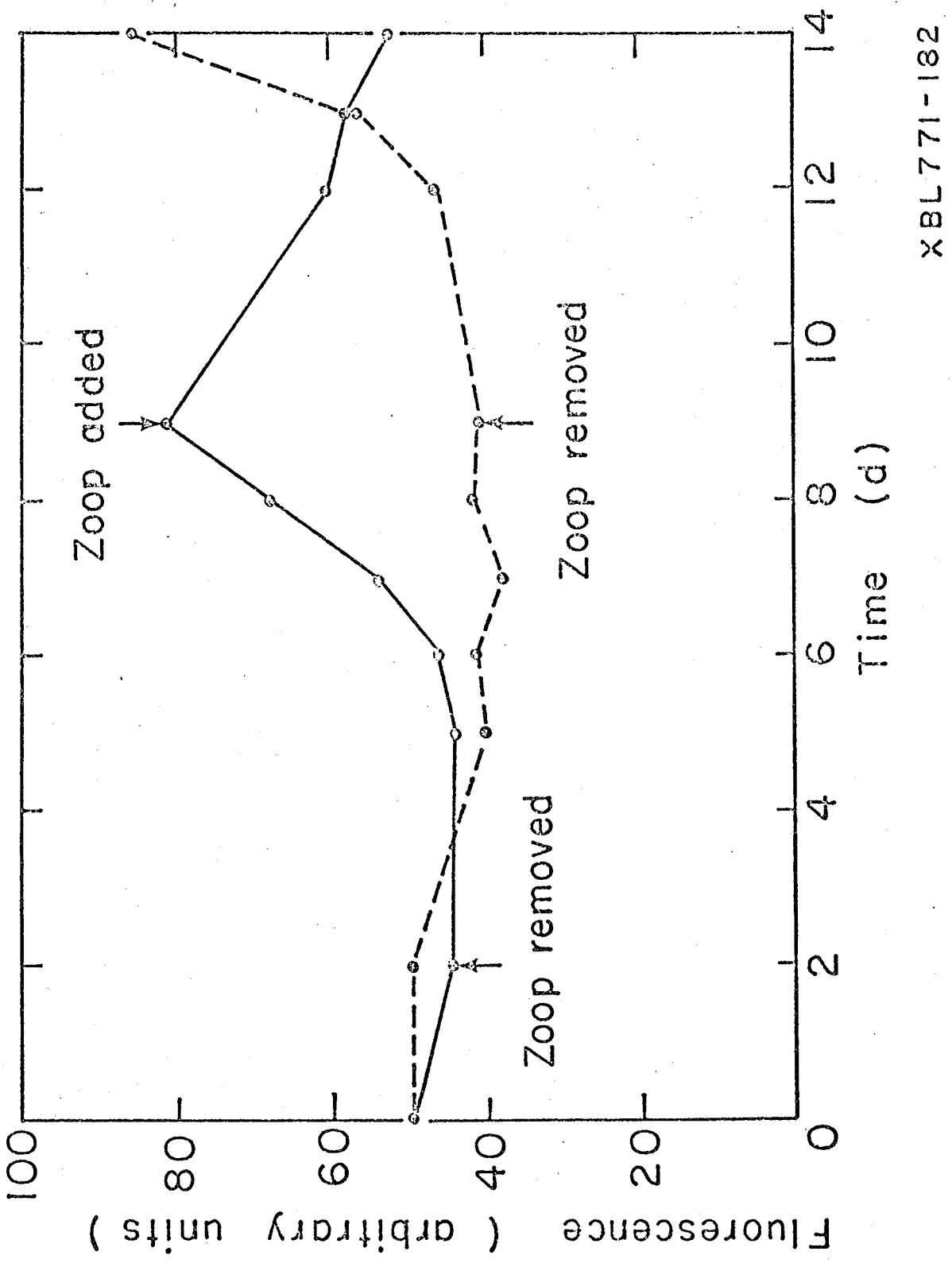
XBL771-183

Figure VI


(a)
Phytoplankton volume
Volume (mm³ liter⁻¹)
5.0
4.0
3.0
2.0
1.0
0.0
(b)
Protozoa
Rotifera x 10
Crustacea
Volume (mm³ liter⁻¹)
0
20
40
60
80
100
120
140
160
180
200
Time (d)
XBL771-184

0 0 0 0 4 7 0 6 6 6 6

Figure VII



This report was done with support from the United States Energy Research and Development Administration. Any conclusions or opinions expressed in this report represent solely those of the author(s) and not necessarily those of The Regents of the University of California, the Lawrence Berkeley Laboratory or the United States Energy Research and Development Administration.

0 0 0 0 4 5 0 2 2 9 4