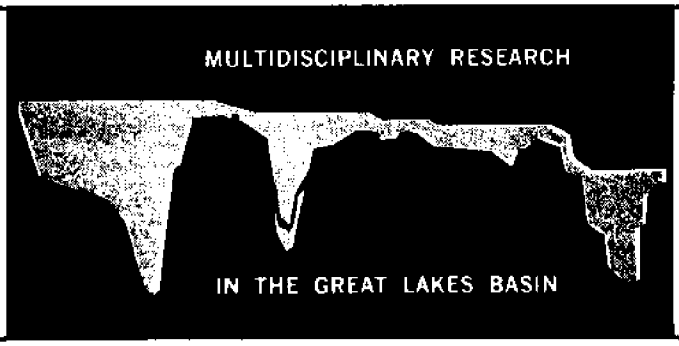

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PREDATOR PREY INTERACTIONS IN AN EXPERIMENTAL PROTOZOAN COMMUNITY

(Tetrahymena pyriformis - Aerobacter aerogenes)

by
Thomas D. Lustig

School of Natural Resources
Department of Resource Planning & Conservation

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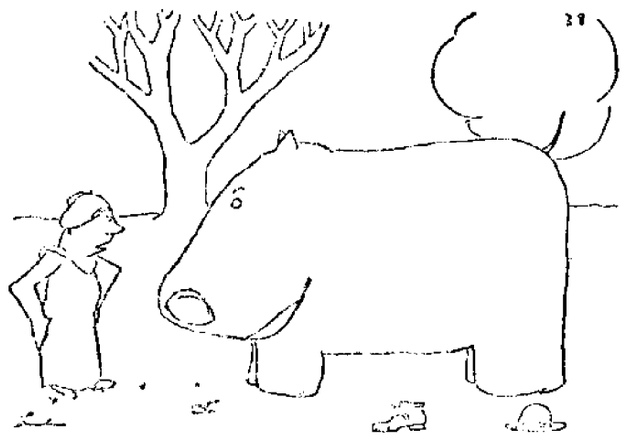
PREFACE

Predation, as depicted by Mr. Thurber's drawing, has long been an absorbing concern of men. Sharp-toothed secondary consumers, whether prey, predators, or competitors of men, gave impetus to an area of study which can now claim legitimacy from a new source, even for people whose only wildlife encounter is the mosquito. Wealth and leisure now allow the luxury of environmental concern, whether from heuristic, aesthetic, or quality considerations. Although it may be difficult to appreciate the aesthetics of a pond full of protozoans, their study, as a hint to the behavior of other predators and as a factor in maintaining the quality of natural waters, is both self-motivating and justifying.



"What Have You Done with Dr. Millbuck?" [1]

Predators (secondary consumers), their prey (primary consumers), and the supporting vegetation (primary producers), form a basic aggregate in the study of environmental regulators, and information on their

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[1] James Thurber, The Thurber Carnival.

interactions, as will be reported in this paper, is a fundamental tool of ecosystem modeling. Prey-predator population dynamics, in combination with mathematical formulations of additional biotic and abiotic elements of an ecosystem's food web, help synthesize a mathematical description of complex natural interactions; once formulated, an ecological model can provide both behavioral and predictive information to assist decision-making in regulating natural communities [Canale]. Thus, the State of Michigan's legislative decision to limit the amount of phosphates discharged into state waters (which some claim to be unnecessary and inconsequential) could be supported or objected to on the basis of a mathematical model of freshwater communities. This research provided population data for a mathematical description of one phase of freshwater ecosystems, and attempted to discern some fundamental properties of predator-prey communities.

Although the experimental work presented here involved the examination of an artifical laboratory community of a predator protozoan, a prey bacterium, and a plant-derived prey food source, the approach of this report is broader. In an attempt to provide a better perspective on the critical factors in our laboratory community, some time is taken to present a discussion of the biotic and abiotic influents on natural and artificial predator-prey communities. Hopefully a review of the natural factors which regulate such a community will provide a better understanding of the limitations and advantages of a laboratory ecosystem. In general, as naturally occurring factors are eliminated or made constant in an experimental system, specific "man-choosen" factors

become more influential and/or more accessible to study. The sacrifice of reality in an experimental ecosystem is hopefully mitigated by the increased insight the laboratory provides.

As mentioned earlier, the discussion and data reported herein is only a part of a broader attempt to describe environmental quality regulators in the language of mathematics. Work is continuing in our laboratory in both additional animal studies and further efforts to translate population kinetics into more malleable mathematics.

ACKNOWLEDGMENTS

This research involved counting over 10 million protozoa who insisted upon growing regardless of weekends or the fact that it was four in the morning. The study would have been impossible without the dedication, support, and enthusiasm of those who worked with me.

My most important support was from Professor Ray Canale, who initiated this series of experiments. Professor Canale's dedication went well beyond the invaluable direction, suggestions, and advice he provided. There was no time of day or night when Dr. Canale wasn't willing to take over the lab chores, help solve another disastrous problem, or help me escape the conviction of futility with his indefatigable enthusiasm.

The success of the investigation is due to the accuracy, patience, and dedication of Frank Cheng, Eric Nelson, Burr Nelson, and Rich Valentine, who despite my persistent interference, unfailingly took readings around the clock and performed the myriad of measurements endemic to our laboratory. In addition, Frank, Eric, and Burr were indispensable in their assistance with the data presentation: it was they who spent hours over an intransigent keypunch so we could use machine analysis of the data.

Certainly the protozoa and I could not have coexisted without the constant cheerful assistance and moral support of Lillian Wilder, Jane Lesfstad, Jan Garvey, and Carol Berdinka. Jan's calm perseverance through thousands of illegible and unspelled words brought the manuscript to a respectable state; and Mrs. Wilder's savvy and good nature made the battle of the purchase order a bearable ordeal.

I am also grateful to Dr. Sally Allen, who was kind enough to continually provide us with protozoa from her own cultures, and to the Sea Grant Program at The University of Michigan, which funded the research.

Finally, I am indebted to Ralph Stemp, who when all was going wrong, assured me that things couldn't get any worse.

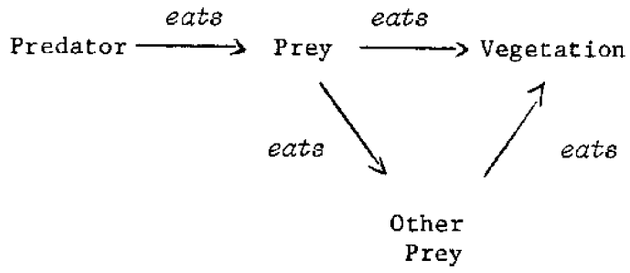
T. D. L.

CHAPTER I

INFLUENTS ON PREDATOR-PREY COMMUNITIES

Obtaining a better understanding of the factors which control groups of predators and their prey is an inexact procedure. The taxonomy presented here, in which community influents are separated, can only be arbitrary in its divisions. The overwhelming influence on any community is the interaction of all components. Dividing regulators into independent influents is most appropriate as an analytical approach rather than an accurate definition of the ways things occur.

A complete concept of a community is superfluous to the scope of this paper. The necessary orientation rests on three definitions: first, that a predator is an organism which captures and consumes other organisms for its food; second, prey are living organisms which derive their food from either other animals or vegetation and in turn comprise the diet of a predator; and third, a predator-prey community is a group of predators feeding on prey who ultimately depend on vegetation. The predator-prey community may be schematically represented as:



Such a simplification, ignoring food web structures and recycling phenomena

(such as decaying predators providing nutrition for vegetation) is nonetheless complete enough to begin an examination of the influents on such a community.

The factors which regulate a predator-prey community may be divided into two general types. First are those originating from outside of the animal population:

I. Energy flow
II. Availability of abiotic materials

The next class of influents originate within the animal populations:

III. Prey availability
IV. Phagic preference of the predators
V. Population densities and competition
VI. Conditions and talents of the populations
VII. Results of predation on the populations

Energy Flow

All animals, and accordingly all communities, are ultimately dependent on the sun as a source of life. However, variations and alterations from this source are more germane to regulating a predator-prey community. Populations are dependent on stored available energy (standing crop), on the rate of the flow of energy into the community, on the efficiency of conversion from energy to mass, and on concomitant washout effects of energy input. The amount of available edible biomass is a measure of how much food there is for heterotrophic prey and predators and determines the size of the feeding populations. A simple example is obtained from experimental work where the yield (as dry weight or biomass) of bacteria ultimately produced is directly proportional to the initial amount of food (sucrose concentration) available, provided

toxicants are not limiting. Similarly, the terminal biomass of a predatory protozoan feeding on the sucrose-grown bacteria was directly proportional to the initial bacterial mass [Curds, 1968a]. A more complete yet less precise measure of the amount of energy determining the amount of prey who in turn fix the mass of predators concerns the mackerel catches off the West Cornish coast. It has been found that the amount of sunlight during February and March is linearly correlated with the size of the mackerel catch in May. The connection seems to be that the amount of sunlight determines the productivity and, therefore, the amount of phytoplankton. The abundance of phytoplankton determines the population size of the phytotrophic zooplankton (the prey). Finally, large zooplankton masses attract and produce concomitantly larger amounts of the zooplankton-feeding mackerel [Allee].

Available biomass is a misleading measure, for although it indicates the amount of predators and/or prey that can be "created," it offers no information as to how these populations can be "supported." The biomass of organisms which can be supported at a trophic level depends not on the amount of fixed energy at the level just below it, but upon the rate at which food is being produced [Odum]. Unless there is a continuous input of energy into a community, the plant and animal populations will increase until they have exhausted the energy supply and then perish. The fact that populations do not increase without limit, even though there is a continuous natural supply, implies that not all energy is utilized for producing more organisms. That the critical factor regulating populations is indeed the rate of supply rather than the amount supplied was demonstrated by adding sterile nutrient food to

natural river populations of prey bacteria and predator protozoa. In the two instances the total amount of added food was constant, however in one instance food was added in infrequent large amounts; in the other food was supplied frequently in small amounts. The results were very different: where food was added regularly in small amounts, populations of prey and predators rose to a fixed level and remained there. Where more food was added with considerably more time between additions, populations of prey (and then predators), would build up, exhaust their food, die off significantly, and then repeat the process at the next food addition [Jensen].

Communities are controlled not only by the rate of energy flow, but also by the efficiency of conversion from one trophic level to another. Although there is a progressive increase in the percentage of lost energy from lower to higher trophic levels (due to respiration), consumers are more efficient in their use of a food supply as the trophic level increases [Allee]. On Isle Royale the efficiency of conversion from one trophic source to another is well illustrated. Each year 762 pounds of moose browse are consumed for each 59 pounds of moose, which in turn are consumed for each 1 pound of the moose-feeding wolf. On a mass basis this means that 7.7% of the vegetation is converted to moose mass and 1.7% of moose mass is changed into wolves. Thus, only .13% of the initially consumed vegetation ends up at the highest trophic level in the form of wolf mass [Mech]. Similarly, near Tilden Park, California, the seed (vegetation) crop weighed 200 times more than the mouse population it supported, and the peak mouse population weighed 17 times more than the predatory carnivore population [Pearson, 1964].

In some natural and artificial communities, a high rate of food flow (energy) into the system may cause a decrease in the population supported in that community. Such a result is caused by washout and may be illustrated by a pond in which food flows in through an inlet stream, and the pond is drained by an outlet stream. If the food is supplied by the inlet stream (say it's rich in untreated sewage), micro-organisms will grow in the pond at a rate proportional to the inflow of food, provided that the input rate does not exceed a critical point equal to the maximum growth rate of the organisms. When that point is passed organisms will be washed out of the community (the pond) via the outlet stream faster than they can be replaced through growth, and the total population and biomass will decline. This natural washout effect has been verified in an experimental bacterial culture [Herbert]. Even in such cases where the food supply remains constant (say growth of attached flora in the pond independent of the inflow rate) unattached prey and predators will be washed out proportionately to the inflow rate.

Predator-prey communities are regulated by energy amounts, flow rates, and conversion efficiencies. Yet though a basic factor, energy alone is only a partial determinant of community size and structure.

Abiotic Materials

Eutrophication and the concept of limiting factors have become so publicized that the influence of certain "non-energy" "non-animal" (abiotic) factors on communities is almost obvious. Elements and compounds, such as phosphates, nitrates, oxygen, and carbon dioxide all

limit the growth of certain populations when these substances are available in insufficient amounts. Some substances, although not essential to the populations, nonetheless have enhancing effects. For instance, a natural grass extract was found to stimulate ingestion of bacteria and reproduction among protozoa [Ducoff]. Some populations may require certain materials to aid their existence such as grit for the gizzards of certain birds or dust baths as a parasite inhibitor for some mammals.

The antithesis of materials available in only limited amounts are factors which increase to the point of becoming deleterious. Temperature, beyond certain limits, easily curtails growth, and the accumulation of metabolites from community secretions may be the principle cause of a low population level. Gause reported cessation of the growth of yeast cells before the exhaustion of the nutritive and energetic resources of the medium, due to inhibition caused by the accumulation of ethyl alcohol [Gause]. Garbage and pollution, both a type of metabolite accumulation, have begun to inhibit the human population growth in more densely populated areas.

Prey Availability and Susceptibility

The amount of predation which occurs in any community is in part controlled by the number of prey available to the predator and of those available, how many are susceptible to the predator's attack. Prey become available to a predator when there is no cover in which to hide or they are forced from this refuge to feed, migrate, or mate. For the prey, the essential protection is a secure biotope or hiding place. In Gause's classic experiments with *Didinum* preying on *Paramecium* all of

the prey were consistently destroyed by the predator until a refuge was introduced into the test tube which could be penetrated by the prey but not by the predator. When the refuge was present, prey maintained themselves within the refuge successfully emerging only when the predators, due to exhausted food supply, had become extinct [Gause]. In studies of such prey as marmots, wood rats, pocket gophers, and beavers, the victims of predation were those animals who were ill-situated and found themselves too far from suitable refuge at the time of enemy attack [Errington, 1946]. Indeed, the concept of prey security density developed to explain that the amount of predation on bobwhite quail was not a function of prey density, but rather related to the number of quail coveys which could not find suitable cover [Errington, 1934]. Similar instances of the relation between the amount of predation and the availability of a refuge have been reported for many natural populations. In reporting on the successful predation of oysters, Lewis Carroll noted that the only surviving oyster remained in a secure refuge.

> 'O oyster come and walk with us!'
> The Walrus did beseech.
> 'A pleasant walk, a pleasant talk,
> Along the briny beach . . . '
> The eldest oyster winked his eye,
> And shook his heavy head--
> Meaning to say he did not choose
> To leave the oyster bed.[2]

The predatory tiger-fish *Hydrocyon vittatus* forces small fish to remain almost exclusively in the cover of vegetation and shallow water until the prey are large enough to lose the interest of the predator [Jackson]. Kingfishers preying on several species of estuary and river

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[2]Lewis Carroll, Through the Looking Glass.

fish were found to secure a high percentage of those prey who used the cover of water inadequately by remaining near the surface, and only a small number of those prey fish who were comparatively unavailable through keeping to deeper water [White]. Similarly, in studies of water bug distribution in an initially predator free pond into which the insect-feeding trout was introduced it was found that the water insects, who were initially ubiquitous over the pond surface, were confined to the marginal "impenetrable" zones after the predator introduction. The predatory trout consumed all the insects except those who were able to locate in the vegetation refuge [Macan]. Finally, in studying wolf-moose interactions on Isle Royale, it was found that deep ponds, thick swampy brush, or deep snow could be used as "escape cover" by moose for either avoiding or outrunning wolves [Mech].

In laboratory studies with predator and prey mites who feed on oranges arranged in a complex maze of barriers and bridges, it was discovered that the notion of a refuge as escape cover could be extended to include a heterogeneous spatial distribution of prey given limited searching abilities of the predator. In the mite experiments, the predator would completely exterminate the prey until sufficient complexity (though not a predator impenetrable refuge) was introduced to the ecosystem to insure that there would always be some prey who would not be discovered by the predator [Huffaker].

Conspicuous is an accurate description of those prey who are most subject to attack by a predator. While the fact that a lack of suitable refuge can leave animals conspicuous and available to predation, conspicu-

ous prey are also those animals who are in some way different or more susceptible to predation.

When wolves hunted moose on Isle Royale, there were some cases when wolves commenced attack immediately rather than using their usual testing procedure to find if they could overpower a moose. From these observations it was concluded that wolves could detect inferiority or weakness from the behavior of a moose, and that such conspicuous behavior prompted an attack. In addition, examination of the remains of wolf-killed moose revealed that at least 39% of the moose had debilitating conditions [Mech]. These wolf victims were an example of susceptible prey, who although they had as much refuge as the rest of the herd, were singled out for attack. A similar situation of attack on a different, susceptible, and therefore conspicuous individual was reported in wild dog hunts against Thompson's gazelles in Africa. The dog rushed into the herd and attacked only the conspicuous gazelle that became panic-stricken and ran while the others stood their ground [Wright].

Susceptibility of prey is most obvious when it concerns size or defensive mechanisms. Many predators have a size range within which they will attack the prey. Tiger-fish, who swallow their prey whole, are limited to prey who are no larger than about 50% of their size [Jackson], while songbirds preying on insects tended to select the larger insects, presumably because they were more conspicuous [Tinbergen]. When prey are protected by some type of defense or covering, those prey with the least defense will be most susceptible and consequently subject to greater predation. For instance, it has been demonstrated that fish

consume mollusks who possess comparatively weak outer coverings more actively than those mollusks with stronger coverings [Ivlev].

Phagic Preferences of the Predator

Even if prey are available because of lack of cover relative to their density, and susceptible and conspicuous due to a debilitating condition, deviant behavior, proper size, or poor defense, predation is dependent on the disposition of the predator: given the availability and susceptibility of several kinds or sizes of prey, the predator must assert a perference. The most simple differentiation of predator's food preferences are between stenophagous predators such as the Everglades Kite *(Rostrahamus sociabilie)* which feeds only on the snail *Pomacea* [Salt] and the more typical euryphagous predator which feeds on a number of prey types. In central Tanganyika lions feed on Wildebeest (49%), zebras (15%), Thompson's gazelle (10%), buffalo (5%), and fourteen other types of prey [Wright]. Isle Royale wolves, although feeding predominantly on moose (75.9%) also consumed beaver grass, and snowshoe hare in amounts greater than 3% [Mech].

A more precise examination of predatory food preferences shows that they are determined by a number of factors including the preference for a type of prey, their abundance, accessibility, size, and the degree of satiation of the predator. Even further complexity is available through combinations of these determinants, although enough separate factors combined yield simply the type of prey upon which the predator normally feeds.

Small carnivores in California continually selected *Microtus* in preference to harvest mice *(Reithrodontomys)*. This clear example of preference as a determining feeding factor was most evident in months when *Microtus* and *Reithrodontomys* were present in approximately equal numbers: at these times the carnivores ate six times as many *Microtus* as harvest mice [Pearson, 1966].

Usually, predators feed on the most abundant prey type, and when one species shows signs of scarcity, the predators switch their feeding preferences [Slobodkin]. Resident hawks and owls in Superior Township, Michigan varied their diet with the density of various prey species. In the fall and winter when meadow voles were frequent and small birds were scarce, the predatory birds ate 82.7% voles and only 1.2% birds. Then, in the spring and when the voles were scarce and the small birds common, the predator's diet switched to 29.7% voles and 36.8% birds [Craighead]. Similar results between prey abundance and feeding preferences were reported for kingfishers feeding on at least twelve types of prey fish [White]. In working with carp as a predator of chironomid larvae, amphipods, freshwater isopods, and mollusks, it was found that when the density of all prey species was increased so the prey was still present in the same relative percentage amounts, the carp increased the percentage of preferred prey in their diet over what it had been before all prey types were increased. When the biomass of the preferred prey type was reduced, the predator's selection for that prey type increased until a lower threshold limit in the density of the preferred food item was reached. At that point the carp declined to feed on the favorite food type and switched to substitute prey [Ivlev]. Thus abundance of

a prey type in combination with the predator's initial preference for that prey will help determine the amount of that prey which is consumed.

Insect-hunting birds, particularly crested and great tits, were found to show a distinct preference for larger insects and larvae. When choice tests were given to the birds it was demonstrated that the controlling influence was a definite predilection for the larger prey [Tinbergen].

In working with carp and bleak, Ivlev demonstrated that the food type consumed by a predator is not constant, but undergoes substantial changes during his progressive satiation. In a predator like carp where taste is the guiding factor, the more satiated the animal is the more discriminating he becomes, until finally, when the carp is almost completely full, it eats only those food items which it likes the best. However, predatory fish like bleak, which are guided in their prey selection by a firmly instilled visual reflex reaction, do not alter the intensity of feeding on a prey type as they become more satiated [Ivlev]. Consequently, predators who depend on taste for guidance in their feeding are subject to alteration in prey preferences as they become less hungry.

