Estimation of food limitation in Daphnia Pulex from Boulder Basin, Lake Mead

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ESTIMATION OF FOOD LIMITATION
IN DAPHNIA PULEX
FROM BOULDER BASIN,
LAKE MEAD

by

Thomas Mark Bartanen

A thesis submitted in partial fulfillment
of the requirements for the degree of

Master of Science
in
the Biological Sciences

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University of Nevada, Las Vegas
August, 1987
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ABSTRACT

In February, 1982 I began a year-long study to determine if growth and reproduction in *Daphnia pulex* were limited by the amount of food available in Boulder Basin, Lake Mead. To determine this, I made monthly collections of *Daphnia pulex* and natural lake seston from an already established station in Boulder Basin. I cultured the *Daphnia pulex* under simulated field conditions in a flow-through feeding apparatus using four different food regimes; 1-natural lake seston filtered through 80 μm mesh to remove other zooplankton, 2-lake seston (as above) with an enrichment of $10^3$ cells·ml$^{-1}$ of *Chlamydomonas reinhardtii*, 3-lake seston (as above) with an enrichment of $10^5$ cells·ml$^{-1}$ of *Chlamydomonas reinhardtii*, 4-0.45 μm (HA Millipore) filtered lake water with $10^5$ cells·ml$^{-1}$ of *Chlamydomonas reinhardtii*. The last two food regimes contain amounts of algae in excess of the incipient limiting concentration and therefore, growth and reproduction should not be limited by lack of food.

For each of the 8 months that I was able to run successful experiments, I found that animals fed only lake seston grew more slowly and reproduced less than the animals fed enriched food regimes. There were some months (February, May 1982 and January 1983) when the amount of
extra food in the lowest enrichment (10^3 cells·ml^-1 of Chlamydomonas reinhardtii) was not enough to improve growth or reproduction significantly. These were the months when phytoplankton was most abundant in Boulder Basin and 10^3 cells·ml^-1 provided relatively less enrichment than in other months. The months for which data could not be obtained (July-October) coincide with the lowest abundance of *Daphnia pulex* in Boulder Basin. The low levels of *Daphnia pulex* and moderate levels of chlorophyll-a present in Boulder Basin, indicate that the animals may have been already food limited during those months. From this, I concluded that during the entire year, *Daphnia pulex* is food limited in Boulder Basin, Lake Mead. Comparison of this study with other studies confirms that food limitation of *Daphnia pulex* is primarily due to low phytoplankton abundance (as indicated by low concentrations of chlorophyll-a) although the food quality of the seston may also contribute to the food limitation.
Acknowledgements

There are many people whose aid proved invaluable in the completion of my thesis. To them I give my profound thanks and gratitude. To Dr. Paulson for mentioning that no one had yet studied food limitation in zooplankton at Lake Mead and thus provided me with the subject for this thesis and provided me with information on Lake conditions. The members of the Lake Mead Limnological Research Center, who collected many of my samples for me in addition to their already full schedule. Dr. Deacon for his guidance and comments as a member of my thesis committee. Dr. Yfantis for his assistance with the statistical analyses and to Dr. Wiede who was gracious enough to take Dr. Yfantis' place as my outside committee member. Patrick Fitzgibbon for his assistance with plotting the figures. To all the members of the Department of Biological Sciences for their patience and to my family and all my friends who helped keep me going throughout this project. My deepest thanks go to Dr. Starkweather for his patience, guidance and assistance in my research and course work. Once again, my thanks to you all.
INTRODUCTION

There are many trophic interactions which contribute to the structure of freshwater, suspension-feeding, zooplankton communities. Predation, by fish and by other zooplankton, plays an important role in establishing the diversity and seasonal succession of zooplankton groups (Gliwicz et al. 1981, Benndorf and Horn 1985, Gophen and Pollingher 1985). Competition is important as it may cause some organisms to exclude other organisms in ways which appear to be driven by food limitation (Gliwicz 1980, Mateev 1985, Gilbert 1985, Orcutt 1985, Edmondson 1985, Geller 1985, Kerfoot et al. 1985, Romanovsky and Feinova 1985). Recent reviews detail the complexity of such interactions in freshwater, limnetic ecosystems (Kerfoot 1980, Meyers and Strickler 1984, Lampert 1985). What these studies have as common themes are the concepts of food (or nutrient) limitation and the behavioral and evolutionary strategies that organisms have developed to avoid it.

All aquatic organisms are involved in predation as either predator or prey. Whether it is Daphnia feeding on nannoplankton (Gliwicz and Hillbricht-Illkowska 1972, McCauley and Kalff 1981) or fish feeding on Daphnia (Paulson and Baker 1983), all aquatic organisms attempt to maximize their input of energy and avoid the effects of food limitation, often at the expense of other organisms.
In the recent literature there are many studies of the effects of food limitation (Lampert 1980, Threkeld 1985, Ghilarov 1985, Larsson et al. 1985, Lampert and Muck 1985). A common species subject to probable food limitation, and a frequent subject of such studies is the cladoceran, *Daphnia pulex*.

**Daphnia Feeding Behavior**

*Daphnia pulex* is a suspension feeding cladoceran which uses its thoracic appendages for both feeding and gas exchange (Cannon 1933). Particles are collected on the thoracic limb setae and setules (Figure 1), concentrated in the food groove, and delivered to the mouthparts (Geller and Muller 1981, Porter et al. 1982). The filtering rate (or filtration rate, Rigler 1971) is defined as the volume of water filtered, containing the number of cells eaten by the animal, in a given time. It does not assume that all particles are removed; that those removed are ingested or even that the volume of water actually sieved by the appendages, is known. Feeding rate is the amount of food collected and ingested. Both filtering rate and filtration rate are dependent on the motions of the thoracic feeding appendages. After food is collected in the food groove it is formed into a bolus and ingested (Cannon 1933). Sometimes the food groove becomes clogged with algae, the bolus is too large to be ingested.
Figure 1. Anterior view of *Daphnia pulex* showing 1st and 2nd antennules, mouthparts and 5 pairs of thoracic appendages.
or it contains chemically unacceptable food items (Porter and Orcutt, 1980). When this happens, rejection occurs. Vigorous movements of the postabdominal claw dislodge the bolus or other obstructions. However, the rejection process causes the loss of the food collected and the energy used to collect the food.

Studies indicate that the filtering rate is constant and maximal at low concentrations of particles (McMahon and Rigler, 1963). Above a certain concentration of particles, termed the "incipient limiting concentration", filtering rate gradually declines asymptotically (Burns and Rigler, 1967). The filtering rate never reaches zero, since movement of the filtering appendages is necessary for respiration (Rigler 1961). Filtering rates can be affected by the nutritional state of Daphnia. Ryther (1954) found that starved animals maintained higher filtering and feeding rates than would be expected in high food concentrations. After a period of time, the animals would acclimate to the new concentration, and filtering rates would decline. McMahon and Rigler (1965) indicated that the filtering rate is mediated by the amount of food in the gut, which explains why filtering rates in starved animals return to normal after a few minutes. The filtering rate is also dependent upon environmental factors such as temperature (Burns 1968), pH (Ivanova 1969, Kring and O'Brien 1976) and oxygen (Green 1956).
Different species of zooplankton, show different optimal ranges for these variables. Above or below the optimal range, the filtering rate declines.

Filtering rates determined for animals feeding on pure cultures were found to be higher than those for animals feeding in natural suspensions (Ryther 1954; Burns and Rigler 1967; Burns 1968; Crowley 1973). Subsequent research showed that this reduction in filtering rate is caused by the interference large algal filaments with the collection of food (Burns 1968, Arnold 1971; Crowley 1973; Webster and Peters 1978; Gliwicz and Siedlar 1980). Many investigations have found that increasing concentrations of filamentous algae (such as cyanobacteria) resulted in higher rejection rates (Gliwicz 1980; Porter and Orcutt 1980, Webster and Peters 1978). Porter and McDonough (1984) found that this increase in rejection is associated with increased respiration rates. They suggest that the decreased food intake and increased energy expenditure, reduces significantly the energy available for growth and reproduction. Arnold (1971) found that *Daphnia pulex*, fed on several different species of cyanobacteria, had levels of ingestion, assimilation, survivorship and reproduction that were lower than those in *Daphnia pulex* fed on green algae. Webster and Peters (1978) found lower filtering rates occurred, and smaller brood sizes were produced, as the concentration of algal filaments increased. Porter
and Orcutt (1980) observed similar results when *Daphnia magna* were fed *Anabaena flos-aquae*. Gliwicz (1980) found that different species of Cladocerans vary in their ability to regulate carapace gape (a mechanism to exclude large filaments). Porter and Orcutt (1980) suggest that smaller species of cladocerans are less disturbed by abundant net plankton since their carapace gap is already small enough to exclude large algae.

Closely related to the filtering rate is the feeding or ingestion rate. Rigler (1961) found that below the incipient limiting concentration, the ingestion rate is limited by the filtering rate and is directly proportional to the food concentration. Above the incipient limiting concentration, feeding rate is relatively constant and maximum, limited only by gut retention time or digestion rates. Not all particles in the seston are collected and ingested by *Daphnia*. Depending on the species, much of the seston biomass is unsuitable because it is either: 1-too large (colonies and filaments, Gliwicz and Siedlar 1980), 2-too small (bacteria, Geller and Muller 1981), 3-contains toxins like cyanobacteria (Crowley 1973) and senescent algae (Ryther 1954, McMahon and Rigler 1965) or 4-is resistant to digestion (Porter and Orcutt 1980, Infante 1973). Porter (1977), suggests that particles from 0.8 µm up to 45 µm in diameter can be ingested by the largest *Daphnia*. Geller and Muller (1981) used scanning
electron microscopy to measure intersetular gaps and found that depending on the species, cladocerans should be able to utilize particles down to 0.2 μm in diameter. Subsequent work by DeMott (1985) and Brendelberger (1985) supports the hypothesis that capture efficiency is related to filter mesh size. Crowley (1973) and Lampert (1978), suggested that the upper limit of 45μm-50μm diameter particles for Daphnia pulex is too high and that 35 μm is a better approximation. Larger particles can be collected if they are elongated or flexible and even short segments of filamentous algae can be consumed. The major food source for Daphnia and other zooplankton thus appears to be nannoplankton (Gliwicz and Hillbricht-Illkowska 1972, Porter 1977, McCauley and Kalff 1981). This nannoplankton can be, and often is, supplemented by other food sources such as bacteria (McMahon and Rigler 1965, Pace et al. 1983) and detritus (Pavylutin 1975 and Hrbacek 1985). There is no standard definition of what phytoplankton size fraction constitutes the nannoplankton (Janik 1984), so for comparison purposes, I will define it as cells whose equivalent spherical diameter is less than 64μm.

Berman and Richman (1974) found that Daphnia pulex can select food primarily, but not exclusively, on the basis of biomass. It is probable that Daphnia pulex can select particles of a given size from a suspension of different sized particles when they are pre-fed particles.
of the size that they later select for. Berman and Richman (1974) in another experiment, found that when the biomass of all particles was equal, selection was for small particles present in high numbers. In contrast to selectivity in *D. pulex*, DeMott 1982 found that *D. rosea* showed no selectivity when presented two different sized particles (*Chlamydomonas reinhardtii* and bacteria), but that *Bosmina longirostris* preferentially selected *Chlamydomonas*. Meise et al. 1985, found that *Daphnia pulex* can modify its behavior to feed on a small high-quality alga, over a slightly larger but closely related alga (of lower food value) or intermediately sized latex spheres. Clearly, Cladocerans differ in their ability to discriminate among food sources.

When *Daphnia* are placed in enclosed containers they exhibit two types of swimming behavior while feeding. When suspended food levels are high the animals feed via the hop-and-sink method also termed "grazing" (McMahon and Rigler 1963, Burns 1969). When levels of food are low or the animals are unfed they forage on the bottom of their containers, a behavior termed "browsing". Browsing is thought to dislodge detritus from the bottom of the containers which is then captured and consumed. Horton et al. (1978) found that as food levels increased the proportion of animals browsing on the bottom decreased and that only above the incipient limiting concentration did
the animals spend more than 50 percent of their time grazing. Even at the lowest food levels, animals occasionally swam into the open water, which Horton et al. (1978) assumed represented the animals "sampling" the upper layers in search of higher food concentrations. Browsing behavior is not just limited to the laboratory. During a field study in a small temperate lake, Meyers (1985) found that when levels of ingestible phytoplankton were low, Daphnia pulex (normally a limnetic "grazer") would invade the littoral zone to feed within beds of macrophytes. Browsing, while an important behavior in my experiments, should not be important in large, limnetic areas like Boulder Basin where there are no container walls or nearby littoral zones.

Temporal variations in the feeding behavior of Daphnia have also been noted and are discussed in detail in Starkweather (1983). Another important diel behavior of Daphnia and other zooplankton is vertical migration. Many zooplankton spend the day deep in the water column and rise to the surface at night. This behavior has very important ramifications for zooplankton populations. During the vertical migrations, they are subjected to variations in temperature, predation and food availability.

Effects of Temperature and Food Limitation
Green (1956) studied the relationship between growth, size and reproduction in the Cladocera, in relation to food and temperature and found that, as temperature increased, the rate of growth increased and the interval between instars (developmental stages) decreased. He found that the growth rate decreased as the animals got older and that low levels of food slowed growth. He also found that at low temperatures growth was slowed, but the final length of the animals was larger. Higher temperatures also decreased the size of primiparous animals and the size of the young. Bottrell (1975a, 1975b) found a similar decrease in the development time with increased temperature for epiphytic cladocerans (and other zooplankton) in the Thames River, England. The studies also found differences between the species in the amount of increase brought about by increased temperature. Munro and White (1975) found that in *Daphnia longispina*, growth increased and egg development time decreased with increasing temperature. Lei and Armitage (1980) confirmed that for *Daphnia ambigua*, instar duration decreased with increased temperature. Comparing animals in the laboratory and in the field, they found that development time was shorter, brood size was larger and more total young were produced in the lab animals fed high concentrations of food. Vijverberg (1976) and Lampert (1978) also found that increased temperature led to
increased egg production and that the curves were similar to those found for increasing food concentrations. These studies show that temperature is an important variable in controlling growth and reproduction in zooplankton. As temperature increases, the rate of growth and reproduction can also increase, given that sufficient food is present.

Food quality, not just quantity is important in regulating the pattern of reproduction in *Daphnia*. According to Gulati et al. (1985), food limitation can be caused by two factors, low food abundance and poor food quality. Low abundance of food can be due to strong grazing of the phytoplankton by large numbers of zooplankton or by low trophic status when there are few phytoplankton to begin with. Low food quality can be due to mechanical interference with filtering, algal toxins, or poor assimilability of the algae. Zhdanova and Frinovskaya (1975), Vijverberg (1976) and Schwartz and Balinger (1980) found that the pattern of reproduction in various Cladocera varied with food quality. Schwartz and Balinger (1980) found that animals fed nutritious, high quality foods grew and reproduced faster than animals fed on foods of lesser quality, but, because the animals fed high quality food had shorter lifespans, the two groups were similar in the total amount of reproduction. Schwartz and Balinger (1980) found two patterns of reproduction in *D. pulex*, early maturity with high
fecundity and short lifespan and delayed maturity with lower fecundity and longer lifespan. They speculated that *D. pulex* could take advantage of an increase in high quality food by greatly increasing its reproduction over the short term. While in less favorable environments, reproduction is limited, but lifespan is lengthened (possible due to decreased stress from reproduction). Taylor (1985) found diminished growth and reproduction at low concentrations of food.

**Objectives**

For my study, I wanted to determine whether or not *Daphnia pulex* in Lake Mead were food limited and, if so, at what times of the year. Because my experiments were to take place in the laboratory, several major factors that affect natural populations would not be present in my study. Predation pressure would be lifted and so would competition with other species. Temperature is an important variable because I wanted to duplicate field conditions as closely as possible. The variations in the phytoplankton would also be important. Because the presence of large net plankton can have a profound effect on growth and reproduction, the make up of the seston, not just the abundance will have an effect on my experiments. Finally, the light regime the animals are exposed to must be regulated to avoid responses that would not be
appropriate to the season being studied (Starkweather, 1983).

MATERIALS AND METHODS

Site Description

Lake Mead is a large reservoir located in the Mojave Desert along the border between southeastern Nevada and northwestern Arizona. Formed in 1935 by the construction of Hoover Dam, it is, by volume, the largest reservoir in North America (Baker and Paulson, 1980). The lake is divided into two large basins (upper and lower) by Boulder Canyon (Paulson 1981). The site for my study was Boulder Basin, a subdivision of the lower basin that receives water from the Colorado River via the Upper Basin and from the Las Vegas Wash (Fig. 2 from Paulson 1981 used with permission). I chose Boulder Basin for the site of this study because it is typical of the lake as a whole (personal communication, L. J. Paulson) and because a number of other studies have been conducted at that site. These have included Burke's 1977 study of the relationship of the zooplankton to the metalimnetic oxygen minimum, Janik's 1984 study of the the role of the nannoplankton in the phytoplankton dynamics in Lake Mead, and Wilde's 1984 study of the seasonal and spatial heterogeneity in the limnetic zooplankton community of Lake Mead.
Figure 2. Map of Lake Mead showing location of sampling station (underlined).
Recent studies of Lake Mead have shown that there has been a considerable decrease in: 1- the nutrients available to phytoplankton (Paulson et al. 1980, Paulson and Baker 1983), 2- the concentration of chlorophyll-a (Paulson et al. 1980 and Janik 1984), 3- zooplankton densities (Wilde 1984) and 4- the abundance of threadfin shad (Paulson and Baker 1983). This collective information indicates that the productivity of Lake Mead is declining. Paulson et al. (1980) found that the production of phytoplankton in Lake Mead is limited due to low nitrogen and phosphorus levels throughout the year and classify the lake as being oligotrophic to mesotrophic on the basis of chlorophyll-a concentrations. The Inner Las Vegas Bay may be slightly eutrophic due to inputs of sewage. Chlorophyll-a concentrations in Boulder Basin have been decreasing in recent years due to the removal of nutrients in the lake via the hypolimnetic discharge from Boulder Dam. The depleting effect is reinforced by the Las Vegas Wash density current which keeps the nutrient input from the Wash at a depth where it is unavailable to phytoplankton most of the year (Baker and Paulson 1980, Paulson and Baker 1983). Janik (1984) classifies the lake as oligotrophic on the basis of phytoplankton biomass and invokes similar limitation of the phytoplankton due to low levels of inorganic nutrients. Examining historical zooplankton densities, Wilde (1984) found that there has
been a 90 percent reduction in total zooplankton density in the lake since 1971. Paulson and Baker (1983) suggest that the reduced levels of zooplankton may be responsible for lower densities of threadfin shad, a planktivore normally abundant in the limnetic areas of Lake Mead. The lower densities of zooplankton in summer (especially Daphnia) might be due to intense predation alone, if it were not for the fact that threadfin shad are now rare in the limnetic areas of the Lake and predation by shad is therefore, minimal (Paulson and Baker, 1983). This information coupled with the fact that phytoplankton growth is limited in the lake suggests that the zooplankton in most of Lake Mead are food limited.

Few studies on zooplankton in Lake Mead have been done other than those describing the species of zooplankton present, their abundance, and their seasonal and spatial heterogenity (Burke 1976, Paulson et al. 1980 and Wilde 1984). Various zooplankton species are dominant during different times of the year in Lake Mead. I chose Daphnia pulex (a filter feeding cladoceran) as the organism for my study for a number of reasons. Daphnia pulex is present in Lake Mead throughout the entire year, which allowed a full year study with a single species. Thus, I avoid having to compare growth and reproduction in different species, which may have quite different responses to seasonal changes in the phytoplankton.
Daphnia pulex is also relatively easy to culture and maintain in the lab and there is a wealth of information on the factors affecting the feeding behavior, growth and reproduction of the Daphnidae both in situ and in laboratory studies.