Predator food preferences can have a dominant effect on communities. If the predation intensity is unselective for prey type, then the outcome of the competition between two prey species may be upset allowing either coexistence (which would not be possible without predation) or upsetting a tenuous equilibrium [Slobodkin]. Conversely, selective predation may alter the prey composition of a community in favor of the non-preferred prey.

Population Densities and Competition

1. Prey Density

The density of prey populations is responsible for both their own growth and for the support and size of the predators who feed on them. Predation which decreases the density of a prey community may have one of two effects. In one case, predation merely substitutes one mortality cause for another, and as a consequence produces no physiological change in the prey population. Predation on muskrats was at least partly inter-compensatory in that victims of predation simply missed becoming victims of some other agency [Errington, 1946]. Likewise, although the reduction of excess quail in Wisconsin was commonly brought down to carrying capacity by predation, the quail population was determined not by the amount of predation, but by the carrying capacity of the community: had predators not been present excess quail would have been removed by other factors [Errington, 1934].

In the other instance, predation significantly reduces prey survival at some specific time or age. In this situation, prey competition will be lessened with a consequent increase in fecundity or survival of fellow prey [Slobodkin]. For instance, heavy predator losses of young muskrats early in the breeding season was offset by the birth of extra litters or higher survival rates [Errington, 1946]. However, extreme or highly selective predation (which might remove only prey of one type) may cause a loss in the recovery rate of the prey. The recovery rate of six-spotted mites after severe attack from a predatory mite was much slower than the growth rate of the initial colony of the same size.

The reason was caused in part by predators leaving very few females. [Huffaker].

Predators must consume prey in order to survive. If a predator eats less than a fixed amount it must become smaller. Conversely, if it consumes more than this amount it will use the surplus for growth, reproduction, or both [Salt]. The consequence of predatory reliance on prey density forces a direct correspondence between the amount of prey and the breeding rate of the predator. The predator *Syrphus* distributed its eggs on leaves in Australia in direct proportion to the number of prey psyllids present [Clark]. In Barrow, Alaska in 1953, the growth of the lemming population could not keep pace with the immigration of avian predators, and as a result of this prey shortage there was a breakdown in the nesting of jaegers [Pitelka]. The direct correspondence between prey and predator density does not necessarily hold over the entire concentration span of the populations. *Woodruffia* preying on *Paramecium* exhibit an on-off threshold response to the prey density--when the density is below a certain level the predator encysts and becomes dormant. When the prey density is above the critical level the *Woodruffia* are completely unresponsive to any additional prey increase [Salt]. Likewise the predator *Syrphus* did not attack the prey psyllid nymphs until they reached a concentration of at least ten per leaf surface [Clark].

Through changes in migration, growth, natality, or mortality most predator densities are directly proportional to the number of prey (as mentioned earlier, some predators like *Woodruffia metabolica*, exhibit

a density independent threshold response to prey numbers). In many invertebrate populations, the frequency of encounter, which is much a function of prey numbers, is the major determinant of the amount of predation [Errington, 1946]. In studying avian predators on the larch sawfly it was found that predators on adult sawflies showed a direct numerical response to changes in the adult prey populations, predators on larvae to changes in the larval populations, and predators of both to changes in either the larval populations or to the populations of both larvae and adults [Buckner]. Density and composition of raptor populations in part of Michigan was dependent on the density of the prey species [Craighead]. In a study area of California, the correlation between prey and predator was illustrated by the fact that the number of *Microtus* eaten per month varied directly with the number of carnivores present [Pearson, 1966]. The link between number of prey and number of predators is a subset of the relation between the energy input to a community and the amount of biomass which can be supported.

Prey density may affect both the size of the predator population and the predator's feeding preferences. The fact that predators usually prefer to feed on the most abundant prey species was discussed earlier, but with some species this correlation is over-simplified unless a threshold concept is introduced. The percentage of predation by crested and great tits on the larvae of *Lepidopterous* and *Hymenopterous* insects increased in proportion to the relative abundance of these larvae until a point was reached where the percentage of prey type in the bird diet did not increase, even though the relative abundance of that prey type

rose. The surmise is that this limiting feeding action above a certain prey density is to assure that the predator has a sufficiently varied diet [Tinbergen].

2. Predator Density

While the abundance of prey usually determines the size of the predator population, the converse where the predators control the number of prey is unproven in many communities and the subject of much controversy in domestic and wildlife management schemes. In some communities the predator clearly regulates the number of prey. In Cultus Lake, Canada, the removal of predatory fish such as squawfish, trout, char, and coho produced a rapid and continued increase in the number of prey (sockeye salmon). Further investigation demonstrated that it was the removal of the predators, rather than indirect changes in other factors, which allowed the increase of the salmon [Foerster]. Another example of predator regulation of prey numbers is Barrow, Alaska, where avian predators deal "a stupendous blow" to the lemming population by reducing the mid-July lemming density to 1/10 or 1/20 of the early-June population [Pitelka]. In laboratory cultures of *Tetrahymena pyriformis* feeding on bacterial populations it was found that the gross feeding rate of the ciliates kept increasing until very low absolute concentrations of bacteria were reached [Curds, 1968a].

Though the three previous communities illustrated predators, controlling the numbers of prey, in other populations the predators don't have a chance. The increase in *Microtus* near Tilden Park, California was far above the capacity of the carnivores to control. Although, the

number of carnivores increased after the prey density grew, at no time of high *Microtus* numbers were the predators prey-limiting [Pearson, 1966]. In Binalong, Australia the percentage mortality of psyllid (prey) caused by birds, ants, and parasites tended to decrease substantially with increasing psyllid density. The concomitant increase in predation by *Syrphus* on the psyllid served only to compensate to some extent for the decrease in percentage mortality caused by the other predators [Clark]. In short, none of the predators was able to regulate the psyllid growth.

In some communities interspecific predator competition results from several types of predators all trying to consume a limited number of prey. An extreme example is the fact that the arctic fox, snowy owl, least weasel, red fox, short-tail weasel, short-eared owl, pomarine jaeger, glaucous gull, parasitic jaeger, and long-tailed jaeger all prey on lemmings near Barrow, Alaska [Pitelka]. More than one type of predator hunting in a community can result either in differences between the predators which serve to reduce the intensity of the interspecies competition, or disoperative relations between the species [Salt].

In studying competition among five species of predatory warblers in a coniferous forest, it was found that overt interspecific competition was avoided through the use of distinct hunting differences. Warblers differed in their food searching by looking for the same prey in different places (each species being confined to a somewhat separate zone in a tree), hunting for the same prey at different times, and by searching for different types of prey [MacArthur]. The selection of a different time, place, or food by the warblers minimized conflict. In another

example of avian interspecific competition it was observed that the movement of any one raptor influenced the movement of others, which tended to distribute the hunting pressure in proportion to the prey vulnerability. This alleviated direct competition for food among the raptors and allowed maximum hunting pressure on a parcel of land [Craighead].

More often, an avoidance of competition between differing predators does not take place for one of two reasons. First, because there is no alternative prey species available for one of the predators to turn to (as is the case with the predators on lemmings in Alaska), and second, because a single prey species is the preferred food of two or more predators [Salt]. In such cases competition and strife arise between the predators. In Alaska, jaegers persistently harassed short-eared owls during high predator population densities. This harassment of a competing predator possibly caused nest losses among the owls and often forced them to fly distances well beyond the reasonable limits of a local living territory [Pitelka]. Similarly, in East Africa interspecific strife operated as a minor mortality factor. Hyaenas were frequently caught and killed by lions who were defending their prey [Wright].

In addition to interspecific competition among predators, increased predator density can exert an effect on the community through either intraspecific cooperation of competition. Presumably there exits a critical predator density below which the effects are either nonexistent or salubrious and above which the population density becomes inhibitory.

Cooperation among individuals of the same species is apparent for those predators which hunt in groups. Wolves hunting moose on Isle Royale had to work as a team to affect a kill. For instance, when attempting to separate a calf from the cow moose, some wolves would harass the cow while others remained beside the calf. As soon as the cow would charge a wolf, those guarding the calf would close in. It was only rarely that a lone wolf could kill a moose, and then it seemed likely that the moose had already been weakened by other wolves [Mech]. During porpoise predation on a school of sardines, the porpoises cooperated by aligning themselves in a crescent and swimming through the school while feeding, during which time other porpoises would dive beneath the sardines or continually circle the school to keep the prey from diving or dispersing [Fink]. Some marine birds such as pelicans and cormorants search in groups even though the attacks are individual. The cooperative effort provides a larger perceptual field in searching for the prey [Salt].

Beyond a certain density for cooperative species, and presumably at a different density for more individualistic predators, increasing numbers of predators produce increasing inhibition and competition. An excellent example of intraspecific competition for a limited resource occurs with the tiger-fish, *Hydrocyon*. The fact that the tiger-fish consumes its prey whole means that the larger predators eat both large and small prey fish, while the small *Hydrocyon* are confined to swallowing only small prey. Therefore, at high predator density (relative to prey availability) the large *Hydrocyon* eat both the large and small prey,

forcing the smaller predators to turn to an insect diet [Jackson]. In fixed or sedentary predators space is likely to be in short supply. A fixed predator (such as an intertidal anemone) who is surrounded by a group of his fellows is less likely to find prey surviving the trip through the encircling competitors and entering his "trapping area" [Salt]. The feeding rate of the ciliate *T. pyriformis* on bacteria decreased as their own density increased [Curds, 1968a] and *Woodruffia* preying on *Paramecium* hunted less and captured less prey per unit time as their own numbers increased [Salt].

Although increasing predators may be either prey-regulating or non prey-regulating an increase in the predator's density may have more subtle effects. At higher carp densities the individuals began to interfere with each other by trying to seize the prey which was another's share. As a result, each fish begins to choose its food less fastidiously, and thereby broadened its "feeding preference" over what it had been at a lower predator density [Ivlev]. In predation by *Woodruffia*, an increase in the predator's density resulted in a decrease in the number of prey eaten per unit time, in the time spent hunting, and in the number of *Paramecium* consumed per new *Woodruffia* produced. In other words, the intensity of predation decreased with increasing predator density [Salt].

3. Predator-Prey Interaction

The ability to explain and demonstrate ecological oscillations and cycles has long been pursued as the vindication of a mathematical ecologist. In the simpliest ecosystem, where one predator feeds on one

prey species, their coactions are theoretically related in the following manner: an initial increase of the prey density results in an out-of-phase increase in the number of predators due to either reproduction, immigration, or decreased predator mortality. At some point the prey population reaches the maximum density which can be supported by the vegetation, and with the increased predator population still feeding on the prey, the prey population decreases. Finally, the shortage of food (decreased prey density) forces a decline in the predator's density. The remaining prey, no longer subject to intense predation, increase and so the cycle begins again [Slobodkin, Gause]. Although this description is a great oversimplification, and unjustified in its insinuations as to cause and effect, cycles in both natural and laboratory communities are apparent.

In the laboratory, cycles as described above have been demonstrated for such predator-prey pairs as ameba-bacteria, *Woodruffia-Paramecium* and predator and prey mites [Drake, Salt, Huffaker]. Some laboratory communities have been able to maintain cyclic population densities for over four years *(Blattisocius-Anagasta)* [Flanders]. Obtaining enduring laboratory oscillations often requires more than just placing predators and prey in the same bottle. In Huffaker's work with mites and Flander's work with moths, considerable experimentation was necessary before a suitable "prey refuge" was developed to prevent the annihilation of the prey when the predators reached their peak density. Basically, so long as all the prey are not available and susceptible to predation, but yet

there remain enough prey who are so disposed to continually support the predators, some type of laboratory oscillation is possible.

The longest natural cycle has been the report of the variation in the number of lynx in Canada. Those cycles, based upon the return of furs to the Hudson Bay Company, have persisted for over 200 years [Elton]. Other notorious natural oscillations occur among lemmings and *Microtus*, where local peaks of these prey are reached every three to four years. Even more dramatic is the 9-10 year cycle in the number of ruffed grouse and snowshoe hares. These prey variations are ubiquitous in the boreal forests and therefore suggest some cosmic regulator.

The existence of cycles in the numbers of predators and prey seems easier to demonstrate than to explain. At one time or another it has been maintained that the 9-10 year grouse and hare cycle was caused by shock disease, forest fires, sunspots, ozone, and finally that it wasn't really occurring at all but just the misinterpreted results of random variation. The 3-4 year lemming-*Microtus* cycle has been attributed to self-regulation from overcrowding, the buildup of marginal genetic traits, the persistence of genetically aggressive territorial animals, and interactions with phosphorus, vegetation, and perma-frost. Only one explanation of the cause of these 3-4 year cycles considered predators as a regulator. This theory is that predators themselves do not destroy the "top" of the mouse cycle, but that they bring the prey down to very low levels and by persisting in predation (made possible by a few catchable prey and the existence of some supplementary "maintenance" prey) retard the recovery of the prey. All this predator regulation

occurs after some other influence has helped to remove the peak mouse population [Pearson, 1966]. Thus in natural vertebrate cycles it appears that although predators may assist, they are certainly not the sole cause of the periodic oscillations.

In a more controlled investigation of cyclic phenomena between the prey *(Anagasta)*, a predator *(Blattisocius)* and a parasite *(Exidechthis)* it was found the predators and prey did in fact regulate their own cycles provided that (1) there was an overlapping of prey generations, insured by renewed allotments of food, which permitted continuity in predator reproduction; and (2) that there was sufficient refuge for prey to insure that deaths equalled births annually, with the replacement of parents by an equal number of progeny [Flanders]. The previously mentioned laboratory cycles in *Woodruffia* and ameba are also examples of predators and prey regulating their own numbers.

Laboratory verification of the existence of prey-predator regulated cyclic phenomena does not insure that the oscillations continue indefinitely. For instance, oscillations between ameba feeding on bacteria who were supported by glucose continued for two weeks. At that time a steady state was attained in which the numbers of prey and predators stopped fluctuating and remained constant [Drake]. Possibly prey were being produced at just the rate they were being consumed by the predators and washed out of the system. Similarly the predators were reproducing at a rate just fast enough to replace themselves. In working with laboratory cycles of the azuki bean weevil and the parasitic braconid wasp (in which the cyclic community was maintained for

over six years) it was found that after the twenty-fifth generation the fluctuations in population density diminished in violence and approached a relatively stable level. Utida was able to mathematically predict such damped oscillations in an interacting host-parasite system [Utida]. Such a parasite-host community is similar enough to predator-prey communities to propose that similar damped oscillations may be possible.

When considering the constraints of population density on communities of predators and prey, the limitations on growth were well summed up by a famous Porkypine who said, "We have met the enemy and he is us."

Conditions and Talents of the Populations

Communities of prey and predators are affected by the conditions and talents of their members. A prey individual who is diseased or unfit is more subject to attack, and several predator traits have a notable impact on the prey. For example, the protozoan *T. pyriformis* increases its feeding rate only to a fixed level, and then does not consume prey any more rapidly, regardless of their concentration [Curds, 1968a]. The ciliate's inherent self-restraint is certainly salubrious for the bacterial prey. Another microbial habit is that the predator *Woodruffia* uses no more than one-quarter of his time for hunting, spending most of his time in digestion, assimilation, formation of new protein, and cell division [Salt]. Obviously the predator's size influences the community. The tiger-fish, which must swallow its prey whole, forced it to consume prey who are less than 50 percent of its own length.

This condition insures that large enough prey are immune to predation [Jackson]. When the praying mantes consumes flies, though the time taken to eat each prey and the weight of each fly taken were unaffected by the mantes' hunger, the hungrier the mantes was, the greater was the distance to the prey which would elicit a stalking or striking response from the predator [Holling].

One of the predator's most important talents is his ability to capture prey. The rate of capture is a function of prey availability (prey density, distribution, and concealment), the predator's searching rate, the frequency of attack, and the percentage of attacks which are successful [Salt]. The efficiency of the predator in consuming prey can be the most influential determinant in establishing cyclic populations. In Utida's weevil-wasp (host-parasite) system, fluctuations were terminated not because of environmental homogeneity, but due to the inefficiency of the parasite in finding a host. Parasites who were either extremely inefficient or efficient would cause extermination by starving to death or annihilating their hosts [Utida]. The "inefficiency" of *T. pyriformis* was demonstrated by the fact that even when the ciliates had reached a maximum population of 40,000/cc and cleared the medium of any visible bacterial (prey) turbidity, there was still an ample supply of food organisms remaining [Ducoff].

Predator attack is a complex process varying with both the type of predator and prey. One particular method of pursuit has been postulated for several species of predatory birds feeding on insects, in

which specific prey characteristics are assimilated by the birds in a kind of learning process. By this method, each bird adopts a "specific searching image" which assists the bird in efficiently catching one particular type of prey. It was this "searching image" and not prey availability, food preferences, or feeding techniques that determined the individual differences in the birds' diets [Tinbergen].

Predators are not overwhelmingly impressive in their ability to capure prey. Although Ospreys have been reported to be 89% efficient when diving for fish [Lambert], most predators cannot maintain that high an average. Mantids were able to capture only 13% of flies that were airborn, compared to a 63% success in capturing grounded flies [Holling]. *Woodruffia* eating *Paramecium* are not much better than the mantid: only 14% of the attempts made on the prey resulted in a successful capture [Salt]. Finally, Isle Royale offers a detailed breakdown of wolf efficiency. Wolves detected 82% of moose within range; of those detected they tested 59%; then wolves were able to wound only 9% of the moose they tested. Since the wolves killed only 6 moose out of the 77 they tested, they had an overall efficiency of only 7-8% [Mech].

Results of Predation

Predation may alter the growth, natality, mortality, or migration which occurs within a community. In addition, the aftermath of predation may affect such things as an individual's size, shape, feeding rate, or well-being. There may be a substantial difference between prey biomass being converted to new predators or just enlarging the existing predators.

Size alterations as the result of vicissitudes in the food supply are easily observed in microbial communities. Both Gause and Beers noted that the predator *Didinium* became smaller as the food supply declined [Salt]. Specifically, at low food levels the *Didinium* continued to multiply intensely at the expense of a vast decrease in the individual's size [Gause]. Alternately, during the growth phase of ciliates feeding on bacteria the number of predators repeatedly fluctuated, while the biomass increased steadily until the available food was exhausted [Curds, 1968b]. The fact that a predator or prey individual may vary its size does not imply that his impact on the supporting trophic level will alter accordingly.

Continued predation may, through changes in the predator's own density or the concentration of the prey, affect the feeding rate of the predator. The predator *Woodruffia* decreases its individual feeding rate on *Paramecium* as its own density increases [Salt]. Likewise, the fact that beyond a certain ciliate concentration a further increase in the number of predators has little effect on the number of viable bacteria consumed, suggests that the feeding rate of an individual ciliate decreases as the total predator biomass increases [Curds, 1968a]. The effect of increasing prey on a predator is likely to be the reverse of the previous examples. As the number of *Microtus* (prey) rose in a California field, the carnivores present almost immediately increased their daily consumption of mice, although several months elapsed before many more carnivores appeared. [Pearson, 1966].

Since most natural predator-prey communities are neither isolated nor impenetrable, predators and prey may migrate to escape enemies or

seek food. When the food is exhausted many populations have the choice of moving elsewhere. Some avian and ungulate populations have yearly migrations based on the impetus of food, space, or climate. The concept of migration need not be of movement over relative large distances, but may be the result of normal peripateticisms within a sufficiently heterogeneous system, as was the case with Huffaker's mites in a complex food-vaseline barrier community.

Predation, as a regulator of the amount of food, will directly affect the fecundity or natality of the populations. As the density of the prey drops there will be more available resources per prey individual. This increase in welfare factors (like food or refuge) for each prey will result in a higher birth rate. This was illustrated earlier with Errington's observation that predator decimated muskrat populations produced more litters, returning the population to its original size. As a consequence of continuing predation, the predators become healthier and suffer decreased mortality or increased natality. The increase in protozoan populations is due to an increase in the division rate as opposed to a mortality change [Salt]. Alterations in predator natality may depend on both the amount and duration of an increased prey supply. The oviposition rate of predaceous phytoseiid mites was relatively unaffected by anything but their immediate nutritional history, while the number of predator eggs laid per day was a direct function of the number of prey eaten, up to the maximum oviposition rate [Chant].