**Experimental Procedure**

I collected the animals and seston for this study from Station 8 (BC8 Paulson and Baker, 1983), Boulder Basin, Lake Mead (Fig. 2). I collected animals for the experiments once each month and seston twice a month when possible. I used an 80μm mesh Wisconsin net to make at least 6 vertical tows from 40m to the surface. I placed the live zooplankton into 4 liter Cubitainers and then placed the containers in an ice chest filled with either lake water or lake water and a small quantity of ice to keep the animals as close to the ambient temperature of the lake as possible during transportation to the lab. I collected natural lake seston from a depth of three meters with a 6 liter Van Dorn bottle, filtered the lake water through an 80μm mesh Wisconsin net (to remove the zooplankton) and placed the filtered water into 5 gallon carboys and transported them to the lab.

In the laboratory, I placed the seston in a temperature controlled room in relative darkness (Berman and Richman, 1974) for the duration of each month's study.
I aerated the carboys containing the lake water gently in the hope that it would prolong the useful life of the seston. I filtered one carboy of the lake water through 0.45μm (HA) Milipore filters to remove all algae and detritus from the water. Before removing water for feeding suspensions, the carboys still containing seston were gently shaken to resuspend any settled seston.

For each monthly run, I fed groups of animals on the following 4 food regimes: Chamber 1, 80μm filtered lake water containing natural nannoplanktonic seston; Chamber 2, lake water as in Chamber 1 plus an additional 10^3 cells·ml⁻¹ of *Chlamydomonas reinhardtii*; Chamber 3, lake water as in Chamber 1 and an enrichment of 10^5 cells·ml⁻¹ of *C. reinhardtii*; and Chamber 4 animals were fed on 0.45μm HA Millipore filtered lake water and 10^5 cells·ml⁻¹ *C. reinhardtii*. I chose an enrichment concentration of 10^3 cells·ml⁻¹ because based on chlorophyll-a concentrations in the lake during 1981, this regime should approximately double the amount of food available to the animals. I chose 10^5 cells·ml⁻¹ because this concentration is above the incipient limiting food concentration for *Daphnia pulex* (P.L. Starkweather personal communication), and young animals fed at this concentration should not be food limited. There has been some question as to the suitability of *Chlamydomonas reinhardtii* as food for *Daphnia pulex* (Taub and Dollar 1968) but this idea has
since been discarded (Porter and Orcutt 1980 and Gerritsen et al. 1985) and the alga is a common food for culturing Daphnia. Comparing the data on growth and reproduction in Chambers 1 and 2, I could determine whether Daphnia pulex can increase growth and/or reproduction with extra food in chamber 2. I could also detect any negative effects due to the algal enrichment (senescence or chemical contamination of the enrichment suspension for example). By comparing Chambers 2 and 3, I could determine if there is any enhancement or detrimental effects of a further increase in the food supply. I could also detect if there is a detrimental effect of the enrichment that appears at high concentrations but not at the lower levels. For example there may be toxic effects of the enrichment that do not appear when only a small amount of C. reinhardtii is used but that become significant when large quantities of the alga are present. By comparing growth and reproduction in Chambers 3 and 4 I could detect any inhibitory effects of the seston. Growth and reproduction in the two chambers should be the same since they are subjected to the same conditions, the only difference being the presence of nannoplankton seston in Chamber 3. Finally, by comparing chambers 1 and 4 I could compare the growth and reproduction of Daphnia pulex fed natural lake seston (devoid of other zooplankton and netplankton >80 μm) and a unialgal food.
For each experiment, I separated the *Daphnia* sp. from the other zooplankton in the sample and removed only the smallest individuals for use in the experiments. When delays occurred in starting the experiments, the animals were kept from starving by additions of *C. reinhardtii*. Whenever possible I tried to use animals less than 1mm in length. I used 40 animals for each experiment, 10 in each chamber. If forty animals of suitable size were not available, gravid females from the rest of the sample were isolated until their next molt. These neonates were then pooled with the other juveniles. When sufficient total young were present, I removed each one using a wide mouth pipette and placed it in a well slide for observation. I removed most of the water from around the animal to immobilize it and examined it to make sure that it was a female *Daphnia pulex* (Brooks 1953). After measuring its length from the top of the helmet to the base of the caudal spine with an ocular micrometer, I placed the animal into its appropriate 500 ml feeding chamber. Arnold (1971) found that this technique did not affect the subsequent behavior of the animals.

After separating the animals, I prepared the feeding suspensions. I established, prior to collection of samples, an axenic culture of *Chlamydomonas reinhardtii* in the lab. I suspended cultures between 6 and 14 days old in 0.45μm (HA) Millepore filtered lake water. I then
placed the algae in a 125ml Erhrlenmyer flask with a magnetic stirrer to facilitate the breakup of algal clumps. After there were no visible clumps left I passed the suspension through a 20μm Nitex filter mesh and a small sample was counted in an electronic particle counter to determine the number of cells (or small cell clusters) ml⁻¹. The resulting value was then used to determine the necessary dilutions to give the final feeding concentrations. Four liters of each feeding suspension were then placed in separate plastic Cubitainers and were continuously and vigorously aerated with aquarium bubblers to keep the algae suspended and the water well oxygenated.

The flow-through feeding apparatus I used for the experiments (Figure 3) is similar to that used by Lampert (1975). No water bath was used but I kept the entire apparatus in a temperature controlled room. I connected all 4 food reservoirs and feeding chambers through one, four-channel peristaltic pump. I calibrated the pump to provide a flow rate of 1 liter per day to each chamber so that the entire contents of each chamber would be replaced twice each day. I screened both the inlets and outlets of each 500ml feeding chamber with 80um Nitex mesh to keep the Daphnia in the chambers. I removed the animals from the chambers three times a week to take measurements. I took length measurements for each animal and counted the number of eggs present (if any). I removed and counted
Figure 3. Diagram of flow-through feeding apparatus.
any neonates present in each chamber. I then placed the animals in clean chambers with their proper food medium. Any dead animals were given a final length measurement, examined for signs of trauma and discarded. After all the measurements were completed all the chambers were reconnected and the pump restarted. On average the entire process of measurements caused an interruption of flow to the chambers for 2 hours. I illuminated the chambers with two fluorescent lamps at an intensity of 300 lux and covered each chamber with a cardboard sleeve so that light would enter only at the top of each chamber and not the sides. In this way I hoped to minimize behavioral aberrations in the animals caused by their orientation to the light source (Young et al., 1984). To maintain conditions in the lab as close to those in the lake as possible, I adjusted the temperature and photoperiod weekly (when necessary) to match conditions in the lake (Table 1). Due to the limits of the timer for controlling the lights, changes to the Light/Dark cycle were made in 15 minute increments. As noted above, I tried to collect fresh lake water every two weeks when possible. This was done so that during experiment the animals would be exposed to the natural changes in the seston of the lake. But sometimes, I only used one collection of seston for the duration of that month's experiment (see Table 1).
Table 1. Collection dates and comparison of field and laboratory temperature and light regimes.

<table>
<thead>
<tr>
<th>MONTH</th>
<th>START</th>
<th>END</th>
<th>TEMP (°C)</th>
<th>PHOTOPERIOD</th>
<th>SESTON COLLECTION</th>
<th>Dates</th>
</tr>
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<tbody>
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<td>2-17</td>
<td>12/12</td>
<td>10:15/13:45</td>
<td>10:15/13:45</td>
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<td></td>
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<td>11:00/13:00</td>
<td></td>
<td></td>
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<tr>
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<td>3-25</td>
<td>13/13</td>
<td>11:37/12:23</td>
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<td></td>
<td>12:32/11:28</td>
<td>12:30/11:30</td>
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</tr>
<tr>
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<td>14/14</td>
<td>12:44/11:16</td>
<td>12:45/11:15</td>
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<td>13:00/11:00</td>
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<td></td>
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<td>15/15</td>
<td>13:44/10:16</td>
<td>13:45/10:15</td>
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<td></td>
<td>5-4</td>
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<td>6-28</td>
<td>18/18</td>
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<td>14:45/9:15</td>
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<td>14:42/9:18</td>
<td>14:45/9:15</td>
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<td></td>
</tr>
<tr>
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<td>11-17</td>
<td>12-4</td>
<td>18/22*</td>
<td>9:41/14:19</td>
<td>9:45/14:15</td>
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<td>9:17/14:43</td>
<td>9:15/14:45</td>
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<td>10:13/13:47</td>
<td>10:15/13:45</td>
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<td></td>
<td></td>
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<td>1-6</td>
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<td>10:30/13:30</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Temperature was reduced to 18°C on Day 2.
I ran the experiments until all the animals in one chamber were dead, there was no more lake seston or *Chlamydomonas* left for feeding or to prepare for the next month's experiment, whichever came first. I ran one experiment each month starting with the collection of animals on January 22, 1982 and terminated the last experiment on January 23, 1983. For a complete listing of the temperatures, L/D cycles and experimental deviations, see Tables 1 and 2.
Table 2. Departures from experimental procedure.

<table>
<thead>
<tr>
<th>MONTH</th>
<th>Day of Experiment</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>February 1982</td>
<td>Day 18</td>
<td>- Color change in chamber 3 due to bacteria (last day of experiment)</td>
</tr>
<tr>
<td>March</td>
<td>Day 5</td>
<td>- Problem with apparatus, Chamber 1 restarted.</td>
</tr>
<tr>
<td></td>
<td>Day 15</td>
<td>- Experiment terminated to begin March run. Chamber 1 did not run as long as chambers 2-4 this month.</td>
</tr>
<tr>
<td>April</td>
<td>Day 5</td>
<td>- Vorticella on debris in chambers 3 and 4. Chambers were cleaned.</td>
</tr>
<tr>
<td>May</td>
<td></td>
<td>- 12 animals used in each chamber.</td>
</tr>
<tr>
<td>June</td>
<td>Day 2</td>
<td>- Peristaltic pump broke, flow interrupted for approximately 5 hours.</td>
</tr>
<tr>
<td></td>
<td>Day 20</td>
<td>- Chamber 1 leaked, reducing available food. Only 1 animal died.</td>
</tr>
<tr>
<td>November</td>
<td>Day 2</td>
<td>- Temperature reduced by 4 degrees to match lake conditions.</td>
</tr>
<tr>
<td></td>
<td>Day 8</td>
<td>- Flow of feeding suspensions to chambers reduced by half for 5 hours while preparing food suspensions.</td>
</tr>
<tr>
<td></td>
<td>Day 12</td>
<td>- Aerator were above level of suspension and algae settled. Concentration of food to chambers was not uniform</td>
</tr>
<tr>
<td></td>
<td>Day 13</td>
<td>- Flow interrupted for 10 hrs while waiting for new food suspensions. Food delivery to chambers not</td>
</tr>
</tbody>
</table>
uniform.

December

Day 8  - Flow reduced by half for 4 hours while preparing new food suspensions.

Day 9  - Flow interrupted for 3.5 hours due to power outage.

January 1983

Day 8  - Flow interrupt for over 20 hrs due to broken peristaltic pump.

Day 10 - Flow interrupted for 5 hours while preparing food suspensions.
RESULTS

Food Supply

Figure 4 shows the amount of Chlorophyll-a (in μg·l⁻¹) at the Boulder Basin station for 1981 and 1982 (Strickland/Parsons uncorrected chlorophyll-a, Kellar et al. 1980). The horizontal lines show the amount of chlorophyll-a (μg·l⁻¹) present in suspensions of 10³ and 10⁵ cells·ml⁻¹ of Chlamydomonas reinhardtii (the lowest level of food enrichment). During 1982, there was an average 1.33 μg of Chlorophyll-a·l⁻¹ present at the Boulder Basin (BC8) station. Comparison shows that the highest relative enrichment occurred in the November study when the Chlorophyll-a in the 10³ cells·ml⁻¹ C.reinhardtii suspension accounted for a relative enrichment of 131.5 percent based upon the Chlorophyll-a present in the seston. The lowest relative enrichment was in February when the enrichment only amounted to 29.5 percent of the natural seston Chlorophyll-a. On average, my enrichment of 10³ cells·ml⁻¹ C.reinhardtii added 75.9 percent more chlorophyll-a to what was naturally present in the lake seston (but with substantial variation). The chambers receiving 10⁵ cells·ml⁻¹ of C.reinhardtii were assumed to be essentially unlimited by food and received an average of 66.9 times the amount of food present in the seston (88.81μg·l⁻¹ chlorophyll-a).
Figure 4. Concentration of chlorophyll-a in Boulder Basin during 1981 and 1982.
Explanation of Figures and Tables

Figures 5, 6 and 7 show the survivorship over time for treatment and each experimental run. Included in the graphs are decreases due to animals lost and those that were discovered to be males. I did not consider them to be mortalities for statistical tests, but the decrease in the number of animals in each chamber is noted in the figures. Each month, I tested the hypotheses: \( H_0 \) = survivorship is constant over the course of the experiment in each chamber and \( H_0 \) = survivorship is the same in all four treatments. For this I used a Kolmogorov-Smirnov goodness of fit analysis with a level of significance 5 percent (Zar 1974). I did this to check for differences in mortality that might be due to collection trauma (high mortality at the beginning of the experiment, little or none thereafter), due to the treatments (food-limited animals starving) or toxic effects due to contamination of the feeding suspensions.

Figures 8, 9 and 10 show the increase in average body length over time for each experimental treatment (± 1 standard error). To correct for differences in average initial length of the animals in each of the four treatments, I subtracted the average initial length for the animals in a given chamber from all subsequent average length determinations for that chamber. Therefore, Figure 4 (and subsequent figures) show the increase in average
length above the average initial length (0 on the graphs) for each treatment. I feel that this technique allows easier comparisons of growth in the chambers. Especially in those months when there was a significant difference in the average initial lengths of the animals in the four treatments. I used a single factor analysis of variance (One-Way ANOVA) with a 5 percent level of significance to test if there was a significant difference in the initial lengths of the animals (Zar 1974).

Figures 11, 12 and 13 show the average number of neonates produced per female, per day (# \cdot \bar{q}^{-1} \cdot d^{-1}). For the months of April 1982, December 1982 and January 1983 the number of neonates produced was extremely low. To better illustrate reproductive activity, I plotted these months as the number of eggs per female per day (# \cdot \bar{q}^{-1} \cdot d^{-1}). Days where data were unavailable are marked with breaks in the graph lines.

Table 1 lists the starting and ending dates for the experiments, temperature in the field and the temperature used in the lab runs and dates of seston collections. Also listed are the natural light/dark cycle and the approximation used in the lab. Table 2 lists deviations from the established methods during each month. Table 3 list the results of Kolmogorov-Smirnov goodness of fit analysis testing \( H_0: \) mortality is constant over the duration of the experiment in a given chamber.
Patterns In Survivorship

I examined the patterns of survivorship over the eight experiments to see if there were any significant differences in the timing of mortalities or the amount of mortality in a given treatment. The pattern of survivorship seen in a given chamber or within a given treatment provides clues to the cause of the mortality. When many animals die at the beginning of the experiment, mortality is most likely due to trauma to the animals during collection and sorting. If the animals in the lake were already in poor condition due to an inadequate food supply, trauma during collection would be even more likely to result in early deaths among the experimental animals. Many deaths among the animals towards the end of the experiment might be due to toxic effects of senescent algae in the food enriched regimes, or due to decreasing food quality of the collected seston over time. Mortalities not due to either the food regimes or collection trauma should be randomly distributed over the course of the experiment.

I used a Kolmogorov-Smirnov goodness of fit analysis (Zar 1974) and a Heterogenity Chi-square analysis (Zar 1974) to test the null hypothesis: The amount of mortality in each treatment (C1-C4) was the same. Neither test showed any significant difference between the four treatments. Kolmogorov-Smirnov (overall), D=.09426 n=122
I next tested the null hypothesis: The mortality in each month of my study is constant within a given treatment (in chamber 1, mortality in January = February = March, etc.). There was no significant difference in any treatment except chamber 4. (Chamber 1, D=.10119 n=42 P > .5; Chamber 2, D=.14286 n=28 P > .5; Chamber 3, D=.09052 n=29 P > .5; Chamber 4, D=.28261 n=23 .05> P > .02). In the December run, chamber 4, all mortality occurred in the first 4 days of the experiment after which there were no mortalities in that chamber. In March and May, there was no mortality at all (the decreased numbers in the figures are due to missing animals). The other months showed no significant changes in mortality over time (see Table 3).

A Kolmogorov-Smirnov goodness of fit test (Zar 1974) on the survivorship curves for the February run showed no significant difference in the survivorship of the animals in the 4 treatments (D=.16667, n=12, P>.05). Testing H<sub>0</sub>: Mortality is constant over the duration of the experiment in a given chamber with a Kolmogorov-Smirnov test for goodness of fit, I found that mortality was not constant over the course of the experiment in C1 (D=.71429, .02<P<.05). All mortality occurred in the last 5 days of the experiment and may have been due to low
food value of the seston since the lake water was 3 weeks old by this time (Fig. 5). There were no significant changes in mortality over time in the other chambers (Table 3).

Figure 5 shows the survivorship curves for the March experiment. There were no significant differences in survivorship either between the treatments (D=.30000, n=10, .5> P>.2) or within the chambers over the course of the experiment (Table 3).

Survivorship curves for April are shown in Figure 5. I found no significant differences in the survivorship over the course of the experiment in any of the treatments (Table 3). Neither is there any significant difference between the treatments (D=.16667, n=15, P>.05).

Analysis of survivorship (Fig. 6) for May showed no significant differences within the chambers over time (Table 3). Kolmogorov-Smirnov goodness of fit showed no significant difference in the amount of mortality between the 4 treatments (D=.25, n=8, .5> P>.2). A heterogeneity Chi-square analysis (Zar 1974) did show a significant difference (χ²=8.000 d.f.=3 <7.815 a=0.05). This is the only month that a Heterogeneity Chi-square analysis found a significant difference in the survivorship in the four treatments (all χ² calculations at the 5 percent level of significance with 3 d.f.). Chambers 2 and 3 did show a decrease in survivorship in the period just after the new
Figure 5. Survivorship curves for February, March and April 1982.
Figure 6. Survivorship curves for May and June 1982.
TABLE 3  Results of Kolmogorov-Smirnov goodness of fit analysis on *Daphnia* mortality.

<table>
<thead>
<tr>
<th>Month</th>
<th>Class</th>
<th>Number of Mortalities</th>
<th>Dn</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
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<td>C1</td>
<td>.71429</td>
<td>3</td>
<td>.05 &gt; P &gt; .02</td>
</tr>
<tr>
<td></td>
<td>C2</td>
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<tr>
<td></td>
<td>C3</td>
<td>.23817</td>
<td>6</td>
<td>P &gt; .5</td>
</tr>
<tr>
<td></td>
<td>C4</td>
<td>.57143</td>
<td>1</td>
<td>P &gt; .5</td>
</tr>
<tr>
<td>MARCH</td>
<td>C1</td>
<td>.55000</td>
<td>5</td>
<td>.1 &gt; P &gt; .05</td>
</tr>
<tr>
<td></td>
<td>C2</td>
<td>.33333</td>
<td>3</td>
<td>P &gt; .5</td>
</tr>
<tr>
<td></td>
<td>C3</td>
<td>.33333</td>
<td>2</td>
<td>P &gt; .5</td>
</tr>
<tr>
<td></td>
<td>C4</td>
<td>0</td>
<td>0</td>
<td>---</td>
</tr>
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<td>C4</td>
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<tr>
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</tr>
<tr>
<td></td>
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<td>0</td>
<td>---</td>
</tr>
<tr>
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<tr>
<td></td>
<td>C4</td>
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<td>---</td>
</tr>
<tr>
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<td>.002 &gt; P &gt; .001</td>
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<tr>
<td></td>
<td>C3</td>
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<td>6</td>
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<td></td>
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<td>C3</td>
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<td>P &gt; .5</td>
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<tr>
<td></td>
<td>C4</td>
<td>.17857</td>
<td>4</td>
<td>P &gt; .5</td>
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</table>
Lake seston was first used. The link between the seston and the mortality in chambers 3 and 4 is not clear since survivorship in chambers 1 and 4 were not affected. Chamber 1 should have been more susceptible to seston changes since it does not have the buffering effect of extra food. If the effect was due to the enrichment food, chamber 4 should have been the most affected. If the effect were due to water chemistry all chambers should have been affected.