If predation has diminished the food supply and emigration is not possible, the only alternative for most predators is an increase

in their mortality rate. Mortality, however, becomes an amorphous concept in describing some ciliate populations. *Woodruffia metabolica* suffered no true mortality, but would become dormant, ready for resurrection at a later time, by encysting [Salt].

Finally, due to the desultory aspects of food supply and abiotic materials, prey populations often violently oscillate in their numbers. The introduction of an adequate predator may reduce the severity and period of these oscillations. Avian predators at Barrow, Alaska who are not themselves culpable for the fluctuations and cycles in lemming numbers, nonetheless successfully depress lemming populations, at least in the upswing portion of the cycle, to the extent that the fluctuations of the prey are dampened and protracted [Pitelka]. Predation has also been found to alter the normal outcome of competition between two prey species. When brown and green hydra were raised in the same tank, the green hydra invariably eliminated the brown. By removing a fixed percentage of the newborn animals of each species (a form of unselective predation), both populations were able to persist in the same tank [Slobodkin].

Conclusion

It was initially cautioned that an academic differentiation of those determinants controlling a community of prey and predators would be arbitrary if not misleading. That caveat should now appear obvious: each influent discussed is tenaciously linked to every other factor in the community. The number of refuges depends on the prey density which

depends on the feeding preferences of the predator which depends on the results of past predation and so on and so forth. In an attempt to escape part of the morass of interrelations, the next section observes the effect of isolated laboratory influents on a select community of predators and their prey.

CHAPTER II

THE EXPERIMENTAL COMMUNITY

The time has come for those of us who are impressed by our own confusion to abandon natural communities; and guided by trepidation to search for causes and effects in a less complex place. Simplicity, lacking the fortitude to provide its own justification, must compensate with information of acuity, quantity, and accuracy. But although humble and even defensive about a laboratory predator prey community, we need not be apologetic: the losses of reality and generality are well replaced through gains in understanding and certainty.

Design

The purpose of these experiments was to observe the numerical, rather than the functional results of the interaction in a predator prey community consisting of one prey food source, one prey species, and one predator species. Accordingly, four measurements were repeatedly made at different times in the community's history. The examined variables were: (1) the amount of "vegetation" in the community; (2) the concentration of the prey; (3) the concentration of the predator; and (4) the rate at which the food was flowing into and out of the community.

In order to simplify the community, several influents were eliminated or held constant. First, since there was only one predator species, one prey species, and one food type, there was neither interspecific competition nor any food preference option available to the predator. Second, abiotic materials such as nutrients, minerals, and oxygen were kept present in abundance to insure they would not become limiting. Similarly, so as to avoid abiotic inhibition temperature and pH were maintained comfortably constant. Third, since the community was kept uniform and closed there were neither refuges for the prey nor was there any possibility of immigration. Finally, it was assumed that the results of predation would be realized in the alteration of the number, size, or mass of the species rather than a functional response such as the predators being in better spirits when food was easily available.

While refuges, migration, interspecific competition, and food types were held constant and abiotic factors were non-limiting and constant, several factors were varied in the community. The experimenter had the ability to directly alter only two influents. First, he could change the concentration of the food-vegetation (provided as sucrose concentration) and second, he could alter the rate at which food was supplied to the community. As a result of these controlled variations, a number of other factors altered. They included (1) the rate at which the predator and prey were washed out of the community; (2) the conditions of the populations; (3) the densities of the populations and the resulting intraspecific competition; and (4) the results of the predation. As noted earlier, some of these variables were not

measured directly, it being assumed that their effects would be reflected in numerical and size alterations.

Two types of experiments were conducted to observe predator-prey interactions. Batch experiments, in which a fixed amount of food at a known initial concentration was provided in a sealed reactor, were used to observe behavior in a non-replenishing food-limited environment. These experiments, since they did not provide a continuing supply of food, permitted the growth of the populations to only a certain concentration followed by a consequent die-off due to depletion of the food supply. The essential ecological implication of this series of experiments was that a continuing supply, rather than a fixed amount of food, is necessary for continuing support of animal populations.

The second type of experiment, continuous runs, provided a constant and continuing flow of food into the community. In these replenishing food-limited environments, where sterile prey food of uniform concentration was pumped into the reactor at a constant and controllable rate, the populations could grow and be sustained at a certain food-limited level. The continuous reactor or chemostat, in which there is an inflow of food and an outflow (through an overflow tube) of food-depleted media and "washed-out" prey and predators (since they do not attach themselves to the reactor), is similar to a water ecosystem. A lake which receives water input through a river or precipitation, and maintains a constant volume by overflowing through an outlet stream, has basically identical flow characteristics as our continuous predator-prey reactor.

Methods

1. Species

The prey was a heterotrophic bacterium, *Aerobacter aerogenes*, obtained from Professor L. L. Kempe, Department of Chemical Engineering, University of Michigan. The bacterium was stored on refrigerated slants of Difco bacto nutrient agar. Before use, a tube of sterile sucrose was inoculated with the bacterium, diluted with sterile water, then plated on the same agar. All colonies appeared uniform, but for certainty new agar slant tubes were started from a single colony as a standard uniform source of the prey.

The predator used was the protozoan, *Tetrahymena pyriformis*, Syngen 1, Strain D (D-20693), obtained fresh for each run in axenic media from Dr. Sally Allen, Department of Botany, University of Michigan (Figure 1). This protozoan was ideal for our purposes in that it could thrive by feeding exclusively on *Aerobacter aerogenes*. To insure that the ciliate could not grow on the sucrose food media which supported the prey, axenically cultured *T. pyriformis* 1/D was inoculated into a reactor containing only the sterile sucrose food media. Counts of the number and size of the ciliates per mℓ. were taken for several days. The fact that the initial concentration after inoculation did not increase assured that the predators could grow only on the prey and not on the prey's sucrose food media.

2. Food Media

The food media was designed to fulfill three requirements. It had to provide a "vegetation-derived" energy source for the prey but

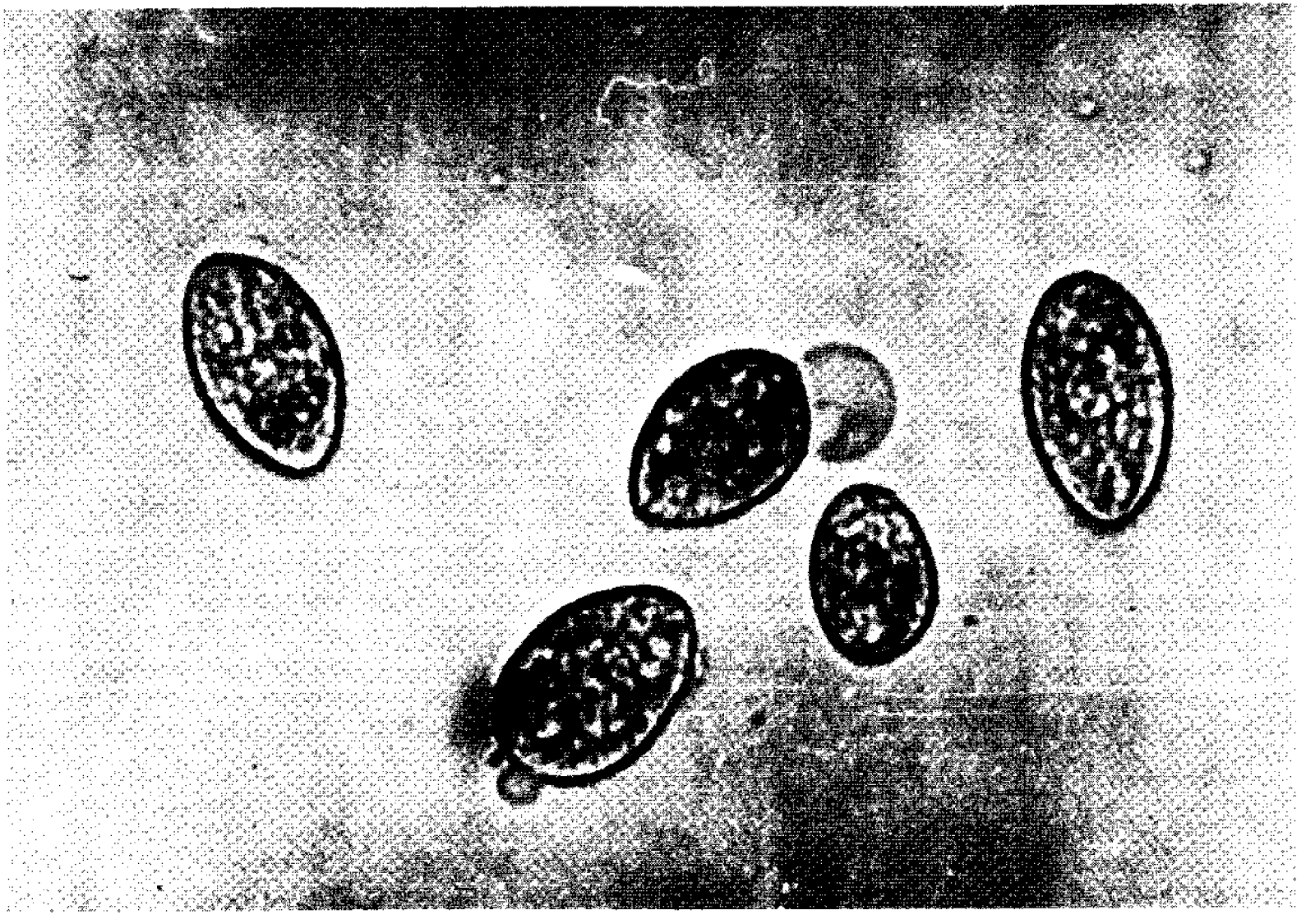


Figure 1: The predator, *Tetrahymena pyriformis* 1/D.

not the predator; it had to provide abiotic materials to insure that food, rather than some other non-living factor, would limit the growth of the prey; and it had to have the capacity to absorb secretions of the species so that these metabolites would not inhibit growth.

Accordingly, four types of ingredients were used. The first was analytical grade sucrose ($C_{12}H_{22}O_{11}$) in solution in distilled water. In order to vary the amount of food available to the prey, the concentration (in mg./ℓ.) of sucrose could be varied.

The second ingredient was a type of vitamin for the predatory protozoan. It was found that the presence of Cerophyl, a powdered form of dehydrated cereal grass leaves (Cerophyl Laboratories, Inc., Kansas City, Mo.), would aid the *Tetrahymena* in reproduction and prey ingestion [Ducoff]. Cerophyl extract was prepared to a final concentration of 30-35 mg./ℓ. (measured as sucrose carbohydrate) by boiling 120 g. of Cerophyl in 800 mℓ. of distilled water, filtering first once through Sargent #500 paper, then twice through Whatman #5 paper, and finally adding 40 mℓ. of the autoclaved extract to a 121 ℓ. food jug. Since it was found that the prey bacterium could use the Cerophyl extract as a food source, the carbohydrate concentrations of both the Cerophyl extract and the sucrose were used in determining the final carbohydrate concentration of the food media.

The major ingredient was distilled water, buffered to a pH of 7.3 with Sorensen's phosphate buffer. The stock buffer was prepared by dissolving 72.62 g. of KH_2PO_4 in 1 ℓ. of distilled water, and 76.01 g. of anhydrous Na_2HPO_4 in 1 ℓ. of distilled water. Approximately 42 mℓ.

of stock KH_2PO_4 solution and 105 mℓ. of stock Na_2HPO_4 solution were added to 12 ℓ. of distilled water to bring the pH to 7.3.

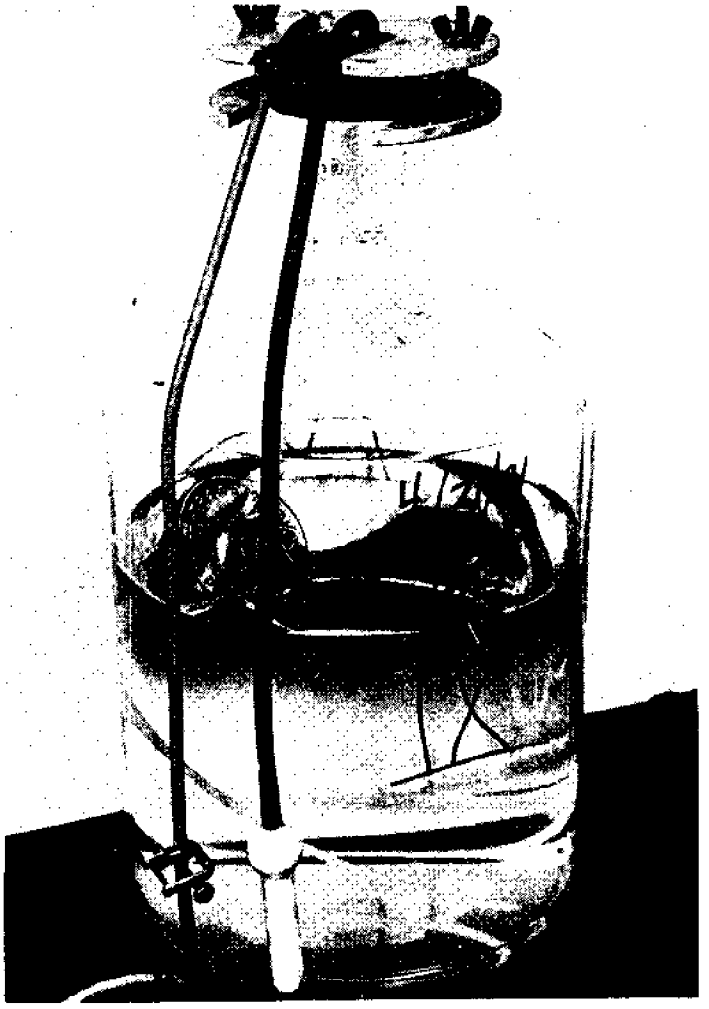
The final ingredient was a stock solution of inorganics as follows:

(in g./ℓ.)

	stock solution	final medium
KCL	7.48	0.037
NaCl	23.40	0.117
$MgSO_4 \cdot 7H_2O$	26.64	0.123
$CaCl_2$	2.78	0.014
$FeSO_4 \cdot 7H_2O$	2.78	0.014
$MnCl_4 \cdot 4H_2O$	0.40	0.002
NH_4Cl	38.22	0.191
EDTA	10.00	0.50

NaOH was added to the stock inorganics until the pH was 7.3. The stock inorganics were added to the final media in the proportion of 5 mℓ. of stock inorganics to 1 ℓ. of food media.

The chief difficulty in preparing and handling the food media was that it had to be autoclaved to insure that it was completely sterile. It was found that the completed food media, if allowed to sit for several days after sterilizing, would turn cloudy with bacterial turbidity if any contaminant organisms were present. Accordingly, to insure that the media was contaminant free all food jugs and food-filled reactors were allowed to sit several days before inoculating our predator and prey organisms.



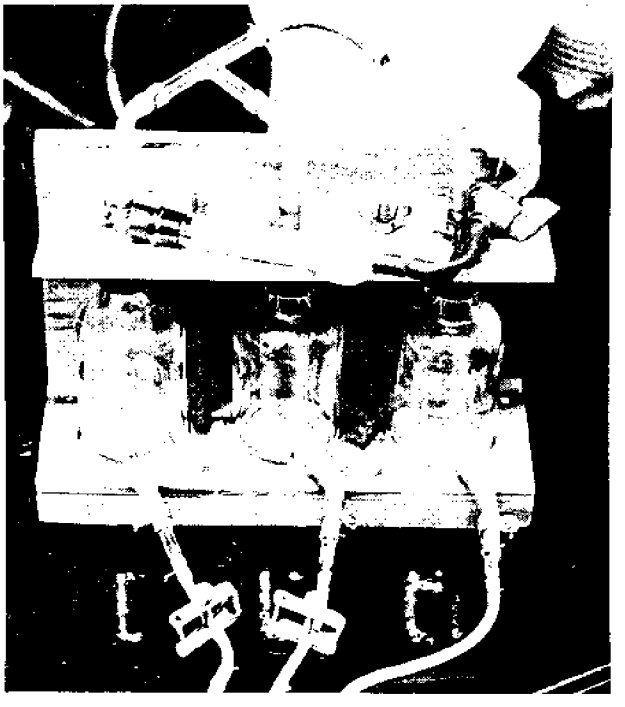


Figure 2: A completed sterile food media jug containing sucrose, Cerophyl extract, inorganics, and buffered water (above). The "jug-making apparatus" used to mix the ingredients in a cool sterile environment (right).

The food media was prepared and stored in sterilly sealed 12 ℓ. pyrex jugs. Air was allowed to enter the jug through a cotton plug, and the media was pumped out through sterile rubber tubing (Figure 2). It was found that if all the ingredients were mixed in the jug, and the jug then autoclaved for the necessary four hours to insure sterility, insoluble precipitates would form at the high temperatures and the sucrose would begin to oxidize. To avoid this problem the ingredients were autoclaved separately and then pumped through sterile tubing into the jug, where they were mixed at room temperature. Once we began using this "jug-mixing apparatus" (Figure 2) the number of jugs contaminated or containing precipitates dropped to zero.

To assure as much uniformity as possible between the jugs, the sucrose, Cerophyl extract, inorganics, and buffer solution were all made in stock solutions and then incrementally added to the jugs. In addition, autoclaving temperatures and times were kept nearly constant.

3. Reactor Design

The batch reactor (Figure 3) was constructed from a 2 ℓ. flat-bottom boiling flask. The flask contained a magnetic stirrer to insure thorough mixing, and was immersed in a water bath kept at 25° C. ± 1°C. Air was bubbled into the reactor through a fritted glass tube, after being passed through a distilled water bubbler to provide humidity (thus preventing volume loss in the reactor) and a drying tube of sterile cotton to screen out any contaminant organisms. Effluent air was released through a piece of glass tubing connected to another sterile cotton tube located at the top of the flask neck. The sterile cotton

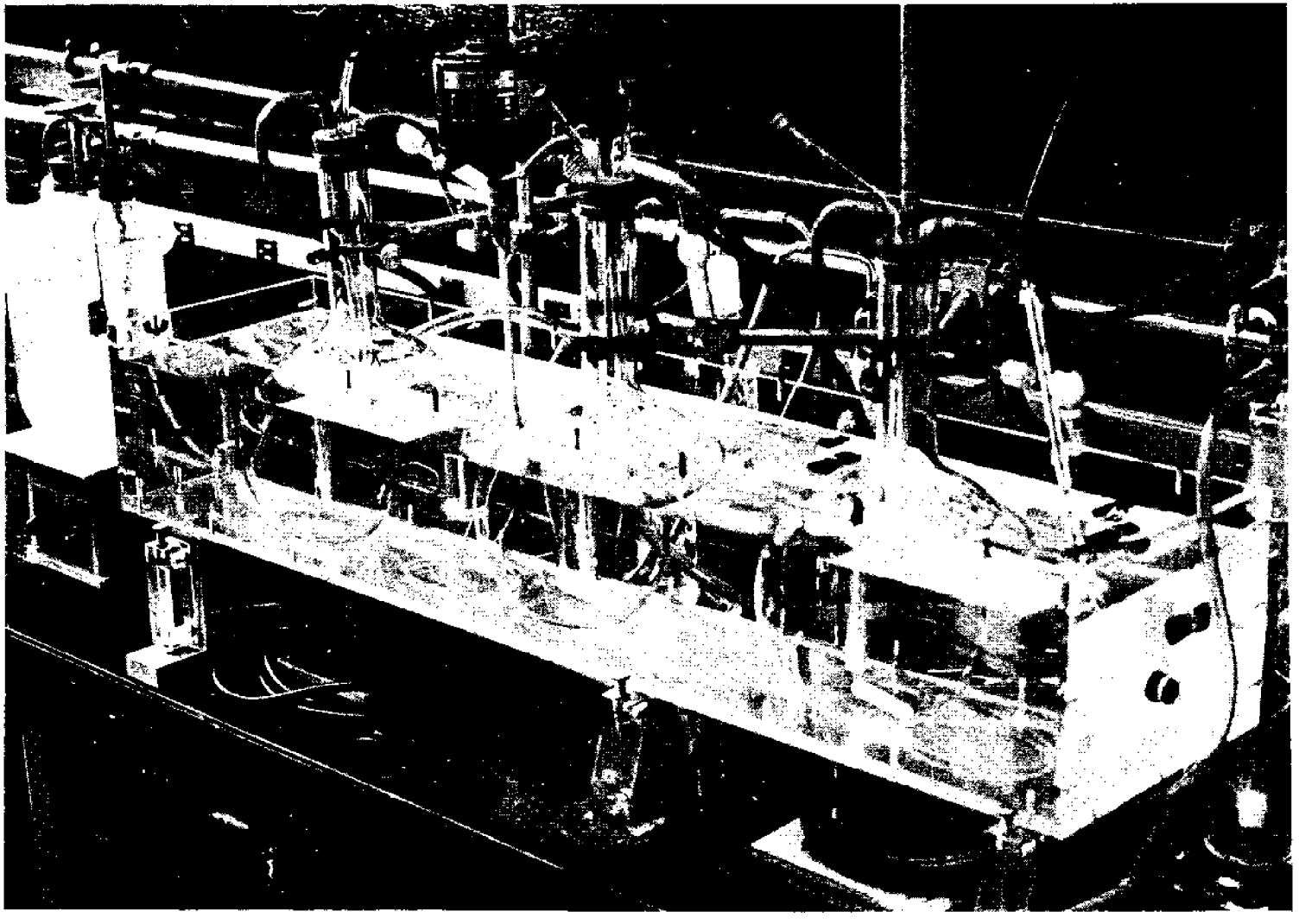


Figure 3: A series of temperature controlled batch reactors used for non-replenishing food-limited studies.

in both the influent and effluent air lines prevented any contamination from entering the reactor. Air was bubbled into the reactor at 0.75 liters per minute, providing non-limiting dissolved oxygen and mixing assistance. The reactor was penetrated by two pieces of capillary tubing, each capped on the exterior with a rubber septum. The reactor was inoculated with the animals by use of a sterile syringe and needle, which was pushed through the flamed rubber septum. The inoculum was injected from the syringe, through the capillary tubing, and into the reactor. Likewise, samples were removed from the second septum-covered capillary by a syringe and sterile needle. The sample capillary tube withdrew the media from the bottom of the reactor just above the magnetic stirrer. The practice of using sterile needles and flaming the septum before penetration made the entrance of contaminant species unlikely. The contents of batch runs VIII and IX were plated on agar at their termination, and the fact that all colonies appeared uniform reassured us that the inoculating-sampling technique prohibited contaminant organisms.