I found that survivorship in June was significantly different (Table 3) in Chambers 2 and 3 over the 20 day run of the experiment, with most of the mortality occurring in the first 5 days of the experiment (Figure 6). I did not find any significant difference in survivorship between the four chambers (D=.15909, n=22, P>.5).

Examining the November survivorship data (Fig. 7) I found that mortality increased dramatically after Day 6. This constant loss of large animals depressed the average length in chamber 1 and kept the total increase in body length low. Death of individual animals occurred in chamber 1 until Day 17 when I found 3 dead animals in the chamber and the last, obviously dying. The mortality was not due to any physical damage that I could detect. I found that the animals in chamber 2 showed a pattern of growth and mortality similar to that in chamber 1. I also
Figure 7. Survivorship curves for November, December 1982 and January 1983.
noted that chambers 3 and 4 did not show the same patterns of survivorship as chambers 1 and 2. After day 6, there was almost no mortality in either chamber 3 or 4 (Figure 7). Survivorship did not change significantly over time in chambers 1, 2 and 4 (Table 3), but was significantly different for chamber 3. In chamber 3, there was high mortality the first 6 days of the experiment. This could be due to poor condition of the collected animals, but the other chambers did not show similar early mortality. Comparing the 4 chambers to each other, I did not find any significant differences in the amount of mortality ($D=0.23913$, $n=23, 0.02 > P > 0.01$).

Figure 7 shows the survivorship data for December. Chambers 2 and 4 had similar survivorship patterns and in both of these chambers survivorship was not constant over time (see Table 3). Most of the mortality occurred during the first four days and may be due to poor condition of the collected animals. Chambers 1 and 3 were also similar to each other and showed no significant differences in mortality over time. I found no significant differences in survivorship among the 4 treatments ($D=0.11364$, $n=22, P>0.5$). The flow interruptions (Table 2) did not seem to have an effect on survivorship.

In January (Fig. 7) my statistical analysis did not find any significant differences in survivorship over the
course of the experiment (Table 3) or in the mortality between the treatments ($D = .15$, $n=10$, $P>.5$).

Survivorship was variable in the all treatments and showed no discernible pattern within a month or season. In most of the experiments, there was no significant difference in the amount of mortality over time (Table 3). This is probably due to the small sample sizes of only 10-12 individuals per treatment. Changes in mortality that might be important, do not test as statistically significant.

Growth

The growth of *Daphnia pulex* for each of the four treatments in the eight experimental runs are shown in Figures 8, 9 and 10. In every experiment, the animals fed the $10^5$ cells·ml$^{-1}$ enrichment (chambers 3 and 4) showed substantially more growth than the animals fed only lake seston (chamber 1). I found similar results with the $10^3$ cells·ml$^{-1}$ enrichment, but the increase in growth was considerably less. I expected this because there is a 100 fold difference in the enrichment (based on chlorophyll-a concentrations) between chambers 2 and 3.

In chambers 1 and 2, there was very little difference in the amount of growth during the winter runs (February and December 1982, January 1983, Figures 8 and 10). I expected growth in chamber 2 to be higher than in chamber
l because of the enrichment. During February 1982, levels of chlorophyll-a in Boulder Basin were at their highest point (Fig. 4). During this month, the $10^3$ cells·ml$^{-1}$ of *Chlamydomonas reinhardtii* provided a relative enrichment of less than 30 percent, which was not enough to enhance the growth of *D. pulex*. In December, the animals in chamber 2 showed better growth than those in chamber 1, except at the very end of the experiment. The large error bars for chamber 1 on Day 12 are due to a difference in size of over 0.5 mm between the two surviving individuals. Overall, growth in Chamber 2 seems to be better than that in Chamber 1, but it is not significant due to the large variation in the length of the animals in Chamber 1 ($F=2.05$ d.f.$=1,6; .25>P>.1$). In January, chamber 1 actually shows more growth than chamber 2 during the first two-thirds of the experiment. Mortality among large individuals does not account for the slower growth in Chamber 2. Senescence of the *Chlamydomonas* is also unlikely since growth in all regimes receiving enrichment should have been affected, and chamber 3 showed no such effect. A possible explanation is that the chamber was contaminated by improper cleaning.

In the March experiment (Fig. 8), drawing conclusions that growth is better in chamber 2 than in chamber 1 must be made carefully. Because of problems with the apparatus, the duration of the experiment was shorter in
Figure 8. Growth in *Daphnia pulex* during February, March and April 1982.
chamber 1. Without enrichment, growth in animals fed only lake seston seems to be extremely slow. The small length increase seen in Chamber 1 may be due in part to the smaller initial length of the animals compared to the other chambers. Because the slope of the curve for chamber 1 is less than for chamber 2, it seems likely that the animals in chamber 1 are food limited.

In November (Fig. 10), the animals in Chamber 1 showed steady growth until Day 6 when the amount of growth abruptly slowed. These animals increased in length only slightly during the rest of the experiment and reproduction (after Day 8) was also low. Examining the survivorship data (Fig. 7) I found that mortality increased dramatically after Day 6. This constant loss of large animals depressed the average length in chamber 1 and kept the total increase in body length low. From Figure 4, I determined that November was the month of the lowest chlorophyll-a concentration in Boulder Basin. As mentioned previously, the relative enrichment in chamber 2 this month (based on chlorophyll-a) is 131.5 percent. The low food value of the seston coupled with the interruption of flow that occurred on days 8 and 12 (Table 2) may have increased the degree of food limitation in chambers 1 and 2. Any decrease in the food value of the seston after collection would increase the food limitation even more. The mortality in these chambers at the end of the
experiment is probably due to decreased food value of the collected seston. I also noted that chambers 3 and 4 did not show the same patterns of growth and survivorship as chambers 1 and 2 (Fig. 7). Growth in chambers 3 and 4 was fairly steady over the 17 days and after day 6 there was almost no mortality in either chamber.

Growth in chamber 3 (lake seston and $10^5$ cells·ml$^{-1}$ *Chlamydomonas reinhardtii*) was considerably greater than that in chamber 2 in every run except March 1982. The decreased growth in chamber 3 (Fig. 8) during this month is probably not due to senescence in *C. reinhardtii* since growth in chamber 4 was not affected. It is possible that chamber 3 was not properly cleaned and some toxin was introduced. It is also possible that there is some factor in the seston that inhibits ingestion or interferes with feeding so that the animals in chamber 3 cannot take full advantage of the enrichment.

Throughout the study, both chambers 3 and 4 were clearly less food limited than chambers 1 and 2. There was very little difference in the amount of growth between the two chambers in most months. The most obvious exceptions were in March (see above) and December. In March, the growth of animals was affected in chamber 3, but chamber 4 showed no inhibition of growth, which seems to point to some factor in the seston. In December, the animals in chamber 3 did show better growth than those in
chamber 2, but less than in chamber 4. This would seem to indicate that there is some factor in the seston that prevents full use of the enrichment by animals in chamber 3.

During many of the experiments, chamber 3 exhibited slightly better growth than chamber 4 initially, but by the end of the experiment growth in chamber 4 had equalled that in chamber 3. The greater final length of the chamber 3 animals seen in May and June (Fig. 9) are probably an artifact because so few animals in chamber 4 survived to the end of the experiments. In the May run, chamber 4 had the fastest growth the first 5 days of the experiment, but this changed after Day 5 when chamber 3 showed the greatest increase in length. This changeover corresponds with my use of the newly collected lake seston. Chamber 1 also shows a similar increase in the amount of growth, but chamber 2 does not. This may indicate that either the old seston was losing its food value or that a change had occurred in the natural lake seston that increased its food value over that of just *Chlamydomonas*. Examining Figure 3, I found that chlorophyll-a levels were increasing at this time. The increase in growth from chamber 2 was not as great as in the other chambers receiving lake seston. It is likely that the extra energy was used for reproduction (Arnold, 1971). Growth in chamber 3 was greater than in
Figure 9. Growth in *Daphnia pulex* during May and June 1982.
chamber 4 by the end of the experiment. The lower growth in chamber 4 is not an artifact due to mortality (Figure 9). It is probably not due to senescent algae either, because chamber 3 should have also been affected. It is possible that for larger *Daphnia pulex*, $10^5 \text{cells} \cdot \text{ml}^{-1}$ is no longer a "food unlimited" regime and the extra energy provided by the seston allows more growth. Alternately, the seston may provide nutrients that make the regime in chamber 3 more "complete" and allow better growth.

In January, there are some unusual patterns, not seen in the previous months (Fig. 10). Chamber 3 shows more growth than Chamber 4 throughout most of the experiment. Even more surprising, chamber 4 doesn't exhibit greater growth than chamber 1 (fed only lake seston) for the first two-thirds of the experiment! This fact would point to some toxic effect of the enrichment algae except for the fact that chamber 3 does not show a similar limitation. It is possible that there might have been something in the *Chlamydomonas* (due to senescence perhaps) that offset the benefits the extra food present. But if this were the case chamber 3 should have been affected too. Another explanation, chambers 2 and 4 may not have been cleaned properly and they were subject to some chemical contamination. The point for Chamber 3 not included on the line at Day 11 was due to errors I made measuring the
Figure 10. Growth in *Daphnia pulicaria* during November, December 1982 and January 1983.
animals and so I did not extend the line through the point, but I did include it for illustration.

Reproduction

Reproduction in D. pulex showed a pattern very similar to those seen in growth during each month's experiment. Over the course of the study, Chamber 1 consistently showed the least reproduction. In most months the animals fed only lake seston (chamber 1) showed little, if any reproduction over the duration of the experiment. On those rare occasions when the reproduction of the animals in chamber 1 was slightly higher than in chamber 2, the results are due to synchronicity of reproduction in the animals in one of the two chambers. Reproduction in the unlimited food regimes (chambers 3 and 4) was invariably greater than that in chamber 2 with the sole exception of the month of March 1982. As mentioned previously, this same month showed unusually slow growth in chamber 3, which may have been due to the effects of something in the seston.

The reproduction data for February is similar to the growth data for that month. Figure 11 shows that the animals in chamber 2 were probably less food limited than those in chamber 1 since the first eggs appeared 7 days earlier and the number of neonates produced per female per day ($\# \cdot \frac{1}{d}$) was greater. Examining the
Figure 11. Neonates (or Eggs) produced per female, per day in February, March and April 1982.
chlorophyll-a data (Fig. 4) I found that February was the
month with the lowest relative enrichment (29.5 percent).
It seems that what benefit the animals in chamber 2 derive
from the enrichment is shunted into reproduction, not
growth. This supports the observations of Arnold (1971),
who found that the response to changing food conditions is
principally seen in changes in reproduction. Animals in
chamber 2 did produce a total of 2 ephippia (resting
embryos) on days 11 and 18. Ephippia are normally
produced when crowded or poor food conditions exist.
Conditions are obviously less than optimal in chamber 2,
even with the enrichment. Reproduction started earlier in
chamber 3, but once reproduction began in chamber 4, the
animals produced more $\# \cdot d^{-1}$ than in chamber 3. There
was no significant difference in the average initial
length of animals in these two chambers ($F=0.53 P>0.25$),
so earlier reproduction is probably not due to age
differences of the animals. It would seem that there is
some factor in the seston that allows faster initial
growth and earlier reproduction in Daphnia pulex.

In March, A 100 fold increase in enrichment ($10^5$ as
opposed to $10^3$ cells per ml) failed to increase either
growth or reproduction in chamber 3 over that in chamber
2. In fact, even though reproduction was delayed in
chamber 2, it did show more reproduction than chamber 3 by
experiment's end (Fig. 11). Though the animals did
benefit from the enrichment to a certain degree, there is probably some factor in the seston that prevents the animals from deriving the full benefit of the enrichment in chamber 3. Unexpectedly, a low level of enrichment benefits growth and reproduction of *Daphnia pulex* but a higher enrichment does not. If the effect were due to the enrichment alga, growth and reproduction should have also been affected in chambers 2 and 4, and this was not found. Reproduction data (Fig. 8) shows no reproduction over the 10 day run in Chamber 1 and the most reproduction taking place in chamber 4. Chambers 2 and 3 show a situation similar to that found in Chambers 4 and 3 in February, with reproduction coming earlier in chamber 3 but the delayed onset of reproduction in chamber 2 resulting in more #.♀-1.d-1 being produced. Again, I expected to find greater reproduction in chamber 3 than in chamber 2.

As noted earlier, April's reproduction data are graphed as eggs per female per day since neonate production was low (Fig. 11). Reproduction in the April experiment followed the same pattern seen in February. In Chamber 1 there was no reproduction during the course of the experiment. In Chamber 2 no neonates were produced but eggs were found on the last day of the experiment. The animals in chamber 3 started reproducing slightly earlier than chamber 4 (1 egg in 1 female on day 9), but was out reproduced on the basis of neonates produced per
female per day by chamber 4. The fact that no neonates were produced over the course of the experiment, despite the higher temperature, could be due to the fact that the average initial lengths of the animals in this month's study are slightly smaller than in previous months. Compared with February's data the total amount of growth seen in April is higher.

I found far more reproduction taking place in the chambers in May than in previous months (Fig.12). This is due to a longer experimental duration (20 days) and the higher temperature which increased growth rates and brood sizes and decreased development times (Lei and Armitage, 1980). In fact, starting with Day 9, so many eggs were being produced in chambers 3 and 4, that I could only estimate the number of eggs/female. By the end of the experiment there was no difference in the amount of reproduction taking place in chambers 1 and 2. As can be seen from Figure 12, reproduction began much earlier in chamber 2 so it is safe to assume that the animals are less food limited than those in chamber 1. The increase in reproduction slowed somewhat in chamber 3 and decreased dramatically in chamber 4 on the last day of the experiment. I thought that this might be due to the fact that there were so many animals in the chambers during the latter part of the experiment that the neonates were depleting the food available to the adults even with a
Figure 12. Neonates produced per female per day in May and June 1982.
complete turnover of chamber contents twice each day. I was removing up to 168 neonates from chambers 3 and 4 every 2 days by the end of the experiment. Another explanation might be that synchronicity of reproduction among the animals contributed to the dramatic decrease in the number of neonates per female per day in chamber 4. This is a distinct possibility given the small number of animals in each chamber. I found males among the neonates of chamber 4 on Day 17 and in chamber 3 on day 20. I also found ephippia in Chamber 2 on Days 14 and 20 and in chamber 4 on Days 14, 17, and 20. Both of these phenomena are probably due to the crowded conditions in the chambers. The average production of neonates per female over the entire 20 day period was 45.71 for chamber 3, 39.02 for chamber 4, 1.42 for Chamber 2 and 1.14 for Chamber 1. So the reproductive patterns seem to parallel those of growth this month. It should be noted that May was the month of highest Daphnia pulex abundance in Boulder Basin, Lake Mead in 1982 (Figure 15). Growth and reproduction in chambers 1 and 2, when compared with chambers 3 and 4, show a definite pattern of food limitation this month.

I found from the the data in Figure 12 that the lowest level of reproduction in June was found in Chamber 1. The surviving females did not reproduce soon enough to release neonates by Day 20 although they did contain eggs.
The graph for Chamber 2 looks the way it does due to reproductive synchronicity among the animals. Chamber 3 shows a steady increase in the number of neonates produced per female per day throughout the course of the experiment. The lone individual in chamber 4 neither grew nor produced eggs during the last 6 days of the experiment. Despite the loss of neonates on Day 20, the number of eggs per female in the surviving animals indicates that reproduction was still increasing in Chambers 1-3. These results show a pattern of reproduction similar to that found in May. Reproduction in animals fed only lake seston was extremely low both months. The large peak in chamber 4 is due to reproductive synchrony among the animals in the chamber and the sharp decrease after Day 14 is due to mortality in the chamber where 4 of the remaining 5 animals died.

The reproduction data in November (Fig. 13) shows similar patterns of reproduction in chamber 3 and chamber 4. The sudden decrease in reproduction after day 13 can be explained by synchronicity of reproduction among the animals in the chambers since the number of eggs per female continued to increase in chamber 4, and decreased only slightly in chamber 3. Initially, reproduction was higher in chamber 3. By the end of the experiment, chamber 4 was producing more neonates per female per day than chamber 3. Chamber 1 showed more reproduction than
Figure 13. Neonates (or Eggs) produced per female per day in November, December 1982 and January 1983.
chamber 2, probably due to the fact that the animals were initially larger, and therefore closer to reproductive age than those in Chamber 2. Two days later Chamber 2 was producing more neonates $\varphi^{-1} \cdot \text{day}^{-1}$ than Chamber 1 and continued to do so for the rest of the experiment. The peak on Day 17 for Chamber 1 is a statistical anomaly due to the small number of animals in the chamber. It represents a total of 2 neonates produced over the last two days by the last surviving individual. No ephippia or males were produced by any animals during the experiment.

The animals in chamber 4 were the only ones that produced neonates by the end of the experiment in December. To better illustrate how the animals were reproducing, Figure 13 shows the number of eggs produced per female per day. Chamber 4 shows the highest production of eggs followed by chamber 3 and chamber 2. Again this seems to indicate some inhibitory effect of the seston on the growth and reproduction of Chambers 1-3. I observed no production of ephippia or males during the experiment. Clearly, from this month's data there is definite food limitation and probably negative effects due to some factor in the seston.

Reproduction data for January was also plotted as the number of eggs produced per female per day (Fig. 13). Chambers 3 and 4 were the only ones to produce eggs during the experiment, a result which indicates a definite
pattern of food limitation. As mentioned previously, growth in chamber 4 was extremely slow during the first part of the experiment. Whatever the cause of the slower growth in chamber 4, it did not seem to affect egg production as would be expected if there were a chemical contamination (Figure 13). By the end of the experiment, the animals in chamber 4 were producing more eggs per female per day than in chamber 3. Chamber 3 animals did start producing eggs earlier, a pattern seen before in February, April and November. (For a detailed description of survivorship, growth and reproduction month-by-month, see Appendix 1)

July-October 1982

I was unable to complete any experiments in July, August, September and October due to the extremely low abundance of Daphnia pulex in Boulder Basin, Lake Mead. I was unable to isolate sufficient animals of the proper length (only 10 animals less than 1.4mm length in July) to conduct the experiments. Even using offspring of gravid females to augment collected zooplankton did not provide the 40 animals I needed. When I studied the data for zooplankton abundance in 1982 (Fig. 14) I found that the months in question had among the lowest abundance of zooplankton at this station for the entire year (no data available for September). Further, I found that the
Figure 14. Zooplankton abundance in Boulder Basin, 1982.
Figure 15. Composition of Zooplankton community in Boulder Basin, 1982.
months of August-October are the months when *Daphnia pulex* is least abundant in Boulder Basin (Fig. 15). I attempted to run experiments in each of the months July-October with the few animals available. However, I could not draw any meaningful conclusions from the data due to the small numbers of individuals available, large differences in the average initial lengths of the animals and the short duration of the experiments due to high mortality. However, given the low levels of chlorophyll-a (Fig. 4) and the low abundance of *Daphnia pulex* during these months (Fig. 15), I feel it is safe to assume that *Daphnia pulex* is food limited during this period.