Batch runs were conducted in two phases. After the food-filled reactor had been allowed to sit to insure the environment was sterile, pure prey were inoculated and samples were removed and analyzed until the prey had reached the maxium food-limited density. At that point the protozoan predator was introduced and a second phase of sampling began until the predator had reached its peak density. Shortly after this point the batch run was terminated. Batch runs typically lasted for 4-5 days, not including the initial idle days to insure reactor sterility.

The differences between the batch and continuous reactor (Figure 4) were small but significant. The continuous reactor was temperature controlled by a stainless steel coil and a dial thermometer which were immersed in the reactor. Temperature-controlled water was then pumped through the coil so that the reactor was maintained at 25° C. ± 1° C. Air was bubbled into the reactor at 0.75 liters per minute after being sterilized by passing through a sterile cotton plug. Unlike the batch reactors, thorough mixing was obtained exclusively through the use of air being forced through fritted glass into the bottom of the reactor. Effluent air was removed at the top of the reactor after passing through a high efficiency gas condenser (to prevent volume loss) and then through a sterile cotton plug. As in the batch reactors, the use of sterile cotton plugs on both the influent and effluent lines prevented the penetration of contaminant organisms.

Food media was supplied to the reactor at a constant rate from the sterile food jugs. From the jugs, tubing ran through a peristaltic pump to a needle which penetrated the reactor. By varying the pump speed the experimenter was able to control the rate at which food entered the community. A rough indication of food inflow rate was obtained by noting the drop rate from the influent food needle. Twice during the experiments the influent food needle became clogged by masses of bacteria growing up the food line from the reactor. When this occurred the needle was replaced with a sterile needle by the use of flaming and autoclaving.

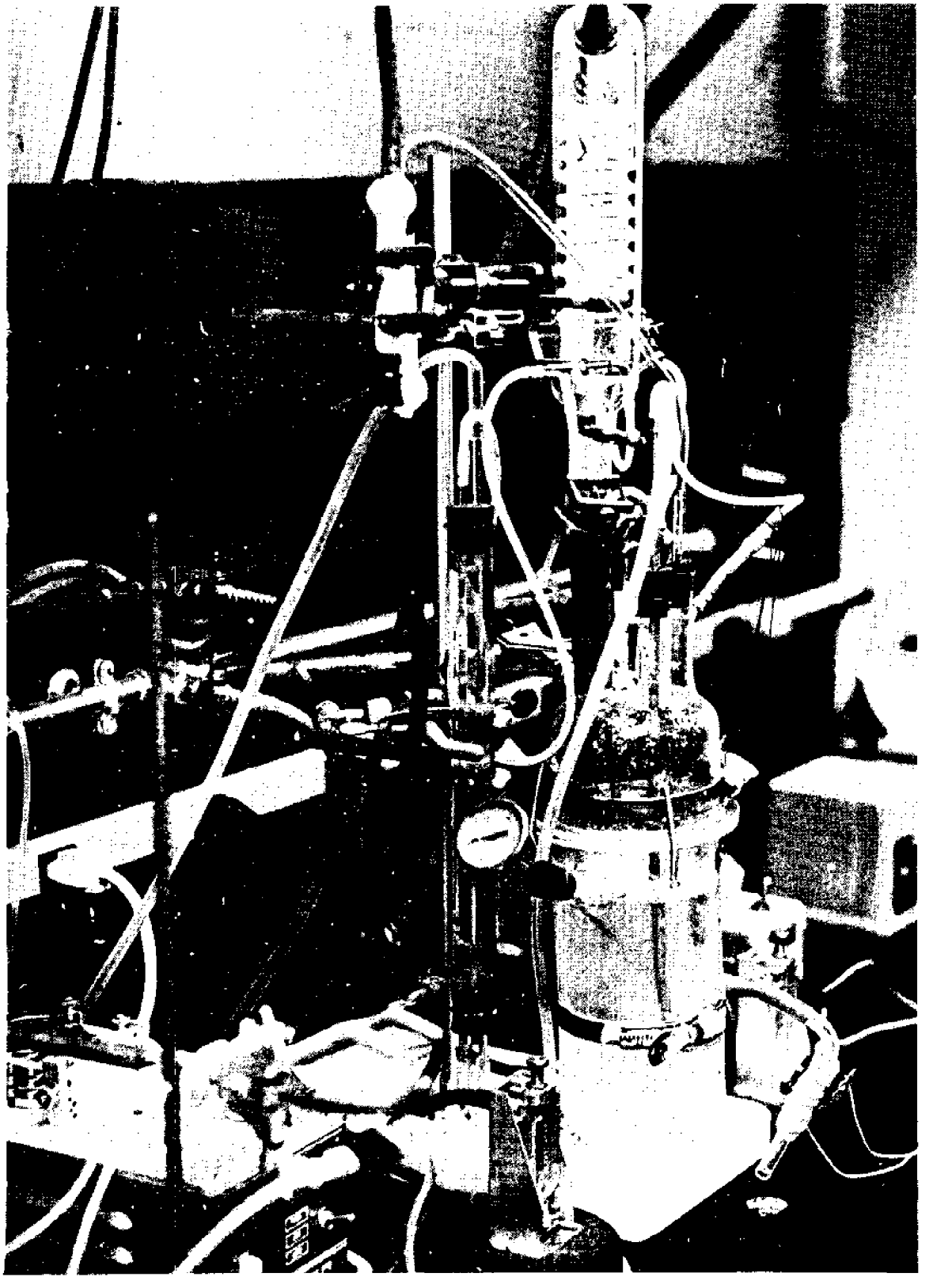


Figure 4: The continuous reactor used. A constant but limited supply of food was continually pumped from the food jugs into the reactor.

Since media was continually being pumped into the reactor, an effluent tube had to be provided for excess liquid to flow out of the community. To accomplish this an "L" shaped overflow tube penetrated the side of the reactor. Externally, the effluent tube was wrapped with an electric heating tape and maintained at a temperature which just missed boiling the effluent liquid as it passed through the effluent tube on its way to the waste jug. Internally, the overflow tube allowed a volume of 1.042 ℓ. of food media.

The continuous reactor, like the batch reactors, was sterilized by autoclaving, pumped full with sterile food, and allowed to sit to insure no contamination had entered. It was then inoculated simultaneously with the predator and prey by means of a sterile syringe through a stopper in the top of the reactor. The food pump was then turned on and the experiment commenced. Samples were collected in 30 mℓ. portions (in two tubes at 15 mℓ. apiece) by turning off the effluent heating tape, allowing the external effluent tube to cool for several minutes, and then collecting the reactor overflow in a graduated centrifuge tube. As soon as we had completed taking the sample the effluent heating tape was again turned on. The continuous reactor ran for a period of 83 days, using 7 different flow rates of food media into (and out of) the community.

4. Censusing

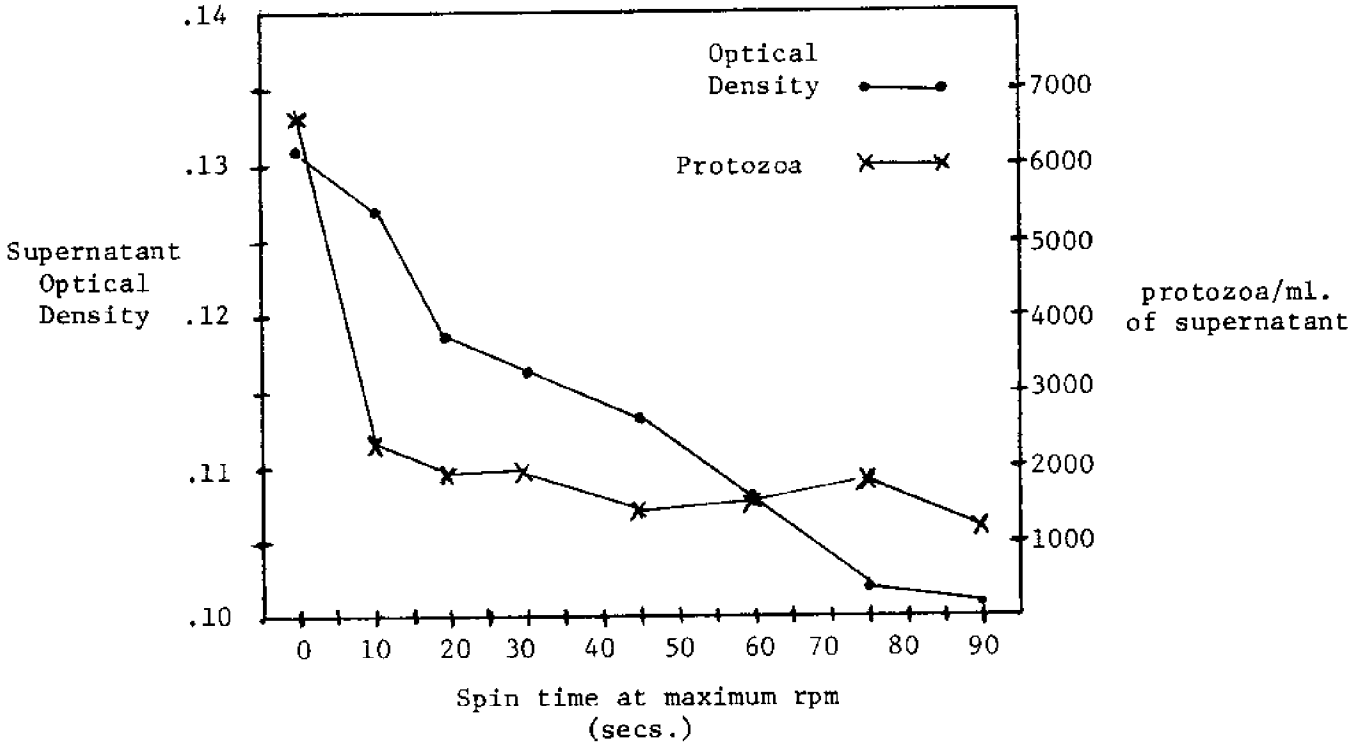
The frequency of sampling varied with the type of reactor and the rate of washout (food pumping rate) or phase of growth. In the continuous run a sample was usually taken every three hours, twenty-four

hours a day, seven days a week. During the prey growth phase of the batch runs, however, samples had to be taken once every forty-five minutes; and at the lower pumping rates in the continuous run samples were reduced to one every four hours.

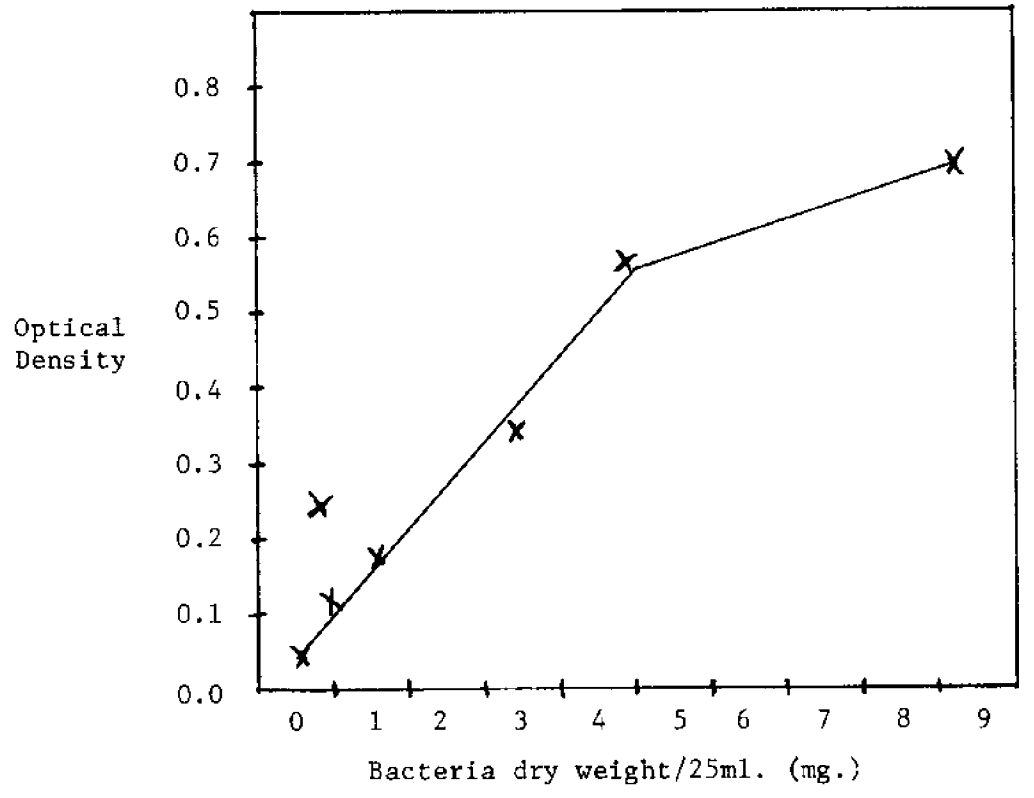
Once the samples were withdrawn (by syringe for batch, by overflow collection for continuous) the censusing procedures were identical for each type of run except that in the continuous run the amount of time it took to collect the 15 mℓ. sample from the overflow tube was recorded. This collection time for the 15 mℓ. sample gave the flow rate into and out of the reactor. Since batch runs had no food being pumped into the reactor, there was no flow rate out of the reactor to be recorded.

The first 15 mℓ. sample was collected in a 15 mℓ. centrifuge tube immersed in a beaker of ice water for the purpose of chilling the protozoa to greatly slow down their movement. Next, the tube and contents were spun in a centrifuge (International Centrifuge, Model CL, max. rpm: 3600) at peak voltage in order to force the more massive protozoan predators to the bottom of the centrifuge tube and leave the prey bacteria in the supernatant at the top of the tube. A series of tests was run to determine the optimum spin time in order to pull down the maximum number of protozoa and the minimum number of bacteria. Graph 1 indicates that a time of 30-45 seconds is suitable. Finally, in order to measure the concentration of the bacterial prey, a syringe was used to draw off 6 mℓ. of supernatant from the spun sample. This 6 mℓ. portion, which was relatively protozoan free, was placed in a cuevette

Graph #1: Spin time duration versus optical density & protozoa density of supernatant.



Graph #2: Optical density versus bacteria dry weight.



and the optical density was measured at 600μ in a Bausch & Lomb Spectronic 20 model 4. The accuracy of the measurement varied inversely with the optical density. Readings are estimated to be accurate within .01 at high optical densities (.1-.3) and within .002 at low optical densities (0.0-0.1). The optical density of the solution, which Graph 2 shows to be directly proportional to the biomass per mℓ. of the bacteria, was the indicator of the prey concentration. Prey concentrations varied from 0.001 optical density units (at extremely low prey concentrations) to 0.70 units (at very high prey concentrations).

The next measurement involved filtering approximately 10 mℓ. of the 15 mℓ. sample through a 0.45μ membrane filter, acidifying the sample to pH 2 with 3 drops of dilute H_2SO_4, and storing it for later analysis at 7° C. The filtration removed all protozoa, bacteria, and debris from the sample. The acid and refrigeration prevented any growth in the sample tube. When a sufficient number of samples had been collected they were analyzed for total carbohydrate equivalent (based on sucrose standards) on an auto analyzer. The total carbohydrate content of the sample gave an indication of the amount of prey food available in the reactor. The inorganics present and metabolite secretions resulted in residual concentrations of 10 mg./ℓ. of carbohydrate (prey food). The precision of the determination varied inversely with concentration, being within 10 mg./ℓ. at high concentrations (150-300) and within 2 mg./ℓ. at low carbohydrate concentrations (0-150).

The third set of measurements concerned censusing the protozoan predators. To accomplish this, the predators were counted and a size distribution was obtained by employing a Coulter Electronic

Particle Counter, Model B, with a 100μ aperture tube (Figure 5). Exactly 15 mℓ. of sample, collected in a clean 15 mℓ. centrifuge tube was mixed in a small beaker with filtered (.45μ filter) 0.9 percent saline. The saline acted as both a dilutant and an electrolyte for the counter and proved innocuous to the protozoa's size and shape. The counter was then used to obtain four replicate counts of the number of protozoan cells per 1/2 mℓ. for each of five different volume thresholds. Individual counts varied by less than 10%, and the replicate counts provided an average estimated to be within 5% of the actual concentration. This method allowed us to obtain a cumulative histogram of the number of predators larger than a fixed size interval for each sample. By calibrating the particle counter with a sphere of known size (lycopodium: 27μ) it was possible to convert the coulter counts at the different size thresholds to a total biovolume per mℓ. In addition to the electronic censusing of the predators, each sample involved microscopic observation of a drop of the sample. Such things as the amount of debris in the solution, the size, shape, and activity of the protozoa, and any anomaly was noted. A large amount of debris, if larger than the smallest protozoan, was inimical to accurate counts and forced us to rely on hand counts with a microscope. Fortunately, this occurred only once, after protozoa were cultured for 100 hours with limited food supply.

The concept of biovolume was critical to the census of predators. The assumption was that a large number of very small predators is equivalent to a small number of very large protozoa. Accordingly a simple numerical count does not provide accurate information of the effect

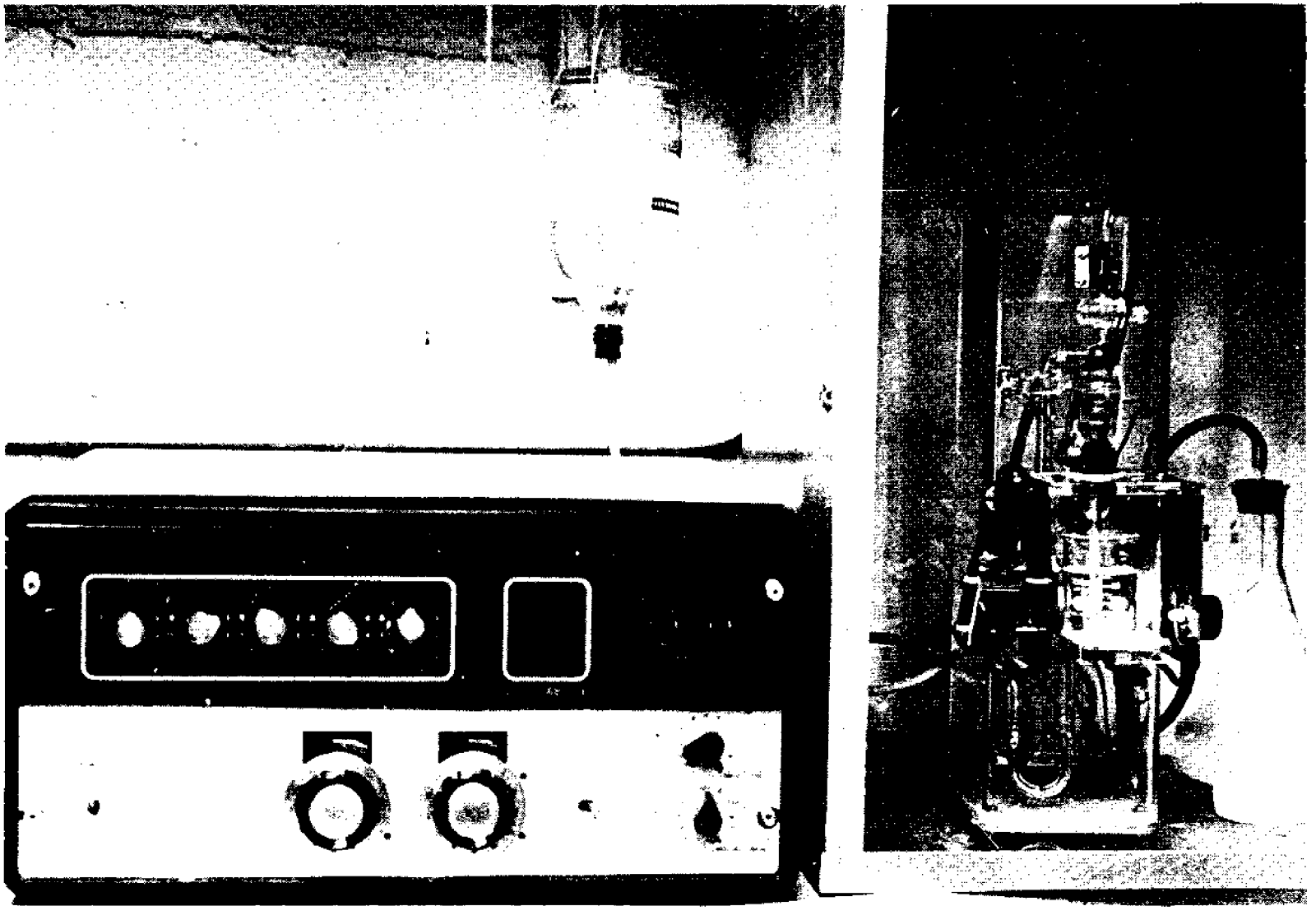


Figure 5: The electronic particle counter used to size and count the protozoan predator.

of the predator on the community: such information can be gathered only through a biomass or biovolume census. Using a more sophisticated particle counter (Coulter Counter, Model Z) we found that a plot of the predator's size distribution appeared very similar to a Poisson distribution. In the computed results, one biovolume unit is roughly equivalent to 10^3 cubic microns. Accordingly a measurement of 1000 biovolumes per mℓ. is approximately equivalent to 10^{-6} mℓ. of protozoa per mℓ. of solution. When certain threshold counts were missing from a sample reading, an approximation of the biovolume was made by assuming a "typical" size distribution for the missing threshold counts.

At a frequency of appoximately once per day, the pH of a sample was checked to insure that pH was not growth inhibiting. The standard pH was about 7.0 with a range of 6.9-7.3. During the continuous run, new food media jugs were made continuously and allowed to sit to insure sterility. Whenever a food jug in use ran low, a new jug was attached to the reactor's food line by using aseptic flaming techniques. Sixteen 12 ℓ. food jugs were used in the continuous run, the first five being pumped out on a rotation basis of 3 hours from each jug, in order to obtain a more uniform mix in the reactor. The last 11 jugs, however, were pumped from until nearly empty before switching to the next jug. Although it was attempted to mix all jugs to a carbohydrate concentration of 250 mg./ℓ., the actual range of variation, as indicated by the anthrone-auto analyzer method, was 230 to 250 mg./ℓ. The relevant carbohydrate jug concentrations for each part of the continuous run are indicated on the graphical plots of the continuous data.

At the conclusion of each run, all data for that run, including prey optical density readings, predator volume threshold counts, carbohydrate values, date and time of the sample, duration of the sample collection (continuous only), and comments on pH, microscope observation, and the state of the reactor were punched into IBM cards. At that point a program written in PL/I was run to list the data, converting all sample times to the number of hours from time zero and all protozoa counts to the predator biovolume. The use of the computer program facilitated graph plotting as well as organizing all data into a machine (card) and human (printout) compatible format. Data is available upon request to Professor R.P. Canale, Department of Civil Engineering, University of Michigan.

Batch Run Results

1. Concentrations of Cerophyl extract were non-limiting

To insure that the Cerophyl extract used to stimulate prey ingestion and reproduction of the protozoa was present in sufficient concentrations and therefore non-limiting, two batch runs were made at approximately equal total carbohydrate concentrations but differing Cerophyl concentration. Batch run VII (Graph #8) contained 92 mg./ℓ. Cerophyl extract at an initial total carbohydrate of 290 mg./ℓ. while batch run V (Graph #6) contained 30 mg./ℓ. Cerophyl extract at 306 mg./ℓ. initial total carbohydrate. Figure 6 indicates that the increased Cerophyl concentration had no effect on the maximum prey growth rate and did not increase the maximum predator growth rate. The differences

BATCH RUN SUMMARY

Run Number	Initial Carbohydrate[1]	Minimum Carbohydrate[1]	Maximum Prey Optical Density	Maximum Protozoa Biovolume[2]	Maximum Prey Growth Rate[3]	Maximum Predator Growth Rate[3]
VIII	50	15	0.050	12,883	0.209	0.118
IV	117	22	0.105	42,828	0.400	0.171
X	122	12	0.170	42,935	0.368	0.184
XI	175	7	0.215	48,919	0.439	0.173
IV	228	14	0.281	61,498	0.512	0.077
VI	265	20	0.265	77,869	0.512	0.124
VII[4]	290	44	0.282	78,963	0.542	0.106
V	306	26	0.367	90,659	0.542	0.119

[1] in mg./ℓ.

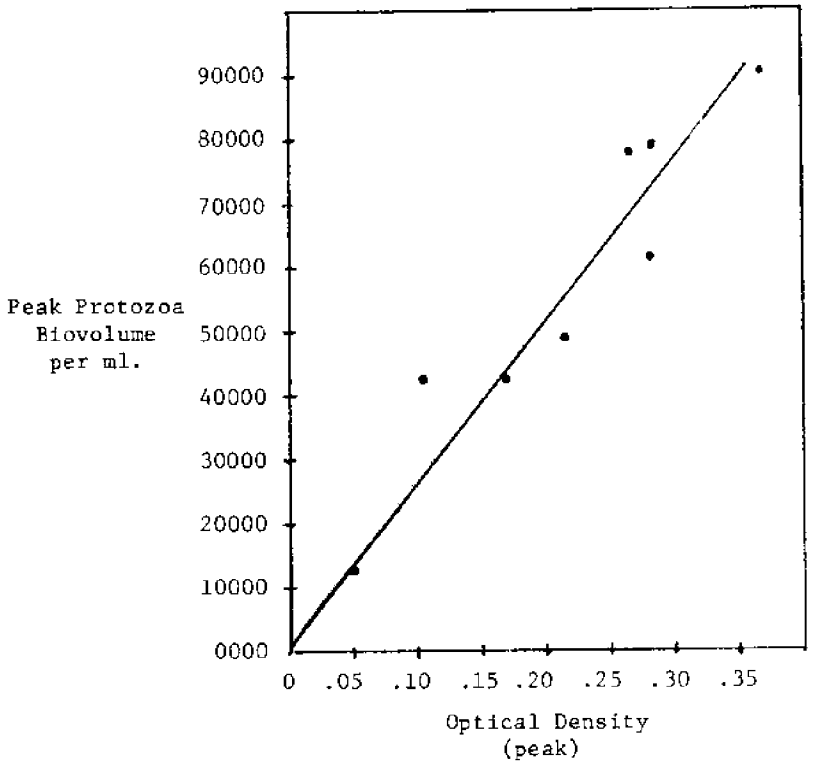
[2] as biovolume per mℓ.

[3] The growth rate is calculated in units per hour by plotting the growth data on semi-log paper and measuring the maximum slope for 1 log cycle (μ_{10}). The growth rate reported (μ_e) is μ_{10} ($\log_e 10$). To convert to doubling time (t_d) = ($\log_e 2$)/μ_e.

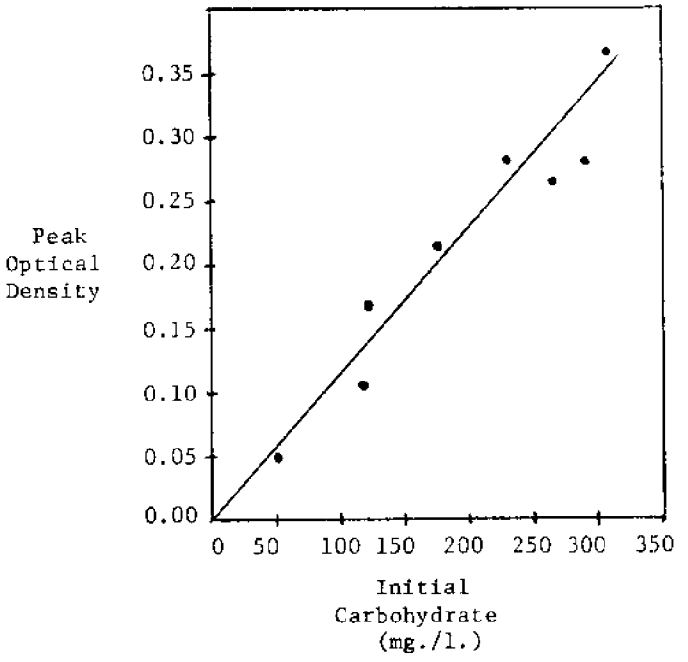
[4] Run at three times the normal Cerophyl concentration.

Figure 6

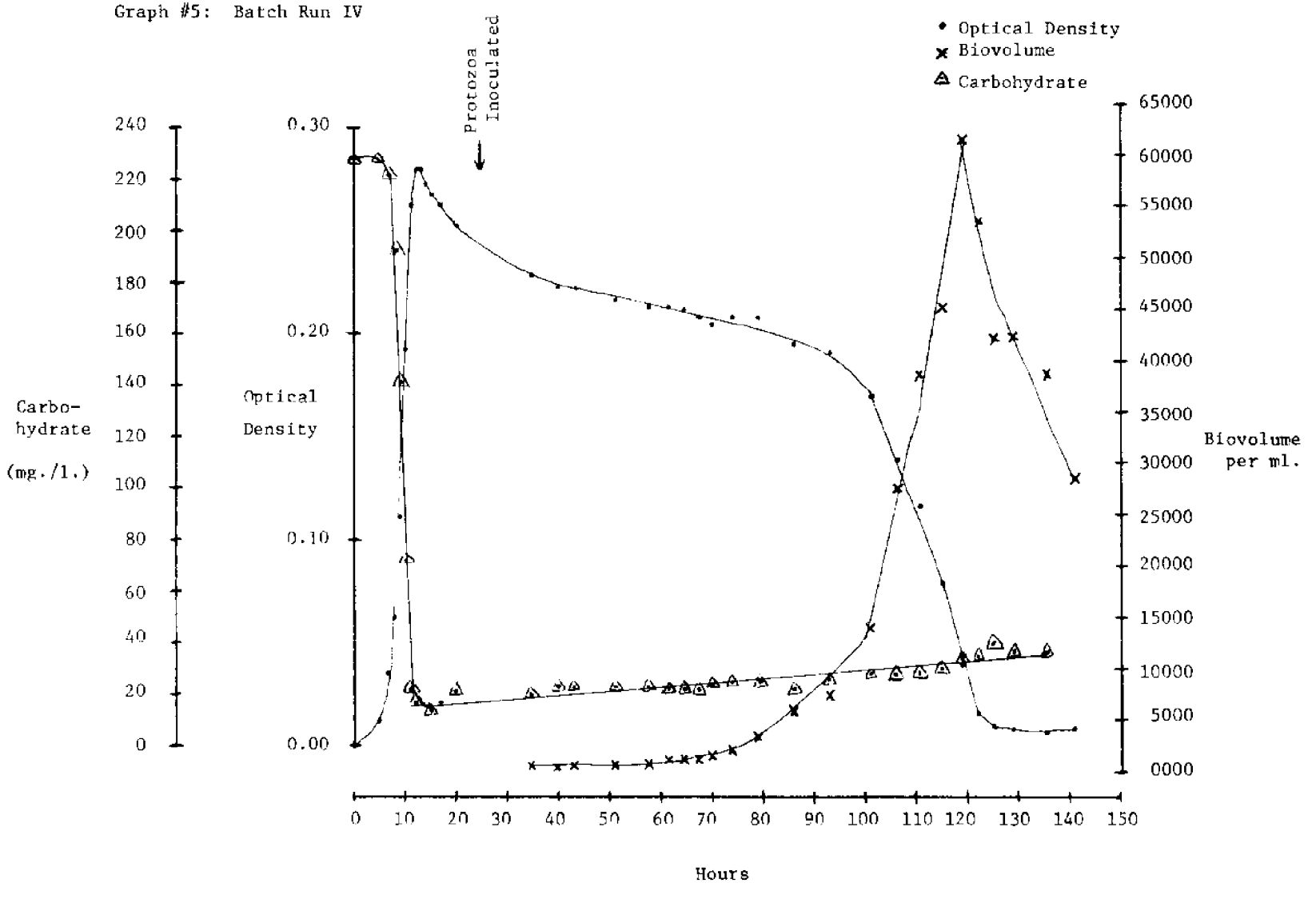
Graph #3: Maximum protozoa biovolume versus maximum prey optical density for batch runs.

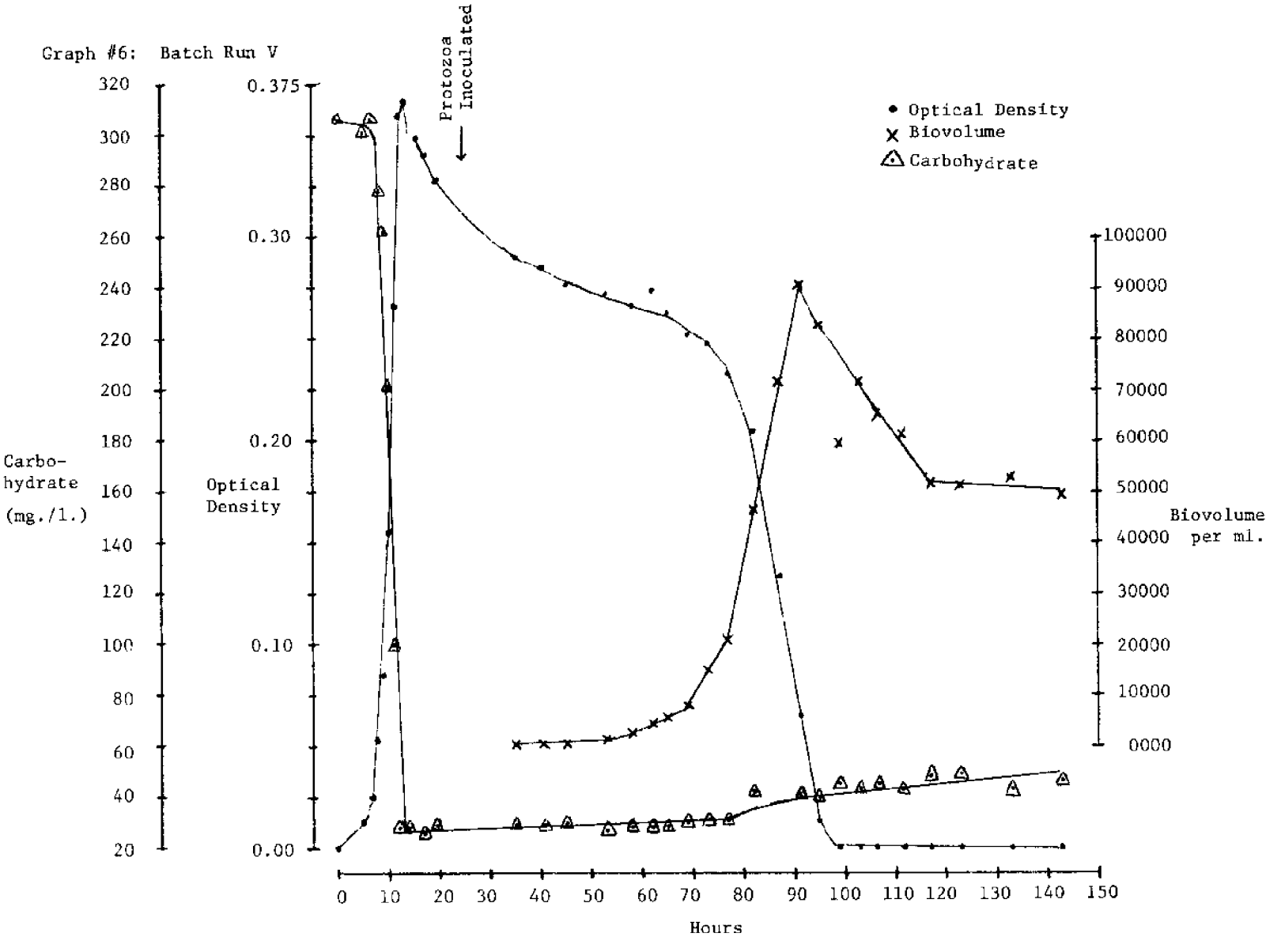


Graph #4: Maximum prey optical density versus initial carbohydrate for batch runs.