**DISCUSSION**

The data I collected from the 8 successful experiments shows a clear pattern of food limitation in *Daphnia pulex* from Boulder Basin, Lake Mead. At no time during my study did growth or reproduction in animals fed only lake seston approach that of the food unlimited animals in chamber 4 (apart from the exception noted for chambers 1 and 4 in January, 1983). It is possible (but not very likely) that the duration of my experiments was too short to allow a proper appraisal of reproduction in the animals fed only lake seston (especially in the March experiment). The life span of *Daphnia pulex* is months, not just weeks long. Even so there is obviously a
considerable delay in the onset of reproduction (possibly more than 16 days depending on the season). No data was obtainable for the months July-October 1982 but given the extremely low levels of *Daphnia pulex* present and the low chlorophyll-a concentrations in the lake during those months, the low abundances of *Daphnia pulex* present in Boulder Basin in the summer (Paulson and Baker 1980, 1983 and Wilde 1984), and the difficulty I had in running experiments during these months, I doubt that the animals would have shown a different pattern. Another sensitive indicator of the nutritional state of cladocerans is the L-0 (lipid-ovary) index of Tessier and Goulden (1982). Cladocerans such as *Daphnia* store their energy reserves as droplets of triglycerides (Tessier and Goulden 1982, Goulden and Hornig 1983). During reproduction these lipid droplets are transferred to the ovary and a droplet of lipid is deposited in each egg. By ranking organisms as to the amount and size of the lipid droplets present, and the opacity of the ovaries, a relative index of the nutritional state of Cladocerans can be determined. I used the L-0 index during the November, December and January runs. I found that the *Daphnia* in chambers 3 and 4 had much higher indices than those in chambers 1 and 2 (Bartanen unpublished). These results very closely paralleled those of growth and reproduction.
The average relative enrichment, based upon chlorophyll-a, was just over 75 percent for chamber 2. It was enough that it did increase the amount of growth and reproduction slightly in most of the months except February 1982, and January 1983 when the animals in chamber 2 actually showed slower growth. I looked to Janik (1984) and his study of phytoplankton succession in Lake Mead for clues as to what might have caused these anomalies. During the winter the phytoplankton is dominated by cryptomonads (>60 percent of phytoplankton biomass by size, Figure 16 (used with permission), from Janik (1984). Almost 80 percent of the biomass present in the lake at this time is <44μm equivalent spherical diameter so there should be adequate amounts of food of appropriate size for Daphnia pulex (Figure 17, from Janik, 1984 (used with permission)). An unanswered question remains, is there any inhibitory effect on the filtering rate of Daphnia pulex when fed cells of borderline size? As noted earlier, cells > 35 μm equivalent spherical diameter, are thought to be too large to be ingested by any but the largest Daphnia pulex. If this is so, then more information is needed on the utilization of cells 35 to 44μm equivalent spherical diameter in Lake Mead.

For most of the study, growth was the same in chambers 3 and 4. In March and December growth was depressed in Chamber 3 indicating that there may have been
some fraction of the seston that inhibited feeding. Examination of Figure 17 shows that during those two months in 1981 (one year earlier) the phytopankton biomass was over 85 percent and 75 percent (respectively) comprised of cells larger than 21μm equivalent spherical diameter and over 65 percent and 45 percent (respectively) of cells greater than 44μm in diameter. Assuming that there is at least a rough correspondence between the phytoplankton succession in 1981 and 1982 would mean that much of the phytoplankton biomass was unavailable to Daphnia pulex. The further effect of these large cells would be to increase the rejection rate and prevent full use of the enrichment by the animals. Further examination of Janik’s data (Fig. 16) reveals that in both of these months the phytoplankton was dominated (over 60 percent of the biomass) by members of the cryptophyta (Cryptomonas and Rhodomonas).

Although Cryptomonas and Rhodomonas are small and digestible, Schindler (1971), Wetzel (1975 p. 449) and Nadine-Hurley and Duncan (1976) indicate that Cryptomonads may not be readily consumed by Daphnia. The results of a study by Sarnelle (1986) indicate that Cryptomonas and Rhodomonas do not support high reproduction in cladocerans. If this is true, it offers another mechanism for the reduction of growth and reproduction in a treatment which should not have been food limited. At
Figure 16. Monthly percentage of phytoplankton biomass represented by various taxa in Lake Mead, 1981-1982.
Figure 17. Percentage of phytoplankton biomass represented in various size fractions in Lake Mead, 1981-1982.
least for some months of the year, my results do seem to confirm those of (Ryther 1954; Burns and Rigler 1967; Burns 1968; Crowley 1973) who found lower growth and reproduction in lake water than in mixed lake water and pure algal enrichment. I also noted a pattern in the onset of reproduction in chambers 3 and 4. Reproduction started earlier in chamber 3, but when reproduction did begin in chamber 4 (usually a couple of days later) the animals produced more neonates per female per day. There may be some nutritional factor in the lake seston that causes a change in the pattern of reproduction.

A note of caution in interpreting these results. The data in Janik (1984) is from March 1981 through February 1982 and the only month our studies overlapped was February 1982. Paulson and Baker (1983) found that productivity in Boulder Basin, Lake Mead was lower in 1982 than in 1981 and this probably had an effect on the phytoplankton succession at this station.

As mentioned above, Paulson and Baker (1983) found decreasing productivity in Boulder Basin, which they attributed to lower phosphorus inputs from the Wash due to the operations of the Advanced Treatment Plant. As I noted in the section on the history of Lake Mead there has been a pattern of declining productivity or abundance at every trophic level. My study shows that the reductions in *Daphnia pulex* abundance are due in part to food
limitation, not solely due to predation or competition with other organisms. Although predation and competition are certainly important in regulating the numbers of Daphnia in the lake. A feeding experiment conducted in 1981 (Paulson and Baker 1983) got results very similar to mine using water from more productive areas of the lake (the Inner and Middle Las Vegas Bay). This shows that the lake is capable of supporting higher growth and reproduction in Daphnia if more nutrients are supplied to phytoplankton. The project currently under way (personal communication with L. J. Paulson, 1987), to fertilize an arm of Lake Mead to improve productivity should yield very interesting results. If the enrichment proves successful to phytoplankton species that are suitable forage for zooplankton like Daphnia, the project should meet with success.
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APPENDIX 1: EXPERIMENTAL CHRONOLOGY

February 1982

Animals for the first experiment (February) were collected on 22 January, 1982 and by 28 January, 1982 I had enough algae cultured to start the experiment. During the interim (as with other months), I added C. reinhardtii to the container with the collected animals to keep them from being starved. This experiment ran for a total of 18 days at a temperature of 12°C. I found the average initial length of animals in chamber 2 to be significantly larger than the other chambers ($F=34.09, P<.0005$).

Figure 8 shows the increase in body length over time for the animals in each treatment. There is very little difference in the amount of growth in chambers 1 and 2. This is unusual since I expected growth in chamber 2 to be higher than in chamber 1. The reproduction data (Figure 11) however, shows that the animals in chamber 2 were probably less food limited since the first eggs appeared 7 days earlier and the number of neonates produced per female per day ($\# \cdot \varphi^{-1} \cdot d^{-1}$) was greater. Animals in chamber 2 did produce a total of 2 ephippia (resting embryos) on days 11 and 18. Epipphia are normally reproduced when crowded or poor food conditions exist. Conditions are obviously less than optimal in chamber 2, even with the enrichment.
Chamber 3 exhibited slightly better growth than chamber 4 initially. By the end of the experiment growth in chamber 4 had equalled that in chamber 3. The decrease in growth on day 11 (chamber 4) and day 18 (chamber 3) was linked to the deaths of 1 or more large individuals in the chambers which lowered the average length of the animals. Reproduction started earlier in chamber 3, but once reproduction began in chamber 4, the animals produced more #. *f*-1 *d*-1 than in chamber 3. There was no significant difference in the average initial length of animals in these two chambers (F=0.53 P>0.25), so earlier reproduction is probably not due to age differences of the animals.

Figure 5 shows the survivorship curves for the first run. A Kolmogorov-Smirnov goodness of fit test (Zar 1974) showed no significant difference in the survivorship of the animals in the 4 treatments (D=.16667, n=12, P>.05). Testing H₀: Mortality is constant over the duration of the experiment in a given chamber with a Kolmogorov-Smirnov test for goodness of fit, I found that mortality was not constant over the course of the experiment in chamber 1 (Table 3). All mortality occurred in the last 5 days of the experiment. There were no significant changes in mortality over time in the other chambers (Table 3).

March 1982
Experiment 2 animals and seston were collected on 19 February and I began the experiment on 10 March. The shorter duration of the experiment in Chamber 1 was due to a problem with the apparatus (Table 2). Chamber 1 was restarted with new animals but could not be run as long as the others to prepare for the April experiment. I had fresh lake water collected on 4 March, so the experiment was not run on old seston (Table 1).

Figure 8 shows the growth data for March. There was a significant difference in the initial length of the animals, with the chamber 1 animals being significantly shorter than the others ($F=4.02 \ P <.0005$). In this experiment the greatest growth was seen in the animals fed only *Chlamydomonas reinhardtii* (chamber 4). The least growth was seen in the animals fed only lake seston (chamber 1). The fact that there appears to be no difference in growth between the enrichments of $10^3$ and $10^5$ cells/ml of *Chlamydomonas reinhardtii* is unexpected. A 100 fold increase in enrichment failed to increase either growth or reproduction in chamber 3 over that in chamber 2. In fact, even though reproduction was delayed in chamber 2, it did show more reproduction than chamber 3 by experiment’s end.

The data (Fig. 11) shows no reproduction over the 10 day run in Chamber 1 and the most reproduction taking place in chamber 4. Chambers 2 and 3 show a situation
similar to that found in chambers 4 and 3 in February, with reproduction coming earlier in chamber 3 but the delayed onset of reproduction in chamber 2, resulting in more \( \# \cdot \varphi^{-1} \cdot d^{-1} \) being produced. Figure 5 shows the survivorship curves for the March experiment. There were no significant differences in survivorship either between the treatments \( (D=.30000, n=10, .5 > P > .2) \) or within the chambers over the course of the experiment (Table 3).

April 1982

Animals and water for the April experiment were collected on 25 March and I began the experiment on 31 March at a temperature of 14° C (Table 1). There was no significant difference in the initial lengths of the animals \( (F=0.22, P>.25) \). The duration of the experiment was 14 days and was terminated due to lack of Chlamydomonas from culture plates. Growth was slowest in Chamber 1, Chamber 2 showed a definite enhancement of growth and Chambers 3 and 4 even more enhancement (Fig. 8). Chambers 3 and 4 show no difference in the amount of growth at the end of the experiment although growth was a little slower initially in chamber 4. This lag in growth can be explained by the high early mortality in chamber 4 which resulted in lower average lengths when compared to chamber 3. The flat portion of the graph between days 7
and 9 for chamber 2 is not an anomaly. There was no growth in the chamber during this period.