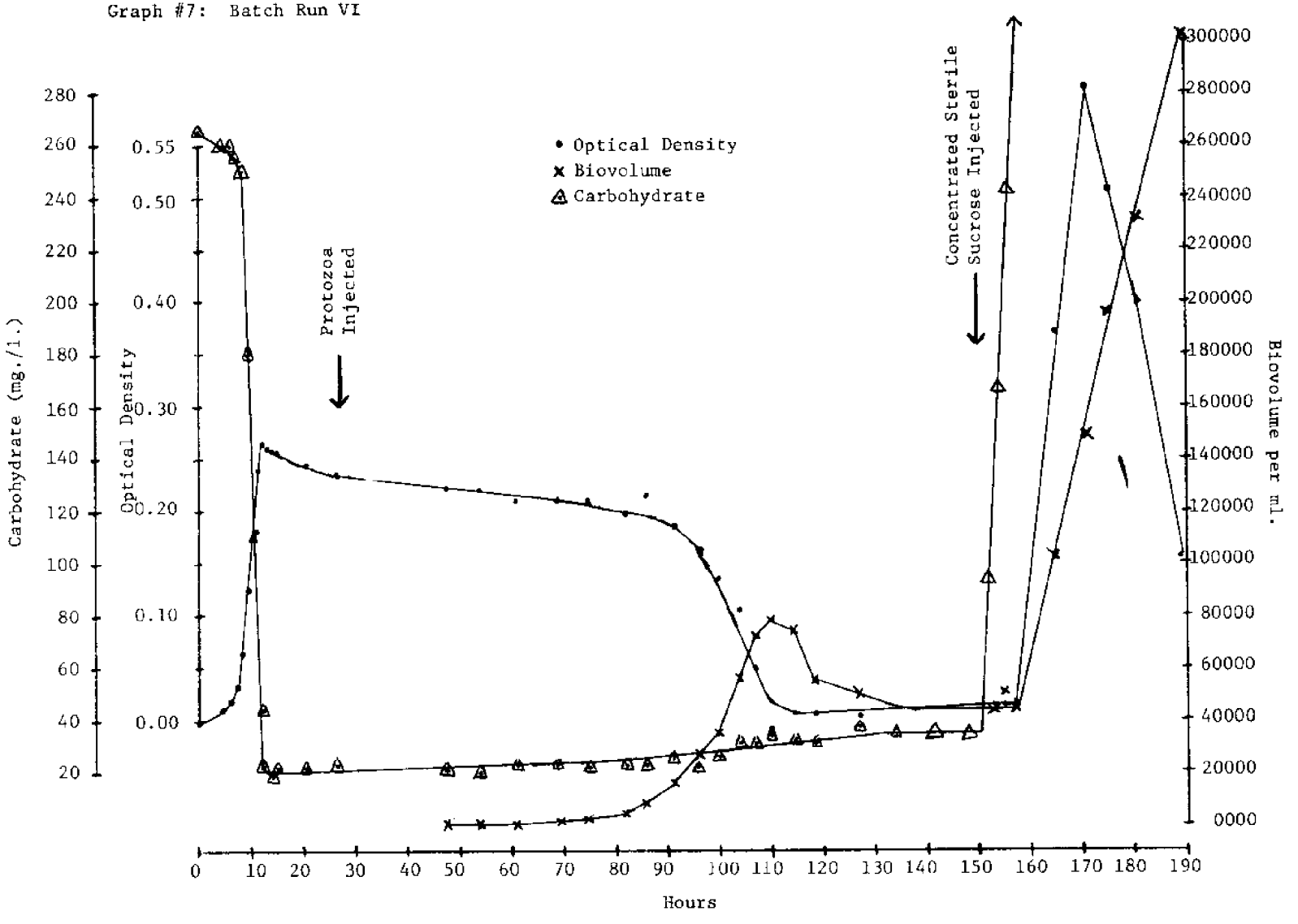


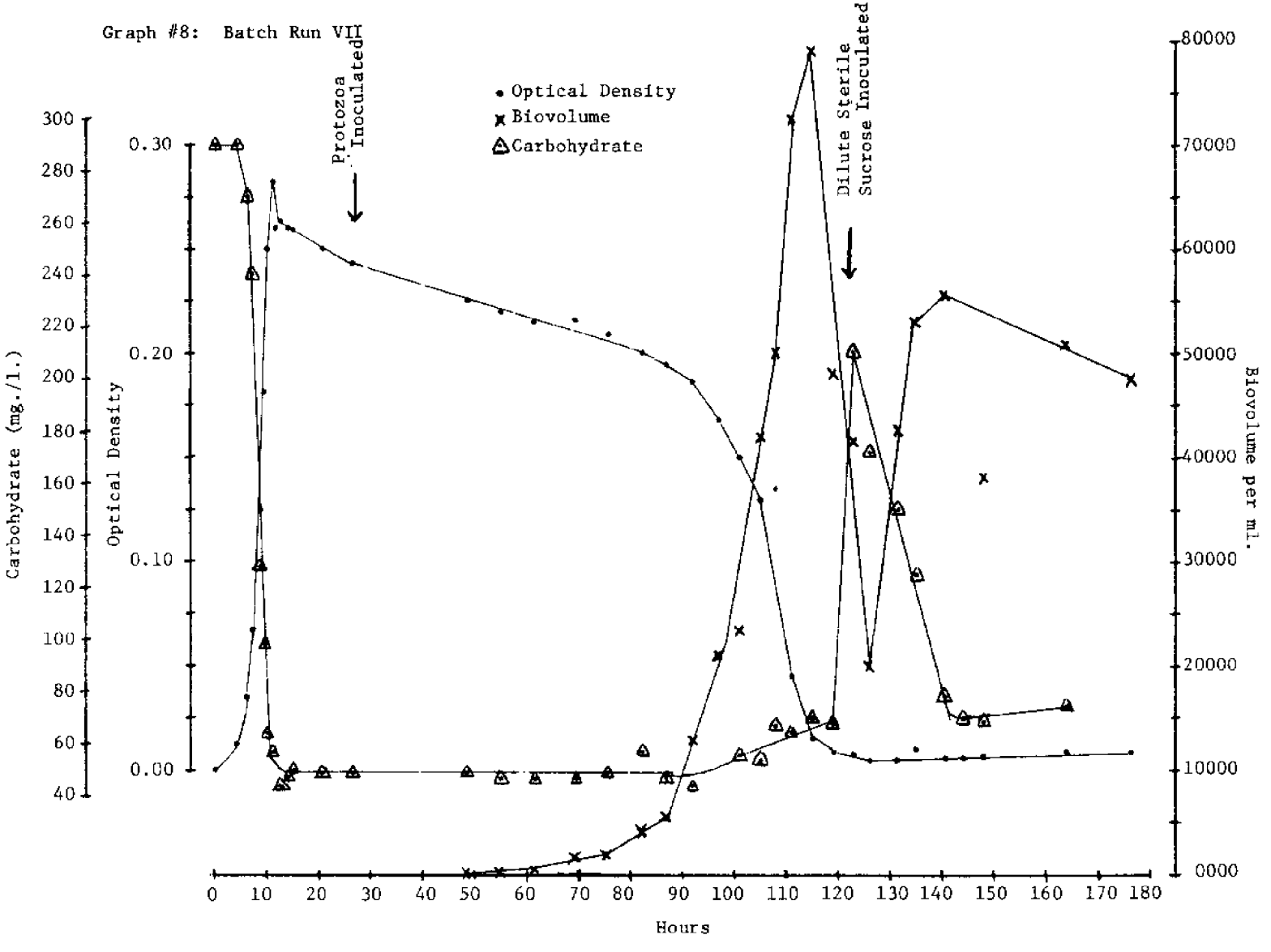
Graph #5: Batch Run IV



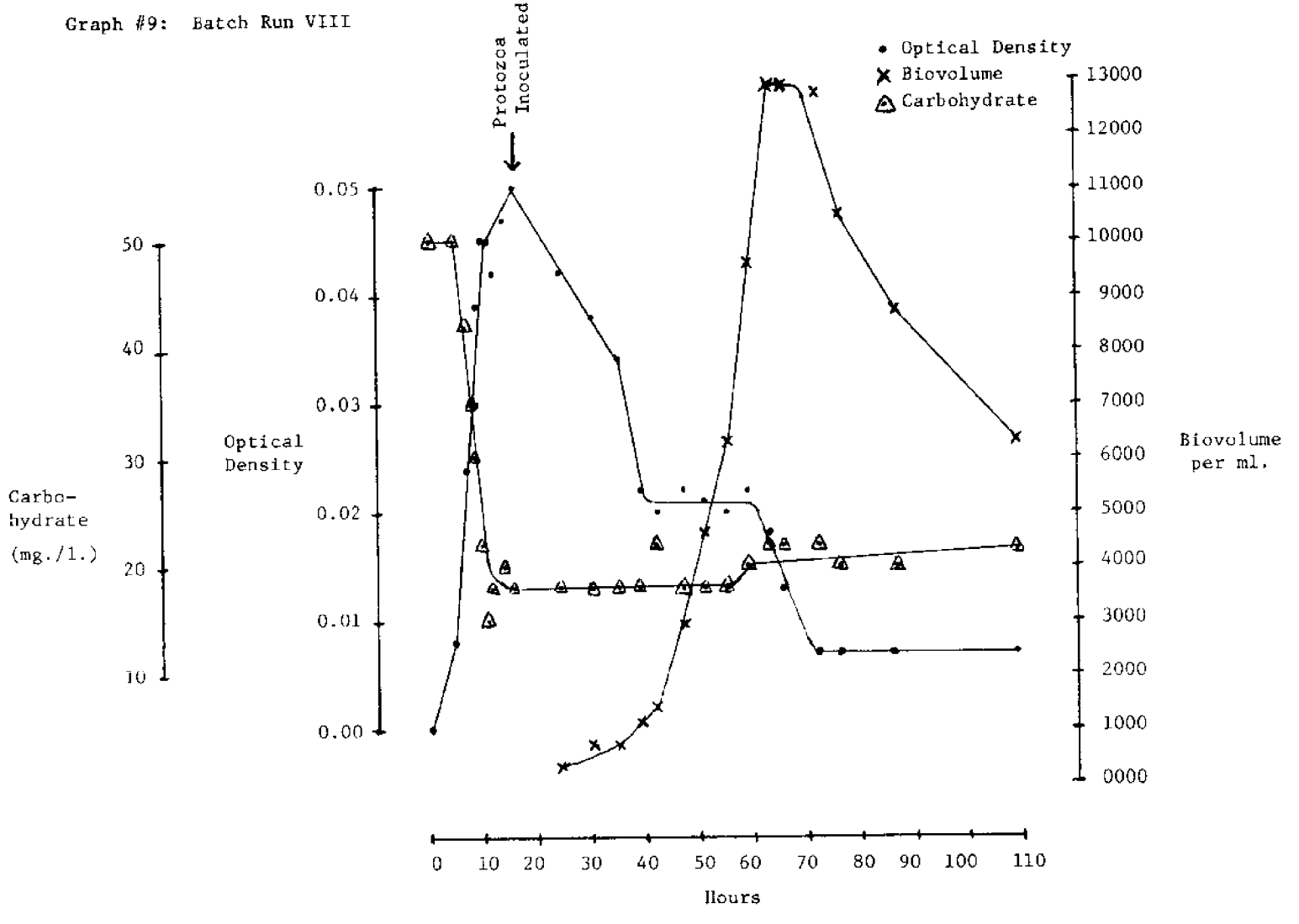

Graph #6: Batch Run V
Protozoa Inoculated
Optical Density
Biovolume
Carbohydrate
Carbo-hydrate (mg./l.)
Optical Density
Biovolume per ml.
320
300
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0.375
0.30
0.20
0.10
0.00
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0000
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150
Hours

Graph #7: Batch Run VI

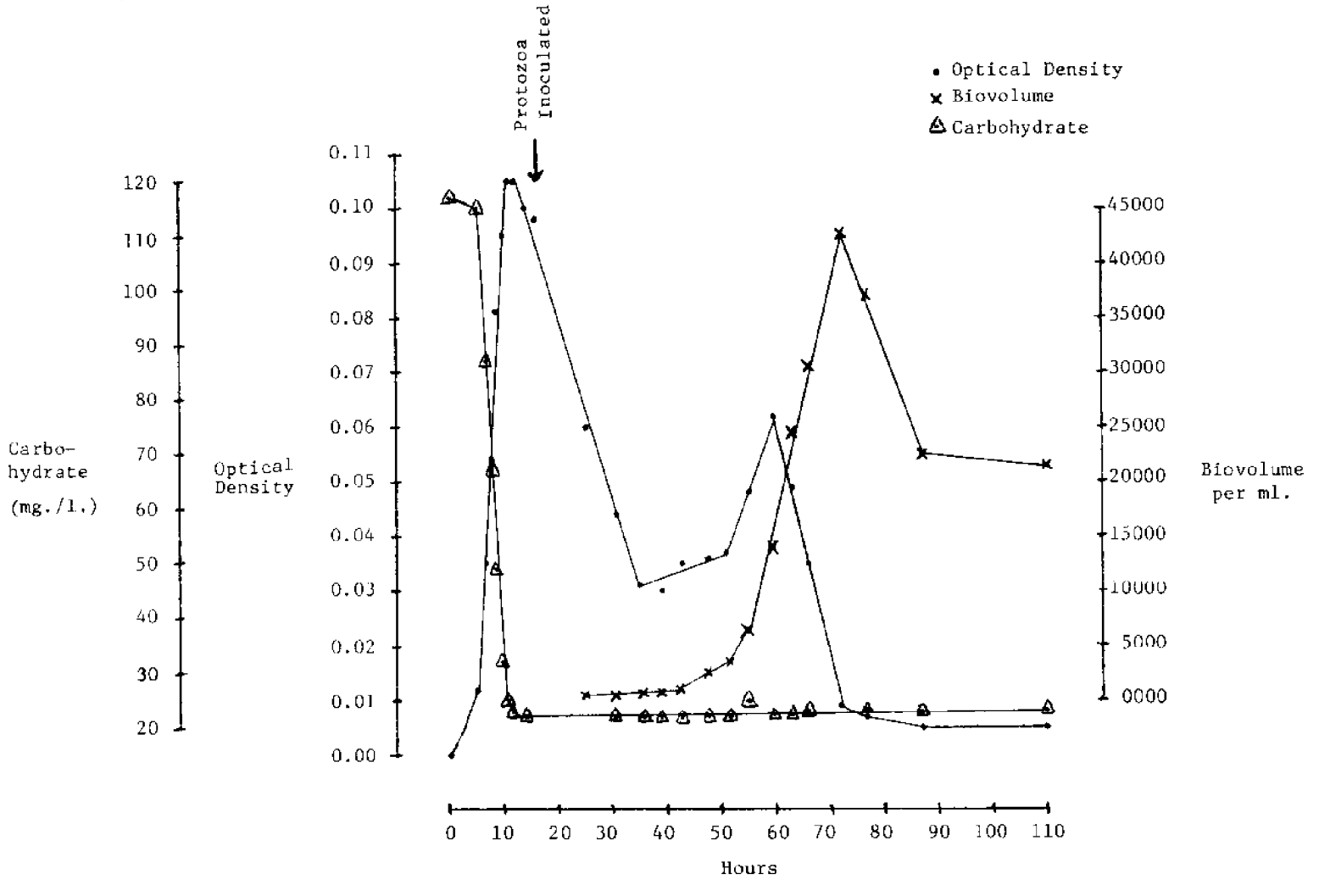



Graph #8: Batch Run VII
Protozoa Inoculated
Dilute Sterile Sucrose Inoculated
Optical Density
Biovolume
Carbohydrate
Carbohydrate (mg./l.)
Optical Density
Biovolume per ml.
Hours
300
280
260
240
220
200
180
160
140
120
100
80
60
40
0.30
0.20
0.10
0.00
80000
70000
60000
50000
40000
30000
20000
10000
0000
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180

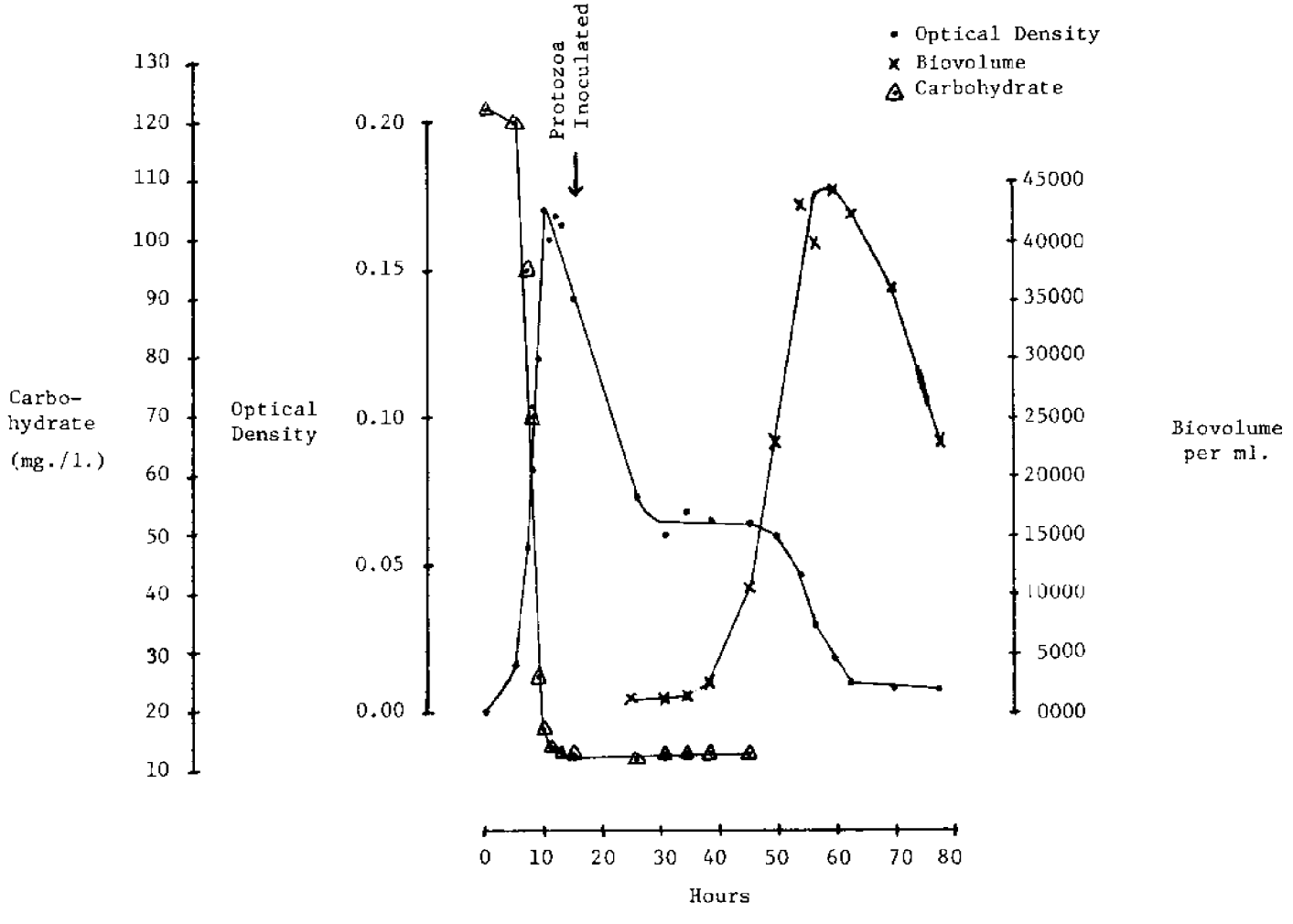
Graph #9: Batch Run VIII



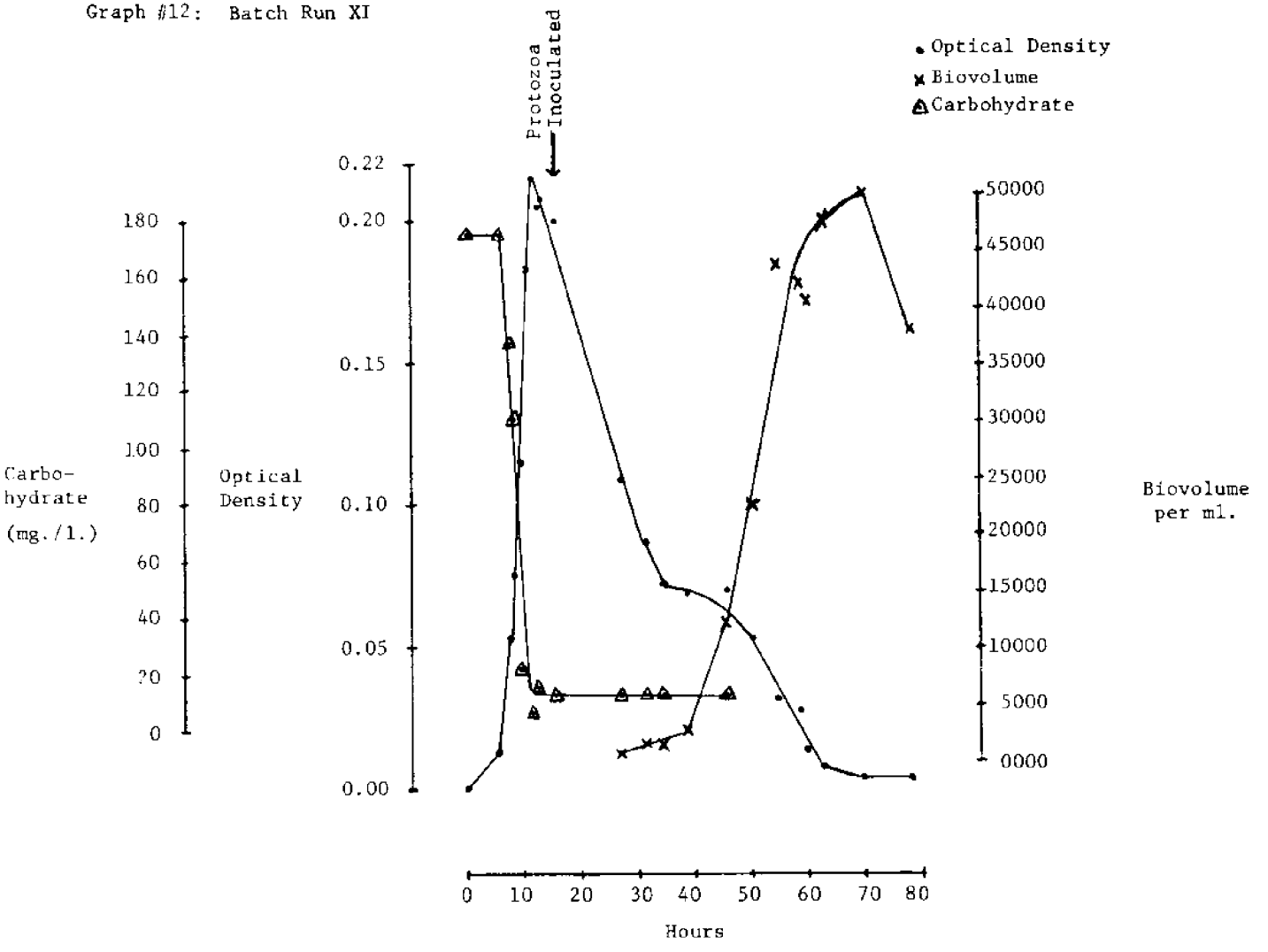
Graph #10: Batch Run IX



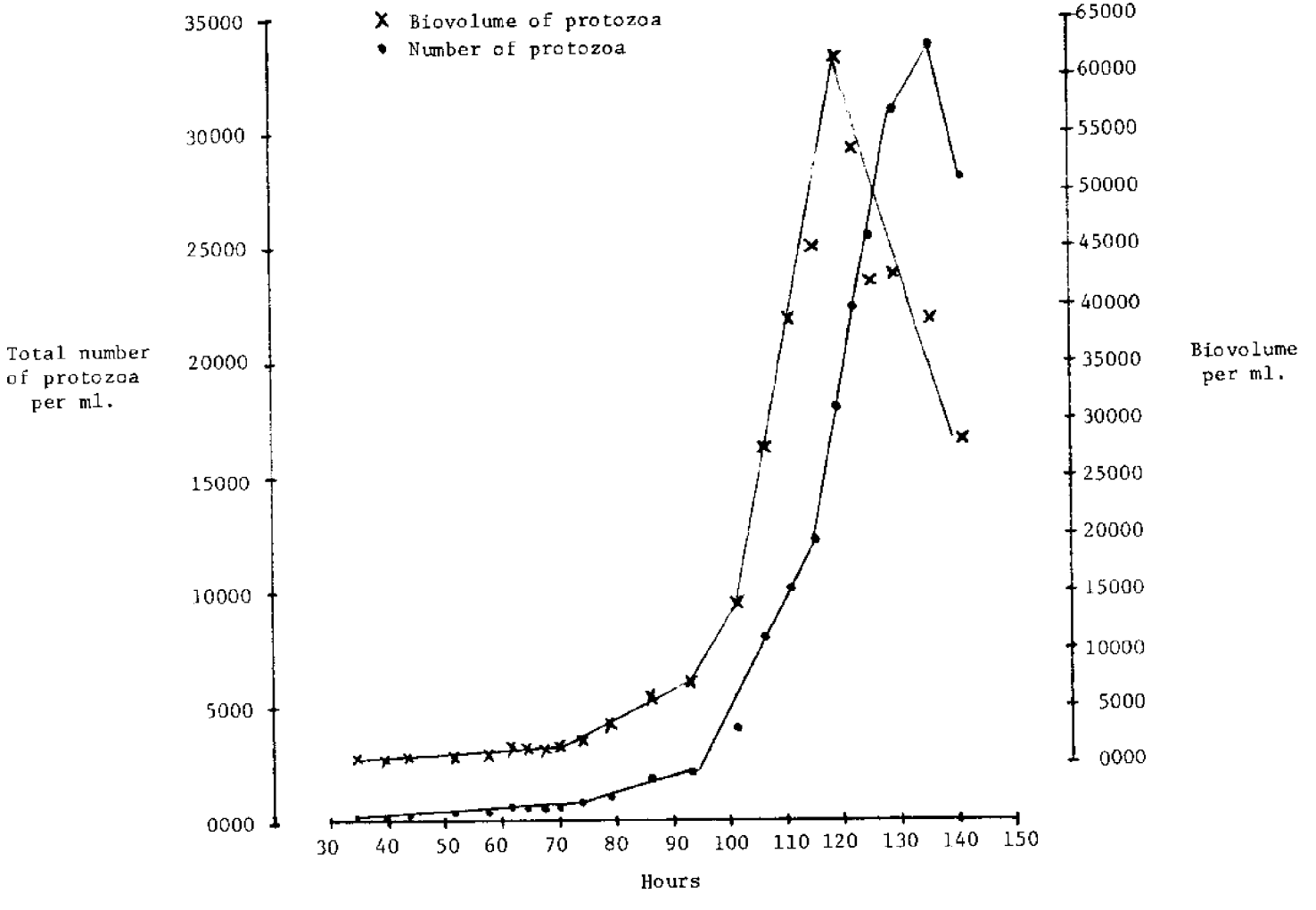
Graph #11: Batch Run X



Graph #12: Batch Run XI



Graph #13: Divergence between growth in number and size (biovolume) for protozoa (batch IV).



between the peak prey density and the peak protozoa biovolume for runs VII and V are to be expected on the basis of differing initial food availability. Since all food media contained at least 30 mg./ℓ. Cerophyl extract, it may be concluded that Cerophyl was at no time predator-limiting.

2. Metabolites were non-limiting

To make certain that cessation of growth was due to the exhaustion of food rather than the buildup of metabolites, batch reactors VI and VII (Graphs 7 & 8) were injected with small amounts of sterile prey food (sucrose) once the predators had passed their peak density and had begun to decline. If metabolite accumulation had caused the growth cessation, then the introduction of a small amount of fresh food would not alter the metabolite concentration and no further growth would take place. In batch VI, where very concentrated sucrose was injected, the immediate response in growth of prey and consequent growth of the predator (Graph #7) demonstrates that it was not toxicants, but rather food depletion which had caused the populations' growth termination. In batch VII a dilute amount of prey food was injected after growth had terminated (Graph #8). The fact that the predator, but not the prey, increased after the dilute food injection showed that predators existed in sufficient concentration to consume new prey as soon as they fed on the additional dilute food and grew (it had previously been demonstrated that the protozoa could not grow on the injected prey food, but only on the prey).

An additional demonstration that metabolites were non-limiting appears in the relation between the initial food available and the maximum size of the feeder population (Graphs 3 & 4). These plots

indicate that doubling the carbohydrate concentration doubles the density of the prey produced (Graph #4) and that doubling the amount of prey doubles the amount of predators (Graph #3). If metabolites had been limiting, then doubling the food concentration would not alter the toxicant concentration or its rate of accumulation, and therefore the feeder population would not have doubled.

3. Predators are incapable of capturing all the prey

Since absolutely no physical refuge was provided for the prey, and the thorough mixing in all reactors was inimical to the notion of a prey refuge, there was an effort to discover if the predators could consume all the prey. The fact that the protozoa were incapable of annihilating the bacteria was demonstrated in batch run VI (Graph #7). Once the predators had passed their peak density, declined due to exhausted food, and cleared the reactor of all bacterial turbudity, the additional prey food (sucrose) was injected. In batch run VI the growth of prey was immediate, proving that there were prey who had not been captured by the predator, despite the fact that the predators had been present in high numbers during extremely low prey densities for 40 hours. Since some prey escaped without a physical refuge, it must be assumed that a biological refuge, in terms of the predator's inefficiency of capturing prey, enabled the bacteria to survive intensive predator numbers.

4. Feeder population is directly proportional to the food concentration

Because the experiments were designed to demonstrate the interactions of food-limited populations, it was important to illustrate

that the initial amount of food in one trophic level was directly related to the amount of feeders produced in the next higher trophic level. Graph #4 shows that as the initial amount of prey food increased, the amount of prey feeding on that food increased proportionately. Similarly, Graph #3 demonstrates that as the biomass of the prey increases (Graph #2 shows biomass directly proportional to optical density within the concentration range of the batch runs), the maximum biovolume of the predator increases. Therefore, there is a one-to-one correspondence between the initial amount of food in a trophic level, and the maximum density of feeders which can be attained on the next higher trophic level.

5. Mass conversion efficiency

Having postulated that the predator was inefficient in hunting at low prey densities, it was attempted to get some idea of the populations' mass conversion efficiency from one trophic level to the next higher level. Given that 1 biovolume unit = 10^{-9} mℓ. of protozoa per mℓ. of solution, and assuming the density of the predator to be the same as that of water (10^{3} mg./mℓ.), which would be a low density estimate, mass conversion efficiencies were calculated for batch runs XI and V. In run XI the initial carbohydrate concentration was.175 mg./ℓ. or.175 mg./mℓ. This produced a peak optical density of .215 (from Graph #2: 1.4 mg./25mℓ.) or .056 mg./mℓ. Finally, the peak protozoa biovolume was 4.9×10^{4} units which is equivalent to 4.9×10^{-5} mℓ. of protozoa per mℓ. of solution, giving .049 mg. of protozoa per mℓ. Thus .175 mg. of carbohydrate produced a .056 mg. peak of bacteria which yielded a .049 mg. peak of

protozoa. This gives a conversion from carbohydrate mass to bacterial mass of 32% and from bacteria mass to protozoa mass of 87%. Similarly in batch run V .306 mg. carbohydrate produced .108 mg. bacteria which produced .091 mg. of protozoa. This gives conversions of 35% for carbohydrate to bacteria and 84% for bacteria to protozoa. These conversion efficiencies are surprisingly high, but follow the general rule of more efficient conversion as the trophic level increases. In addition, the conversions are in terms of mass, while conversions in terms of energy may be lower.

6. Population growth rates

Since one of the major objectives of this research was to provide population data for a mathematical model of predator-prey interaction, it was of interest to determine the maximum growth rate of the bacteria, the protozoa, and any variation with initial food concentration. In order to calculate the maximum growth rate, the growth phase data was plotted on semi-log paper for each batch run. At the beginning of the growth the slope of the growth points tended to increase, while near the end of growth the slope decreased and then went negative as the exhausted food forced the populations to decline. Accordingly the maximum slope was measured ignoring the initial and terminal points, converted to base e, and reported in Figure 6. Prey growth seems dependent on a threshold of initial carbohydrate concentration. Once the initial concentration is above 228 mg./ℓ., growth of the prey does not vary significantly with initial carbohydrate. Below 228 mg./ℓ., the growth of the prey tends to vary directly with increasing initial

maximum carbohydrate concentration. The predator growth rate varies independently of the peak prey density, while the cause of this variance is unknown.

7. Predator growth at the expense of size

T. pyriformis behaves like Gause's *Didinium* once it has exhausted the major proportion of the prey: namely the predator population continues growing in numbers at the expense of a decrease in the size of an individual. The first hint of this phenomenon is microscopic observation, which reveals that the protozoa are fat, roundish, and inactive during high prey concentrations. At low prey concentrations, however, one notices thin elliptical predators swimming about frantically. This increased activity becomes apparent in the number and size distributions indicated on the particle counter. The frantic increase of activity of the starving protozoa results in a large use of energy with no food to replace it. As a result the total biovolume, as indicated in every batch run, drops dramatically once the prey reach low densities. However, though the biovolume and number of large predators drop sharply, the number of small protozoa continues to increase for some time. This divergence between size and number is illustrated in Graph #13. In short, when food has been exhausted there is growth in the number and activity of the predator at the expense of the total predator biovolume. Large predators become scarce while smaller protozoa become more common.

8. Carbohydrate, prey density, and predator density are directly related

Since the experiments were designed to allow only one food source for each type of feeder, and because there was no food replenishment

involved in the batch runs, it was to be expected that the concentration at each trophic level would be dependent upon the concentration of food and/or feeder at the level below or above. This result was evident in all of the batch runs (Graphs #5-12). The mechanism was that the inoculated bacteria increased their density while the carbohydrate, upon which the prey were feeding, dropped in concentration. As soon as the prey had reached their peak density by consuming all of the available edible carbohydrate, the amount of bacteria began to decline due to the fact that the prey had to continue to use energy (even though they did not grow) without any source of replacement. This prey "die-off" occurred immediately after the peak density, and in most cases reached a temporary stable plateau.

As soon as the prey began to decline the predatory protozoan was introduced and after an initial lag increased while feeding on the prey. As a consequence, as the protozoa biovolume increased, the prey density dropped rapidly to a uniformly low level of about .01 units. During this portion of the run, which due to the predator's slower growth rate occurred more slowly than the bacterial growth phase, the carbohydrate concentration tended to creep up from the accumulation of organic animal wastes. Although the rise in carbohydrate was slight, it was observable in most of the batch runs. Finally, when the prey were depleted by the predators, the protozoa burned up their newly-gained volume in a search for the infrequent remaining prey. This energy use, without a compensating supply of food, caused a quick drop-off in the predator biovolume.

An anomaly appears in batch run IX (Graph #10) where the prey density, after declining and reaching a plateau, rises after the predator inoculation before decreasing when the protozoa begin to grow rapidly. It is suspected that this unusual prey peak was caused by unused carbohydrate in the protozoan inoculum, but the fact that this suspected increased carbohydrate was not indicated in the samples preceding the anomalous prey peak, makes such a conclusion tenuous.

Continuous Run Results

1. Predator density is dependent on the washout rate

Since the protozoa and bacteria who live in the continuous reactor do not attach themselves to any fixed object, they are susceptible to being washed out of the community. The thorough mixing which insures that the organisms are uniformly distributed also necessitates that they be washed out at the same rate that media flows out of the reactor, this being equal to the rate of food inflow due to the constant reactor volume. The washout becomes a type of forced emigration, and since no immigration is possible, the organisms can only maintain themselves in the reactor by continued growth and replacement. It follows that when the washout rate exceeds the organisms' growth rate, their density will continually decrease. Conversely, when the washout rate is less than the growth rate, emigration will not overwhelm the organisms' growth, and their density may increase.

In the continuous studies 7 different flow (washout) rates were used. These are specified in Figure 7 and are indicated in the continuous data plots (Graphs #14-18). The dependency of the populations on the washout rate is best illustrated in Graph #14, in which three different pumping rates were alternated with intervals when the pump was off and no washout took place. It is apparent in Graph #14 that the density of the protozoa is intimately dependent on the washout rate. In each case of flows A, B, and C the result was identical: the predators, given their past momentum, continued to grow or hold their ground for a short time after the pump was turned on, then, since flows A, B, and C were greater than the growth rate, the protozoa were washed out of the community faster than they could replace themselves and their density (biovolume) decreased. Conversely, during the three periods when the pump was turned off, no forced emigration occurred and the predator enjoyed unrestricted growth until the pump was again turned on and washout began.

Although washout also affects the bacteria, their growth rate was constantly greater than the pumping rate and washout was therefore unimportant. This is illustrated during flow A (Graph #14) where the prey increased directly as the predator declined, regardless of the fact that this was the highest flow rate used.

Graphs #15, 16, 17, and 18 each illustrates the community's behavior during a different uniform flow rate. Since protozoa were able to increase their density while the pump was on, it may be concluded that flows D, E, F, and G were all less than the predator's growth rate, and consequently allowed internal factors to govern the community, rather than the external regulation by forced emigration through washout.

CONTINUOUS RUN PARAMETERS

Flow Rate	Sample Numbers	Duration (hours)	Average Flow Rate (ml./min.)
A	1-14	38	3.07
**	15-20	24	pump off
B	21-54	103	2.36
**	55-59	15	pump off
C	60-76	71	1.80
**	**	100	pump off
D	77-207	393	1.43
E	208-315	427	1.24
F	316-394	312	1.03
G	395-486	442	0.82

Sample Numbers	Jug Used	Carbohydrate Concentration (mg./ℓ.)
1-76	1-5[1]	240[2]
76-104	6-9[1]	242[3]
105-144	7	230
145-171	8	250
172-199	9	240
200-217	6	250
218-255	10	240
256-290	12	240
291-326	11	240
327-371	13	240
372-405	14	230
406-459	15	240
460-486	16	250

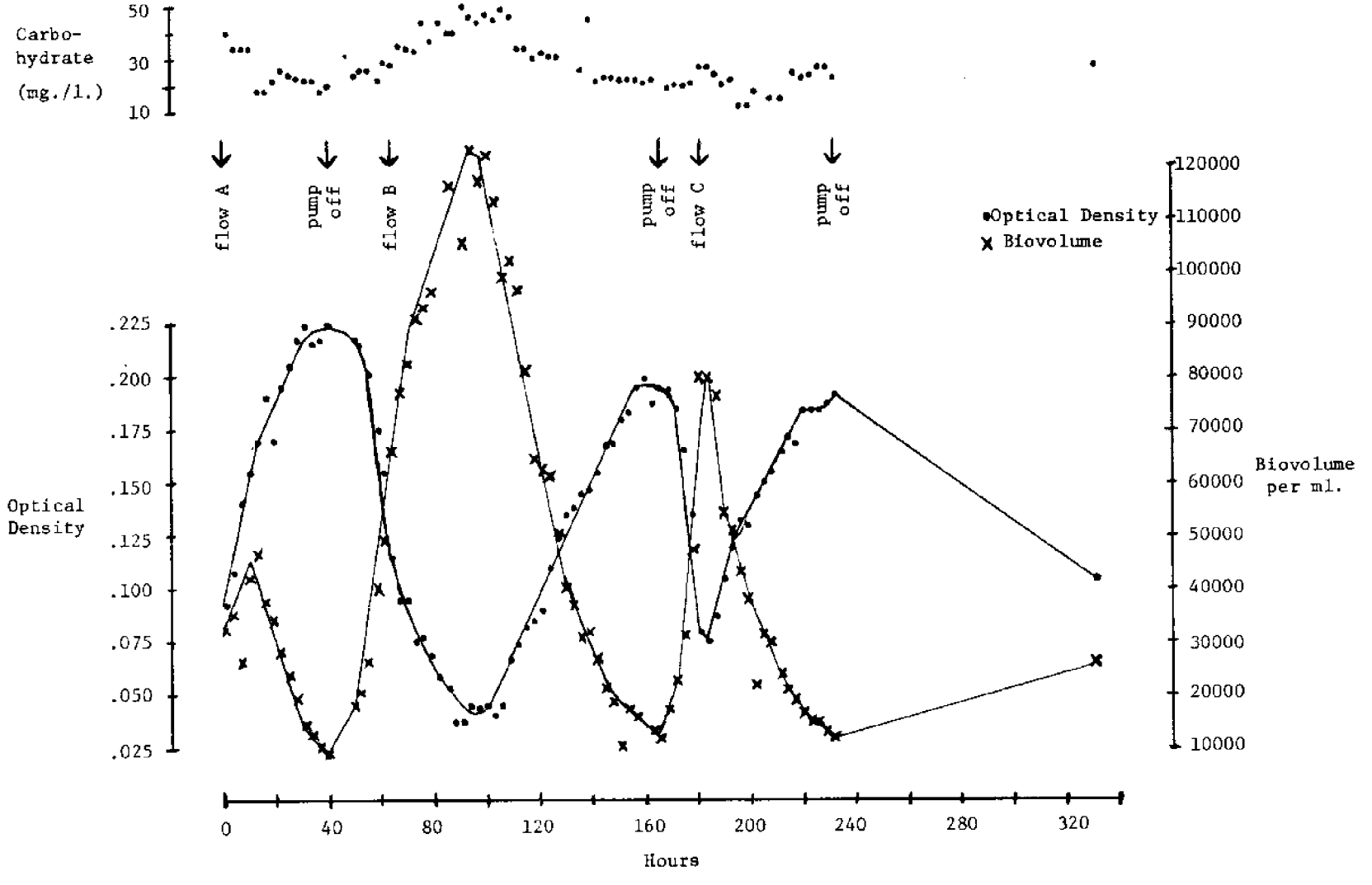
[1]These jugs were rotated: each was used for three hours before switching to the next jug. (1→2→3→4→5→1)

[2]Measured by analysis of a mixture of equal amounts of media from each jug.

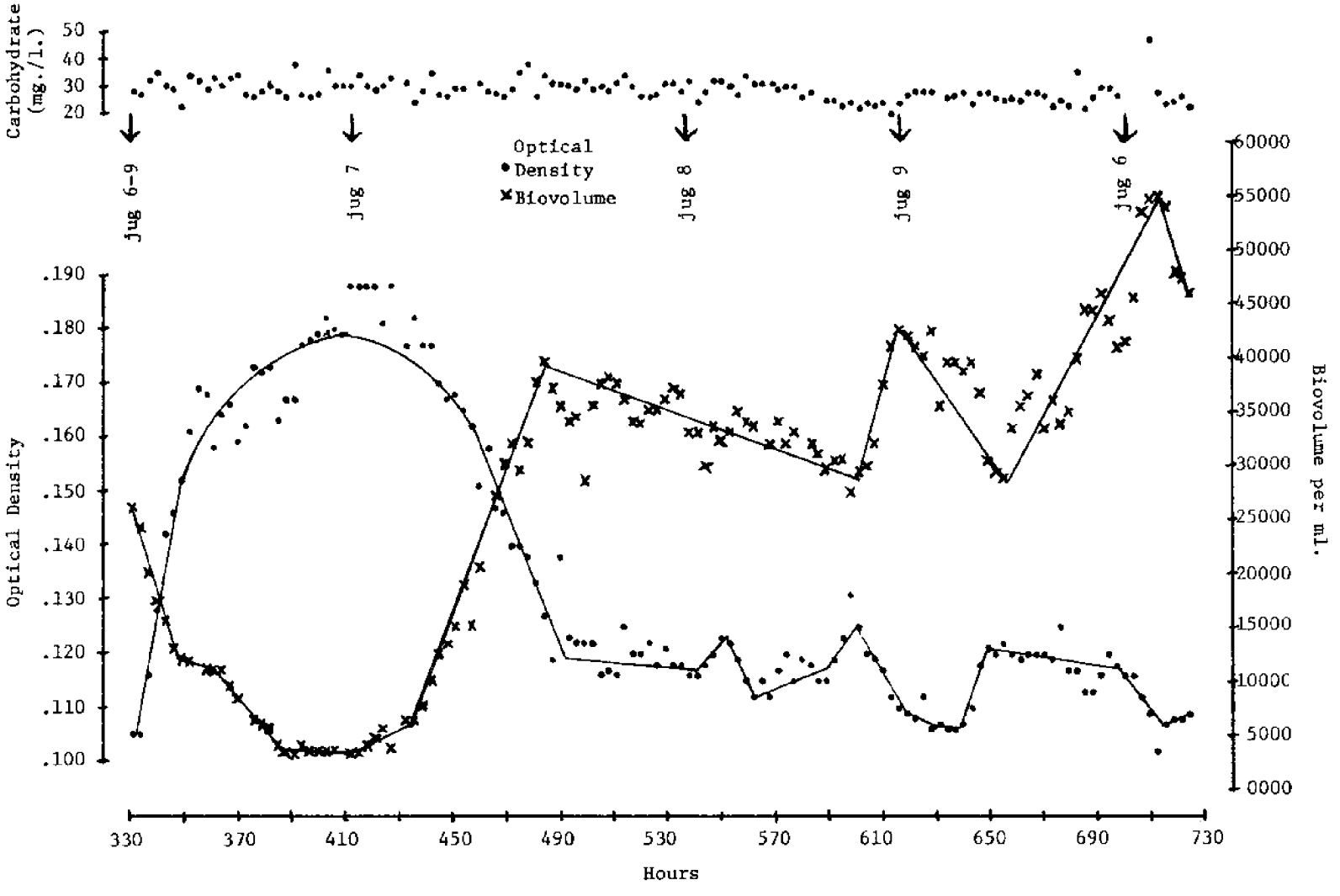
[3]Calculated by averaging the concentrations of jugs 6-9.