This month's reproduction data are graphed as eggs per female per day since neonate production was low (Fig. 11). Reproduction in the April experiment followed the same pattern seen in February. In Chamber 1 there was no reproduction during the course of the experiment. In Chamber 2 no neonates were produced but eggs were found on the last day of the experiment. The animals in chamber 3 started reproducing slightly earlier than chamber 4 (1 egg in 1 female on day 9), but was out reproduced on the basis of neonates produced per female per day by chamber 4. Survivorship curves are shown in Figure 5. I found no significant difference in the survivorship over the course of the experiment (Table 3), nor was there any significant difference between the treatments (D=.16667, n=15, P>.05).

May 1982

I collected the animals on 22 April and began the experiment on 30 April at a starting temperature of 15° C. I received fresh seston on 4 May which was used until 20 May, the end of the experiment (see Table 1). The average initial lengths of the animals in the four treatments were significantly different (F=3.08, .05<P<.25). The animals in chamber 2 were slightly longer than the others.
I found that chamber 1 animals had the least growth followed by chamber 2, chamber 4 and chamber 3 (Fig. 9). Over the first 5 days of the experiment, chamber 4 had the fastest growth but this changed after Day 5 when chamber 3 showed the greatest increase in length. Chamber 1 also shows a similar increase in the amount of growth, but chamber 2 does not. Growth in chamber 3 was greater than in chamber 4 by the end of the experiment. The lower growth in chamber 4 is not an artifact due to mortality (Figure 6). It is probably not due to senescent algae, since chamber 3 should have also been affected.

Starting with Day 9, so many eggs were being produced in chambers 3 and 4, that I could only estimate the number of eggs/female. The increase in reproduction slowed somewhat in chamber 3 and decreased dramatically in chamber 4 on the last day of the experiment (Fig. 9). I thought that this might be due to the fact that there were so many animals in the chambers during the latter part of the experiment that the neonates were depleting the food available to the adults even with a complete turnover of chamber contents twice each day. I was removing up to 168 neonates from chambers 3 and 4 every 2 days by the end of the experiment. Another explanation might be that synchronicity of reproduction among the animals contributed to the dramatic decrease in the number of neonates per female per day in chamber 4. This is a distinct
possibility given the small number of animals in each chamber.

To estimate the filtering rate of *Daphnia pulex*, I used the formula derived by Burns (1969). I calculated the filtering rates per day for neonates 0.5 mm and 1.0 mm in length at 15°C. I did not determine lengths for neonates removed from the chambers so I chose 0.5mm as the length of a neonate since it was the shortest initial length of any animal used for my experiments. I also included the figure for animals of 1 mm length. Animals this long are probably larger than any neonates but they are approximately the starting length of the experimental animals. In calculating the total volume filtered for the largest number of animals in a given chamber, I included the volume filtered by the adult animals (using the average length of the adults that day). The filtering rate is 396 ml·day⁻¹ (40 percent of the 1 liter of suspension delivered to the chamber each day) for neonates assumed .5mm in length. For neonates assumed 1.0mm length, the filtering per day was 875 ml. The actual figure is probably somewhere between these two extremes. Therefore, the animals are not totally depleting the food suspension, but they are filtering a significant portion of it. Unfortunately, I had to terminate the experiment before I could determine whether reproduction recovered.
I found males among the neonates of chamber 4 on Day 17 and in chamber 3 on day 20. I also found ephippia in Chamber 2 on Days 14 and 20 and in chamber 4 on Days 14, 17, and 20. The average production of neonates per female over the entire 20 day period was 45.71 for chamber 3, 39.02 for chamber 4, 1.42 for Chamber 2 and 1.14 for Chamber 1.

Analysis of survivorship (Fig.6) for May showed no significant differences within the chambers over time (Table 3). Kolmogorov-Smirnov goodness of fit showed no significant difference in the amount of mortality between the 4 treatments (D=.25, n=8, .5> P>.2). A heterogeneity Chi-square analysis (Zar 1974) did show a significant difference ($\chi^2=8.000$ d.f.=3 <7.815 $a=0.05$). This is the only month that a Heterogeneity Chi-square analysis found a significant difference in the survivorship in the four treatments (all $\chi^2$ calculations at the 5 percent level of significance with 3 d.f.). Chambers 2 and 3 did show a decrease in survivorship in the period just after the new lake seston was first used.

June 1982

I collected the animals for the experiment on 3 June and began the experiment on 8 June at a starting temperature of 18° C (see Table 1). I found no significant difference in the starting lengths of the
animals (F=2.39, .1 > P > .05). Figure 9 shows the increase in body length for the June experiment. In a situation similar to May, I found that Chamber 1 showed the least amount of growth and that the enrichment in chamber 2 increased growth. There doesn't seem to be much difference in amount of growth in chambers 3 and 4. There was no growth in chamber 4 over the last 3 days of the experiment is because there was only 1 animal in the chamber which did not molt in that period of time. This is also why the last two points on the curve for chamber 4 have no error bars.

I found from the the data in Figure 12 that the lowest level of reproduction was found in Chamber 1. The surviving females did not reproduce soon enough to release neonates by Day 20 although they did contain eggs. The graph for Chamber 2 looks the way it does due to reproductive synchronicity among the animals. Chamber 3 shows a steady increase in the number of neonates produced per female per day through out the course of the experiment. The lone individual in chamber 4 neither grew nor produced eggs during the last 6 days of the experiment. The apparent growth in chamber 4 for days 14-17 was due to the deaths of some shorter individuals. Despite the loss of neonates on Day 20, the number of eggs per female in the surviving animals indicates that reproduction was still increasing in Chambers 1-3.
I found that survivorship was significantly different (Table 3) in Chambers 2 and 3 over the 20 day run of the experiment, with most of the mortality occurring in the first 5 days of the experiment (Figure 6). I did not find any significant difference in survivorship between the four chambers (D=.15909, n=22, P>.5).

November 1982

I collected the animals for this experiment on 11 November and started the actual experiment at 18°C on 17 November (see Table 1). I found that there was a significant difference in the average initial length of the animals with those individuals in Chamber 1 being longer (F=4.02, .025>P>.01).

The increase in body length for the four treatments is shown in Figure 10. The animals in Chamber 1 showed steady growth until Day 6 when the amount of growth abruptly slowed. These animals increased in length only slightly during the rest of the experiment and reproduction (after Day 8) was also low. Examining the survivorship data (Fig. 7) I found that mortality increased dramatically after Day 6. Death of individual animals occurred in chamber 1 until Day 17 when I found 3 dead animals in the chamber and the last, obviously dying. The mortality was not due to any physical damage that I
could detect. The decrease in the rate of growth did not occur until after day 8 in chamber 2.

Survivorship did not change significantly over time in chambers 1, 2 and 4 (Table 3), but was significantly different for chamber 3 ($D= .71429, n=5,.005 > P > .002$). In chamber 3, there was high mortality the first 6 days of the experiment. Comparing the 4 chambers to each other, I did not find any significant differences in the amount of mortality ($D=.23913, n=23,.02 > P > .01$).

The reproduction data (Fig. 13) shows similar patterns of reproduction in chamber 3 and chamber 4. The peak on Day 17 for Chamber 1 is a statistical anomaly due to the small number of animals in the chamber. It represents a total of 2 neonates produced over the last two days by the last surviving individual. No ephippia or males were produced by any animals during the experiment.

**December 1982**

I ran the December experiment for a total of 12 days at a starting temperature of $14^\circ$ C. I collected animals and seston on 16 December and started the experiment on 18 December. It was terminated when I ran out of lake seston (see Table 1). There was a significant difference in the average initial lengths of the animals in the 4 treatments ($F=4.02,.025 > P > .01$)
Figure 10 shows the growth of the animals in each of the treatments for this month. In Chamber 1, the slight decrease in average length on Day 10 was due to the death of 1 large individual and the large error bars on Day 12 are due to a difference in size of over 0.5 mm between the two surviving individuals. Growth in Chamber 2 seems to be better than in Chamber 1 but it is not significant due to the large variation in the length of the animals in Chamber 1 (F=2.05 d.f.=1,6 .25>P> .1). Chamber 4 seems to have better growth than chamber 3. The growth response in chambers 2 and 3 is similar to that seen in March, except that this month chamber 3 does seem to benefit from the hundredfold increase in enrichment.

The animals in chamber 4 were the only ones that produced neonates by the end of the experiment. Figure 13 shows the number of eggs produced per female per day. Chamber 4 shows the highest production of eggs followed by chamber 3 and chamber 2. I observed no production of ophiopodia or males during the experiment. Figure 7 shows the survivorship data for December. Chambers 2 and 4 had similar survivorship patterns and in both of these chambers survivorship was not constant over time (Table 3). Most of the mortality occurred during the first four days and may be due to poor condition of the collected animals. Chambers 1 and 3 were also similar to each other and showed no significant differences in mortality over
time. I found no significant differences in survivorship among the 4 treatments ($D=.11364, n=22, P>.5$). The flow interruptions (Table 2) did not seem to have an effect on survivorship.

January 1983

I collected the animals for this final experiment on 6 January and started the experiment the next day on 7 January. I ran the experiment for 16 days at a constant temperature of 12°C (see Table 1). The average initial length of the animals in each of the treatments were not significantly different ($F=0.24, P>.25$).

In Figure 10, I have plotted the growth data for this month. Chamber 1 shows more growth than chamber 2 during the first two-thirds of the experiment. Mortality (Fig. 7) among large individuals does not account for the slow growth in Chamber 2. Chamber 3 also shows more growth than Chamber 4 throughout most of the experiment. Chamber 4 doesn't exhibit greater growth than chamber 1 (fed only lake seston) for the first two-thirds of the experiment. This fact would point to some toxic effect of the enrichment algae except for the fact that chamber 3 does not show a similar limitation. The point for Chamber 3 not included on the line at Day 11 was due to errors I made measuring the animals and so I did not extend the
line through the point, but I did include it for illustration.

The final average length in chamber 4 did not differ from that in chamber 3. It did not seem to affect egg production as would be expected if there were a chemical contamination (Figure 13). By the end of the experiment, the animals in chamber 4 were producing more eggs per female per day than in chamber 3. Chamber 3 animals did start producing eggs earlier, a pattern seen before in February, April and November. My statistical analysis did not find any significant differences in survivorship over the course of the experiment (Table 3) or in the mortality between the treatments (D=.15, n=10, P>.5).