Figure 7

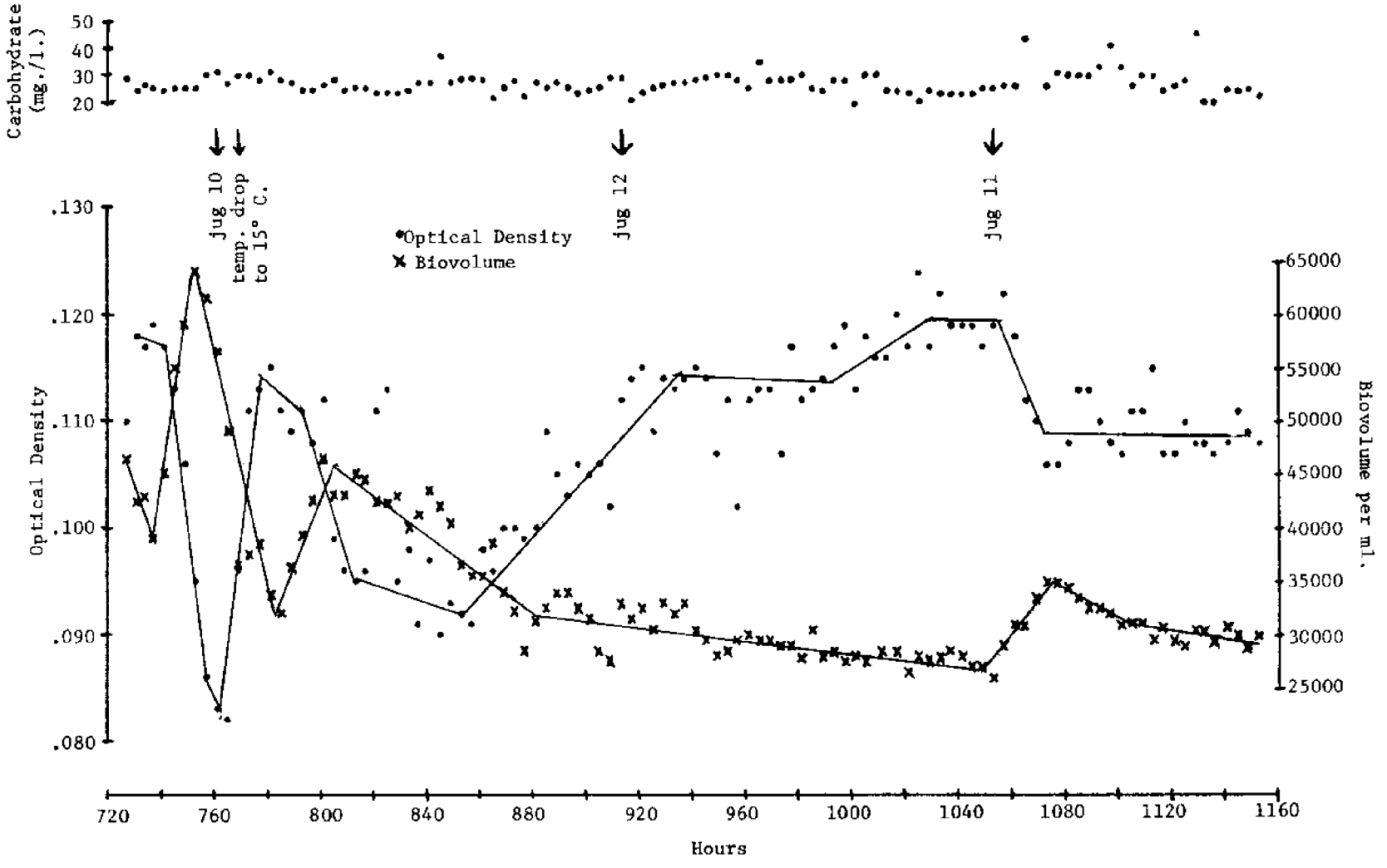
Graph #14: Continuous run III; Samples 1-76; Jugs 1-5.



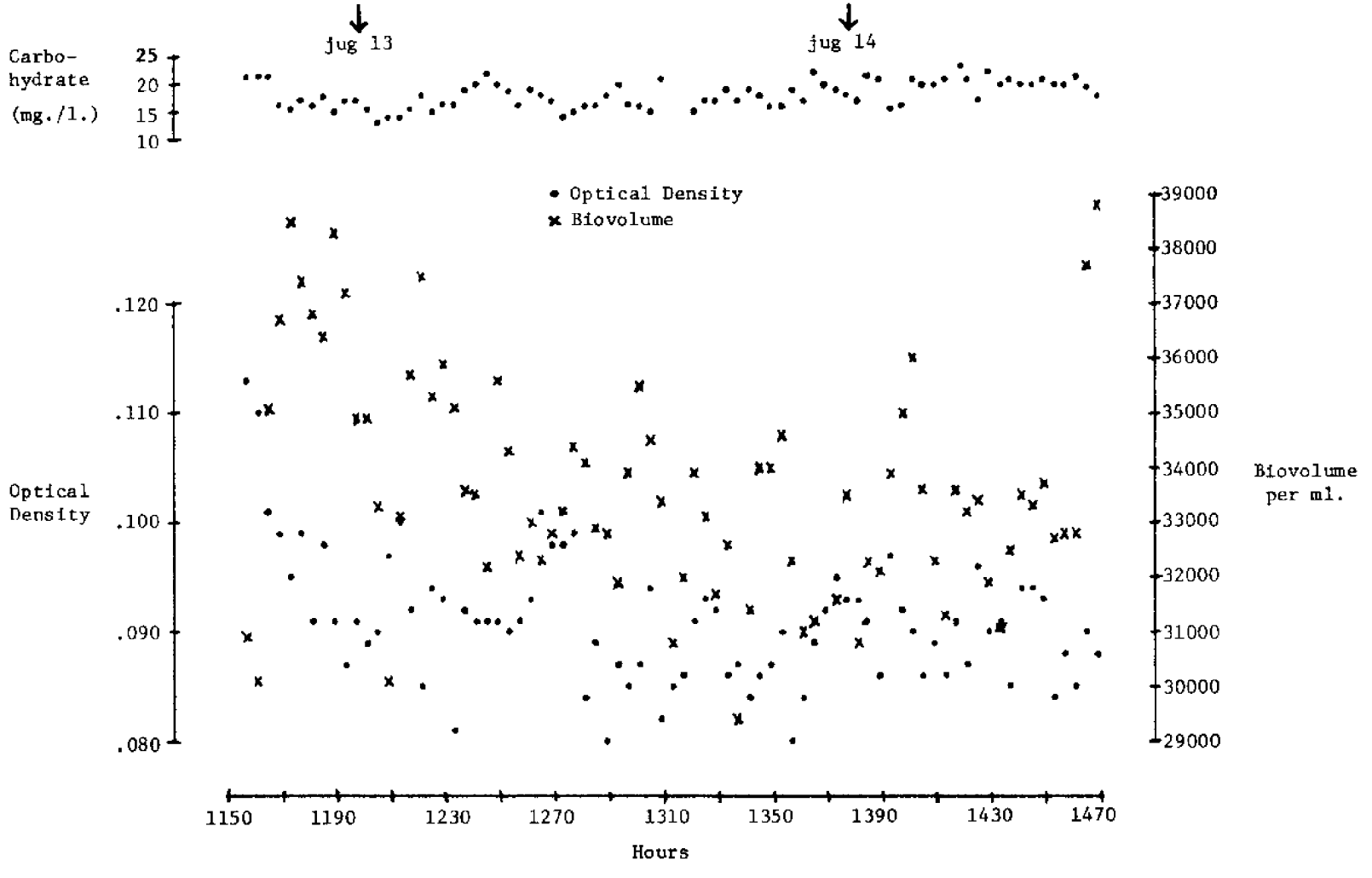
Graph #15: Continuous run III (continued); Samples 77-207; Flow D.



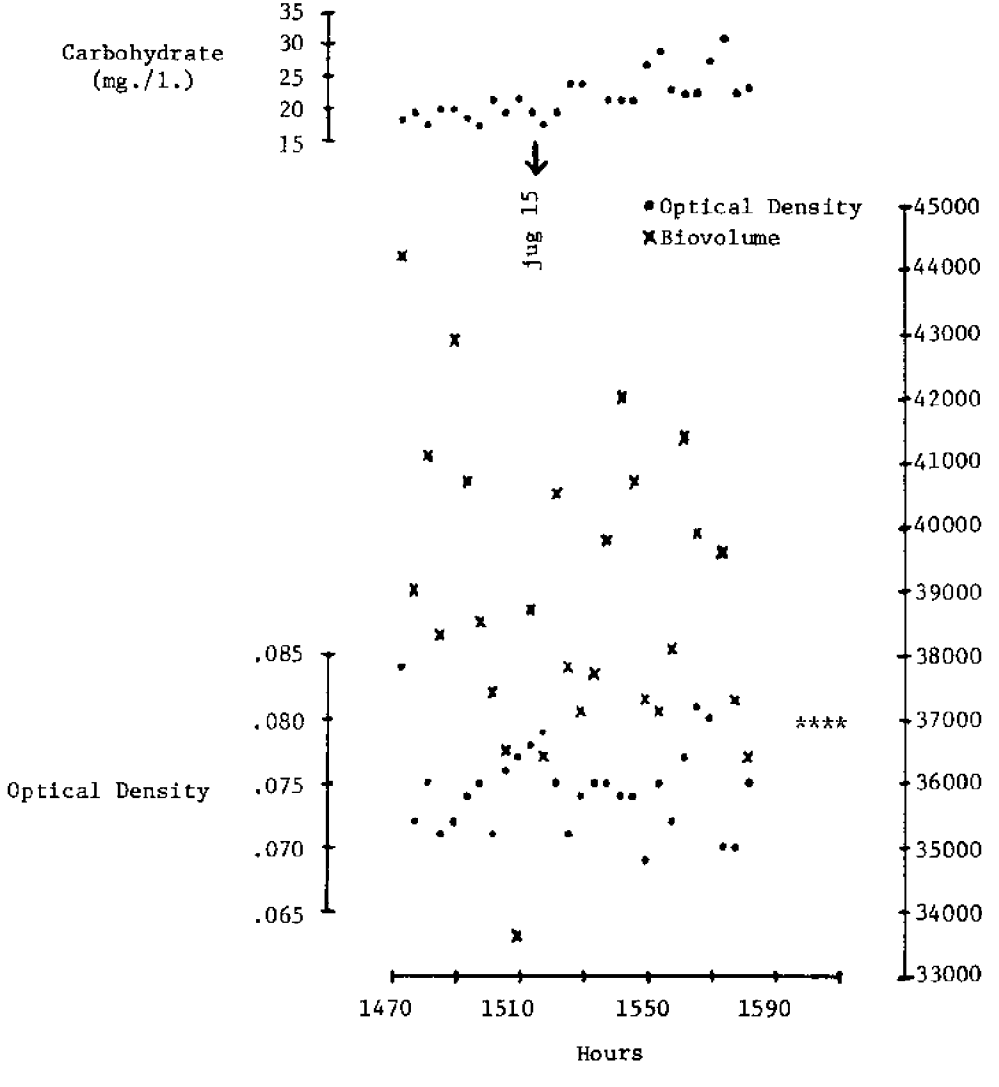
Graph #16: Continuous run III (continued); Samples 207-315; Flow E.



Graph #17: Continuous run III (continued); Samples 316-394; Flow F.



Graph #18: Continuous run III (continued); Samples 395-422; Flow G.



****microscope examination showed protozoa lysis occurred about 1573 hrs. making the duration of readings(to 1915 hrs.) unreliable

There are two observations concerning the fluctuations in biovolume and optical density, apparent in the four last flows, which should be appropriate now. First, it appears that the magnitude of the fluctuations decreases as the flow rate decreases. This observation is made by examining the range of the biovolume scales for each graph and noting that the lower the flow-washout rate, the smaller the range of the biovolume scale that had to be employed. The situation is identical for the prey optical density: as the flow decreases, so does the range of prey variation. This direct relationship between amplitude and flow rate suggests that the community's propensity is to reach a uniform state. As the external disturbance of washout decreases, the compensating reactions of the organisms will also decrease.

The second observation concerning fluctuations is that the first three graphs (14, 15, 16) are deceptive in that the scale used for the predator biovolume eliminates much of the normal variation inherent in the overflow sample technique used. To illustrate the normal variance in biovolume measurements, the last two graphs (17 and 18) were plotted using an expanded biovolume scale. On this enlarged scale, the variation between one sample and the next is obvious. It is important to note that if the last two graphs had been plotted with the biovolume scale used in the earlier graphs, the result would have been a straight line with almost no fluctuation. These normal vicissitudes in protozoa biovolume pose no problem so long as conclusions are based on a substantial number of observations in the same range. No attempt was made to draw

a line between the points in the expanded graphs of 17 & 18, since the non-fluctuating uniformity of the protozoan is evident in the small range of the biovolume scale.

2. Insufficient washout will cause protozoa lysis

There may come a time in the life of every *T. pyriformis* 1/D, when having been cultured for about 100 hours at low prey densities lysis will occur. Such protozoa lysis took place in two different continuous runs when the washout rate was so low that the predators built up to very high densities, consumed most of the prey, and suffered the consequence by the fact that a majority of the predators exploded. The fact that the protozoa lysed is not abnormal, for predatory populations which have no food are forced to reduce their numbers, but the problem occurs in our censusing, which could not give accurate prey optical density readings or protozoa counts when the fine debris from ruptured protozoa was ubiquitous in the reactor. As a result, protozoa lysis forced the termination of continuous run I after 160 hours, and continuous run III after 1915 hours.

It is unusual that our maintenance problem concerned the predator. Most experimental communities are troubled by the predator eating all the prey and then starving because no food remained, rather than by predator die-off terminating the experiment before all the prey were consumed.

3. When washout is not dominant, populations tend toward homeostasis

The graphs of the continuous run (14-18) reinforce some of the fundamentals demonstrated in the batch runs, and suggest some mechanisms of predator-prey interactions. First, as in the batch runs, the density

of predator and prey are directly related: a peak or rise in the protozoa biovolume is matched by a trough or decline in the bacteria optical density. Further, the uniform predator biovolume at flow rates F and G (Graphs #17 and 18), which as mentioned earlier varies little when plotted on a smaller scale, is accompanied by a relatively uniform optical density.

Second, carbohydrate varies with the density of the prey, provided the prey fluctuations are large enough. For the majority of the run the variance in the prey was not large enough to be reflected in a reciprocal deviation in the carbohydrate concentration, and as a result the carbohydrate concentration remained relatively constant. However, at flow B in Graph #14 the variation in the prey density was great enough to show a change in the carbohydrate concentration: as the prey decreased the prey food (carbohydrate) increased and vice versa. No where other than flow B was the prey variation great enough to show a concomitant carbohydrate alteration.

Examination of flow rates D, E, F, and G (Graph #15, 16, 17 and 18) indicates that when washout is not dominant the predator and prey tend to reach a uniform state, or homeostatic condition. This steady condition is characterized by deviations and fluctuations from the "norm" (as at 610 and 670 hours in Graph #15), but as noted earlier, the magnitude of the fluctuations decreases as the washout rate decreases. Finally, at very low washout rates, the populations reach and maintain themselves at a steady level. The steady state causes a change in one organism's density to be eliminated by a compensating alteration in the other organism's density. This homeostasis, or system of predator-prey checks and balances,

tends to produce uniform population levels whose average density is proportionate to the predator washout rate. The two final flow rates indicate that there is a good deal of minor variation during the steady state (some of it due to sampling techniques), but the gross trend is one of uniformity.

It is of further interest to speculate on the method by which this steady state is attained. Graphs #15 and 16 indicate that the final uniform population level is arrived at after a series of damped oscillations. Indeed, the entire concept of homeostasis is well supported by a method of damped oscillations in which a disturbance in the community, such as the change of the pump flow-washout rate, causes a series of fluctuations of diminishing intensity until a new uniform state has been achieved.

Within a range of washout rates low enough so as not to dominate the predator density but high enough to prevent prey depletion and protozoa buildup to the point of lysis, homeostasis causes the populations to arrive at a steady state condition. Further, after a disturbance this uniform state is attained through a series of damped oscillations and the magnitude of these oscillations and additional fluctuations is proportional to the rate of washout.

4. The probability of a prey mutation

When the reactor was opened at the termination of the continuous run, some of the contents were plated on agar to determine if the prey were still pure. Two distinct bacterial colonies appeared on the plates,

one colony type of the *Aerobacter aerogenes*, a white-gray translucent amorphous colony, and another colony type which was white-yellow with distinct borders. It is not known if this colony was an alien bacterium or a mutation of the prey used. Given that the prey went through over 770 divisions (based on a doubling time of 2.48 hours calculated from the batch runs) the anomalous colony, which comprised about 40% of the total colonies, could well have been a mutation.

Conclusion

The most essential requirement of this research is that the results and observations derived from the experiments be applied in solving environmental problems. As noted earlier, this research is but the first phase in the development of an ecological mathematical model which would help predict the consequences of environmental alterations. It seems germane to illustrate some conclusions based on the results of these experiments which could be made concerning a human environmental alteration.

Suppose that a legislature is trying to determine if a law should be passed requiring municipalities above a specified size to increase their waste treatment from 35% removal of organic material to at least 95% removal. One of the primary motivations behind such action is to eliminate the food supply of the odor-producing bacteria. However, it is too complex and costly to conduct experiments in all the state's waterways to determine which microorganisms comprise the fauna or if the organic load reduction will result in the anticipated bacteria

elimination. At this point, using the protozoan and bacterium in these experiments as indicator organisms of the natural river populations, a mathematical model based on the results of the batch and continuous runs can provide an inexpensive answer to the effects of an organic waste diminution.

For instance, the results of these experiments might apply to the organic waste reduction problem. First, based on the fact that predatory ciliates which feed on bacteria can improve water quality by reducing the bacterial concentration, it might be discovered that there was an optimal organic waste load. Below this concentration the prey bacteria would become so scarce that the predatory ciliates, which these experiments show are inefficient at capture and survival at low prey densities, would perish and deprive the community of their natural cleansing-feeding habits. Thus, the state might obtain cleaner water by eliminating only 80% of the organic wastes in order to support enough prey to maintain a thriving predatory population.

Second, the experimental results in a model might indicate that the bacteria are regulated not by the amount of food (organic wastes) but by the density of the predator. The predator density has been demonstrated to be dependent on the flow rate, and as a consequence eliminating organic municipal wastes might not be as important as controlling the river's flow.

These problems and solutions, though hypothetical, are not impossible. They are questions which are difficult or impossible to answer without further laboratory studies of basic ecological phenomena.

Similar problems concerning thermal and nutrient pollution are equally susceptible to laboratory-modeling solutions. In fact, given the complexity of natural ecosystems, a laboratory model may be the only method which is able to provide an answer.

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